Proficiency test for the metals copper and zinc in compound feed

D.P.K.H. Pereboom, H. Brust and M. de Nijs

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Project leader: M. de Nijs
Scientist: H. Brust

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P.O. Box 230, 6700 AE Wageningen, The Netherlands, T +31 (0)317 48 02 56, E info.wfsr@wur.nl, www.wur.eu/food-safety-research. WFSR is part of Wageningen University & Research.

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Distribution list:
- Participating laboratories

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Summary

A proficiency test (PT) for the determination of copper and zinc in compound feed was organised by Wageningen Food Safety Research (WFSR) between January and May 2021 in accordance with ISO/IEC 17043. WFSR is accredited for the organisation of proficiency tests in the field of contaminants, pesticides, mycotoxins, plant toxins and veterinary drugs in feed and feed ingredients according to ISO/IEC 17043 (R013). The primary goal of this proficiency test was to give participants the opportunity to evaluate and demonstrate their competence for the analysis of copper and zinc in compound feed.

For this proficiency test, two materials were prepared and dispatched. The consensus value (based on moisture content of 12%) of the metals in each material are given in Table 1.

Table 1 *Consensus values and RSD_R of the copper and zinc in the proficiency materials.*

	Material A (feed sows)		Material B (feed piglets)	
Compound	**Consensus value mg/kg**	**RSD_R (%)**	**Consensus value mg/kg**	**RSD_R (%)**
Copper	20.4	12	133	10
Zinc	96.7	7	128	7

Forty-five participants subscribed for the participation in this PT, of which 44 reported results. One participant submitted the results seven days after the closing date of reporting, but the results were nevertheless included in the evaluation. One participant was unable to report results.

Materials A and B were prepared by grinding and extensive mixing of two commercially obtained piglet and sow compound feeds. Both materials were sufficiently homogeneous and stable during the PT. Each participant received one test sample of each material.

All participants submitted results for copper and zinc. One participant analysed only material A.

For both materials (A and B), 88% of the copper and zinc results were rated with satisfactory z-scores ($|z| \leq 2$), 5% of the results fell in the range of questionable results with $2<|z|<3$, and 7% of the results fell in the range of unsatisfactory results with $|z| \geq 3$.

Thirty-three participants achieved optimal performance for both materials by detecting both metals copper and zinc with the correct quantification, the absence of false negative results and reporting within the indicated deadline. Eleven participants reported questionable or unsatisfactory z-scores. No false negative results were reported. The results of this PT on copper and zinc are summarized in Table 2.

Table 2 *Summarized performance of laboratories reporting results in the proficiency test on copper and zinc in materials A and B.*

Compound	# of results	Satisfactory performance (%)
	Material A	
Copper	44	89
Zinc	44	86
	Material B	
Copper	43	91
Zinc	43	86

Based on the results submitted by the participants of this PT it can be concluded the participants are capable of satisfactory quantification of copper and zinc in piglet and sow compound feed. The interlaboratory reproducibility (RSD_R) ranged from 7 – 12%. The satisfactory results for copper and zinc varied from 86 to 91%.

1 Introduction

Proficiency testing is conducted to provide participants with a powerful tool to evaluate and demonstrate the reliability of the data that are produced by the laboratory. Proficiency testing is an important requirement and demanded by ISO/IEC 17025:2017 [1].

The preparation of the materials, including the homogeneity and stability testing of the materials, and the evaluation of the quantitative results were carried out under accreditation according to ISO/IEC 17043:2010 [2] accreditation by the Dutch Accreditation Board (R013).

The maximum levels of the metals copper and zinc are regulated in Regulation (EC) No 1334/2003 [9] and amendments thereof. The maximum level (ML) for copper in complete feed for piglets, suckling and weaned up to four weeks after weaning is 150 mg/kg and from the 5th week after weaning up to 8 weeks after weaning 100 mg/kg. The ML for copper in sow compound feed is 25 mg/kg. For zinc, the MLs for both the piglet feed and sow compound feed are 150 mg/kg.

The aim of this proficiency test was to give participants the opportunity to evaluate or demonstrate their competence for the analysis of copper and zinc in piglets and sow compound feed.

2 Material and methods

2.1 Scope of the proficiency test

This proficiency test (PT) focused on the metals copper and zinc in compound feed, using commercially obtained piglet and sow compound feed as representative matrices. The target concentrations aimed for are presented in Table 3 and took the regulatory limits into account.

Table 3 *Target concentrations mg/kg of copper and zinc in the PT materials.*

	Target concentrations (mg/kg)	
Compound	**Material A (sow feed)**	**Material B (piglet feed)**
Copper	25	150
Zinc	100	150

2.2 Material preparation

For preparation of the two PT materials A and B, respectively, commercial piglet and sow compound feed were used. For each material, three kilograms were milled using a centrifugal mill (ZM 200, Retsch, Haan) to obtain a particle size of 500 µm. The materials were homogenised by extensive mixing using a Stephan Cutter UM12 according to the in-house standard operating procedure [3].

2.3 Sample identification

After homogenization, materials A and B were divided into sub-portions of approximately 25 grams and stored in polypropylene, airtight closed tubes of 50 ml at room temperature until use.

The samples for the participants were randomly selected and coded using a web application designed for PTs. The code used was "2021/metals/compound feed/000", in which the three digit number of the code was automatically generated by the WFSR Laboratory Quality Services web application. One sample set was prepared for each participant. Each sample set consisted of one randomly selected sample of material A and one of material B. The codes of the samples for each sample set are presented in Annex 1. The samples for homogeneity and stability testing were also randomly selected tubes of material A and B.

2.4 Homogeneity study

To verify the homogeneity of the PT materials, ten containers of material A and B were analysed in duplicate for copper and zinc.

The homogeneity of both materials was evaluated according to The International Harmonized Protocol for Proficiency Testing of Analytical Laboratories [6] and ISO 13528:2015 [4] taking into account the insights discussed by Thompson [5] regarding the Horwitz equation. With this procedure the between-sample standard deviation (s_s) and the within-sample standard deviation (s_w) were compared with the standard deviation for proficiency assessment. The method applied for homogeneity testing is considered suitable if $s_w<0.5*\sigma_P$ and a material is considered adequately homogeneous if $s_s<0.3*\sigma_P$.

Ten containers of material A and B were analysed in duplicate to determine the homogeneity of the materials. Both materials proved to be sufficiently homogeneous for this PT. The results of the homogeneity study, grand means with the corresponding RSD, are presented in Table 4. The results of the statistical evaluation of materials A and B are presented in Annex 3.

Table 4 *Concentration of copper and zinc in materials A and B obtained during homogeneity testing.*

Material code	Material A		Material B	
	Concentration mg/kg	RSD %	Concentration mg/kg	RSD %
Copper	20.1	2.77	131	2.15
Zinc	98.2	2.22	132	1.86

2.5 Stability of the materials

The stability of copper and zinc in the PT materials was assessed according to the procedures [5, 7]. On March 29th, 2021, the day of distribution of the PT samples, six randomly selected tubes of each material A and B were stored at -20°C. Under these conditions it is assumed that copper and zinc are stable in the materials. In addition, six samples of each material were stored at room temperature.

On May 17th, 2021, 49 days after distribution of the samples, six samples of materials A and B, that were stored at -20°C and at room temperature, were analysed. For each set of test samples, the average of the results and the standard deviation were calculated.

It was determined whether a consequential instability of the analytes had occurred [5, 7] in the materials stored at room temperature. A consequential instability is observed when the average value of an analyte in the samples stored at room temperature is more than $0.3\sigma_P$ below the average value of the analyte in the samples stored at -20°C. If so, the instability has a significant influence on the calculated z-scores.

The results of the stability of materials A and B in this PT are presented in Annex 4. None of the tested storage conditions caused a consequential difference for the analytes in both materials. Copper and zinc in the materials were, therefore, considered stable for the duration of the PT.

3 Organisational details

3.1 Participants

Forty-five participants registered for the participation in the PT and 44 participants reported their results. Of the laboratories 39 were situated in Europe, one in Oceania and five in Asia. One participant was unable to report result due to custom clearance issues of the samples. Each participant was free to use their method of choice reflecting their routine procedures. The participants were asked to report the results through an existing web application designed for proficiency tests organised by WFSR.

3.2 Material distribution and instructions

Each participant received a randomly assigned laboratory code, generated by the web application. The sets of samples with the corresponding number, consisting of two coded samples (Annex 1) were sent to the PT participants on the 29th of March 2021. The sets of samples were dispatched by courier to the participants in carton boxes. The participants were asked to store the samples at room temperature and to analyse the samples according to their routine practice. As reported by the participants, all parcels were received in good order. One parcel took 5 weeks to reach the laboratory.

The samples were accompanied by a letter with instructions for the requested analysis (Annex 2) and an acknowledgement of receipt form. In addition, by e-mail, each participant received instructions on how to use the web application to report the results. Results should be reported as mg/kg product (relative to a feed with a moisture content of 12%). Participants were asked to provide information on their analytical method (sample preparation procedure, internal standards used, detection technique, limit of detection, limit of quantification).

A single analysis result for both the metals copper and zinc in each sample was requested. The deadline for submitting the quantitative results was the 10th of May 2021, allowing the participants six weeks for analysis of the test samples. All results, except one, were submitted within the deadline. Participant PT8863 was unable to report results in time due to the long delivery time of the sample. The participant reported the results 1.5 week later than the target deadline. This was not be seen as exceeding the deadline.

4 Statistical evaluation

The statistical evaluation was carried out according to the International Harmonized Protocol for the Proficiency Testing of Analytical Laboratories [6], elaborated by ISO, IUPAC and AOAC and ISO 13528:2015 [4] in combination with the insights published by the Analytical Methods Committee [7,8] regarding robust statistics.

For the evaluation of the quantitative results, the consensus value, the uncertainty of the consensus value, the standard deviation for proficiency assessment and z-scores were calculated according to in-house standard operating procedure [11].

4.1 Calculation of the consensus value

The consensus value (X) was determined using robust statistics [4, 7, 8]. The advantage of robust statistics is that all values are taken into account: outlying observations are retained, but given less weight. Furthermore, it is not expected to receive normally distributed data in a proficiency test. When using robust statistics, the data do not have to be normally distributed in contrast to conventional outlier elimination methods.

The robust mean of the reported results of all participants, calculated from an iterative process that starts at the median of the reported results using a cut-off value depending on the number of results, was used as the consensus value [4, 7].

4.2 Calculation of the uncertainty of the consensus value

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

The uncertainty of the consensus value (the robust mean) is calculated from the estimation of the standard deviation of the consensus value and the number of values used for the calculation of the consensus value [4] and is calculated using the formula:

$$u = 1.25 * \frac{\hat{\sigma}}{\sqrt{n}}$$

where:

u = Uncertainty of the consensus value;

n = Number of values used to calculate the consensus value;

$\hat{\sigma}$ = The estimate of the standard deviation of the consensus value resulting from robust statistics.

According to ISO 13528:2015 [4] the uncertainty of the consensus value (u) is negligible and therefore does not have to be included in the statistical evaluation if:

$u \leq 0.3\sigma_P$

where:
u = The uncertainty of the consensus value;
σ_P = Standard deviation for proficiency assessment (§3.3).

In case the uncertainty of the consensus value does not comply with this criterion, the uncertainty of the consensus value should be taken into account when evaluating the performance of the participants regarding the accuracy (§3.4). In case the uncertainty is > $0.7\sigma_P$ the calculated z-scores should not be used for evaluation of participants performance and are presented for information only.

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

According to Commission Decision 2002/657/EC [10], the coefficient of variation for the repeated analysis of a reference or fortified material under reproducibility conditions, shall not exceed the level calculated by the Horwitz equation. The Horwitz equation, $\sigma_H = 0.02c^{0.8495}$, presents a useful and widespread applied relation between the expected relative standard deviation of a singular analysis result under reproducibility conditions, and the concentration, c (g/g). It expresses inter-laboratory precision expected in inter-laboratory trials. Therefore, this relation is suitable for calculating the standard deviation for proficiency assessment in proficiency tests (σ_P).

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

For analyte concentrations <120 μg/kg:

$\sigma_P = 0.22c$

For analyte concentrations >138 g/kg:

$\sigma_P = 0.01c^{0.5}$

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

4.4 Performance characteristics with regard to the accuracy

For illustrating the performance of the participating participants with regard to the accuracy a z_a-score is calculated. For the evaluation of the performance of the participants, ISO 13528:2015 [4] is applied. According to these guidelines z_a-scores are classified as presented in Table 5.

Table 5 *Classification of z_a-scores.*

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

If the calculated uncertainty of the consensus value complies with the criterion mentioned in §3.2, the uncertainty is negligible. In this case the accuracy z-score is calculated from equation I:

$$z_a = \frac{\bar{x} - X}{\sigma_P} \qquad \text{Equation I}$$

where:

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

However, if the uncertainty of the consensus value does not comply with the criterion mentioned in §3.2, it could influence the evaluation of the participants. Although, according to ISO 13528 in this case no z-scores can be calculated, we feel that evaluation of the participating participants is of main importance justifying the participating participants' effort. Therefore in this case, the uncertainty is taken into account by calculating the accuracy z-score [4] using equation II:

$$z'_a = \frac{\bar{x} - X}{\sqrt{\sigma_P^2 + u^2}} \qquad \text{Equation II}$$

where:

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

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

In the case of a consequential instability the accuracy z-score for the participants that reported an amount below the consensus value is corrected for this instability using equation III:

$$z_{ai} = \frac{\bar{x} - X}{\sqrt{\sigma_P^2 + \Delta^2}} \qquad \text{Equation III}$$

where:

z_{ai} = Accuracy z-score taking into account the instability of the consensus value;
$\bar{x}$ = The average result of the laboratory;
X = Consensus value;
σ_P = Standard deviation for proficiency assessment;
Δ = Difference between average concentration of compound stored at -20°C and stored at room temperature.

In some cases the uncertainty of the consensus value does not comply with the criterion in §3.2 and a consequential instability is observed. In this case the z'_a-score for the participants that reported an amount below the consensus value is corrected for this instability using equation IV:

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

where:

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

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

X = Consensus value;

σ_P = Standard deviation for proficiency assessment;

Δ = Difference between average concentration of compound stored at -20°C and stored at room temperature;

u = Uncertainty of the consensus value.

5 Methods and results

5.1 Scope and LOQ

This PT was dedicated to copper and zinc in piglets and sow compound feed. Ranges for the reported limits of detection (LODs) and limits of quantifications (LOQs) for copper and zinc are presented in Annex 5. Ten participants provided no details of the LODs and LOQs of the method used.

All the participants determined and quantified copper and zinc as was requested. One participant analysed only material A.

The LODs reported by the participants ranged from 0.0044 to 4 mg/kg for copper and 0.00053 to 5.6 mg/kg for zinc. LOQs provided by the participants ranged from 0.02 to 12.5 mg/kg for copper and from 0.005 to 20 mg/kg for zinc.

5.2 Methods of analysis applied by participants

An overview of the information provided by the participants regarding the methods applied in this PT is presented in Annex 5. Each participant was free to use their method of choice reflecting their routine procedures. The information provided was not always complete. Six participants provided no information at all.

Eighteen laboratories applied ICP-MS (inductively coupled plasma mass spectrometry) for the identification and quantification of the metals, nine laboratories applied ICP-AES (inductively coupled plasma atomic emission spectroscopy) also referred as inductively coupled plasma optical emission spectrometry (ICP-OES), eight laboratories applied FAAS (flame atomic absorption spectroscopy). One laboratory applied ICP-MS as well as AAS (atomic absorption spectroscopy), one applied multi-element photo detector, while seven laboratories did not report the detection technique.

Twenty-three laboratories used microwave digestion for sample preparation and therefore different acid digestion procedures were employed for the determination of elements in compound feed. Five laboratories carried out the acid digestions with a mixture of nitric acid and hydrogen peroxide to bring the sample in the form of a solution in order to introduce it into the analyser, three laboratories used nitric acid and hydrochloric acid, three laboratories used only nitric acid and 12 laboratories used microwave digestion but without further specifications. Eight laboratories dry-ashed the sample. One participant digested the sample with a mixture of nitric acid and hydrogen peroxide and diluted the extract for measurement. Two laboratories digested the sample with a mixture of nitric acid and hydrogen peroxide without further specifications. Ten laboratories provided no details on the sample preparation conditions they used.

Out of 44 participants, 17 laboratories used one or more internal standards for copper and zinc quantification and 10 did not use an internal standard. The internal standards used were: beryllium, gallium, germanium, indium, iridium, lithium, rhodium, scandium and ytterbium. One participant used a standard of zinc as standard reference material (SRM) And one used a certified reference sample of tomato leaves to calculate recovery. Sixteen laboratories provided no information.

5.3 Performance assessment

The quantitative performance was assessed through z-scores. The individual z-scores obtained by each participant, including their graphical representation, for copper and zinc in materials A and B are summarised in Annex 6. A summary of the performance of the participants in this PT is provided in Annex 7.

A summary of the statistical evaluation of the PT results is presented in Table 6. This table includes all relevant parameters: the consensus value (CV), the uncertainty of the assigned value (u), the standard deviation for proficiency assessment (σ_p) and the robust (relative) standard deviation, based on participants' results.

Table 6 *Parameters of copper and zinc and summary in material A and B.*

	Material A		Material B	
	Copper	Zinc	Copper	Zinc
CV (mg/kg)	20.4	96.7	133	128
u (mg/kg)	0.448	1.23	2.62	1.74
σ_p (mg/kg) suggested by Horwitz	2.07	7.77	10.2	9.85
σ_p (%)	10.2	8.04	7.66	7.71
u>0.3σ_p	No	No	No	No
robust σ (mg/kg)	2.38	6.55	13.8	9.15
robust σ (%) (RSD_R)	11.7	6.77	10.3	7.16
# reported	44	44	43	43
# quantitative results	44	44	43	43
|z|≤ 2	39	38	39	37
2<|z|<3	3	2	2	2
|z|≥ 3	2	4	2	4
Satisfactory z-scores (%)	89	86	91	86

The consensus values for copper and zinc in material A were respectively 20.4 and 96.7 mg/kg and in material B respectively 133 and 128 µg/kg.

For copper and zinc in both materials, the uncertainty of the consensus value did comply with the criterion u≤0.3σ_p and was, therefore, considered as negligible in the evaluation of the z-scores.

For material A, three of the reported results for copper were questionable results (PT8860, PT8863 and PT8883) and two results were unsatisfactory (PT8857 and PT8876). For zinc two results were questionable (PT8875 and PT8879) and four results were unsatisfactory (PT8857, PT8867, PT8876 and PT8890). For material B, two of the reported results for copper were questionable results (PT8859 and PT8867) and two results were unsatisfactory (PT8857 and PT8875). For zinc two results were questionable (PT8847 and PT8879) and four results were unsatisfactory (PT8857, PT8867, PT8875 and PT8890).

The robust relative standard deviation (RSD_R) was calculated according to ISO13528:2015 [4] for informative purposes only. In this study it was used as a good estimation of the interlaboratory variability. The RSD_R values for copper and zinc in both materials are shown in Annex 6 and in Table 6. For both materials (A and B), the robust standard deviations (RSD_R) of the reported results were comparable with standard deviation suggested by Horwitz (σ_p). The RSD_R for copper and zinc in material A were respectively 12% and 7% and the σ_p were respectively 10% and 8%. In material B the RSD_R for copper and zinc were respectively 10% and 7% and the σ_p were for both metals 8%. The lower RSD_R for zinc in both materials shows that the laboratories' performance for zinc was slightly better than for copper.

For the individual metals in material A, the percentage of satisfactory results for copper was 89% and for zinc 86%. For the combined results of copper and zinc in material A, 88% of the results were rated with satisfactory z-scores ($|z| \leq 2$), 5.7% of the results felt into the questionable range with $2<|z|<3$ and 6.8% of the results fell into the unsatisfactory range with $|z| \geq 3$.

For the individual metals in material B, the percentage of satisfactory results for copper was 91% and for zinc 86%. For the combined results of copper and zinc in material A, 88% of the results were rated with satisfactory z-scores ($|z| \leq 2$), 5% of the results felt into the questionable range with $2<|z|<3$ and 7% of the results fell into the unsatisfactory range with $|z| \geq 3$.

Overall, 88% of the copper and zinc results obtained for both materials (A and B) were rated with satisfactory z-scores $|z| \leq 2$, 5% of the results felt into the questionable range with $2<|z|<3$ and 7% of the results fell into the unsatisfactory range with $|z| \geq 3$.

Participant PT8857 reported unsatisfactory results for copper and zinc in material A. Based on the results of participant PT8857, it can be speculated that the results for copper and zinc have been interchanged by the participant. Participant PT8876 analysed only material A and reported very high results for material A. Based on these results, it can be speculated that the batch numbers of the samples during reporting on the web application have been changed by the participant.

In Annex 7 an overview of the overall performance of each participant in this PT is summarised. For the two materials combined, a maximum of 4 satisfactory z-scores could be obtained, and '4 out of 4' therefore reflects an optimal performance in terms of scope and capability for quantitative determination. All the participants analysed the materials for both copper and zinc. Out of 44 participants, 33 participants achieved optimal performance for both materials by detecting copper and zinc with correct quantification, the absence of false positive and/or false negative results, and reporting all results within the set deadline.

6 Discussion and conclusions

Forty-five participants subscribed for the proficiency test on copper and zinc in compound for piglets and sows and 44 participants reported their results.

Two materials were sent to each participant. The metals copper and zinc were homogeneously distributed in both materials. An overview of each participant's performance is shown in Annex 7 and a summary of the results is presented in Table 6.

For the individual metals in material A, the percentage of satisfactory results for copper was 89% and for zinc 86%. The robust standard deviations (RSD_R) of the reported results were comparable with the standard deviation suggested by Horwitz.

For the individual metals in material B, the percentage of satisfactory results for copper was 91% and for zinc 86%. The robust standard deviations (RSD_R) of the reported results were comparable with the standard deviation suggested by Horwitz. One participant did not analyse copper and zinc in material B.

Overall, for copper and zinc in both materials combined, 88% of the results were rated with satisfactory z-scores $|z| \leq 2$, 5% of the results fell into the questionable range with $2<|z|<3$ and 7% of the results fell into the unsatisfactory range with $|z| \geq 3$. Out of 44 participants 33 showed optimal performance for both materials by detecting copper and zinc with a correct quantification, the absence of false positive or false negative results and reporting within the deadline. Eleven participants reported questionable or unsatisfactory z-scores. A total of 9 questionable z-scores and 12 unsatisfactory z-scores was reported.

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

- The satisfactory results for copper and zinc varied from 86-91% in this proficiency test.
- The interlaboratory reproducibility (RSD_R) ranged from 7 – 12% which is comparable with the standard deviation suggested by Horwitz (8 – 10%).
- Overall results of this PT: 75% of the participants showed optimal performance and are capable of satisfactory determination of copper and zinc in compound feed for piglets and for sows.
- The LOQs as provided by the participants varied widely, from 0.00053 to 50 mg/kg. This variation is probably caused by the different combinations of sample preparation and detection technique.

References

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10 Commission Decision 2002/657/EC. 12 August 2002. Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Official Journal. L 221:67A-76A.

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Annex 1 Codification of the samples

Participants code	Last three digits of codes Material A*	Last three digits of codes Material B*
PT8845	147	740
PT8847	349	458
PT8848	184	206
PT8849	234	766
PT8850	221	215
PT8851	196	209
PT8852	764	381
PT8853	735	814
PT8854	178	440
PT8855	446	172
PT8856	461	120
PT8857	266	513
PT8858	151	253
PT8859	157	473
PT8860	718	114
PT8861	237	171
PT8862	988	103
PT8863	399	416
PT8864	471	122
PT8865	854	722
PT8866	173	883
PT8867	957	408
PT8868	749	825
PT8869	762	734
PT8870	926	798
PT8871	438	932
PT8872	817	829
PT8873	442	633
PT8874	931	498
PT8875	262	571
PT8876	449	199
PT8878	879	756
PT8879	613	362
PT8880	779	818
PT8881	493	397
PT8882	697	675
PT8883	410	469
PT8885	522	673
PT8886	945	623
PT8887	676	997
PT8888	388	116
PT8889	572	679
PT8890	325	508
PT8891	464	288

* All sample codes start with 2021/metals/compound feed/

Annex 2 Instruction letter

WAGENINGEN
UNIVERSITY & RESEARCH

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

Wageningen Food Safety Research

DATE
March 29, 2021

SUBJECT
Instructions proficiency test metals copper and zinc in compound feed.

OUR REFERENCE
2112554/WFSR

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

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

INTERNET
www.wur.nl

CoC NUMBER
09098104

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

TELEPHONE
+31 (0)614 323 017

EMAIL
pt.wfsr@wur.nl

Dear Madam, Sir,

Thank you very much for your interest in the proficiency test for the analysis of the metals copper and zinc in compound feed. Hereby I send you a parcel containing two randomly coded samples. Each sample consists of approximately 25 grams of test material.

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

Instructions:

- After arrival store the samples at room temperature.
- Before analysis, homogenise them according to your laboratory's procedure.
- Treat the test material as if it was a sample for routine analysis.
- Please use the web application for entering your results (https://crlwebshop.wur.nl/apex/f?p=307:LOGIN). Information about the use of this web application was sent to you earlier by e-mail.
- Report all results relative to a feed with a moisture content of 12%. When a metal is not within your scope, please report 'nt' (not tested) in the web application. Do not use the option 'detected' from the web application. When a metal is 'not detected' or the result is below your LOQ, report the result as <LOQ-value and specify the value (e.g. <20 mg/kg).
- The deadline for submitting test-results for this test is **10th of May 2021.**

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

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

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

With kind regards,

D. Pereboom

Diana Pereboom
Organiser proficiency tests

Wageningen Research Foundation/Wageningen Food Safety Research (WFSR) is part of Wageningen University & Research. WFSR carries out research and analysis contributing to the safety and reliability of food and feed. WFSR is ISO 17025 and ISO 17043 accredited. The accredited tests are described on www.rva.nl (no. L014, L235 and R013).

Annex 3 Statistical evaluation of homogeneity data

	Copper in material A (mg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/A001	20.9	21.4
Hom/A002	19.7	20.9
Hom/A003	19.5	19.7
Hom/A004	20.1	19.5
Hom/A005	19.9	20.4
Hom/A006	20.0	19.4
Hom/A007	20.3	20.8
Hom/A008	19.8	19.7
Hom/A009	19.8	19.7
Hom/A010	19.5	20.1
Grand mean	20.0	
Cochran's test		
C	0.464	
C_{crit}	0.602	
$C < C_{crit}$?	NO OUTLIERS	
Target s = σ_P	2.04	
s_x	0.492	
s_w	0.391	
s_s	0.407	
Critical= 0.3 σ_P	0.613	
s_s < critical?	ACCEPTED	
$s_w < 0.5\ \sigma_P$?	ACCEPTED	

s_x = Standard deviation of the sample averages.

s_w = Within-sample standard deviation.

s_s = Between-sample standard deviation.

	zinc in material A (mg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/A001	99.6	99.4
Hom/A002	96.2	100
Hom/A003	96.4	96.5
Hom/A004	97.8	95.5
Hom/A005	94.1	99.7
Hom/A006	96.9	99.8
Hom/A007	98.1	97.7
Hom/A008	104	98.4
Hom/A009	99.2	96.7
Hom/A010	100.0	98.4
Grand mean	98.2	
Cochran's test		
C	0.329	
C_{crit}	0.602	
$C < C_{crit}$?	NO OUTLIERS	
Target s = σ_P	7.88	
s_x	1.47	
s_w	2.26	
s_s	0.00	
Critical= 0.3 σ_P	2.36	
s_s < critical?	ACCEPTED	
$s_w < 0.5\ \sigma_P$?	ACCEPTED	

Sample No.	Copper in material B (mg/kg)	
	Replicate 1	Replicate 2
Hom/A001	128	133
Hom/A002	128	131
Hom/A003	131	125
Hom/A004	132	134
Hom/A005	126	134
Hom/A006	132	128
Hom/A007	129	132
Hom/A008	131	129
Hom/A009	126	131
Hom/A010	125	130
Grand mean	130	
Cochran's test		
C	0.506	
C_{crit}	0.602	
C < C_{crit}?	NO OUTLIERS	
Target s = σ_P	10.1	
s_x	1.62	
s_w	3.23	
s_s	0.000	
Critical= 0.3 σ_P	3.03	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

Sample No.	Zinc in material B (mg/kg)	
	Replicate 1	Replicate 2
Hom/A001	130	131
Hom/A002	134	134
Hom/A003	136	129
Hom/A004	136	133
Hom/A005	130	137
Hom/A006	134	130
Hom/A007	130	132
Hom/A008	133	131
Hom/A009	130	133
Hom/A010	128	130
Grand mean	132	
Cochran's test		
C	0.318	
C_{crit}	0.602	
C < C_{crit}?	NO OUTLIERS	
Target s = σ_P	10.1	
s_x	1.61	
s_w	2.60	
s_s	0.000	
Critical= 0.3 σ_P	3.04	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

Annex 4 Statistical evaluation of stability data

*Statistical evaluation for **copper in material A.***

Storage temperature	-20 °C	room temperature
Time (days)	0	49
Calculated amounts (mg/kg)	20.6	20.4
	20.5	20.2
	20.8	20.2
	20.2	20.1
	19.6	20.0
	20.4	20.8
Average amount (mg/kg)	20.3	20.3
n	6	6
st. dev (mg/kg)	0.416	0.291
Difference		0.064
$0.3*\sigma_P$		0.621
Consequential difference? Diff < $0.3*\sigma_P$		No

*Statistical evaluation for **zinc in material A.***

Storage temperature	-20 °C	room temperature
Time (days)	0	49
Calculated amounts (mg/kg)	102	99.4
	100	99.4
	99.9	101
	103	101
	98.6	99.0
	98.9	99.1
Average amount (mg/kg)	100	99.8
n	6	6
st. dev (mg/kg)	1.66	0.928
Difference		0.604
$0.3*\sigma_P$		2.41
Consequential difference? Diff < $0.3*\sigma_P$		No

*Statistical evaluation for **copper in material B.***

Storage temperature	-20°C	room temperature
Time (days)	0	49
Calculated amounts (mg/kg)	134	136
	138	138
	135	135
	138	137
	140	137
	139	136
Average amount (mg/kg)	137	137
n	6	6
st. dev (mg/kg)	2.16	1.03
Difference		0.776
$0.3*\sigma_P$		3.14
Consequential difference? Diff < $0.3*\sigma_P$		No

*Statistical evaluation for **zinc in material B.***

Storage temperature	-20°C	room temperature
Time (days)	0	49
Calculated amounts (mg/kg)	135	134
	135	136
	131	135
	133	132
	133	137
	131	136
Average amount (mg/kg)	133	135
n	6	6
st. dev (mg/kg)	1.99	1.93
Difference		-1.64
$0.3*\sigma_P$		3.06
Consequential difference? Diff < $0.3*\sigma_P$		No

Annex 5 Overview of the applied methods

Lab	Sample purification	Internal standard	LOD mg/kg		LOQ mg/kg		Detection method
			copper	zinc	copper	zinc	
PT8845	Microwave digestion with HNO3 and HCL	No	0.043		0.13	0.5	Multielement photo detector
PT8847	Microwave digestion system		0.08 ppm	0.05 ppm			FAAS
PT8848	Microwave digestion with HNO3 and HCl, oxidation agent H2O2	No	0.02	0.006			ICP-OES
PT8849							
PT8850	Pressure digestion followed by dilution	No	0.35	0.35	1	1	ICP-OES
PT8851					0.7	0.4	ICP-OES
PT8852	HNO3+H2O2	45Sc	0.1	0.1	2	2	ICP-MS
PT8853	Microwave destruction	Rh, Sc, Ge en Ir			1	5	ICP-MS
PT8854							
PT8855		In					ICP-MS
PT8856	Dry ashing/ microwave digestion		0.3	0.05	1.0	0.15	FAAS
PT8857	Dry ashing	No					FAAS
PT8858	High pressure microwave digestion. Sample solubilised in a mixture of concentrated HNO3 and HCL.	Rhodium	0.02	0.04	0.06	0.12	ICP-MS
PT8859	Dry ashing, dilution in HCL	Standard Zn Merck 1000 mg/l (NIST SRM 3168a)					FAAS
PT8860	Samples were digested in a microwave using 6ml of HNO3 and 2ml of H2O2	No	0.01	0.01	0.025	0.05	ICP-MS
PT8861	Microwave Digestion	Yes	0.1	0.1	5	20	ICP-OES
PT8862	Microwave digestion				0.1	0.1	ICP-MS
PT8863	Microwave-assisted acid digestion	No	4	2	12.5	6.25	FAAS
PT8864	Digestion of 0.5g sample + 3 ml HNO3 + 0,5 ml H2O2 + 2 ml H2O. Dilution to 50 g with H2O.				3.7	18	ICP-OES
PT8865			0.16	0.54	0.54	1.79	
PT8866	Closed microwave		0.0044	0.00053	0.05	0.005	ICP-OES
PT8867	Microwave Digestion	Scandium					ICP-MS

Lab	Sample purification	Internal standard	LOD mg/kg		LOQ mg/kg		Detection method
			copper	zinc	copper	zinc	
PT8868	Closed microwave		0.5	2	1.7	6.7	ICP-MS
PT8869	A test portion is dissolved in HCL after ashing in a muffle furnace. Any silica compounds present are removed by precipitation and filtration.	No	1.18	1.62	4	5	FAAS
PT8870					10	20	
PT8871	Dry and incineration the sample	No					FAAS
PT8872	Pressure digestion	Ga 71	0.062	0.11	0.21	0.37	Q-ICP-MS
PT8873	Microwave Digestion with HNO3 at 260 °C for 25 minutes	Ytterbium	1.7	5.6	5	16.7	ICP-OES
PT8874	Dry ashing procedure. Dissolution with HCl.		1.6	1.6	3.3	4.2	FAAS
PT8875					0.02	0.1	
PT8876		Yes			0.05	0.05	ICP-MS
PT8878							
PT8879	HNO3/H2O2	Rh	0.02	0.05	0.05	0.1	ICP-MS
PT8880	Microwave mineralization with HNO3, addition to volume 50ml with distilled water, filtration	No	3	3	5	5	ICP-OES
PT8881	Microwave acid digestion	Ir, In, Ge			3	50	ICP-MS
PT8882	Dicrowave	No	0.3	0.3	1	1	AAS; ICP MS
PT8883			0.013	0.063	0.02	1	
PT8885	dry ashing	Rhodium	2	2	4	4	ICP-MS
PT8886	Microwave closed-vessel digestion using HNO3 and H2O2 as oxidative reagents	indium			0.05	0.2	ICP-MS
PT8887	Microwave oven wet-digestion: weight 0.3-0.4 g of sample, add 6 mL ultrapure HNO3 and 2 mL H2O2	Ge	0.3	1.5	1	5	ICP-MS
PT8888	Acid Digestion after dry ashed	103 Rh					ICP-MS
PT8889	Microwave destruction						ICP-MS
PT8890	Samples are digested with concentrated HNO3 under pressure.	Rh	3.3	3.3	10	10	ICP-MS
PT8891	Droge verassing	Beryllium, Lithium	0.5	0.5	1	1	ICP-OES

Annex 6 Results material A and B

Lab code	Material A Copper CV: 20.4 mg/kg u: 0.448 mg/kg σp: 2.07 mg/kg robust σ: 2.38 mg/kg (11.7%)		Material A Zinc CV: 96.7 mg/kg u: 1.23 mg/kg σp: 7.77 mg/kg robust σ: 6.55 mg/kg (6.77%)		Material B Copper CV: 133 mg/kg u: 2.62 mg/kg σp: 10.2 mg/kg robust σ: 13.8 mg/kg (10.3%)		Material B Zinc CV: 128 mg/kg u: 1.74 mg/kg σp: 9.85 mg/kg robust σ: 9.15 mg/kg (7.16%)	
	Result (mg/kg)	z_a-score	Result (mg/kg)	z_a-score	Result (mg/kg)	z_a-score	Result (mg/kg)	z_a-score
PT8845	20.7	0.16	98.3	0.21	142	0.87	135	0.73
PT8847	22	0.79	88.7	-1.02	135.6	0.24	106.8	**-2.13**
PT8848	21.77	0.68	100.56	0.50	141.37	0.81	129.33	0.15
PT8849	20.1	-0.13	97.4	0.10	136.3	0.31	134.1	0.64
PT8850	20.6	0.11	105.8	1.18	139.6	0.63	131.2	0.34
PT8851	17	-1.63	98	0.17	115	-1.78	121	-0.69
PT8852	18.82	-0.75	86.65	-1.29	127.21	-0.58	117.07	-1.09
PT8853	18.4	-0.95	89.6	-0.91	125	-0.80	129	0.12
PT8854	21.2	0.40	95.2	-0.19	143	0.97	124	-0.39
PT8855	19.37	-0.48	90.67	-0.77	118.82	-1.40	115.76	-1.22
PT8856	20	-0.18	89	-0.98	114	-1.88	120	-0.79
PT8857	86.376	**31.88**	12.822	**-10.79**	98.397	**-3.41**	93.035	**-3.53**
PT8858	22.7	1.13	104	0.95	150	1.65	144	1.64
PT8859	16.8	-1.72	96.9	0.03	111	**-2.17**	115	-1.30
PT8860	24.7	**2.09**	97.7	0.13	151	1.75	140	1.24
PT8861	22.161	0.86	91.037	-0.72	148.637	1.52	127.344	-0.05
PT8862	20.21	-0.08	105.86	1.18	137.29	0.41	142.37	1.48
PT8863	25.45	**2.45**	97.09	0.06	145.36	1.20	128.22	0.04
PT8864	21	0.30	101	0.56	134	0.09	135	0.73
PT8865	20.8	0.21	99.4	0.35	146	1.26	137	0.93
PT8866	18.74	-0.79	91.25	-0.70	130.34	-0.27	120.27	-0.77
PT8867	24	1.75	124	**3.52**	158	**2.44**	161	**3.37**
PT8868	20.1	-0.13	93	-0.47	136	0.28	124	-0.39
PT8869	19.97	-0.19	101.67	0.65	114.78	-1.80	127.83	0.00
PT8870	19.1	-0.61	93	-0.47	127	-0.60	128	0.02
PT8871	21.9	0.74	96.51	-0.02	140.06	0.68	117.6	-1.04
PT8872	18	-1.15	92	-0.60	119	-1.39	124	-0.39
PT8873	20.1	-0.13	99.1	0.31	145	1.16	134	0.63
PT8874	23	1.27	101	0.56	121	-1.19	128	0.02
PT8875	24.1	1.80	118.6	**2.82**	176.3	**4.23**	161.5	**3.42**
PT8876	156	**65.51**	150	**6.86**				
PT8878	20.6	0.11	92	-0.60	140	0.67	129	0.12
PT8879	23.5	1.51	118	**2.75**	146	1.26	152	**2.45**
PT8880	21.3	0.45	96.59	-0.01	142.8	0.95	129.7	0.19
PT8881	21	0.30	88	-1.11	138	0.48	128	0.02
PT8882	17.2	-1.53	99.6	0.38	124	-0.89	123	-0.49
PT8883	15.81	**-2.20**	97.75	0.14	114.73	-1.80	126.16	-0.17
PT8885	17	-1.63	96	-0.08	118	-1.48	127	-0.08
PT8886	18.1	-1.10	96.4	-0.03	133	-0.01	130	0.22
PT8887	19	-0.66	93	-0.47	131	-0.21	129	0.12
PT8888	18.7	-0.81	103	0.82	132	-0.11	133	0.53
PT8889	19.4	-0.47	96.1	-0.07	135	0.18	129	0.12
PT8890	20	-0.18	46.2	**-6.49**	129	-0.40	77.9	**-5.07**
PT8891	17.9	-1.19	94	-0.34	124.5	-0.85	119	-0.89

CV = consensus value (robust mean)

u = uncertainty of consensus value

σ_p = target standard deviation for proficiency

robust σ = robust (relative) standard deviation based on participants' results

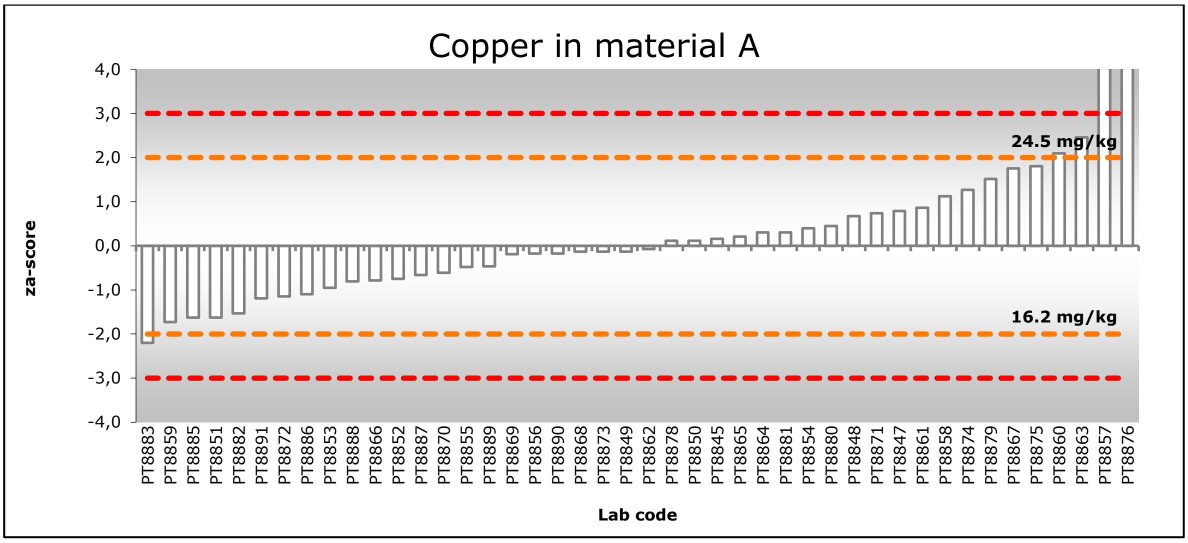

Figure a *Graphical representation of the z_a-scores for copper in material A. The X ± $2\sigma_P$ lines (dotted) are calculated according to equation I in §4.4.*

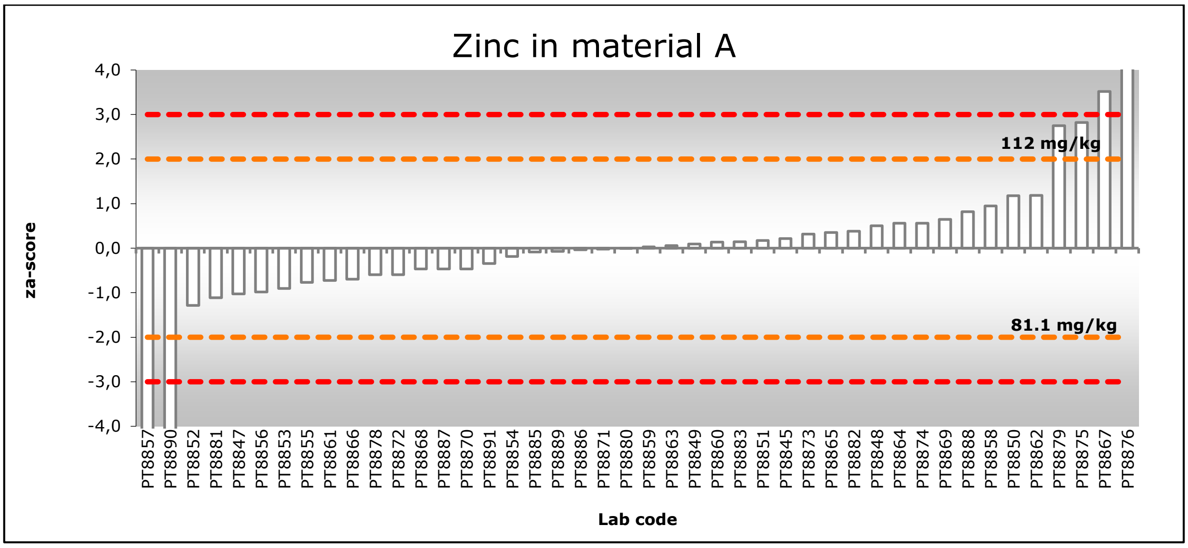

Figure b *Graphical representation of the z_a-scores for zinc in material A. The X ± $2\sigma_P$ lines (dotted) are calculated according to equation I in §4.4.*

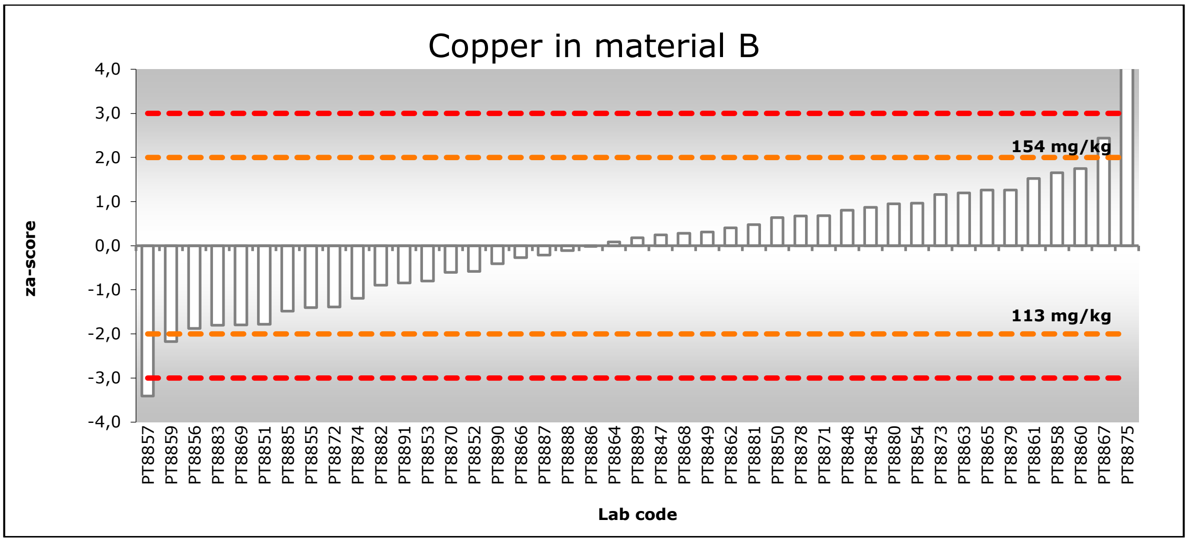

Figure c *Graphical representation of the z_a-scores for copper in material B. The $X \pm 2\sigma_P$ lines (dotted) are calculated according to equation I in §4.4.*

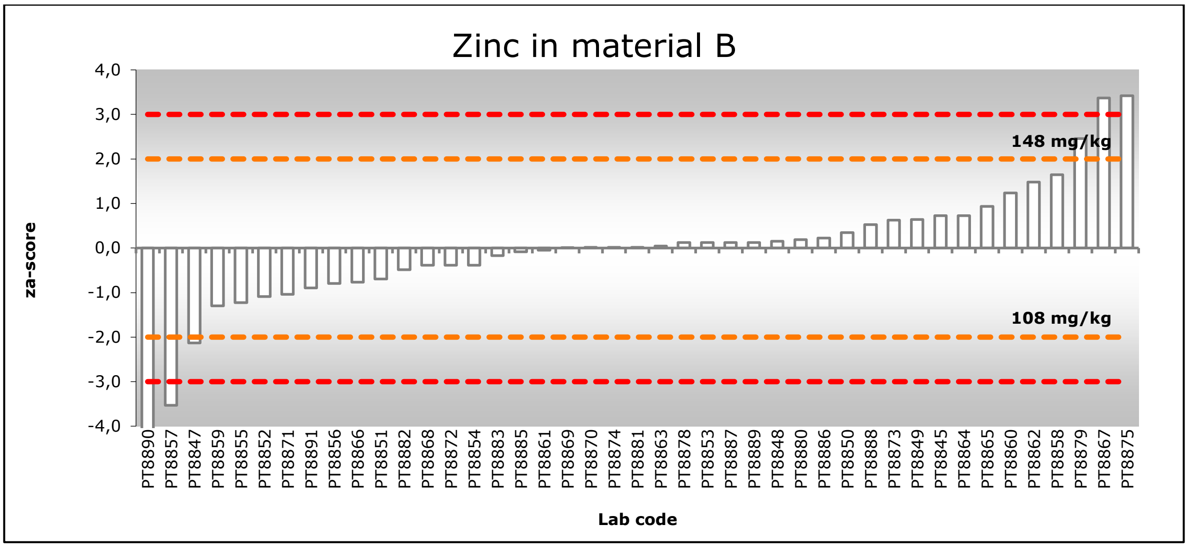

Figure d *Graphical representation of the z_a-scores for zinc in material B. The $X \pm 2\sigma_P$ lines (dotted) are calculated according to equation I in §4.4.*

Annex 7 Overview performance per laboratory

Laboratory code	Satisfactory performance
PT8845	4 out of 4, **optimal performance**
PT8847	3 out of 4
PT8848	4 out of 4, **optimal performance**
PT8849	4 out of 4, **optimal performance**
PT8850	4 out of 4, **optimal performance**
PT8851	4 out of 4, **optimal performance**
PT8852	4 out of 4, **optimal performance**
PT8853	4 out of 4, **optimal performance**
PT8854	4 out of 4, **optimal performance**
PT8855	4 out of 4, **optimal performance**
PT8856	4 out of 4, **optimal performance**
PT8857	0 out of 4
PT8858	4 out of 4, **optimal performance**
PT8859	3 out of 4
PT8860	3 out of 4
PT8861	4 out of 4, **optimal performance**
PT8862	4 out of 4, **optimal performance**
PT8863	3 out of 4
PT8864	4 out of 4, **optimal performance**
PT8865	4 out of 4, **optimal performance**
PT8866	4 out of 4, **optimal performance**
PT8867	1 out of 4
PT8868	4 out of 4, **optimal performance**
PT8869	4 out of 4, **optimal performance**
PT8870	4 out of 4, **optimal performance**
PT8871	4 out of 4, **optimal performance**
PT8872	4 out of 4, **optimal performance**
PT8873	4 out of 4, **optimal performance**
PT8874	4 out of 4, **optimal performance**
PT8875	1 out of 4
PT8876	0 out of 2*
PT8878	4 out of 4, **optimal performance**
PT8879	2 out of 4
PT8880	4 out of 4, **optimal performance**
PT8881	4 out of 4, **optimal performance**
PT8882	4 out of 4, **optimal performance**
PT8883	3 out of 4
PT8885	4 out of 4, **optimal performance**
PT8886	4 out of 4, **optimal performance**
PT8887	4 out of 4, **optimal performance**
PT8888	4 out of 4, **optimal performance**
PT8889	4 out of 4, **optimal performance**
PT8890	2 out of 4
PT8891	4 out of 4, **optimal performance**

*Participant PT8876 analyse only one sample

Wageningen Food Safety Research
P.O. Box 230
6700 AE Wageningen
The Netherlands
T +31 (0)317 48 02 56
www.wur.eu/food-safety-research

WFSR report 2021.015

The mission of Wageningen University & Research is “To explore the potential of nature to improve the quality of life”. Under the banner Wageningen University & Research, Wageningen University and the specialised research institutes of the Wageningen Research Foundation have joined forces in contributing to finding solutions to important questions in the domain of healthy food and living environment. With its roughly 30 branches, 6,800 employees (6,000 fte) and 12,900 students, Wageningen University & Research is one of the leading organisations in its domain. The unique Wageningen approach lies in its integrated approach to issues and the collaboration between different disciplines.

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

Wageningen Food Safety Research
P.O. Box 230
6700 AE Wageningen
The Netherlands
T +31 (0)317 48 02 56
www.wur.eu/food-safety-research

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