DLA
Dienstleistung
Lebensmittel
Analytik GbR

**Evaluation Report**
proficiency test

**DLA 13/2014**

# Fumonisins in Corn

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# Contents

# 1. Introduction

The participation in proficiency testing schemes is an essential element of the quality-management-system of every laboratory testing food and feed. The implementation of proficiency tests enables the participating laboratories to prove their own analytical competence under realistic conditions. At the same time they receive valuable data regarding the validity of the particular testing method.
The purpose of DLA is to offer proficiency tests for selected parameters in concentrations with practical relevance.
Realisation and evaluation of the present proficiency test follows the technical requirements of DIN EN ISO/IEC 17043 (2010) and DIN ISO 13528:2009.

# 2. Realisation

## 2.1 Test material

The test material was customary in commerce cornflour with a natural content of fumonisins and with an added glucose content of 1,7% for the homogeneity test. Approximately 2 kg of the material were homogenized and then packaged lightproof in portions to approximately 50 g. The portions were numbered chronologically. The material was checked for homogeneity.

### 2.1.1 Homogeneity

The homogeneity was examined with glucose (HPLC-method/ 5-fold determination) and is considered verified with a standard deviation of 0,9%. The results are given in the documentation.
The calculation of the repeatability standard deviation of the participants for total-fumonisins was used additionally as an indicator of homogeneity. The result is similar to the repeatability standard deviation of the German official method ASU § 64 LFGB 15.05-3 for corn flour (14). The repeatability standard deviation of the participants is given in the documentation.
In the documentation the portion numbers are graphically assigned to the results of total fumonisins. There is no trend recognizable in the results which could suggest inhomogeneity.

## 2.2 Test

Two portions of test material were sent to every participating laboratory in the 18th week of 2014. The testing method was optional. The tests should be finished at june 13th 2013 the latest.

## 2.3 Results

The participants submitted their results in standard forms, which have been handed out with the samples (by email). The finally calculated concentrations of fumonisin $B_1$,fumonisin $B_2$ and total fumonisins as average of duplicate determinations of both numbered samples was used for the statistical evaluation.
Queried and documented were single results, recovery, fumonisin $B_1$, $B_2$,

and the used testing method.
All participants submitted their results in time. Two participants submitted only the results for total fumonisins.

## 3. Evaluation

### 3.1 Assigned value

Because the analysed material was no certified reference material the robust mean of the submitted results was used as assigned value X (6).
The distribution of submitted results showed no hint for bimodal distribution or other reasons for a higher variability.

### 3.2 Standard deviation

For comparison to the target standard deviation a robust standard deviation ($S_x$) was calculated (6).

### 3.3 Outliers

Statistical outliers were determined by Mandel´s-H-Statistic (significance level: 5%) (5). Detected outliers were stated for information only, when z-score simultaneously was < -2 or > 2.

### 3.4 Target standard deviation

The target standard deviation of the assigned value is determined according to the following methods.

#### 3.4.1 General model (Horwitz)

The relative target standard deviation in % of the assigned value is calculated according to the following equation.

$$\hat{\sigma} \ (\%) = 2^{(1-0,5\log X)}$$

Out of this is calculated the target standard deviation in µg/kg

$$\hat{\sigma} = X * \hat{\sigma} \ (\%) / 100.$$

The target standard deviation according to Horwitz was used in this PT.

#### 3.4.2 Precision experiment

Using the reproducibility standard deviation $\sigma_R$ and the repeatability standard deviation $\sigma_r$ of a precision experiment the between-laboratories standard deviation ( $\sigma_L$) can be calculated :

$$\sigma_L = \sqrt{(\sigma_R^2 - \sigma_r^2)} \ .$$

And then, using the number of replicate measurements n, each participant is to perform, the standard deviation for proficiency assessment is

calculated:

$$\hat{\sigma} = \sqrt{(\sigma_L^2 + (\sigma_r^2/n))} \quad .$$

The German official method ASU § 64 LFGB 15.05-3 gave a relative target standard deviation of 19,6 % for fumonisin $B_1$ and 18,5 % for fumonisin $B_2$ for a comparable fumonisin content in corn flour (14). These target standard deviations are listed for information in the evaluation.

### 3.5 z-Score

To assess the results of the participants the z-score is used. It indicates about which multiple of the target standard deviation ( $\hat{\sigma}$ ) the result (x) of the participant is deviating from the assigned value (X)(6).

Participants' z-scores were derived as:

$$z = (x - X) / \hat{\sigma} \quad ;$$

the requirements for the analytical performance are generally considered as fulfilled if

$$-2 \leq z \leq 2.$$

### 3.6 Quotient $S^x/\hat{\sigma}$

Following the Horrat-value the results of a proficiency-test (PT) can be considered convincing, if the quotient of robust standard deviation and target standard deviation does not exceed the value of 2.
A value > 2 means an insufficient precision, i.e. the analytical method is too variable, or the variation between the test participants is higher than estimated. Thus the comparability of the results is not given.

For this PT the results show a sufficient comparability.

### 3.7 Standard uncertainty

The assigned value X has a standard uncertainty $u_X$ that depends on the analytical method, differences between the analytical methods used, the test material, the number of participant laboratories and perhaps on other factors. The standard uncertainty ( $u_X$ ) for this PT is calculated as follows (6).

$$u_X = 1{,}25 * S^x/\sqrt{(p)}$$

If $u_X \leq 0{,}3 * \hat{\sigma}$ the standard uncertainty of the assigned value needs not be included in the interpretation of the results of the PT (6). The quotient $u_X/\hat{\sigma}$ is reported in the characteristics of the test.

## 4. Results

All following tables are anonymized. With the delivering of the evaluation-report the participants are informed about their individual evaluation-number.

In the upper table – test - the characteristics are listed:

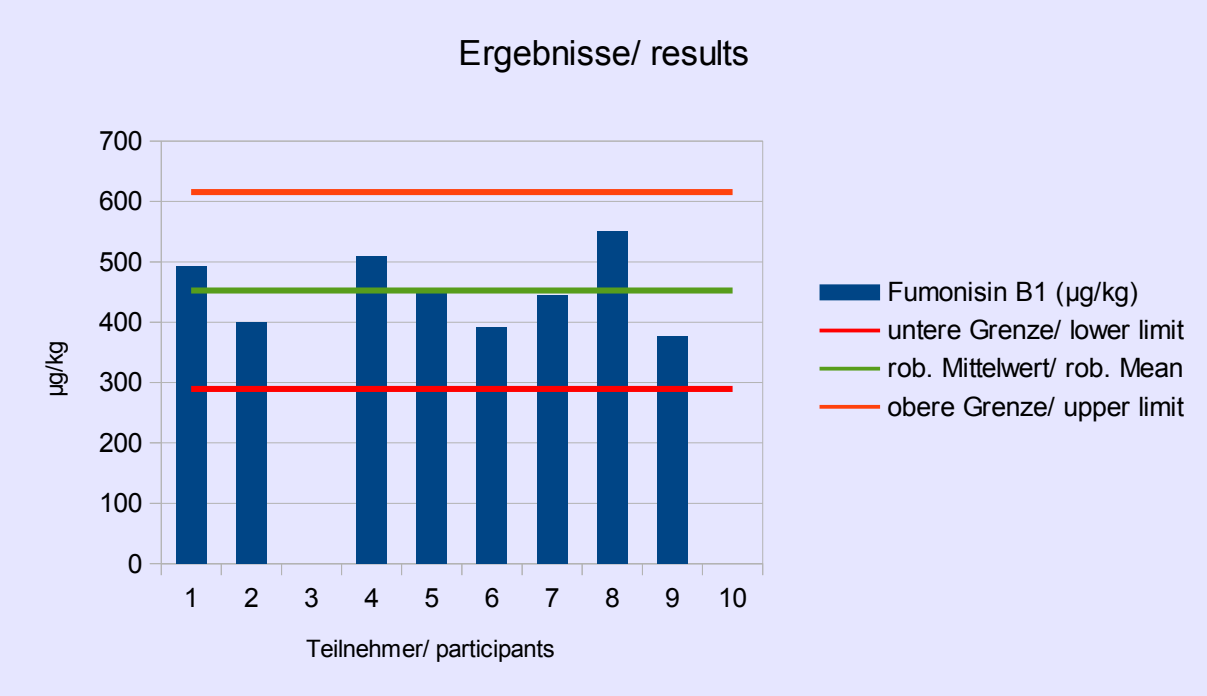
| | |
|---|---|
| Number of results | |
| Number of outliers | |
| Mean | |
| Median | |
| Robust mean (X) | |
| Robust standard deviation ($S^x$) | |
| Target standard deviation( $\hat{\sigma}$ )(Horwitz) | |
| Target standard deviation (ASU §64 LFGB 15.05-3) for information | |
| Lower limit of target range (X - 2 $\hat{\sigma}$ ) | |
| Upper limit of target range (X + 2 $\hat{\sigma}$ ) | |
| Quotient $S^x/\hat{\sigma}$ | |
| Standard uncertainty $u_x$ | |
| Quotient $u_X/\hat{\sigma}$ | |
| Number of results in the target range | |

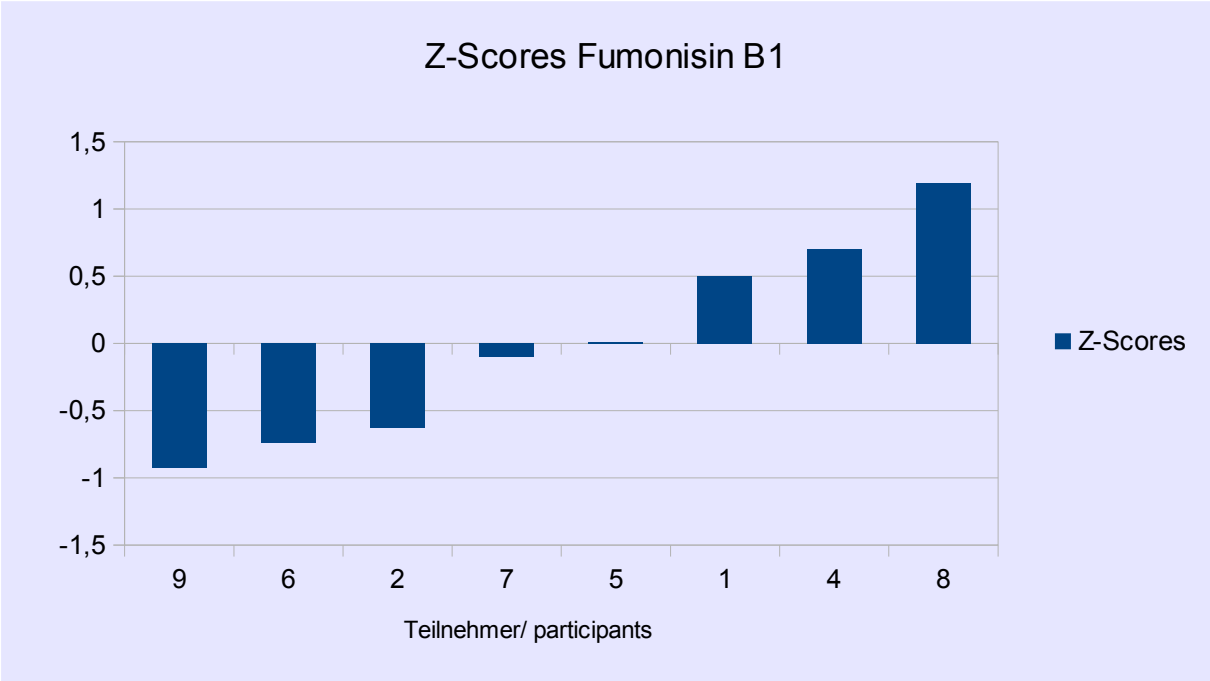
In the lower table - Laboratories - the individual results of the participating laboratories are listed:

| Evaluation number | Result | Deviation | z-Score | Remark |
|---|---|---|---|---|

## 4.1 Fumonisin $B_1$ in µg/kg

| Characteristics | |
|---|---|
| Number of results | 8 |
| Number of outliers | 0 |
| Mean | 452 |
| Median | 449 |
| Robust mean (X) | 452 |
| Robust standard deviation ($S^x$) | 69,8 |
| Target standard deviation( $\hat{\sigma}$ ) | 81,8 |
| Target standard deviation (L 15.05-3 for information) | 88,8 |
| Lower limit of target range (X - 2 $\hat{\sigma}$ ) | 289 |
| Upper limit of target range (X + 2 $\hat{\sigma}$ ) | 615 |
| Quotient $S^x/\hat{\sigma}$ | 0,9 |
| Standard uncertainty $u_x$ | 30,9 |
| Quotient $u_X/\hat{\sigma}$ | 0,38 |
| Number of results in the target range | 8 (100%) |





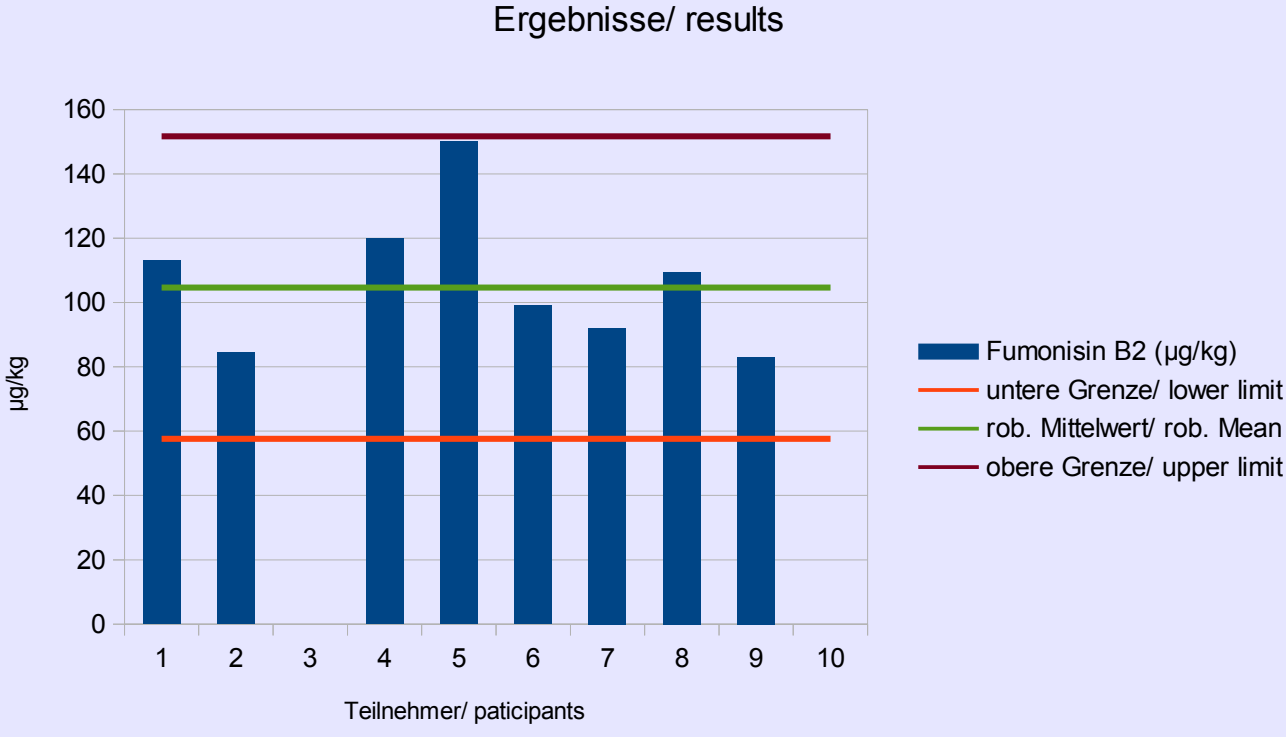

Laboratories

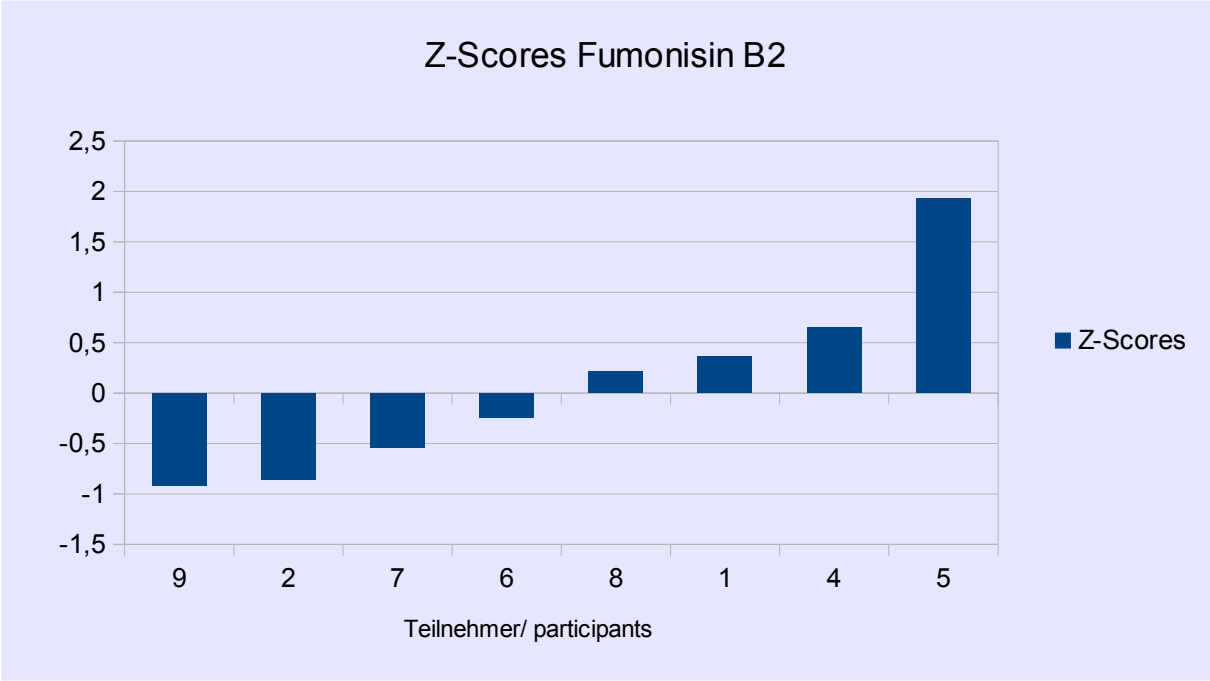
| Teilnehmer/ participants | Fumonisin B1 | Abweichung/ deviation | Z-Scores | Bemerkungen/ remarks |
|---|---|---|---|---|
| | µg/kg | µg/kg | | |
| 1 | 493 | 40,45 | 0,5 | |
| 2 | 400,9 | -51,65 | -0,6 | |
| 3 | | | | |
| 4 | 510 | 57,45 | 0,7 | |
| 5 | 453 | 0,45 | 0,0 | |
| 6 | 392,5* | -60,05 | -0,7 | |
| 7 | 444 | -8,55 | -0,1 | |
| 8 | 550 | 97,45 | 1,2 | |
| 9 | 377 | -75,55 | -0,9 | |
| 10 | | | | |

** = The mean was calculated by DLA*

## 4.2 Fumonisin $B_2$ in µg/kg

| Characteristics | |
|---|---|
| Number of results | 8 |
| Number of outliers | 0 |
| Mean | 106 |
| Median | 104 |
| Robust mean (X) | 105 |
| Robust standard deviation ($S^x$) | 20,9 |
| Target standard deviation( $\hat{\sigma}$ ) | 23,5 |
| Target standard deviation (L 15.05-3 for information) | 19,4 |
| Lower limit of target range (X - 2 $\hat{\sigma}$ ) | 57,6 |
| Upper limit of target range (X + 2 $\hat{\sigma}$ ) | 151,6 |
| Quotient $S^x/\hat{\sigma}$ | 0,9 |
| Standard uncertainty $u_x$ | 9,2 |
| Quotient $u_X/\hat{\sigma}$ | 0,39 |
| Number of results in the target range | 8 (100%) |





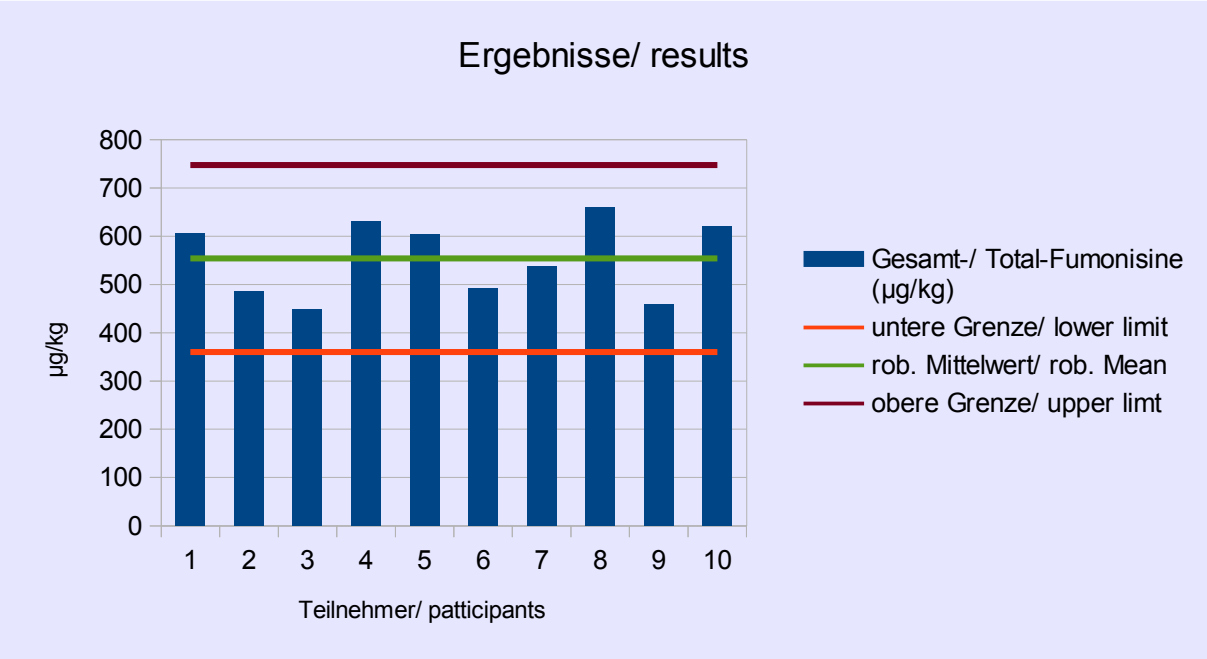

Laboratories

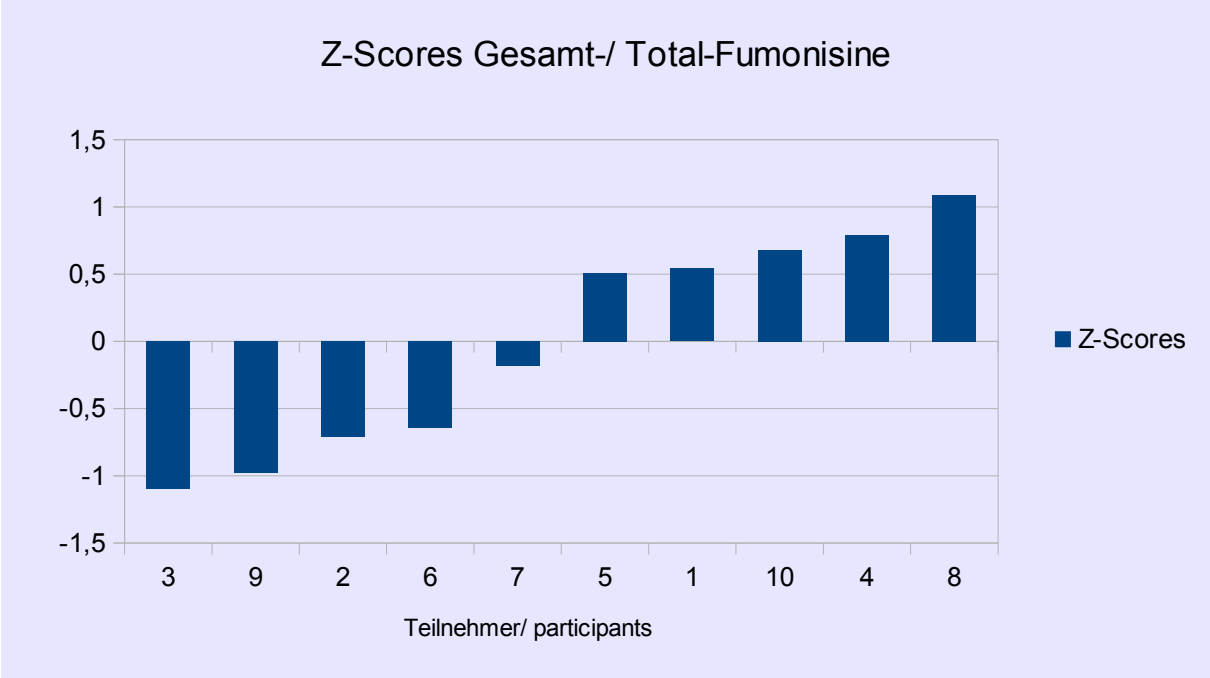
| Teilnehmer/ participants | Fumonisin B2 | Abweichung/ deviation | Z-Scores | Bemerkungen/ remarks |
|---|---|---|---|---|
| | µg/kg | µg/kg | | |
| 1 | 113 | 8,38 | 0,4 | |
| 2 | 84,5 | -20,12 | -0,9 | |
| 3 | | | | |
| 4 | 120 | 15,38 | 0,7 | |
| 5 | 150 | 45,38 | 1,9 | |
| 6 | 99* | -5,62 | -0,2 | |
| 7 | 92 | -12,62 | -0,5 | |
| 8 | 110 | 4,88 | 0,2 | |
| 9 | 83 | -21,62 | -0,9 | |
| 10 | | | | |

** = The mean was calculated by DLA*

## 4.3 Total Fumonisins in µg/kg

| Characteristics | |
|---|---|
| Number of results | 10 |
| Number of outliers | 0 |
| Mean | 554 |
| Median | 570 |
| Robust mean (X) | 554 |
| Robust standard deviation ($S^x$) | 89,2 |
| Target standard deviation( $\hat{\sigma}$ ) | 96,8 |
| Lower limit of target range (X - 2 $\hat{\sigma}$ ) | 360 |
| Upper limit of target range (X + 2 $\hat{\sigma}$ ) | 747 |
| Quotient $S^x/\hat{\sigma}$ | 0,9 |
| Standard uncertainty $u_x$ | 35,3 |
| Quotient $u_X/\hat{\sigma}$ | 0,36 |
| Number of results in the target range | 10 (100%) |






Laboratories

| Teilnehmer/ participant | Gesamt-/ Total-Fumonisine | Abweichung/ deviation | Z-Scores | Bemerkungen/ remarks |
|---|---|---|---|---|
| | µg/kg | µg/kg | | |
| 1 | 606 | 52,23 | 0,5 | |
| 2 | 485,4 | -68,37 | -0,7 | |
| 3 | 447,3* | -106,47 | -1,1 | |
| 4 | 630 | 76,23 | 0,8 | |
| 5 | 603 | 49,23 | 0,5 | |
| 6 | 491,5* | -62,27 | -0,6 | |
| 7 | 536 | -17,77 | -0,2 | |
| 8 | 660 | 105,73 | 1,1 | |
| 9 | 459 | -94,77 | -1,0 | |
| 10 | 620 | 66,23 | 0,7 | |

** = The mean was calculated by DLA*

# 5. Documentation

## 5.1 Primary data

### 5.1.1 Fumonisin $B_1$

| Teilnehmer/ participant | Endergebnis/ final result | DLA-Nr Probe A/ sample A | DLA-Nr Probe B/ sample B | Ergebnis A inkl. WF/ result A incl. recovery | Ergebnis B inkl. WF/ result B incl. recovery | Wiederfin-dungsrate/ recovery |
|---|---|---|---|---|---|---|
| | µg/kg | | | µg/kg | µg/kg | in % |
| 1 | 493 | 7 | 21 | 479 | 506 | 102 |
| 2 | 400,9 | 16 | 34 | 379,5 | 422,2 | 101 |
| | | | | | | |
| 4 | 510 | 22 | 33 | 510 | 510 | |
| 5 | 453 | 3 | 18 | 456 | 450 | 79 |
| 6 | 392,5* | 15 | 30 | 394 | 391 | 106,1 |
| 7 | 444 | 1 | 25 | 425 | 462 | 88 |
| 8 | 550 | 9 | 23 | 554 | 546 | 98 |
| 9 | 377 | 11 | 29 | 395 | 358 | 117 |
| 10 | | 10 | 31 | | | |

### 5.1.2 Fumonisin $B_2$

| Teilnehmer/ participant | Endergebnis/ final result | DLA-Nr Probe A/ sample A | DLA-Nr Probe B/ sample B | Ergebnis A inkl. WF/ result A incl. recovery | Ergebnis B inkl. WF/ result B incl. recovery | Wiederfin-dungsrate/ recovery |
|---|---|---|---|---|---|---|
| | µg/kg | | | µg/kg | µg/kg | in % |
| 1 | 113 | 7 | 21 | 119 | 107 | 99 |
| 2 | 84,5 | 16 | 34 | 84 | 85 | 100 |
| | | | | | | |
| 4 | 120 | 22 | 33 | 120 | 120 | |
| 5 | 150 | 3 | 18 | 137 | 163 | 77 |
| 6 | 99* | 15 | 30 | 93 | 105 | 105,1 |
| 7 | 92 | 1 | 25 | 88 | 96 | 90 |
| 8 | 110 | 09 | 23 | 108 | 111 | 102 |
| 9 | 83 | 11 | 29 | 82 | 83 | 99 |
| 10 | | | | | | |

5.1.3 Total Fumonisins

| Teilnehmer/ participant | Endergebnis/ final result | DLA-Nr Probe A/ sample A | DLA-Nr Probe B/ sample B | Ergebnis A inkl. WF/ result A incl. recovery | Ergebnis B inkl. WF/ result incl. recorery | Wiederfin-dungsrate/ recovery |
|---|---|---|---|---|---|---|
| | µg/kg | | | µg/kg | µg/kg | in % |
| 1 | 606 | 7 | 21 | 598 | 613 | |
| 2 | 485,4 | 16 | 34 | 463,5 | 507,2 | |
| 3 | 447,3* | 8 | 27 | 419,5* | 475* | |
| 4 | 630 | 22 | 33 | 630 | 630 | |
| 5 | 603 | 3 | 18 | 593 | 613 | n.a. |
| 6 | 491,5* | 15 | 30 | 487 | 496 | |
| 7 | 536 | 1 | 25 | 513 | 558 | 88/90 |
| 8 | 660 | 09 | 23 | 662* | 657* | |
| 9 | 459 | 11 | 29 | 477 | 441 | |
| 10 | 620 | 10 | 31 | 620 | 620 | |

** = The mean was calculated by DLA*

## 5.2 Homogeneity

### 5.2.1 Homogeneity test of the sample material before the PT

To verify the homogeneity of the test material glucose was added to the corn flour before homogenisation. The homogeneity was examined with glucose/ HPLC. The data showed no indication of inhomogeneity.

| Probe/ sample | Glucose | | |
|---|---|---|---|
| 1 | 1,710 | g/100g | |
| 2 | 1,680 | g/100g | |
| 3 | 1,720 | g/100g | |
| 4 | 1,705 | g/100g | |
| 5 | 1,690 | g/100g | |
| | | | |
| Mittelwert/ mean | 1,7 | g/100g | |
| Standardabw./ Standard deviation | 0,02 | 0,9 | % |

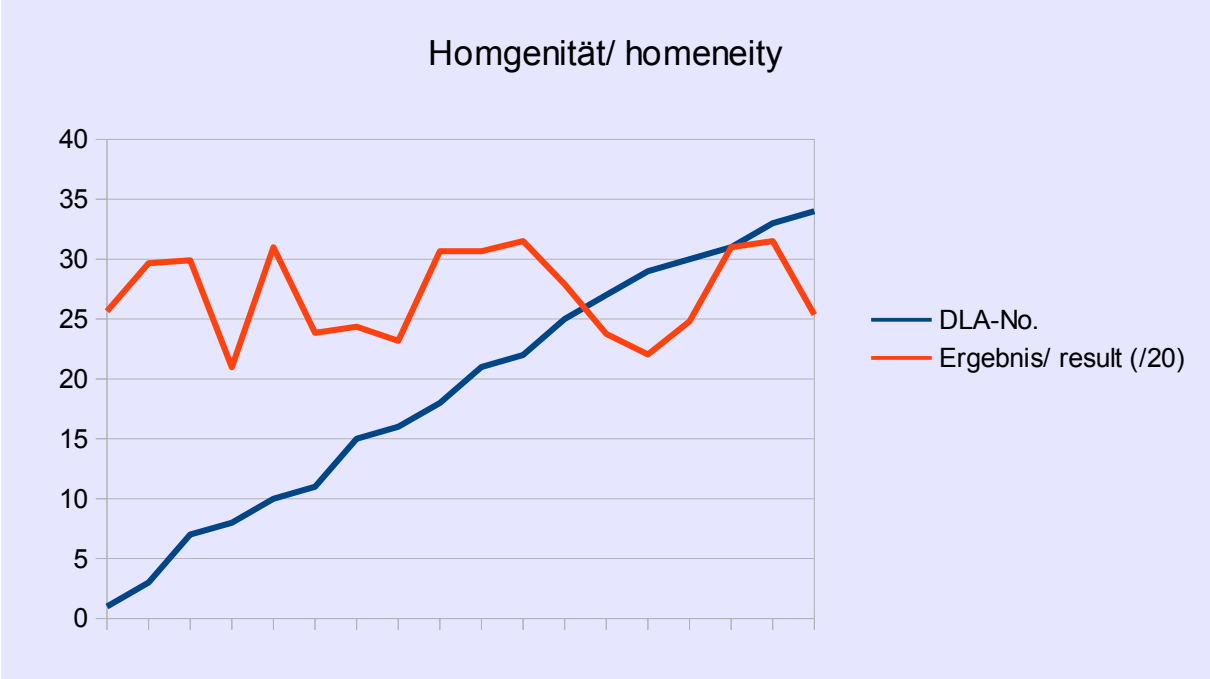
### 5.2.2 Repeatability standard deviation of participants

The repeatability standard deviation of the single results was calculated.
It is 26,0 µg/kg = 5,7 % of X (Fumonisin $B_1$).

In the ASU L15.05-3 the relative repeatability standard deviation of 18,8% for a fumonisin $B_1$ content of 780 µg/kg and of 26,8% for a fumonisin $B_2$ content of 203 µg/kg was determined for corn flour (14).

### 5.2.3 Comparison of sample number/test result

The comparison of the increasing sample-numbers and measured total fumonisins-results (/20) shows a sufficient homogeneity.



## 5.3 Analytical methods

| Teilnehmer/ participant | Methode/ method | Wiederfindung mit gleicher Matrix/ recovery w ith same matrix | Akkreditiert/ accredited | Hinweise/ remarks |
|---|---|---|---|---|
| | | ja / nein | ja / nein | |
| 1 | In house method LC/MS/MS | yes | Yes | |
| 2 | HPLC | no | yes | |
| 3 | Testkit r-biopharm Ridascreen Fast Fumonisin (diviation in the sample preparation: 2g sample weight, 10ml 70% Methanol) | no, it was a sinlge determination | yes | |
| 4 | Fumonisins | no | yes | Standard addition before preparation for the quantifica-tion. |
| 5 | Determination (HPLC-Fluoreszenz) by in house method, PV 805250 | yes | no | - |
| 6 | Determination of the sum of Fumonisin B1 and B2 in compound feed by cleaning with a IAC and RP-HPLC with fluorescence detection after pre- and post-column derivatization | yes | yes | |
| 7 | Fumonisins (HPLC) | no | yes | Recovery with wheat flour |
| 8 | In house method LC/MS/MS | yes | yes | |
| 9 | | yes | yes | |
| 10 | Neogen ELISA Fumonisin HS | no | | |

*IAC = Immuno Affinity Column

## 6. Index of participant laboratories

| Teilnehmer/ participant | Ort/ location |
|---|---|
| | Austria |
| | Switzerland |
| | Germany |
| | Belgium |
| | Germany |
| | Germany |
| | Germany |
| | Germany |
| | Germany |
| | Germany |

*[The address data of the participants were deleted for publication of the evaluation report.]*

## 7. Index of literature


1. DIN EN ISO/IEC 17043:2010; Konformitätsbewertung – Allgemeine Anforderungen an Eignungsprüfungen / Conformity assessment – General requirements for proficiency testing
2. Verordnung / Regulation 882/2004/EU; Verordnung über amtliche Kontrollen / Regulation on official controls
3. DIN EN ISO/IEC 17025:2005; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien / General requirements for the competence of testing and calibration laboratories
4. Richtlinie / Directive 1993/99/EU; über zusätzliche Maßnahmen im Bereich der amtlichen Lebensmittelüberwachung / on additional measures concerning the official control of foodstuffs
5. ASU §64 LFGB: Planung und statistische Auswertung von Ringversuchen zur Methodenvalidierung
6. DIN ISO 13528:2009; Statistische Verfahren für Eignungsprüfungen durch Ringversuche
7. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Laboratories ; J.AOAC Int., 76(4), 926 – 940 (1993)
8. The International Harmonised Protocol for the Proficiency Testing of Ananlytical Chemistry Laboratories ; Pure Appl Chem, 78, 145 – 196 (2006)
9. Evaluation of analytical methods used for regulation of food and drugs; W. Horwitz; Analytical Chemistry, 54, 67-76 (1982)
10. Protocol for the design, conduct and interpretation of method performance studies; W. Horwitz; Pure & Applied Chemistry, 67, 331-343 (1995)
11. ASU §64 LFGB L15.05-2, Bestimmung von Fumonisin B1 und B2 in Mais/ HPLC-Verfahren mit Reinigung durch Festphasenextraktion (Juli 2004)
12. A Horwitz-like funktion describes precision in proficiency test; M. Thompson, P.J. Lowthian; Analyst, 120, 271-272 (1995)
13. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing; M. Thompson; Analyst, 125, 385-386 (2000)
14. ASU §64 LFGB L15.05-3, Bestimmung von Fumonisin B1 und B2 in Maiserzeugnisse, HPLC-Verfahren mit Immunoaffinitätssäulen-Reinigung (September 2006)
15. EG-VO 401-2006 zur Festlegung der Probenahmeverfahren und Analysemethoden für die amtliche Kontrolle des Mykotoxingehalts von Lebensmitteln

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