Proficiency test for mycotoxins in oat meal

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Distribution list:

- Twenty-three participating laboratories

Contents

Summary

In April 2019 a proficiency test for mycotoxins in oat meal was organised by Wageningen Food Safety Research (WFSR), Wageningen University & Research in accordance with ISO 17043. WFSR, part of Wageningen University & Research 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 or demonstrate their competence for the analysis of mycotoxins in oat meal.

Two materials were prepared and dispatched on dry-ice to the participants. The consensus values of the mycotoxins in each material are given in Table 1.

Table 1 *Consensus values of the mycotoxins in the proficiency materials.*

	Material A	Material B
Compound	**Consensus value µg/kg**	**Consensus value µg/kg**
15-acetyl-Deoxynivalenol	161	152
3-acetyl-Deoxynivalenol	613	645
Deoxynivalenol-3-glucoside	1143	1144
Deoxynivalenol	4174	4268
Aflatoxin B1	25	10.3
Enniatin B	121	115
Enniatin B1	65	65
T-2 toxin	133	24
HT-2 toxin	185	61
Zearalenone	293	289
Sum of T-2 and HT-2 toxin	319	83

Material A was prepared by spiking a solution of aflatoxin B1, T-2 toxin and HT-2 toxin to an incurred oat meal to the required target concentrations. Material B was prepared by spiking a solution of aflatoxin B1 to the same incurred oat meal. The materials were mixed with water and the slurry was freeze-dried. The materials were homogenized afterwards.

Homogeneity assessment showed that both materials were sufficiently homogeneous for proficiency testing. The stability test demonstrated no statistically significant loss of the mycotoxins, except for HT-2 toxin in material B. This decrease was accounted for in the calculation of the z-scores.

Twenty-three participants subscribed for the participation in this proficiency test. For calculating of the accuracy z-scores in this test a target standard deviation of 25% was taken to assess proficiency of the participants.

Not all compounds could be statistically evaluated since the number of results submitted by the participants was too low. In both material A and B seven statistical evaluations were possible.

A total of 249 z-scores could be calculated from the submitted results of which thirteen questionable z-scores and four unsatisfactory z-scores were reported. In addition, two false positive and four false negative results were reported. Five participants showed optimal performance by detecting the mycotoxins with a correct quantification/qualification (14 satisfactory z-scores) and the absence of false positive and false negative results. Another ten participants showed suboptimal performance

within their scope by reporting thirteen or less satisfactory z-scores. Eight participants reported qualitative, false negative, false positive, questionable or unsatisfactory z-scores.

Based on the results of this test it can be concluded that all quantitative results for aflatoxin B1 show optimal performance. The performance of deoxynivalenol, 3-acetyl-deoxynivalenol, T-2 toxin and HT-2 toxin needs improvement; satisfactory results vary from 70 to 89%. Only nine participants included one or more of the metabolites of deoxynivalenol in their method, despite the recommendation of the European Commission for monitoring of these mycotoxins.

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 [3] accreditation by the Dutch Accreditation Board (R013).

There is EU harmonised regulation for aflatoxin B1 in animal feed materials at a limit of 0.02 mg/kg (Directive 2002/32/DC and its amendments) [11]. Guidance values for deoxynivalenol and zearalenone in animal feed are laid down in recommendation 2006/576/EC and amendments [12] at, respective 8 mg DON/kg and 2 mg ZEN/kg cereals and cereal products with the exception of maize by-products. For the sum of T2/HT2 indicative levels have been set in recommendation 2013/165/EU of 0.5 mg/kg in cereals and 2 mg/kg for oat milling products (husks).

The aim of this proficiency test was to give participants the opportunity to evaluate or demonstrate their competence for the analysis of mycotoxins in oat meal.

2 Material and methods

2.1 Scope of the proficiency test

This proficiency test (PT) focused on the mycotoxins aflatoxin B1, deoxynivalenol, 3-acetyl-deoxynivalenol, 15-acaetyl-deoxynivalenol, deoxynivalenol-3-glucoside, zearalenone, T-2 toxin, HT-2 toxin, enniatin A, enniatin A1, enniatin B and enniatin B1 in oat meal. The target concentrations for the mycotoxins in this PT are presented in Table 3.

2.2 Participants

Twenty-three participants registered for the participation in the PT and all reported their results. All of these participants are situated in Europe. Each participant was asked *a priori*, to indicate which compounds were included in the scope of their method. The participants were asked to report the results through a web application designed for PTs.

2.3 Material preparation

Two spiked/contaminated oat meals, material A and material B, were prepared for the PT. The starting material was an oat meal which was contaminated with several compounds (Table 3). For material A levels of T-2 toxin and HT-2 toxin were artificially increased and aflatoxin B1 was added by spiking an acetonitrile-solution containing aflatoxin B1, T-2 toxin and HT-2 toxin and material B by adding an acetonitrile-solution containing aflatoxin B1 at levels presented in Table 2. The oat meal samples (2 kg) were mixed with three litres of water and homogenized using a concrete mixer according to in-house standard operating procedures [4]. The fortified slurry was freeze-dried, homogenized and stored in the freezer until use.

Table 2 *Target concentrations of mycotoxins in the proficiency materials.*

	Abbreviation	Oat meal material	Material A	Material B
Compound		Contains (µg/kg)	Target concentration (µg/kg)	Target concentration (µg/kg)
15-acetyl-Deoxynivalenol	15-Ac-DON	20	20	20
3-acetyl-Deoxynivalenol	3-Ac-DON	600	600	600
Deoxynivalenol	DON	3600	3600	3600
Deoxynivalenol-3-glucoside	DON-3G	900	900	900
Aflatoxin B1	AFLA		25	10
Enniatin B	ENN B	110	110	110
Enniatin B1	ENN B1	60	60	60
Zearalenone	ZEN	350	350	350
T-2 toxin	T2	25	150	25
HT-2 toxin	HT2	50	175	50

2.4 Logistic procedure

After homogenization, the samples for the participants were randomly selected and coded using a web application designed for PTs (Annex 1). The code used was 2019/mycotoxins/oats/000, in which the three digit number at the end of the code was automatically generated by the WFSR Laboratory Quality Services web application. In addition, the samples for homogeneity and stability testing were also randomly selected.

Each of the participating participants received a randomly assigned laboratory code, generated by the web application. The sample sets with the corresponding numbers were sent to the participants on April 8, 2019. The sample sets were packed in an insulating box with dry-ice and were dispatched to the participants immediately by courier. The samples were accompanied by a letter describing the requested analysis (Annex 2) and an acknowledgement of receipt form. By e-mail the participants received instructions on how to use the web application to report the results.

The participants were asked to store the samples in the freezer and to analyse the samples according to their routine method. A single analysis result for the mycotoxins in each sample was requested. The deadline for submitting the quantitative results was May 20, 2019, allowing the participants at least five weeks for the analysis.

Results should be reported for mycotoxins as µg/kg product (no correction for moisture). Participants were asked to provide information on their analytical method (extraction solvent, clean-up procedure, internal standards used, detection technique, limit of detection, limit of quantification).

2.5 Homogeneity study

The homogeneity of the materials was tested according to The International Harmonized Protocol for Proficiency Testing of Analytical Laboratories [7] and ISO 13528:2015 [5]. For homogeneity a target standard deviation for proficiency assessment (σ_P) of 25% was used as a fit-for-purpose standard deviation, in line with the target RSD used in proficiency tests on mycotoxins as organised by the EURL mycotoxins & plant toxins [13]. 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 $s_s < 0.3*\sigma_P$. The results of the homogeneity study, the grand mean with the corresponding RSD are presented in Table 4 and the statistical evaluation of material A and material B are presented in Annex 3.

Ten containers of materials A and B were analysed in duplicate for ten mycotoxins (Table 3). All mycotoxins in material A fulfilled the homogeneity-criterion. T-2 toxin in material B was the only analyte for which the criteria did not comply with the criterion for the within-sample standard deviation. Despite the deviation of this compound, material B was considered to be homogeneous, since the other nine mycotoxins fulfilled the homogeneity requirements and were spiked in the same solution.

The mycotoxins in material A and material B demonstrated to be sufficiently homogeneous for use in the proficiency test.

Table 3 *Concentration of mycotoxins in materials A and B obtained during homogeneity testing.*

Material code	Material A		Material B	
	Concentration µg/kg	RSD %	Concentration µg/kg	RSD %
15-Ac-DON	19	4.4	22	10.4
3-Ac-DON	568	5.2	590	4.1
DON	3630	4.8	3866	7.3
DON-3G	629	7.9	649	7.5
AFLA	17	2.5	6.5	6.3
ENN B	86	4.5	84	4.9
ENN B1	48	7.1	47	6.9
ZEN	196	8.2	165	6.3
T2	53	4.3	9.4	11.6
HT2	199	3.2	67	8.4

2.6 Stability of the materials

On April 8, 2019, the day the materials were distributed to the participants, six randomly selected samples of both materials were stored at <-70°C. It is assumed that the mycotoxins are stable at these storage conditions. Another 12 containers remained stored in the freezer. In addition, to mimic a possible thaw situation during transport, six containers were stored at room temperature for two days and then stored again in the freezer.

On May 21, 2019, 43 days after distribution of the samples, six samples of both materials B that were stored at <-70, in the freezer and for two days at room temperature were analysed for mycotoxins. 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 occur [5, 7]. A consequential instability is observed when the average value of an analyte in the samples stored in the freezer or at room temperature for two days is more than $0.3\sigma_P$ below the average value of the analyte in the samples stored at <-70°C. If so, the instability has a significant influence on the calculated z-scores.

For the mycotoxins in material A no consequential instability was observed and therefore, they are considered stable for the duration of the proficiency test. In material B only AFLA, T2 and HT2 were evaluated for stability and a consequential instability was observed for HT2. A decrease of 15% was observed between samples stored at <-70°C and in the freezer/two days at room temperature. This instability was taken into account in the calculation of the z-scores. The results of the stability test are presented in Annex 4.

2.7 Interpretation of the results

A result was assigned as false negative result if a compound was not detected, taking into account the reported scope of the participant, the consensus value and the reported LOQ for the compound by the participant.

*For example: the consensus value of compound A is 70 µg/kg and the participant reported an LOQ= 20 µg/kg for this compound. Taken into account the 25% target standard deviation in this test, the -2z threshold would be at 35 µg/kg (70-(2*25% of 70)). Since the LOQ of this participant is lower than the -2z value, this participant should be able to detect the presence. If the LOQ would have been 40 µg/kg no false negative result would be assigned.*

Also, when no LOQ values were reported by the participants and the compound was reported as not detected (nd), or the compound was not reported but was within the participants' scope, a false negative (FN) result was assigned.

For false positive results it was decided to apply a cut-off level of 2 µg/kg for ochratoxin A, 50 µg/kg or enniatin A(1) and 100 µg/kg for fumonisin B1 and concentrations below these levels were not assigned as false positive results.

3 Statistical evaluation

The statistical evaluation was carried out according to the International Harmonized Protocol for the Proficiency Testing of Analytical Laboratories [7], elaborated by ISO, IUPAC and AOAC and ISO 13528:2015 [5] in combination with the insights published by the Analytical Methods Committee [8,9] 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.

3.1 Calculation of the consensus value

The consensus value (X) was determined using robust statistics [5, 8, 9]. 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 [5, 8].

3.2 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 [5]:

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

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 [5] 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.

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

A target standard deviation for proficiency assessment (σ_P) of 25% was used as a fit-for-purpose standard deviation which is in line with the target RSD used in proficiency tests on mycotoxins as organised by the EURL.

$\sigma_P = 0.25c$

where:
σ_P = Expected standard deviation in proficiency tests for animal feed;
c = Concentration of the analyte (µg/kg).

3.4 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 [5] is applied. According to these guidelines z_a-scores are classified as presented in Table 4.

Table 4 *Classification of z_a-scores.*

$\|z_a\| \leq 2$	Satisfactory
$2 < \|z_a\| < 3$	Questionable
$\|z_a\| \geq 3$	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:

$$z_a = \frac{\overline{x} - X}{\sigma_P}$$ Equation I

where:
z_a = Accuracy z-score;
$\overline{x}$ = 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 [5]:

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

where:
z'_a = Accuracy z-score taking into account the uncertainty of the consensus value;
$\overline{x}$ = 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 by:

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

where:
z_{ai} = Accuracy z-score taking into account the instability of the consensus value;
$\overline{x}$ = 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 <-70 °C, <-18 °C and average concentration at room temperature for two days.

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 by:

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

where:
z'_{ai} = Accuracy z-score taking into account the uncertainty and instability of the consensus value;
$\overline{x}$ = 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 <-70 °C, <-18 °C and average concentration at room temperature for two days;
u = Uncertainty of the consensus value.

4 Methods and results

4.1 Participants

Twenty-three participants registered for the PT. All participants reported that the samples were received in good order and all participants submitted the results. The performance of individual participants is summarized in Annex 9.

4.2 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. The samples were analysed using various extraction methods, purification steps and detection techniques.
Ranges for the reported limits of detection (LODs) and limits of quantification (LOQs) for the mycotoxins are presented in Table 5.

Table 5 *Overview of reported LOD and LOQ reported by the participants.*

Compound	LOD (µg/kg)	LOQ (µg/kg)
15-Ac-DON	1-100	2-500
3-Ac-DON	1-100	2-500
DON	2-151.5	10-500
DON-3-G	61-100	20-500
AFLA	0.07-10	0.2-10
ENN B	0.2-3	2-10
ENN B1	0.2-3	1-10
ZON	0.4-70	2-140
T2	0.4-21	2-50
HT2	1.5-20	4-60

4.3 Performance participants material A

4.3.1 15-acetyl-Deoxynivalenol

Five participants reported quantitative results for 15-Ac-DON. Two participants (PT9615 and PT9618) reported the sum of 15-Ac-DON and 3-Ac-DON.
The lowest concentration reported was 93.6 µg/kg and the highest was 494 µg/kg. The consensus value is 161 µg/kg with a robust standard deviation of 67 µg/kg (resulting in an RSD_R of 42%) expressing the reproducibility within this PT. The robust standard deviation of 67 µg/kg is more than 1.5 times higher than the target standard deviation σ_P of 40 µg/kg (25% of the consensus value) suggested in §3.3 for feed material. With only five laboratories reporting quantitative results, statistic evaluation is not appropriate.

4.3.2 3-acetyl-Deoxynivalenol

Seven participants reported quantitative results for 3-Ac-DON. Two participants (PT9615 and PT9618) reported the sum of 15-Ac-DON and 3-Ac-DON.
The lowest concentration reported is 415 µg/kg and the highest is 664 µg/kg. The consensus value is 613 µg/kg with a robust standard deviation of 56 µg/kg (resulting in an RSD_R of 9.1%) expressing the

reproducibility within this PT. The robust standard deviation of 56 µg/kg is almost 3 times lower than the target standard deviation σ_P of 153 µg/kg. The uncertainty of the consensus value is 26 µg/kg, which does not exceed $0.3\sigma_P$ (§3.2). With respect to the accuracy all results are satisfactory.

4.3.3 Deoxynivalenol-3-glucoside

Four participants reported quantitative results for DON-3G.
The lowest concentration reported is 569 µg/kg and the highest is 1413 µg/kg. The consensus value is 1143 µg/kg with a robust standard deviation of 290 µg/kg (resulting in an RSD_R of 25%) expressing the reproducibility within this PT. The robust standard deviation of 290 µg/kg is comparable to the target standard deviation σ_P of 286 µg/kg. With only four laboratories reporting quantitative results, statistic evaluation is not appropriate.

4.3.4 Deoxynivalenol

Twenty participants reported quantitative results for DON. Participant PT9609 failed to detect the presence of DON and reported a false negative result.
The lowest concentration reported is 2572 µg/kg and the highest is 6860 µg/kg. The consensus value is 4174 µg/kg with a robust standard deviation of 629 µg/kg (resulting in an RSD_R of 15%) expressing the reproducibility within this PT. The robust standard deviation of 629 µg/kg is almost 2 times lower than the target standard deviation σ_P of 1044 µg/kg. The uncertainty of the consensus value is 176 µg/kg, which does not exceed $0.3\sigma_P$ (§3.2). With respect to the accuracy two laboratories reported questionable results (PT9625 and PT9628).

4.3.5 Aflatoxin B1

Twenty-two of the 23 participants reported quantitative results for AFLA.
The lowest concentration reported is 12.4 µg/kg and the highest is 33.0 µg/kg. The consensus value is 25 µg/kg with a robust standard deviation of 5.5 µg/kg (resulting in an RSD_R of 22%) expressing the reproducibility within this PT. The robust standard deviation of 5.5 µg/kg is comparable to the target standard deviation σ_P of 6.2 µg/kg. The uncertainty of the consensus value is 1.5 µg/kg, which does not exceed $0.3\sigma_P$ (§3.2). With respect to the accuracy all results are satisfactory.

4.3.6 Enniatin B

Four participants reported quantitative results for ENN B.
The lowest concentration reported is 75 µg/kg and the highest is 197 µg/kg. The consensus value is 121 µg/kg with a robust standard deviation of 56 µg/kg (resulting in an RSD_R of 38%) expressing the reproducibility within this PT. The robust standard deviation of 46 µg/kg is 1.5 times higher than the target standard deviation σ_P of 30 µg/kg (25% of the consensus value). With only four laboratories reporting quantitative results, statistic evaluation is not appropriate.

4.3.7 Enniatin B1

Four participants reported quantitative results for ENN B1.
The lowest concentration reported is 35.4 µg/kg and the highest is 100.4 µg/kg. The consensus value is 65 µg/kg with a robust standard deviation of 31 µg/kg (resulting in an RSD_R of 48%) expressing the reproducibility within this PT. The robust standard deviation of 31 µg/kg is almost two times higher than the target standard deviation σ_P of 16.2 µg/kg (25% of the consensus value). With only four laboratories reporting quantitative results, statistic evaluation is not appropriate.

4.3.8 Zearalenone

Twenty-one participants reported quantitative results for ZEN.
The lowest concentration reported is 177 µg/kg and the highest is 692 µg/kg. The consensus value is 293 µg/kg with a robust standard deviation of 41 µg/kg (resulting in an RSD_R of 14%) expressing the reproducibility within this PT. The robust standard deviation of 41 µg/kg is almost two times lower

than the target standard deviation σ_P of 73 µg/kg. The uncertainty of the consensus value is 11.3 µg/kg, which does not exceed $0.3\sigma_P$ (§3.2). With respect to the accuracy one result is unsatisfactory (PT9628).

4.3.9 T-2 toxin

Nineteen participants reported quantitative results for T-2 toxin.
The lowest concentration reported is 26.0 µg/kg and the highest is 221 µg/kg. The consensus value is 133 µg/kg with a robust standard deviation of 25 µg/kg (resulting in an RSD_R of 19%) expressing the reproducibility within this PT. The robust standard deviation of 25 µg/kg is comparable to the target standard deviation σ_P of 33 µg/kg. The uncertainty of the consensus value is 7.1 µg/kg, which does not exceed $0.3\sigma_P$ (§3.2). With respect to the accuracy one result is questionable (PT9620) and one is unsatisfactory (PT9627).

4.3.10 HT-2 toxin

Nineteen participants reported quantitative results for HT-2 toxin.
The lowest concentration reported is 71.1 µg/kg and the highest is 259 µg/kg. The consensus value is 185 µg/kg with a robust standard deviation of 42 µg/kg (resulting in an RSD_R of 23%) expressing the reproducibility within this PT. The robust standard deviation of 42 µg/kg is comparable to the target standard deviation σ_P of 46 µg/kg. The uncertainty of the consensus value is 12.1 µg/kg, which does not exceed $0.3\sigma_P$ (§3.2). With respect to the accuracy two results are questionable (PT9609 and PT9627).

4.3.11 Sum of T-2 and HT-2 toxins

Nineteen participants reported quantitative results for T-2 and/or HT-2 toxin. Sum-concentrations were calculated by adding the concentrations.
The lowest sum-concentration reported is 97.1 µg/kg and the highest is 452 µg/kg. The consensus value is 319 µg/kg with a robust standard deviation of 62 µg/kg (resulting in an RSD_R of 19%) expressing the reproducibility within this PT. The robust standard deviation of 62 µg/kg is comparable to the target standard deviation σ_P of 80 µg/kg. The uncertainty of the consensus value is 17.9 µg/kg, which does not exceed $0.3\sigma_P$ (§3.2). With respect to the accuracy one result is questionable (PT9627).

4.3.12 Other mycotoxins

Participant PT9627 reported a false positive result by detecting the presence of 2.8 µg/kg ochratoxin A.

4.4 Performance results participants material B

4.4.1 15-acetyl-Deoxynivalenol

Five participants reported quantitative results for 15-Ac-DON. Two participants (PT9615 and PT9618) reported the sum of 15-Ac-DON and 3-Ac-DON.
The lowest concentration reported is 106 µg/kg and the highest is 514 µg/kg. The consensus value is 152 µg/kg with a robust standard deviation of 48 µg/kg (resulting in an RSD_R of 32%) expressing the reproducibility within this PT. The robust standard deviation of 48 µg/kg is comparable to the target standard deviation σ_P of 38 µg/kg suggested in §3.3 for feed material. With only five laboratories reporting quantitative results, statistic evaluation is not appropriate.

4.4.2 3-acetyl-Deoxynivalenol

Seven participants reported quantitative results for 3-Ac-DON. Two participants (PT9615 and PT9618) reported the sum of 15-Ac-DON and 3-Ac-DON.
The lowest concentration reported is 432 µg/kg and the highest is 713 µg/kg. The consensus value is 645 µg/kg with a robust standard deviation of 69 µg/kg (resulting in an RSD_R of 11%) expressing the reproducibility within this PT. The robust standard deviation of 69 µg/kg is almost 2 times lower than

the target standard deviation σ_P of 161 µg/kg. The uncertainty of the consensus value is 33 µg/kg, which does not exceed $0.3\sigma_P$ (§3.2). With respect to the accuracy all results are satisfactory.

4.4.3 Deoxynivalenol-3-glucoside

Four participants reported quantitative results for DON-3G.
The lowest concentration reported is 726 µg/kg and the highest is 1517 µg/kg. The consensus value is 1144 µg/kg with a robust standard deviation of 418 µg/kg (resulting in an RSD_R of 37%) expressing the reproducibility within this PT. The robust standard deviation of 418 µg/kg is almost 1.5 times higher than the target standard deviation σ_P of 286 µg/kg. With only four laboratories reporting quantitative results, statistic evaluation is not appropriate.

4.4.4 Deoxynivalenol

Nineteen participants reported quantitative results for DON. Participant PT9609 failed to detect the presence of DON and reported a false negative result.
The lowest concentration reported is 2627 µg/kg and the highest is 5682 µg/kg. The consensus value is 4268 µg/kg with a robust standard deviation of 729 µg/kg (resulting in an RSD_R of 17%) expressing the reproducibility within this PT. The robust standard deviation of 729 µg/kg is higher to the target standard deviation σ_P of 549 µg/kg. The uncertainty of the consensus value is 209 µg/kg, which exceeds $0.3\sigma_P$ (§3.2), so the uncertainty is included in the calculation of the z´-scores (Equation II is used). With respect to the accuracy four laboratories reported questionable results (PT9610, PT9624, PT9625 and PT9628).

4.4.5 Aflatoxin B1

Twenty-one of the 23 participants reported quantitative results for AFLA.
The lowest concentration reported is 5.45 µg/kg and the highest is 12.6 µg/kg. The consensus value is 10.3 µg/kg with a robust standard deviation of 1.6 µg/kg (resulting in an RSD_R of 15%) expressing the reproducibility within this PT. The robust standard deviation of 1.6 µg/kg is almost two times lower than the target standard deviation σ_P of 2.6 µg/kg. The uncertainty of the consensus value is 0.43 µg/kg, which does not exceed $0.3\sigma_P$ (§3.2). With respect to the accuracy all results are satisfactory.

4.4.6 Enniatin B

Four participants reported quantitative results for ENN B.
The lowest concentration reported is 74.2 µg/kg and the highest is 211 µg/kg. The consensus value is 115 µg/kg with a robust standard deviation of 39 µg/kg (resulting in an RSD_R of 34%) expressing the reproducibility within this PT. The robust standard deviation of 39 µg/kg is higher than the target standard deviation σ_P of 29 µg/kg (25% of the consensus value). With only four laboratories reporting quantitative results, statistic evaluation is not appropriate.

4.4.7 Enniatin B1

Four participants reported quantitative results for ENN B1.
The lowest concentration reported is 31.4 µg/kg and the highest is 110.3 µg/kg. The consensus value is 65 µg/kg with a robust standard deviation of 35 µg/kg (resulting in an RSD_R of 48%) expressing the reproducibility within this PT. The robust standard deviation of 35 µg/kg is two times higher than the target standard deviation σ_P of 16.2 µg/kg. With only four laboratories reporting quantitative results, statistic evaluation is not appropriate.

4.4.8 Zearalenone

Twenty-two participants reported quantitative results for ZEN.
The lowest concentration reported is 172 µg/kg and the highest is 390 µg/kg. The consensus value is 289 µg/kg with a robust standard deviation of 38 µg/kg (resulting in an RSD_R of 13%) expressing the reproducibility within this PT. The robust standard deviation of 38 µg/kg is almost two times lower than

the target standard deviation σ_P of 72 µg/kg. The uncertainty of the consensus value is 10.2 µg/kg, which does not exceed $0.3\sigma_P$ (§3.2). With respect to the accuracy all results are satisfactory.

4.4.9 T-2 toxin

Sixteen participants reported quantitative results for T-2 toxin. Three participants reported '< …' values of which lab PT9627 reported '< 10 µg/kg'. This is considered a false negative result (see explanation in §2.7).
The lowest concentration reported is 21 µg/kg and the highest is 78.9 µg/kg. The consensus value is 24 µg/kg with a robust standard deviation of 2.4 µg/kg (resulting in an RSD_R of 10%) expressing the reproducibility within this PT. The robust standard deviation of 2.4 µg/kg is more than two times lower than the target standard deviation σ_P of 6.1 µg/kg. The uncertainty of the consensus value is 0.74 µg/kg, which does not exceed $0.3\sigma_P$ (§3.2). With respect to the accuracy two results are unsatisfactory (PT9620 and PT9625).

4.4.10 HT-2 toxin

Eighteen participants reported quantitative results for HT-2 toxin. Participant PT9625 failed to detect the presence of HT-2 toxin and reported a false negative result.
The lowest concentration reported is 23.8 µg/kg and the highest is 90 µg/kg. The consensus value is 61 µg/kg with a robust standard deviation of 9.2 µg/kg (resulting in an RSD_R of 15%) expressing the reproducibility within this PT. The robust standard deviation of 9.2 µg/kg is almost tow times lower than the target standard deviation σ_P of 15.2 µg/kg. The uncertainty of the consensus value is 2.7 µg/kg, which does not exceed $0.3\sigma_P$ (§3.2).). A consequential instability (15% decrease) for HT-2 toxin during storage was observed. With respect to the accuracy one result is questionable (PT9627).

4.4.11 Sum of T-2 and HT-2 toxins

Nineteen participants reported quantitative results for T-2 and/or HT-2 toxin. Sum-concentrations were calculated ('< values' were not taken into account) by adding the concentrations.
The lowest sum-concentration reported is 23.8 µg/kg and the highest is 144.3 µg/kg. The consensus value is 83 µg/kg with a robust standard deviation of 15.0 µg/kg (resulting in an RSD_R of 18%) expressing the reproducibility within this PT. The robust standard deviation of 15.0 µg/kg is lower than the target standard deviation σ_P of 21 µg/kg. The uncertainty of the consensus value is 4.3 µg/kg, which does not exceed $0.3\sigma_P$ (§3.2). A consequential instability (15% decrease) for HT-2 toxin during storage was observed. With respect to the accuracy two participants reported questionable results (PT9620 and PT9627). In case no instability of HT-2 toxin occurred, the z-score for participant PT9627 would still be questionable.

4.4.12 Other mycotoxins

Participant PT9627 reported a false positive result by detecting the presence of 3.5 µg/kg ochratoxin A.

5 Discussion and conclusions

Twenty-three participants subscribed for the proficiency test on mycotoxins in oat meal and all reported results. Each participant was asked to indicate *a priori* which compounds were included in their scope. This allowed the evaluation of the results which regard to the participants' scope.

Two materials were sent to the participants. The mycotoxins were homogeneously distributed in the materials. An overview of each participant's performance is shown in Annex 9 and a summary of the results is presented in Table 6.

Table 6 *Summarized performance of participants reporting results.*

Compound	# results	quantitative result	FN	q z-score	u z-score	used z-score	correct results (%)
Material A							
15-Ac-DON	7	5				not possible	
3-Ac-DON	9	7				z_a	78
DON-3G	4	4				not possible	
DON	21	20	1	2		z_a	86
AFLA	22	22				z_a	100
ENN B	4	4				not possible	
ENN B1	4	4				not possible	
ZEN	21	21			1	z_a	95
T2	19	19		1	1	z_a	89
HT2	19	19		2		z_a	89
Sum T2/HT2	19	19		1		z_a	95
Material B							
15-Ac-DON	7	5				not possible	
3-Ac-DON	9	7				z_a	78
DON-3G	4	4				not possible	
DON	22	19	1	4		z'_a	75
AFLA	21	21				z_a	100
ENN B	4	4				not possible	
ENN B1	4	4				not possible	
ZEN	22	22				z_a	100
T2	20	16			2	z_a	70
HT2	20	18		1		z_{ai}	85
Sum T2/HT2	19	19	2*	2		z_a	89

* false negative results for the individual toxins

FN false negatives

q questionable z-score

u unsatisfactory z-score

Two participants failed to submit results for aflatoxin B1, the regulated mycotoxin in animal feed. Maximum three participants did not submit results for ZEN and DON, which have a guidance value in EU legislation. Four participants analysed for all three DON conjugates, as advised by the Commission. Five participants analysed for 3- and 15-Ac-DON, besides DON, and two laboratories analysed for 3-Ac-DON and DON-3G.

Five participants showed optimal performance by detecting the mycotoxins with a correct quantification/qualification (14 satisfactory z-scores), the absence of false positive and false negative results and reporting within the deadline. Another ten showed suboptimal performance within their

scope by reporting thirteen or less satisfactory z-scores. Eight participants reported FN, FP, questionable or unsatisfactory z-scores. A total of thirteen questionable z-scores, four unsatisfactory z-score, four false positive and two false negative results were reported.

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

- The optimal quantification for the mycotoxins in oat meal varied from 70-100% in this proficiency test. T-2 toxin in material B was the most difficult compound to quantify; all quantitative results for AFLA in both materials were satisfactory.
- The determination of DON, ZEN and T-2/HT-2 toxin should be included in the scope of all participants since guideline values/indicative levels have been set at an EU-level.
- The determination of 15-Ac-DON, 3-Ac-DON and DON-3G should be included in the scope of all participants due to the advice of the Commission and because it is foreseen that these forms of DON will be included in future legislation.

References

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9 Regulation (EC) No 396/2005 of 23 February 2005 on maximum residue levels of mycotoxins in or on food and feed of plant and animal origin and amending Council 91/414/EEC.

10 EC 2014. Compilation of agreed monitoring recommendations as regards the presence of mycotoxins and plant toxins in food. Summary report of the standing committee on plants, animals, food and feed, held in Brussels on 1 JULY 2014 (Section Toxicological Safety of the Food chain, https://ec.europa.eu/food/safety/chemical_safety/contaminants/catalogue/mycotoxins_en)

11 EC. 2002. Directive 2002/32/EC of the European Parliament and of the council of 7 May 2002 on undesirable substances in animal feed (https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1572259187508&uri=CELEX:02002L0032-20171225)

12 EC. 2006. Commission Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding (Text with EEA relevance) (2006/576/EC) https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02006H0576-20160802&qid=1572259308626&from=EN

13 Performance assessment in proficiency tests organised by the EURL mycotoxins & plant toxins in food and feed v1, 2019. EURL mycotoxins & plant toxins, WFSR Wageningen University & Research.

Annex 1 Codification of the samples

Participants code	Material A*	Material B*
PT9604	194	161
PT9607	584	323
PT9608	915	696
PT9609	920	796
PT9610	993	743
PT9611	213	420
PT9612	746	314
PT9613	425	202
PT9614	557	467
PT9615	702	941
PT9616	860	367
PT9617	694	961
PT9618	967	828
PT9619	465	937
PT9620	189	525
PT9621	383	880
PT9622	551	398
PT9623	671	100
PT9624	112	521
PT9625	938	864
PT9626	222	526
PT9627	486	637
PT9628	527	887

* All sample codes start with 2019/mycotoxins/oats/

Annex 2 Instruction letter

WAGENINGEN
UNIVERSITY & RESEARCH

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

RIKILT

Contaminants & Toxins

DATE
April 8, 2019

SUBJECT
proficiency test for analysis of mycotoxins in feed matrix oats

OUR REFERENCE
1921564/RIK

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

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

INTERNET
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COC NUMBER
09098104

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

TELEPHONE
+31 (0)614 323 017

EMAIL
pt.rikilt@wur.nl

Dear Madam/Sir,

Thank you very much for your interest in the proficiency test for the analysis of the mycotoxins in the feed matrix oats. Hereby I send you a parcel containing two randomly coded samples. Each sample consists of approximately 35 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.rikilt@wur.nl)

Instructions:

- After arrival store the samples in the freezer.
- Before analysis, homogenize them according to your laboratory's procedure.
- Treat the test material as if it was a sample for routine analysis. Report one result and not an average of multiple measurements.
- Report all results in **µg/kg** for the product as received (i.e. no correction moisture is needed). When a mycotoxin 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 mycotoxin is 'not detected' or the result is below your LOQ, report the result as <LOQ-value and specify the value (e.g. <20 µg/kg).
- 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.
- The deadline for submitting test-results for this test is **May 20th2019.**

- 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

D.P.K.H. Pereboom - de Fauw
Proficiency tests
Pt.rikilt@wur.nl

100years 1918 — 2018

Wageningen Research Foundation/RIKILT is part of Wageningen University & Research. RIKILT carries out research into the safety and reliability of food and feed. RIKILT is ISO 17025 and ISO 17043 accredited (the accredited tests are described on www.rva.nl (no. L014 and R013).

Annex 3 Statistical evaluation of homogeneity data

	Aflatoxin B1 in material A (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/A001	16.96	16.45
Hom/A002	16.88	16.81
Hom/A003	16.75	16.65
Hom/A004	17.11	16.75
Hom/A005	15.61	16.96
Hom/A006	16.94	16.98
Hom/A007	16.93	16.55
Hom/A008	17.29	17.17
Hom/A009	16.48	16.96
Hom/A010	17.69	16.47
Grand mean	16.8	
Cochran's test		
C	0.443	
C_{crit}	0.602	
$C < C_{crit}$?	NO OUTLIERS	
Target s = σ_P	4.21	
s_x	0.258	
s_w	0.454	
s_s	0.000	
Critical= 0.3 σ_P	1.262	
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.

	3-Acetyl-Deoxynivalenol in material A (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/A001	565.90	616.41
Hom/A002	584.89	598.43
Hom/A003	519.23	553.47
Hom/A004	574.06	555.93
Hom/A005	518.27	557.42
Hom/A006	594.12	577.58
Hom/A007	588.11	561.32
Hom/A008	576.97	549.22
Hom/A009	554.41	512.49
Hom/A010	579.42	617.70
Grand mean	567.78	
Cochran's test		
C	0.237	
C_{crit}	0.602	
$C < C_{crit}$?	NO OUTLIERS	
Target s = σ_P	141.94	
s_x	24.79	
s_w	23.19	
s_s	18.59	
Critical= 0.3 σ_P	42.58	
s_s < critical?	ACCEPTED	
$s_w < 0.5\ \sigma_P$?	ACCEPTED	

	15-Acetyl-Deoxynivalenol in material A (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/A001	19.38	18.90
Hom/A002	18.78	19.52
Hom/A003	19.33	17.94
Hom/A004	18.33	19.56
Hom/A005	17.39	17.79
Hom/A006	19.51	19.82
Hom/A007	18.86	19.70
Hom/A008	19.58	20.34
Hom/A009	18.66	19.66
Hom/A010	20.82	18.92
Grand mean	19.14	
Cochran's test		
C	0.349	
C_{crit}	0.602	
C < C_{crit}?	NO OUTLIERS	
Target s = σ_P	4.78	
s_x	0.683	
s_w	0.719	
s_s	0.456	
Critical= 0.3 σ_P	1.435	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

	Deoxynivalenol in material A (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/A001	3622.39	3482.17
Hom/A002	3704.33	3556.63
Hom/A003	3278.92	3340.25
Hom/A004	3891.11	3864.71
Hom/A005	3554.09	3442.36
Hom/A006	3745.54	3673.19
Hom/A007	3664.93	3739.59
Hom/A008	3775.61	3853.91
Hom/A009	3649.23	3512.61
Hom/A010		
Grand mean	3631	
Cochran's test		
C	0.232	
C_{crit}	0.638	
C < C_{crit}?	NO OUTLIERS	
Target s = σ_P	907.7	
s_x	171.6	
s_w	72.27	
s_s	163.8	
Critical= 0.3 σ_P	272.3	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

	Deoxynivalenol-3-glucoside in material A (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/A001	674.51	706.11
Hom/A002	690.72	665.37
Hom/A003	600.46	584.58
Hom/A004	616.01	626.93
Hom/A005	613.85	711.35
Hom/A006	662.16	610.90
Hom/A007	579.71	574.80
Hom/A008	639.05	623.41
Hom/A009	615.70	505.27
Hom/A010	659.63	619.35
Grand mean	628.99	
Cochran's test		
C	0.432	
C_{crit}	0.602	
C < C_{crit}?	NO OUTLIERS	
Target s = σ_P	157.25	
s_x	42.46	
s_w	37.57	
s_s	33.12	
Critical= 0.3 σ_P	47.17	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

Sample No.	Enniatin B in material A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	85.32	81.32
Hom/A002	87.08	78.46
Hom/A003	85.97	89.31
Hom/A004	87.88	91.67
Hom/A005	87.58	81.61
Hom/A006	83.82	84.69
Hom/A007	79.80	82.46
Hom/A008	88.89	86.56
Hom/A009	87.25	91.50
Hom/A010	92.38	86.70
Grand mean	86.01	
Cochran's test		
C	0.345	
C_{crit}	0.602	
C < C_{crit}?	NO OUTLIERS	
Target s = σ_P	21.50	
s_x	3.164	
s_w	3.278	
s_s	2.154	
Critical= 0.3 σ_P	6.451	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

Sample No.	Enniatin B1 in material A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	46.50	42.78
Hom/A002	48.72	43.98
Hom/A003	47.31	50.72
Hom/A004	49.88	50.65
Hom/A005	51.28	46.49
Hom/A006	45.62	48.93
Hom/A007	40.91	43.22
Hom/A008	44.50	45.48
Hom/A009	52.01	50.24
Hom/A010	52.31	49.79
Grand mean	47.57	
Cochran's test		
C	0.234	
C_{crit}	0.602	
C < C_{crit}?	NO OUTLIERS	
Target s = σ_P	11.89	
s_x	3.036	
s_w	2.216	
s_s	2.601	
Critical= 0.3 σ_P	3.567	
s_s < critical?	NOT ACCEPTED	
s_w < 0.5 σ_P?	NOT ACCEPTED	

Sample No.	HT-2 toxin in material A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	203.66	203.17
Hom/A002	198.40	197.49
Hom/A003	203.23	200.40
Hom/A004	192.75	189.16
Hom/A005	197.28	191.16
Hom/A006	190.25	208.12
Hom/A007	200.81	200.75
Hom/A008	211.84	202.46
Hom/A009	197.30	188.03
Hom/A010	201.01	206.13
Grand mean	199.17	
Cochran's test		
C	0.552	
C_{crit}	0.602	
C < C_{crit}?	NO OUTLIERS	
Target s = σ_P	49.79	
s_x	5.234	
s_w	5.380	
s_s	3.594	
Critical= 0.3 σ_P	14.938	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

Sample No.	T-2 toxin in material A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	52.62	51.64
Hom/A002	48.19	50.40
Hom/A003	51.44	55.61
Hom/A004	53.48	51.95
Hom/A005	52.30	52.97
Hom/A006	48.90	54.90
Hom/A007	56.89	54.56
Hom/A008	56.16	54.00
Hom/A009	53.90	50.69
Hom/A010	52.13	52.36
Grand mean	52.75	
Cochran's test		
C	0.437	
C_{crit}	0.602	
C < C_{crit}?	NO OUTLIERS	
Target s = σ_P	13.19	
s_x	1.776	
s_w	2.029	
s_s	1.046	
Critical= 0.3 σ_P	3.957	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

Sample No.	Zearalenone in material A (µg/kg)	
	Replicate 1	Replicate 2
Hom/A001	212.64	191.65
Hom/A002	215.11	215.57
Hom/A003	223.51	191.28
Hom/A004	187.94	178.22
Hom/A005	197.66	204.69
Hom/A006	190.31	203.57
Hom/A007	223.16	180.24
Hom/A008	185.02	173.75
Hom/A009	185.48	192.98
Hom/A010	167.84	196.52
Grand mean	195.86	
Cochran's test		
C	0.396	
C_{crit}	0.602	
C < C_{crit}?	NO OUTLIERS	
Target s = σ_P	48.96	
s_x	11.93	
s_w	15.24	
s_s	5.12	
Critical= 0.3 σ_P	14.69	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

Sample No.	Aflatoxin B1 in material B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	7.07	5.93
Hom/B002	6.38	6.35
Hom/B003	5.89	6.35
Hom/B004	6.55	6.39
Hom/B005	6.32	7.54
Hom/B006	6.33	6.65
Hom/B007	misinjection	misinjection
Hom/B008	6.35	6.19
Hom/B009	6.57	6.49
Hom/B010	6.52	7.21
Grand mean	6.504	
Cochran's test		
C	0.408	
C_{crit}	0.638	
C < C_{crit}?	NO OUTLIERS	
Target s = σ_P	1.63	
s_x	0.259	
s_w	0.448	
s_s	0.000	
Critical= 0.3 σ_P	0.49	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

	3-Acetyl_Deoxynivalenol in material B (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/B001	569.32	579.06
Hom/B002	566.09	588.06
Hom/B003	596.76	568.37
Hom/B004	606.23	568.18
Hom/B005	608.80	611.63
Hom/B006	572.40	615.03
Hom/B007	564.99	557.79
Hom/B008	546.06	545.88
Hom/B009	604.77	587.56
Hom/B010	537.35	599.09
Grand mean	579.67	
Cochran's test		
C	0.432	
C_{crit}	0.602	
C < C_{crit}?	NO OUTLIERS	
Target s = σ_P	144.9	
s_x	18.58	
s_w	21.00	
s_s	11.16	
Critical= 0.3 σ_P	43.48	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

	15-Acetyl-Deoxynivalenol in material B (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/B001	21.89	21.16
Hom/B002	21.89	22.43
Hom/B003	19.12	22.83
Hom/B004	20.13	21.58
Hom/B005	20.46	27.12
Hom/B006	17.33	24.26
Hom/B007	misinjection	misinjection
Hom/B008	21.78	20.15
Hom/B009	18.64	22.46
Hom/B010	23.54	23.51
Grand mean	21.68	
Cochran's test		
C	0.380	
C_{crit}	0.638	
C < C_{crit}?	NO OUTLIERS	
Target s = σ_P	5.42	
s_x	1.22	
s_w	2.65	
s_s	0.000	
Critical= 0.3 σ_P	1.63	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

	Deoxynivalenol in material B (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/B001	4091.14	3981.28
Hom/B002	3926.88	4034.11
Hom/B003	3273.86	3988.25
Hom/B004	4038.58	4024.94
Hom/B005	3865.14	3961.16
Hom/B006	3239.96	3969.49
Hom/B007	misinjection	misinjection
Hom/B008	3755.26	3816.83
Hom/B009	3563.20	3748.91
Hom/B010	4411.47	3898.94
Grand mean	3866	
Cochran's test		
C	0.387	
C_{crit}	0.638	
C < C_{crit}?	NO OUTLIERS	
Target s = σ_P	966.52	
s_x	202.9	
s_w	276.54	
s_s	54.15	
Critical= 0.3 σ_P	289.96	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

	Deoxynivalenol-3-glucoside in material B (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/B001	638.12	626.25
Hom/B002	639.16	635.07
Hom/B003	684.61	623.81
Hom/B004	673.88	661.70
Hom/B005	688.22	613.91
Hom/B006	658.80	743.43
Hom/B007	707.08	556.62
Hom/B008	681.50	617.21
Hom/B009	711.20	648.10
Hom/B010	546.10	620.98
Grand mean	648.79	
Cochran's test		
C	0.427	
C_{crit}	0.602	
C < C_{crit}?	NO OUTLIERS	
Target s = σ_P	162.20	
s_x	31.70	
s_w	51.50	
s_s	0.000	
Critical= 0.3 σ_P	48.66	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

	Enniatin B in material B (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/B001	92.66	80.67
Hom/B002	85.89	79.92
Hom/B003	83.34	84.92
Hom/B004	87.45	81.88
Hom/B005	91.32	83.09
Hom/B006	86.88	84.59
Hom/B007	misinjection	misinjection
Hom/B008	81.52	79.22
Hom/B009	84.46	82.13
Hom/B010	76.26	85.59
Grand mean	83.99	
Cochran's test		
C	0.375	
C_{crit}	0.638	
C < C_{crit}?	NO OUTLIERS	
Target s = σ_P	21.00	
s_x	2.376	
s_w	4.617	
s_s	0.000	
Critical= 0.3 σ_P	6.299	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

	Enniatin B1 in material B (µg/kg)	
Sample No.	Replicate 1	Replicate 2
Hom/B001	54.78	43.70
Hom/B002	46.62	45.11
Hom/B003	47.63	46.97
Hom/B004	46.61	44.36
Hom/B005	50.63	47.51
Hom/B006	47.90	47.61
Hom/B007	misinjection	misinjection
Hom/B008	42.67	46.20
Hom/B009	47.72	45.68
Hom/B010	39.54	48.62
Grand mean	46.66	
Cochran's test		
C	0.513	
C_{crit}	0.638	
C < C_{crit}?	NO OUTLIERS	
Target s = σ_P	11.66	
s_x	1.860	
s_w	3.647	
s_s	0.000	
Critical= 0.3 σ_P	3.499	
s_s < critical?	ACCEPTED	
s_w < 0.5 σ_P?	ACCEPTED	

Sample No.	HT-2 toxin in material B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	75.27	67.29
Hom/B002	67.09	72.88
Hom/B003	58.15	68.60
Hom/B004	65.06	60.33
Hom/B005	59.13	78.07
Hom/B006	64.14	65.44
Hom/B007	misinjection	misinjection
Hom/B008	61.88	71.86
Hom/B009	63.80	68.02
Hom/B010	64.11	73.50
Grand mean	66.92	
Cochran's test		
C	0.451	
C_{crit}	0.638	
$C < C_{crit}$?	NO OUTLIERS	
Target s = σ_P	16.73	
s_x	2.974	
s_w	6.644	
s_s	0.000	
Critical= 0.3 σ_P	5.019	
s_s < critical?	ACCEPTED	
$s_w < 0.5\ \sigma_P$?	ACCEPTED	

Sample No.	Zearalenone in material B (µg/kg)	
	Replicate 1	Replicate 2
Hom/B001	164.76	165.18
Hom/B002	155.97	150.01
Hom/B003	162.36	152.69
Hom/B004	outlier	outlier
Hom/B005	171.64	173.28
Hom/B006	162.47	159.72
Hom/B007	152.01	163.89
Hom/B008	165.58	160.50
Hom/B009	176.58	164.11
Hom/B010	180.31	190.67
Grand mean	165.1	
Cochran's test		
C	0.273	
C_{crit}	0.638	
$C < C_{crit}$?	NO OUTLIERS	
Target s = σ_P	41.27	
s_x	9.83	
s_w	5.62	
s_s	8.99	
Critical= 0.3 σ_P	12.38	
s_s < critical?	ACCEPTED	
$s_w < 0.5\ \sigma_P$?	ACCEPTED	

Annex 4 Statistical evaluation of stability data

*Statistical evaluation for **aflatoxin B1 in material A.***

Storage temperature	<-70 °C	<-18 °C	2 days RT
Time (days)	0	43	43
Calculated amounts (µg/kg)	16.05	16.02	15.31
	15.65	15.10	16.04
	16.35	15.61	15.23
	15.75	15.86	15.83
	15.71	15.56	16.91
	16.44	16.80	16.56
Average amount (µg/kg)	16.0	15.8	16.0
n	6	6	6
st. dev (µg/kg)	0.342	0.573	0.671
Difference		0.165	0.011
$0.3*\sigma_P$		1.199	1.199
Consequential difference? Diff < $0.3*\sigma_P$		NO	NO

*Statistical evaluation for **3-Ac-DON in material A.***

Storage temperature	<-70 °C	<-18 °C	2 days RT
Time (days)	0	43	43
Calculated amounts (µg/kg)	596.85	616.34	575.36
	567.32	544.86	578.23
	533.71	624.58	574.39
	587.42	577.99	566.25
	597.11	540.52	571.03
	571.26	587.01	576.35
Average amount (µg/kg)	575.6	581.9	573.6
n	6	6	6
st. dev (µg/kg)	24.07	35.025	4.319
Difference		-6.27	2.010
$0.3*\sigma_P$		43.17	43.17
Consequential difference? Diff < $0.3*\sigma_P$		NO	NO

*Statistical evaluation for **15-Ac-DON in material A.***

Storage temperature	<-70 °C	<-18 °C	2 days RT
Time (days)	0	43	43
Calculated amounts (µg/kg)	29.78	30.18	29.61
	31.35	30.86	31.63
	32.55	30.26	20.87
	32.54	34.26	33.57
	28.80	29.89	32.14
	30.56	33.29	32.08
Average amount (µg/kg)	30.9	31.5	30.0
n	6	6	6
st. dev (µg/kg)	1.509	1.848	4.642
Difference		-0.525	0.946
$0.3*\sigma_P$		2.32	2.32
Consequential difference? Diff < $0.3*\sigma_P$		NO	NO

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

Storage temperature	<-70 °C	<-18 °C	2 days RT
Time (days)	0	43	43
Calculated amounts (µg/kg)	3986.21	3992.84	3838.54
	3945.81	3766.77	3947.20
	3878.49	3885.78	3738.05
	3936.03	3867.29	3943.09
	3830.92	3790.93	4180.56
	3859.93	4059.74	3910.11
Average amount (µg/kg)	3906	3894	3926
n	6	6	6
st. dev (µg/kg)	59.054	113.9	147.5
Difference		12.34	-20.03
$0.3*\sigma_P$		293	293
Consequential difference? Diff < $0.3*\sigma_P$		NO	NO

*Statistical evaluation for **DON-3G in material A.***

Storage temperature	<-70 °C	<-18 °C	2 days RT
Time (days)	0	43	43
Calculated amounts (µg/kg)	530.97	601.34	518.55
	528.48	479.29	534.71
	483.25	519.61	507.44
	515.34	577.77	506.85
	574.27	557.70	516.44
	503.02	454.81	525.53
Average amount (µg/kg)	523	532	518
n	6	6	6
st. dev (µg/kg)	30.9	57.4	10.7
Difference		-9.198	4.30
$0.3*\sigma_P$		39.2	39.2
Consequential difference? Diff < $0.3*\sigma_P$		NO	NO

*Statistical evaluation for **enniatin B in material A.***

Storage temperature	<-70 °C	<-18 °C	2 days RT
Time (days)	0	43	43
Calculated amounts (µg/kg)	83.00	83.27	74.09
	81.61	77.97	79.66
	79.78	74.02	69.38
	82.10	77.48	80.36
	78.59	80.47	81.64
	81.67	83.15	81.05
Average amount (µg/kg)	81.1	79.4	77.7
n	6	6	6
st. dev (µg/kg)	1.63	3.60	4.90
Difference		1.73	3.43
$0.3*\sigma_P$		6.08	6.08
Consequential difference? Diff < $0.3*\sigma_P$		NO	NO

*Statistical evaluation for **enniatin B1 in material A.***

Storage temperature	<-70 °C	<-18 °C	2 days RT
Time (days)	0	43	43
Calculated amounts (µg/kg)	49.72	49.35	41.97
	50.18	46.34	47.49
	47.96	41.78	40.52
	50.38	45.46	47.28
	48.38	50.48	48.72
	49.77	53.14	48.47
Average amount (µg/kg)	49.4	47.8	45.7
n	6	6	6
st. dev (µg/kg)	0.991	4.05	3.56
Difference		1.64	3.66
$0.3*\sigma_P$		3.71	3.71
Consequential difference? Diff < $0.3*\sigma_P$		NO	NO

*Statistical evaluation for **HT-2 toxin in material A.***

Storage temperature	<-70 °C	<-18 °C	2 days RT
Time (days)	0	43	43
Calculated amounts (µg/kg)	151.50	147.89	141.84
	154.47	141.63	142.71
	148.09	142.57	135.67
	153.33	140.46	146.50
	153.03	148.93	159.28
	148.32	158.64	146.67
Average amount (µg/kg)	152	147	145
n	6	6	6
st. dev (µg/kg)	2.69	6.79	7.877
Difference		4.77	6.01
$0.3*\sigma_P$		11.4	11.4
Consequential difference? Diff < $0.3*\sigma_P$		NO	NO

*Statistical evaluation for **T-2 toxin in material A.***

Storage temperature	<-70 °C	<-18 °C	2 days RT
Time (days)	0	43	43
Calculated amounts (µg/kg)	102.20	98.63	94.66
	104.19	96.70	100.64
	102.85	98.23	94.54
	105.98	95.84	96.30
	97.61	100.09	105.28
	101.22	106.36	102.95
Average amount (µg/kg)	102.3	99.3	99.1
n	6	6	6
st. dev (µg/kg)	2.85	3.76	4.55
Difference		3.04	3.28
$0.3*\sigma_P$		7.68	7.68
Consequential difference? Diff < $0.3*\sigma_P$		NO	NO

*Statistical evaluation for z**earalenone in material A.***

Storage temperature	<-70 °C	<-18 °C	2 days RT
Time (days)	0	43	43
Calculated amounts (µg/kg)	262.28	262.31	250.97
	253.89	239.10	289.41
	275.56	253.65	252.63
	270.86	256.12	281.84
	259.21	274.93	278.53
	251.77	279.59	260.17
Average amount (µg/kg)	262	261	269
n	6	6	6
st. dev (µg/kg)	9.38	14.8	16.4
Difference		1.31	-6.66
$0.3*\sigma_P$		19.7	19.7
Consequential difference? Diff < $0.3*\sigma_P$		NO	NO

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

Storage temperature	<-70 °C	<-18 °C	2 days RT
Time (days)	0	43	43
Calculated amounts (µg/kg)	6.17	6.04	6.86
	6.50	6.30	6.62
	5.97	6.18	5.95
	6.80	6.65	6.19
	6.73	6.35	6.41
	6.91	6.50	6.38
Average amount (µg/kg)	6.51	6.34	6.40
n	6	6	6
st. dev (µg/kg)	0.373	0.219	0.318
Difference		0.179	0.111
$0.3*\sigma_P$		0.489	0.489
Consequential difference? Diff < $0.3*\sigma_P$		NO	NO

*Statistical evaluation for **HT-2 toxin in material B.***

Storage temperature	<-70 °C	<-18 °C	2 days RT
Time (days)	0	43	43
Calculated amounts (µg/kg)	50.41	46.54	47.32
	50.37	42.85	48.49
	45.36	outlier	45.71
	67.58	46.57	41.93
	50.12	44.89	43.01
	60.07	47.40	49.86
Average amount (µg/kg)	54.0	45.6	46.1
n	6	5	6
st. dev (µg/kg)	0.8.21	1.81	3.11
Difference		8.34	7.93
$0.3*\sigma_P$		4.05	4.05
Consequential difference? Diff < $0.3*\sigma_P$		YES	YES

*Statistical evaluation for **T-2 toxin in material B.***

Storage temperature	<-70 °C	<-18 °C	2 days RT
Time (days)	0	43	43
Calculated amounts (µg/kg)	18.9	17.8	18.1
	17.8	17.4	18.0
	16.2	outlier	19.1
	19.9	18.5	16.7
	18.6	16.8	17.5
	21.7	19.5	17.4
Average amount (µg/kg)	18.8	18.0	17.8
n	6	6	6
st. dev (µg/kg)	1.89	1.02	0.81
Difference		0.8	1.0
$0.3*\sigma_P$		1.41	1.41
Consequential difference? Diff < $0.3*\sigma_P$		NO	NO

Annex 5 Overview of the applied methods

Lab	Compound	Sample purification	Internal standard	Detection technique
PT9604	all	Immunoaffinity	no	Fluorescentie - Fumonisin/zearalenon/aflatoxin/ochratoxin UV - Vomitoxin
PT9607		Extractie met acetonitril/water/mierenzuurmengsel	isotoop gelabelde mycotoxines	triple quadrupool massaspectrometer
PT9608	DON-3G	25g sample + 100ml Acetonitril/Water/Acetic acid (79/20/1); 120 min stirring no clean up only dilution 1:10 "Dilute & Shoot"	fully 13C-labeld analogs of each mycotoxin (Biopure/RomerLabs)	LC-MS/MS LC-QqQ ESI Electro Spray Ionisation 2 MRMs/compound (identification criteria according to Hans Mol)
PT9610		shake with solvent (ACN/H2O/FA:80:20:0.1) centrifuge filter: Oasis Prime HLB Evaporate and reconstitute with ACN/H2O (16:84)	13C internal standard for every analyte.	MS-MS
PT9611	AFLA	add NaCl, add extraction solvent MeOH/H2O (80/20), shake, filtration, clean up by IAC		LC-FLD
PT9611	DON, ZEN, HT2, T2	add extraction solvent ACN/H20 (80/20), shake, dilute	DON-C13, ZAN, HT2-C13, T2-C13	LC-MS/MS
PT9611	ENN	add extraction solvent ACN/H2O (80/20), shake, dilute		LC-MS/MS
PT9611	OTA	add NaHCO3, add extraction solvent MeOH/H2O (80/20), shake, clean up by IAC		LC-FLD
PT9612	AFLA	5g sample. Extraction with methanol/water. Clean-up with immunoaff. column	No	FLD
PT9612	DON	2.5g sample. extraction with water. Clean up with immunoaff. column	No	UV detection
PT9612	OTA	5g sample. extraction with 40ml acetonitrile/water. Clean-up with immunoaff. column	No	FLD
PT9612	T2, HT2	10g sample. Extraction with methanol/water 90/10. Dilution of filtrate with water. pH7.4 Clean up with immunoaff column. Derivatiz. with DMAP and 1-AN	No	FLD
PT9612	ZEN, DON relatives, ENN	5g sample. Extraction with 50ml acetonitrile / water (90/10). Shaking mashine. Filtration. 10ml filtrate is diluted with 40ml of water. 10mldiluted filtrate is passed through a immunoaffinity column. Washing, elution with methanol, evaporation and reconstit	No	Fluorescence detection

Lab	Compound	Sample purification	Internal standard	Detection technique
PT9613		-5g sample + 10mL H2O (soaking time 1 hour) -10mL acidified acetonitrile (0.1% formic acid) -manual shaking 2 minute -add 6.5 g Quechers mixture -manual shaking 2 minuts -Centrifuge at 5000 g 5 minuts -Filter and inject	no internal standard	LC-MS/MS
PT9614		ACN/water no clean-up	several	LCMSMS
PT9616		Different for each parameter	No	UPLC/MS/MS
PT9617		Inmunoafinity column	Not used	LC-MS/MS
PT9618		Extraction solvent: 80% acetonitrile in water 5 gram oat feed, 20 ml solvent. Clean-up: pass through "Bond Elut mycotoxin" SPE-column. 2 ml cleaned extracts is evaporated and dissolved in 1 ml 25% acetonitrile in water.	None. Only instrument suitability standard (zearalanone) added to each sample.	LC-Q-Orbitrap (model: Thermo Q-Exactive) in tSIM-ddMS2 mode.
PT9620		HPLC-ACN 1%FA shake	IS Myc	LC-MSMS
PT9621		Shake with acetonitrile/water, centrifuge and filter	13C labelled stds for each analyte	LCMSMS
PT9622		AcN/Acetic acid/H2) no clean up	Isotopic labelled standards	LCMSMS
PT9623		Extraction with acified acetonitril/water	13C for each component	MSMS
PT9624		QuEChERS	no IS	LC-MS/MS
PT9625		Extraction by shaking with a mixture of acetonitrile and aqueous formic acid solution	13C 15 Deoxynivalenol 13C 17 Aflatoxin B1 13C 20Ochratoxin A	MS detection
PT9627	AFLA, DON relatives, ENN	Extraction solvent: C3H6O:H2O 85:15 Clean-up: IAC	External calibration	FLD
PT9627	DON, DON-3-G, T2, HT2, ZEN	Extraction solvent: ACN:H2O 84:16 Clean-up: SPE	External calibration	LC MSMS
PT9627	OTA	Extraction solvent: MeOH:H2O 50:50 Clean-up: IAC	External calibration	FLD
PT9627	T2, HT2	Extraction solvent: ACN:H2O 84:16 Clean-up: SPE	External calibration	LC MSMS
PT9627	ZEN	Extraction solvent: ACN:H2O 84:16 Clean-up: SPE	External calibration	LC MSMS

Annex 6 Results material A

	15-Ac-DON CV: 161 µg/kg u: 38 µg/kg σ_p: 40 µg/kg robust σ: 67 µg/kg		3-Ac-DON CV: 613 µg/kg u: 26 µg/kg σ_p: 153 µg/kg robust σ: 56 µg/kg		DON-3G CV: 1143 µg/kg u: 181 µg/kg σ_p: 286 µg/kg robust σ: 290 µg/kg		DON CV: 4174 µg/kg u: 176 µg/kg σ_p: 1044 µg/kg robust σ: 629 µg/kg	
Lab code	Result (µg/kg)	z_a-score	Result (µg/kg)	z'_a-score	Result (µg/kg)	z_a-score	Result (µg/kg)	z_a-score
PT9604		NO				NO	4557	0.37
PT9607		STATISTICAL				STATISTICAL	4036	-0.13
PT9608	93.6	EVALUATION	631	0.12	1227	EVALUATION	4277	0.10
PT9609		POSSIBLE				POSSIBLE	FN	
PT9610							2572	-1.54
PT9611	250	TOO	479	-0.87		TOO	3874	-0.29
PT9612		LITTLE				LITTLE	4666.4	0.47
PT9613		RESULTS				RESULTS	3886.78	-0.28
PT9614	494		486	-0.83			4610	0.42
PT9615	422.9 for the sum of 15-Ac-DON and 3-Ac-DON						3657.6	-0.50
PT9616							3009	-1.12
PT9617								
PT9618	1330.5 for the sum of 15-Ac-DON and 3-Ac-DON						4249.8	0.07
PT9619								
PT9620	nd		415	-1.29	1413		3509	-0.64
PT9621	nd		664	0.33	569		4539	0.35
PT9622							3860	-0.30
PT9623							4332	0.15
PT9624	135.84		654.44	0.27	1087.1		5680.16	1.44
PT9625	nd						6458	2.19
PT9626	136		653	0.26			4170	0.00
PT9627							3300	-0.84
PT9628							6860	2.57

CV = consensus value.

u = uncertainty of consensus value.

nd = not detected

	Aflatoxin B1 CV: 25 µg/kg u: 1.5 µg/kg σ_p: 6.2 µg/kg robust σ: 5.5 µg/kg		Enniatin B CV: 121 µg/kg u: 28 µg/kg σ_p: 30 µg/kg robust σ: 46 µg/kg		Enniatin B1 CV: 65 µg/kg u: 19.7 µg/kg σ_p: 16.2 µg/kg robust σ: 31 µg/kg		Zearalenone CV: 293 µg/kg u: 11.3 µg/kg σ_p: 73 µg/kg robust σ: 41 µg/kg	
Lab code	Result (µg/kg)	z_a-score	Result (µg/kg)	z_a-score	Result (µg/kg)	z_a-score	Result (µg/kg)	z_a-score
PT9604	16.2	-1.37		NO		NO	253	-0.54
PT9607	26.96	0.37		STATISTICAL		STATISTICAL	317	0.34
PT9608	29.3	0.75		EVALUATION		EVALUATION	314	0.29
PT9609	31	1.03		POSSIBLE		POSSIBLE	290	-0.03
PT9610	29	0.70					231	-0.84
PT9611	18.9	-0.94	197	TOO	55	TOO	309	0.23
PT9612	25.2	0.08		LITTLE		LITTLE	288.3	-0.06
PT9613	24	-0.11		RESULTS		RESULTS	292.06	-0.01
PT9614	29	0.70					289	-0.05
PT9615	24.1	-0.09					283.4	-0.12
PT9616	13.3	-1.84						
PT9617	29	0.70					177	-1.58
PT9618			117.4		100.4		271.4	-0.29
PT9619	18.75	-0.96						
PT9620	25.35	0.11	75		35.4		273	-0.27
PT9621	25	0.05					284	-0.12
PT9622	26.7	0.33					331	0.53
PT9623	27.7	0.49					376	1.14
PT9624	27.81	0.51	123.54		70.92		363.84	0.98
PT9625	21.5	-0.51					270.1	-0.31
PT9626	19.3	-0.87					360	0.92
PT9627	12.4	-1.99					200	-1.26
PT9628	33.0	1.35					692	5.46

Lab code	T-2 toxin CV: 133 µg/kg u: 7.1 µg/kg σ_p: 33 µg/kg robust σ: 25 µg/kg Result (µg/kg)		HT-2 toxin CV: 185 µg/kg u: 12.1 µg/kg σ_p: 46 µg/kg robust σ: 42 µg/kg Result (µg/kg)	z'_a-score	Sum of T-2 and HT-2 toxin CV: 319 µg/kg u: 17.9 µg/kg σ_p: 80 µg/kg robust σ: 62 µg/kg Result (µg/kg)	z_a-score
PT9604						
PT9607	150	0.50	197	0.25	347	0.35
PT9608	148	0.44	218	0.70	366	0.58
PT9609	110	-0.70	78	-2.32	188	-1.65
PT9610	122	-0.34	259	1.59	381	0.77
PT9611	151	0.53	194	0.18	345	0.32
PT9612	80.5	-1.58	137	-1.05	217.5	-1.28
PT9613	170.69	1.12	137.06	-1.04	307.75	-0.15
PT9614	126	-0.22	184	-0.03	310	-0.12
PT9615	156.2	0.69	220.6	0.76	376.8	0.72
PT9616						
PT9617						
PT9618	144.6	0.34	201.8	0.35	346.4	0.34
PT9619						
PT9620	221	2.63	231	0.98	452	1.66
PT9621	127	-0.19	208	0.49	335	0.20
PT9622	138	0.14	187	0.03	325	0.07
PT9623	146	0.38	228	0.92	374	0.68
PT9624	120.75	-0.38	193.09	0.16	313.84	-0.07
PT9625	87.3	-1.38	130.2	-1.19	217.5	-1.28
PT9626	136	0.08	178	-0.16	314	-0.07
PT9627	26.0	-3.22	71.1	-2.47	97.1	-2.78
PT9628	109	-0.73	162	-0.51	271	-0.61

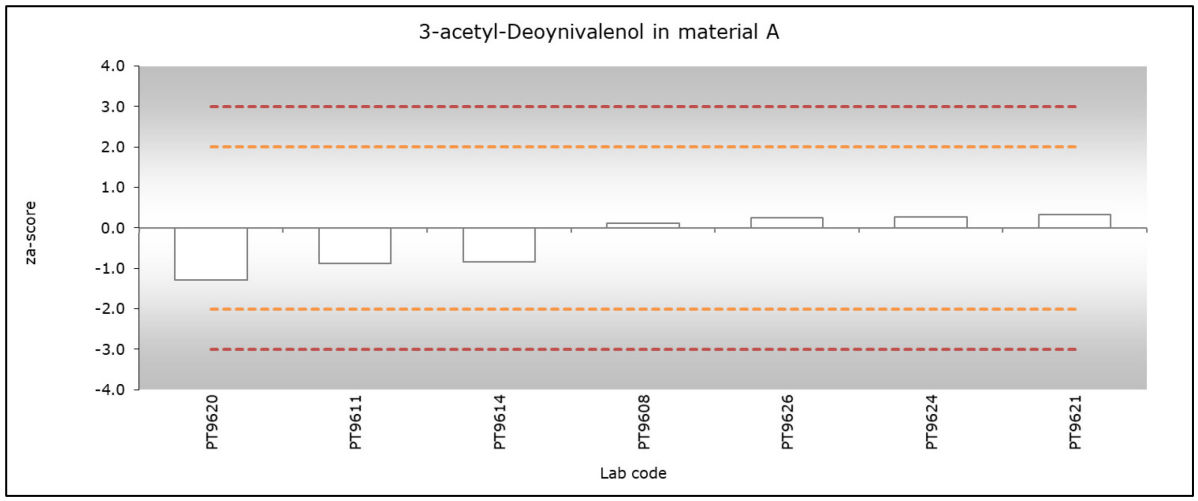

Figure a *Graphical representation of the z_a-scores for 3-acetyl-deoxynivalenol in material A. The $X \pm 2\sigma_P$ lines (dotted) are calculated according to equation I in §3.4.*

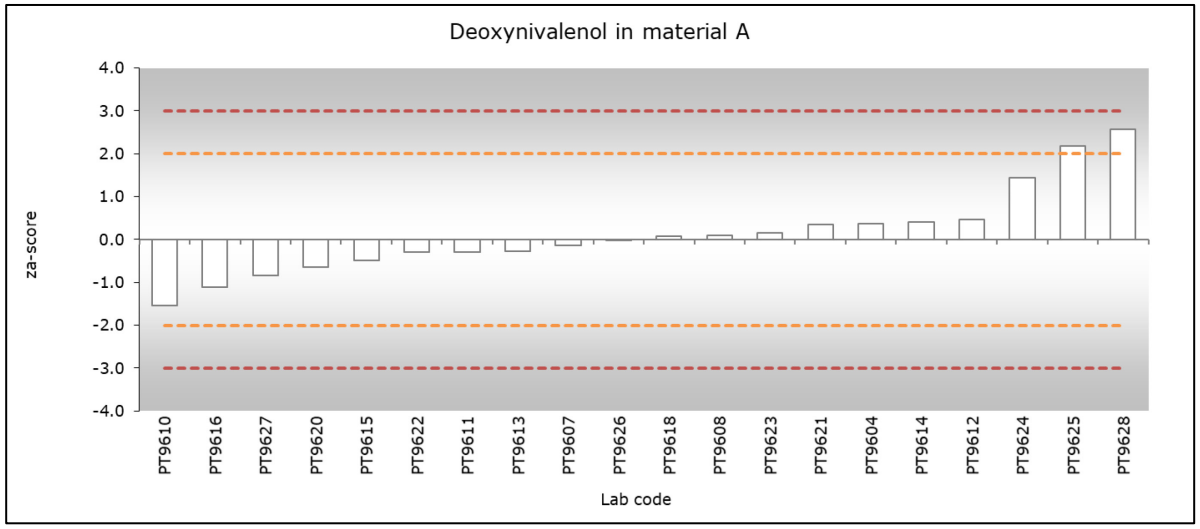

Figure b *Graphical representation of the z_a-scores for deoxynivalenol in material A. The $X \pm 2\sigma_P$ lines (dotted) are calculated according to equation I in §3.4.*

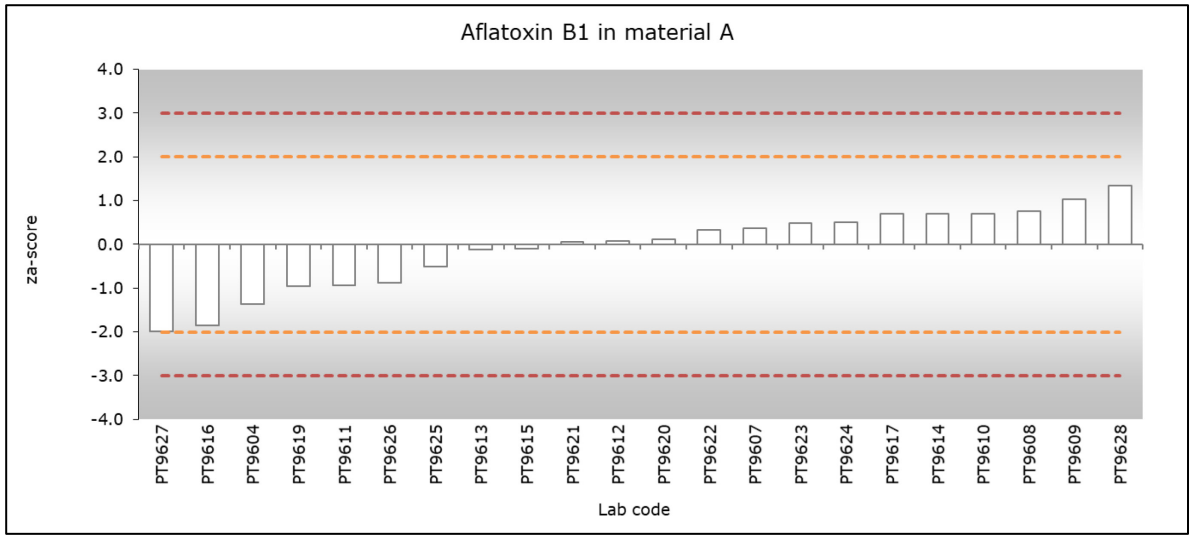

Figure c *Graphical representation of the z_a-scores for aflatoxin B1 in material A. The $X \pm 2\sigma_P$ lines (dotted) are calculated according to equation I in §3.4.*

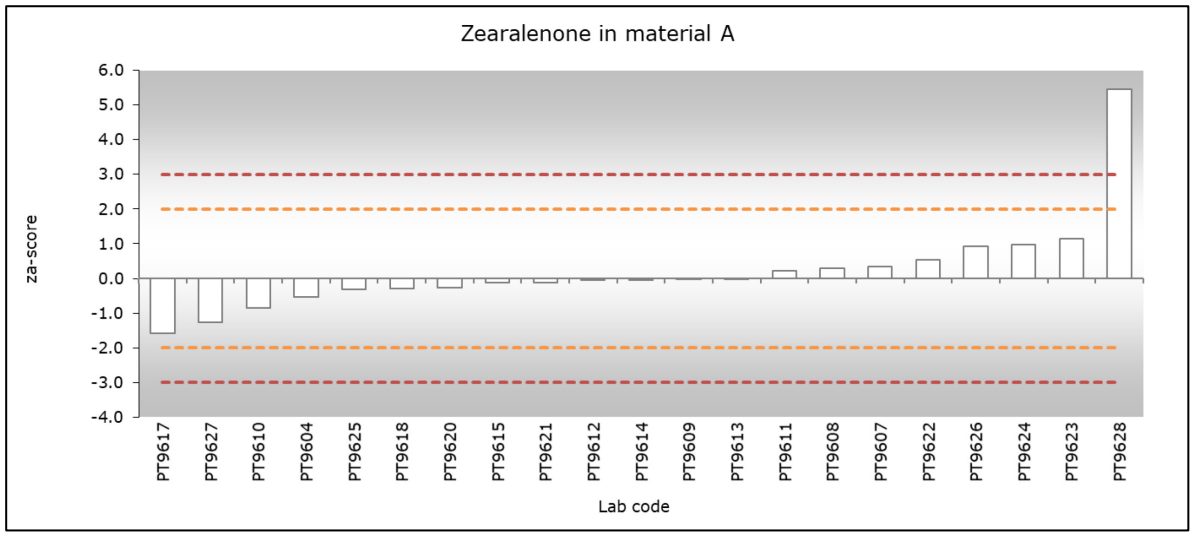

Figure d *Graphical representation of the z_a-scores for zearalenone in material A. The X ± 2σ_P lines (dotted) are calculated according to equation I in §3.4.*

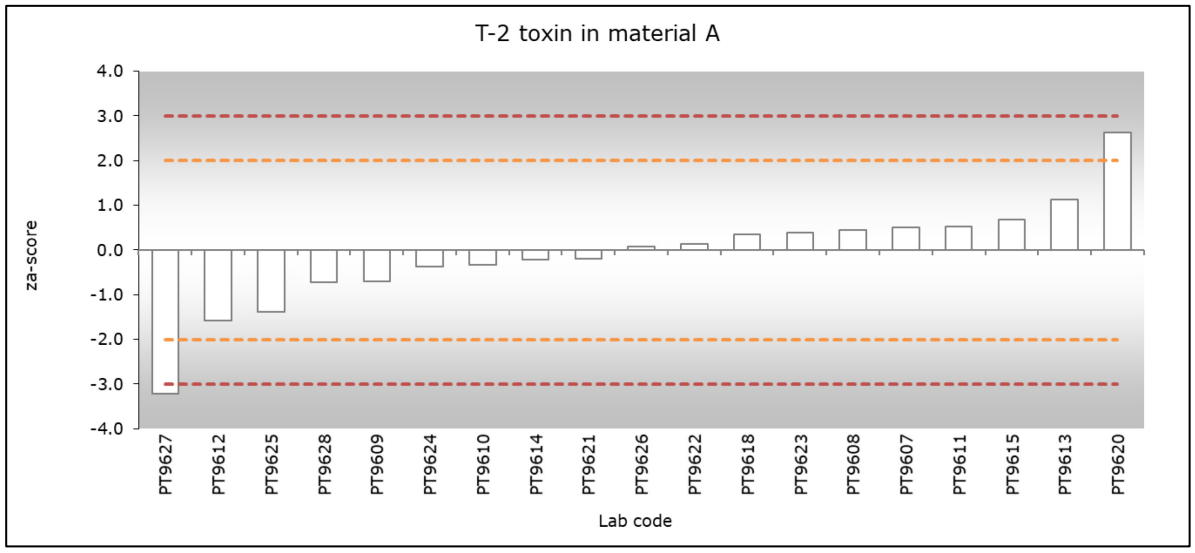

Figure e *Graphical representation of the z_a-scores for T-2 toxin in material A. The X ± 2σ_P lines (dotted) are calculated according to equation I in §3.4.*

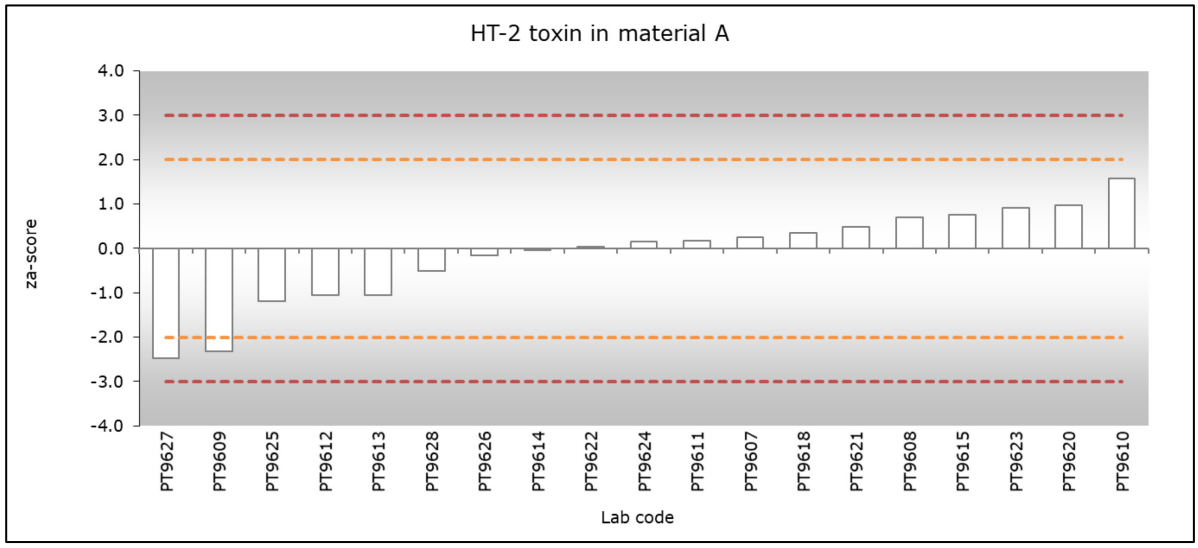

Figure f *Graphical representation of the z_a-scores for HT-2 toxin in material A. The X ± 2σ_P lines (dotted) are calculated according to equation I in §3.4.*

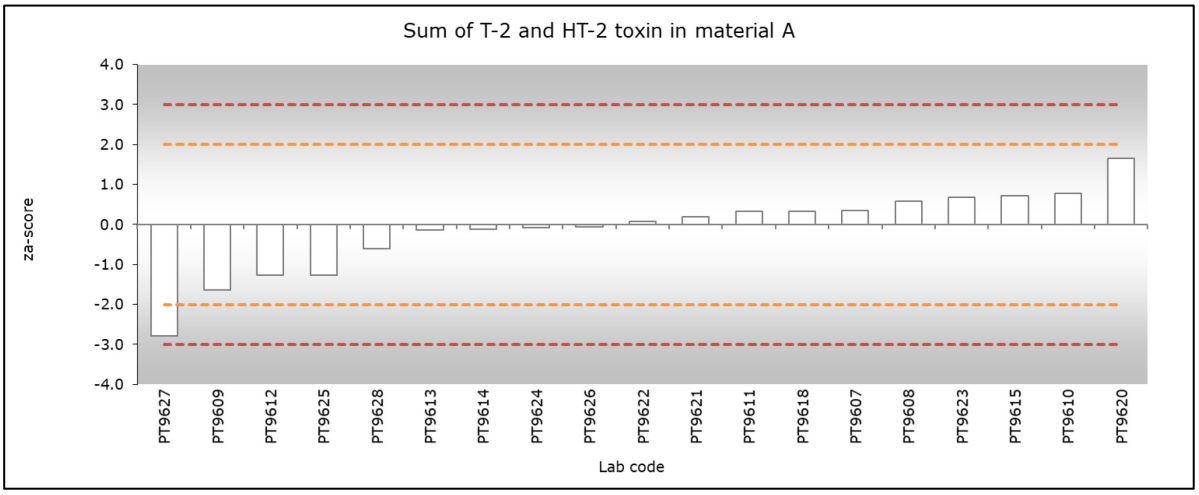

Figure g *Graphical representation of the z_a-scores for the sum of T-2 and HT-2 toxin in material A. The X ± 2σ_P lines (dotted) are calculated according to equation I in §3.4.*

Annex 7 Results material B

	15-Acetyl-DON CV: 152 µg/kg u: 27 µg/kg σ_p: 38 µg/kg robust σ: 48 µg/kg		3-Acetyl-DON CV: 645 µg/kg u: 33 µg/kg σ_p: 110 µg/kg robust σ: 69 µg/kg		DON-3-glucoside CV: 1144 µg/kg u: 261 µg/kg σ_p: 286 µg/kg robust σ: 418 µg/kg		DON CV: 4268 µg/kg u: 209 µg/kg σ_p: 549 µg/kg robust σ: 729 µg/kg	
Lab code	Result (µg/kg)	z_a-score	Result (µg/kg)	z_a-score	Result (µg/kg)	z_a-score	Result (µg/kg)	z'_a-score
PT9604		NO				NO	4530	0.45
PT9607		STATISTICAL				STATISTICAL	3909	-0.61
PT9608	106	EVALUATION	671	0.23	1317	EVALUATION	4249	-0.03
PT9609		POSSIBLE				POSSIBLE	FN	
PT9610							2627	-2.79
PT9611	240	TOO	477	-1.53		TOO	3460	-1.38
PT9612		LITTLE				LITTLE	4747.2	0.82
PT9613		RESULTS				RESULTS	3968.75	-0.51
PT9614	514		509	-1.24			4790	0.89
PT9615	471.4 for the sum of 15-Ac-DON and 3-Ac-DON						3826.6	-0.50
PT9616								
PT9617								
PT9618	1300 for the sum of 15-Ac-DON and 3-Ac-DON						4330.5	0.07
PT9619								
PT9620	nd		432	-1.94	1517		3386	-1.50
PT9621	nd		706	0.55	726		4436	0.29
PT9622							4530	0.45
PT9623							4300	0.05
PT9624	135.73		674.54	0.26	1015.82		5614.24	2.29
PT9625	nd						5682	2.41
PT9626	130		713	0.61			4160	-0.18
PT9627							3180	-1.85
PT9628							5591	2.25

CV = consensus value

u = uncertainty of consensus value

nd = not detected

	Aflatoxin B1 CV: 10.3 µg/kg u: 0.43 µg/kg σ_p: 2.6 µg/kg robust σ: 1.6 µg/kg		Enniatin B CV: 115 µg/kg u: 25 µg/kg σ_p: 29 µg/kg robust σ: 39 µg/kg		Enniatin B1 CV: 65 µg/kg u: 22 µg/kg σ_p: 16.2 µg/kg robust σ: 35 µg/kg		Zearalenone CV: 289 µg/kg u: 10.2 µg/kg σ_p: 72 µg/kg robust σ: 38 µg/kg	
Lab code	Result (µg/kg)	z_a-score	Result (µg/kg)	z_a-score	Result (µg/kg)	z_a-score	Result (µg/kg)	z_a-score
PT9604	6.7	-1.40		NO		NO	314	0.35
PT9607	11.09	0.31		STATISTICAL		STATISTICAL	285	-0.05
PT9608	11.5	0.47		EVALUATION		EVALUATION	313	0.34
PT9609	11	0.27		POSSIBLE		POSSIBLE	280	-0.12
PT9610	12	0.66					258	-0.42
PT9611	7.9	-0.93	211	TOO	58	TOO	299	0.15
PT9612	10.8	0.20		LITTLE		LITTLE	292.9	0.06
PT9613	10.11	-0.07		RESULTS		RESULTS	285.17	-0.05
PT9614	12.6	0.89					293	0.06
PT9615	10.5	0.08					283	-0.08
PT9616							178	-1.53
PT9617	12	0.66					172	-1.62
PT9618			115.3		110.3		275.7	-0.18
PT9619	7.98	-0.90						
PT9620	9.8	-0.19	74.2		31.4		274	-0.20
PT9621	10	-0.12					280	-0.12
PT9622	10.4	0.04					311	0.31
PT9623	11.3	0.39					390	1.41
PT9624	11.59	0.50	115.56		69.74		322.73	0.47
PT9625	9.3	-0.39					259	-0.41
PT9626	6.3	-1.55					364	1.05
PT9627	5.45	-1.88					194	-1.31
PT9628	10.6	0.12					389	1.39

Lab code	T-2 Toxin CV: 24 µg/kg u: 0.74 µg/kg σ_p: 6.1 µg/kg robust σ: 2.4 µg/kg Result (µg/kg)		HT-2 Toxin CV: 61 µg/kg u: 2.7 µg/kg σ_p: 15.2 µg/kg robust σ: 9.2 µg/kg Result (µg/kg)	z_{ai}-score	Sum of T-2 and HT-2 Toxin CV: 83 µg/kg u: 4.3 µg/kg σ_p: 21 µg/kg robust σ: 15.0 µg/kg Result (µg/kg)	z_a-score
PT9604						
PT9607	23.35	-0.16	61.37	0.04	84.72	0.07
PT9608	25.5	0.19	68.4	0.51	93.9	0.51
PT9609	<20		47	-0.77	47	-1.74
PT9610	24	-0.05	90	1.93	114	1.47
PT9611	23	-0.22	53	-0.43	76	-0.35
PT9612	<50.0		< 50.0			
PT9613	27	0.44	36.31	-1.37	63.31	-0.96
PT9614	22.8	-0.25	62.1	0.09	84.9	0.08
PT9615	31.5	1.18	69.8	0.60	101.3	0.87
PT9616	22.6	-0.28	56	-0.26	78.6	-0.23
PT9617						
PT9618	26.2	0.31	61.5	0.05	87.7	0.21
PT9619						
PT9620	78.9	8.98	65.4	0.31	144.3	2.93
PT9621	21	-0.55	64	0.22	85	0.08
PT9622	23.9	-0.07	62.8	0.14	86.7	0.16
PT9623	24	-0.05	64	0.22	88	0.23
PT9624	21.53	-0.46	64.82	0.27	86.35	0.15
PT9625	47	3.73	FN (LOD 15)		47	-1.74
PT9626	23	-0.22	69.1	0.55	92.1	0.42
PT9627	FN (<10)		23.8	-2.07	23.8*	-2.86
PT9628	nd, <42		47.1	-0.76	47.1	-1.74

* taken into account the instability of HT-2 toxin this z-score would still be questionable.

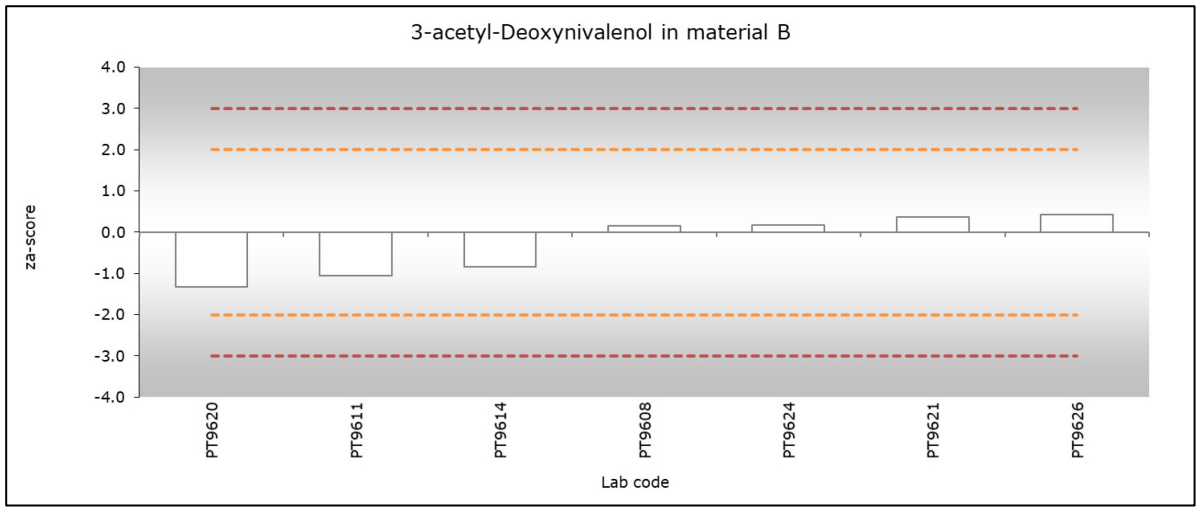

Figure a *Graphical representation of the z_a-scores for 3-acetyl-deoxynivalenol in material B. The $X \pm 2\sigma_P$ lines (dotted) are calculated according to equation I in §3.4.*

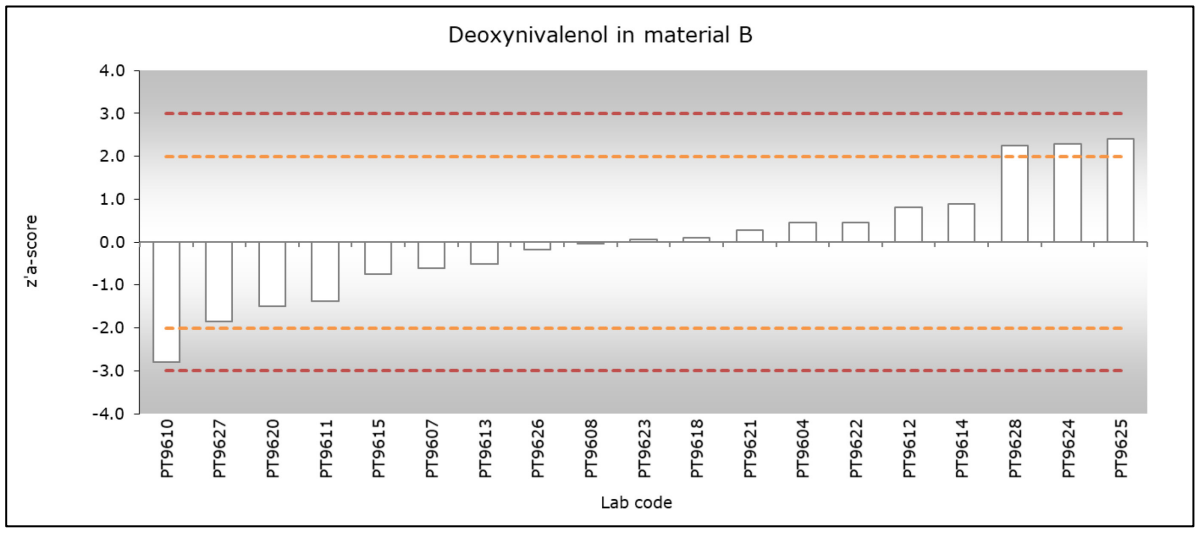

Figure b *Graphical representation of the z'_a-scores for deoxynivalenol in material B. The $X \pm 2\sigma_P$ lines (dotted) are calculated according to equation II in §3.4.*

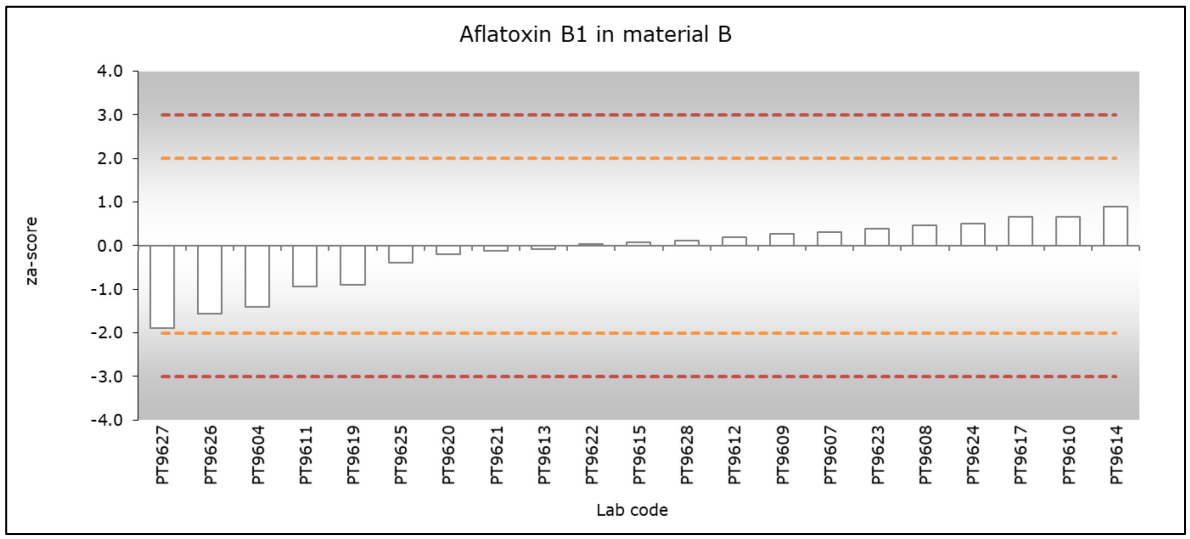

Figure c *Graphical representation of the z_a-scores for aflatoxin B1 in material B. The $X \pm 2\sigma_P$ lines (dotted) are calculated according to equation I in §3.4.*

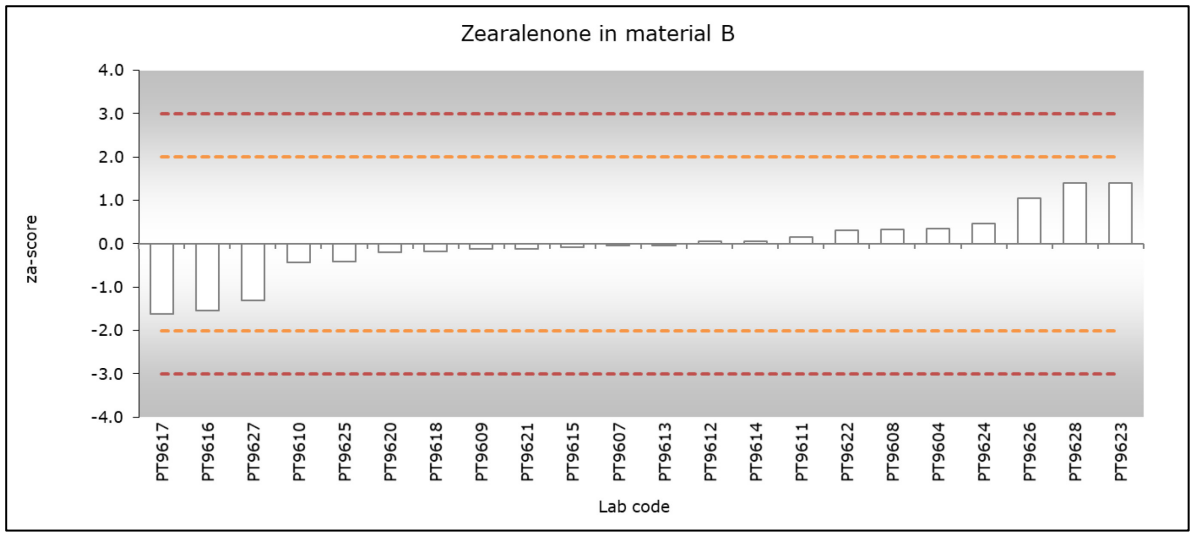

Figure d *Graphical representation of the z_a-scores for zearalenone in material B. The $X \pm 2\sigma_P$ lines (dotted) are calculated according to equation I in §3.4.*

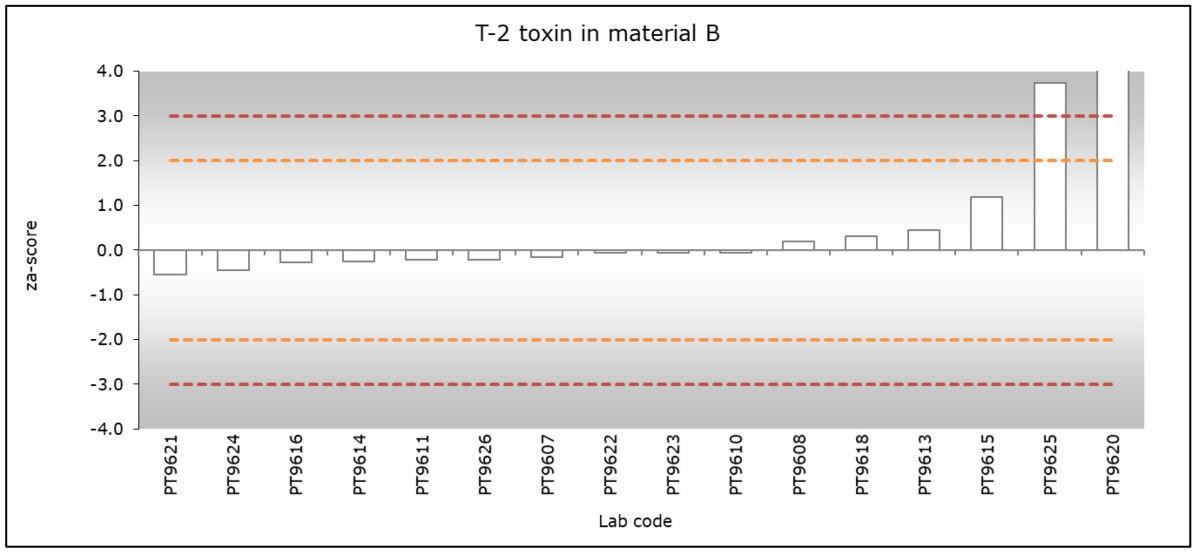

Figure e *Graphical representation of the z_a-scores for T-2 toxin in material B. The $X \pm 2\sigma_P$ lines (dotted) are calculated according to equation I in §3.4.*

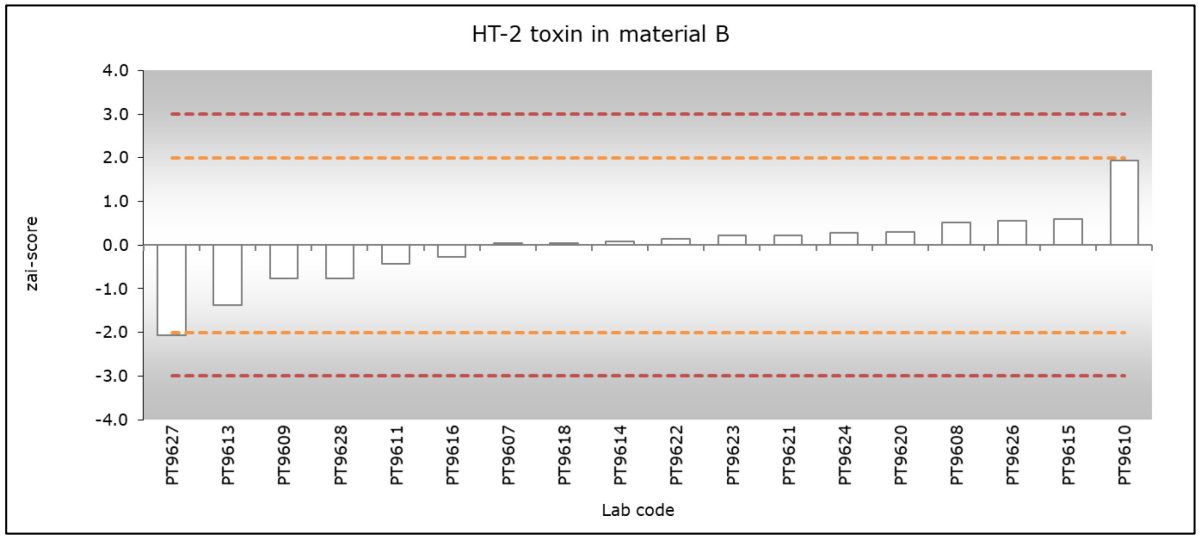

Figure f *Graphical representation of the z_a-scores for HT-2 toxin in material B. The $X \pm 2\sigma_P$ lines (dotted) are calculated according to equation III in §3.4.*

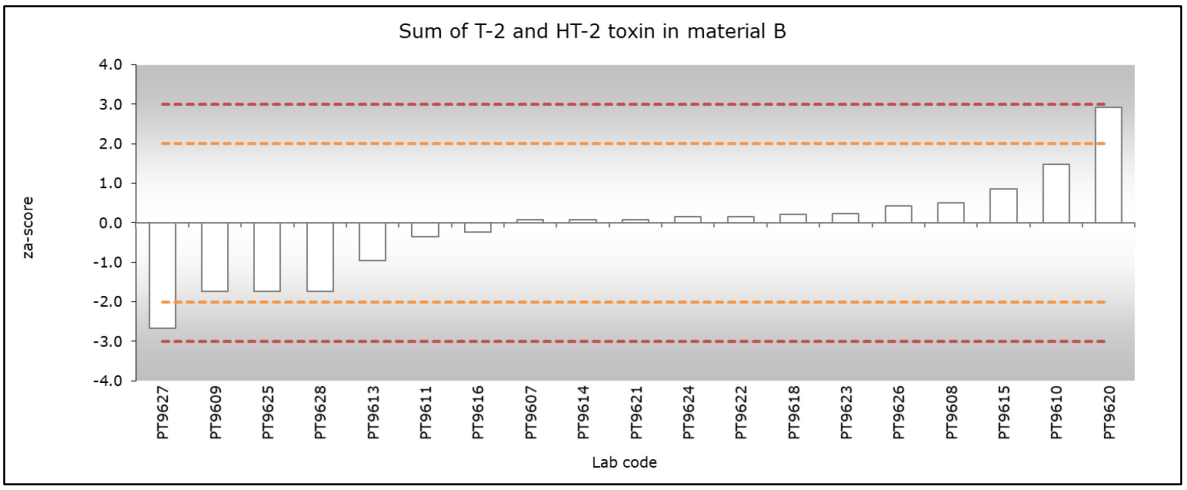

Figure g *Graphical representation of the z_a-scores for the sum of T-2 and HT-2 toxin in material B. The $X \pm 2\sigma_P$ lines (dotted) are calculated according to equation I in §3.4.*

Annex 8 False positive and false negative results

False negative results

Lab code	Material	Compound missed
PT9609	A	DON
PT9609	B	DON
PT9625	B	HT-2 toxin
PT9627	B	T-2 toxin

False positive results

Lab code	Material	Compound detected
PT9627	A	2.8 µg/kg ochratoxin A
PT9627	B	3.5 µg/kg ochratoxin A

Annex 9 Overview performance per laboratory

Laboratory code	Performance
PT9604	6 out of 14 satisfactory results
PT9607	12 out of 14 satisfactory results
PT9608	14 out of 14 satisfactory results, **optimal performance**
PT9609	8 out of 14 satisfactory results, 1 questionable result, 1 qualitative result, 2 false negative results
PT9610	11 out of 14 satisfactory results, 1 questionable result
PT9611	14 out of 14 satisfactory results, **optimal performance**
PT9612	9 out of 14 satisfactory results, two qualitative results
PT9613	12 out of 14 satisfactory results
PT9614	14 out of 14 satisfactory results, **optimal performance**
PT9615	12 out of 14 satisfactory results
PT9616	6 out of 14 satisfactory results
PT9617	4 out of 14 satisfactory results
PT9618	10 out of 14 satisfactory results
PT9619	2 out of 14 satisfactory results
PT9620	11 out of 14 satisfactory results, 2 questionable results, 1 unsatisfactory result
PT9621	14 out of 14 satisfactory results, **optimal performance**
PT9622	12 out of 14 satisfactory results
PT9623	12 out of 14 satisfactory results
PT9624	13 out of 14 satisfactory results, 1 questionable result
PT9625	8 out of 14 satisfactory results, 2 questionable results, 1 unsatisfactory result, 1 false negative result
PT9626	14 out of 14 satisfactory results, **optimal performance**
PT9627	6 out of 14 satisfactory results, 4 questionable results, 1 unsatisfactory result, 1 false negative and two false positive results
PT9628	8 out of 14 satisfactory results, 2 questionable and 1 unsatisfactory result

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