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International interlaboratory study for the determination of the *Fusarium* mycotoxins zearalenone and deoxynivalenol in agricultural commodities

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Twenty-eight laboratories from 12 different countries participated in an interlaboratory study for the determination of the Fusarium *mycotoxin zearalenone (ZON) in maize and deoxynivalenol (DON) in maize and wheat employing their usual in-house methods. The aim of this study was to obtain information about the state-of-the-art of ZON and DON analysis in cereals and to support a knowledge and experience exchange between the participating laboratories in the field of mycotoxin analysis. Eight different sample types were distributed to the participants, 'blank' materials, spiked samples (102 μg/kg ZON in maize and 475 μg/kg DON in wheat) and naturally-contaminated maize and wheat. For the final separation and quantification either gas chromatography (GC), high performance liquid chromatography (HPLC), thin layer chromatography (TLC) or enzyme linked immunosorbent assays (ELISA) were employed by the participating laboratories. Coefficients of variation (CV) between laboratory mean results (outliers rejected) ranged from 28 to 41% for ZON and from 32 to 38% for DON. The results are close to the between laboratory CV criteria of 40% for DON and ZON at concentration levels of >100 μg/kg established by the CEN in 1999. A good trueness was obtained for the wheat samples spiked at 475 μg/kg DON. However, a significant deviation at p = 0.01 from the respective target value was observed for the maize samples spiked at 102 μg/kg ZON. The high CVs can be traced back to problems occurring by determination of the concentration of the participants' own calibrant solutions. Additionally, the variability of the results is strongly influenced by the use of different final separation and quantification procedures.*

Keywords: mycotoxin, deoxynivalenol, zearalenone, analysis, interlaboratory study, CRM, standardization, risk assessment

Introduction

The contamination of food and animal feed by moulds is a worldwide problem. In the more temperate regions of the world the omnipresent moulds of the genera *Fusarium* can cause crop spoilage and mycotoxin formation during agricultural production (Tanaka *et al.* 1988). Numerous surveys and monitoring studies on the presence of fungal metabolites indicate that deoxynivalenol (DON), a member of the type B trichothecenes, and zearalenone (ZON) belong to the most prevalent *Fusarium* mycotoxins in agricultural commodities in Europe (Gareis *et al.* 1989, Mueller *et al.* 1993).

DON leads to symptoms like vomiting, diarrhoea, and lower weight gain and affects the immune system in humans and animals. ZON shows remarkable oestrogenic and anabolic properties and can cause severe reproductive and infertility problems in farm animals. Moreover, exposure to ZON may cause the development of cancers, genetic mutations and birth defects. Since both mycotoxins are potential health risks for humans and animals, several countries regulate ZON and DON in food and feed at maximum tolerable levels (van Egmond and Dekker 1996). Therefore, accurate and precise analytical methodologies are required (Scott 1995, Krska 1998).

An interlaboratory study organized by Schuhmacher *et al.* (1997) has shown an acceptable level of accuracy based on the CEN (1999) criteria of 40% for the between-laboratory CV for DON and ZON, although

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the participants surprisingly had problems in determining ZON and DON in pure organic solvents (e.g. CV = 21% for DON). The good level of accuracy could be traced back to the common calibrant solutions provided which had to be used for calibration by the participants.

A recently performed intercomparison within a Standard, Measurement and Testing (SMT) project of the European Commission organized by Pettersson (1996) for trichothecene analysis in which no common calibrant was provided emphasized this problem, since a high variation was found for the determination of DON. The values for a naturally-contaminated wheat sample ranged from 185 to 1100 µg/kg for DON with a CV of 60% (after rejection of outliers). According to the experience gained during this EC SMT project the major problems seem to be the different molar absorptivities employed for the spectrophotometric measurement of the calibrants and the response enhancement in the presence of the matrix particularly when using GC methods.

In summary, it can be stated that especially for the most relevant and routinely analysed *Fusarium* mycotoxin DON the state-of-the-art in the analysis is still not satisfactory.

Owing to these findings a need was felt to launch another interlaboratory knowledge and experience exchange in the field of DON and ZON analysis, through the organization of this interlaboratory study to improve the quality of analytical data and the comparability of DON and ZON results produced by different laboratories at the international level.

Participants

The announcement of this interlaboratory study found great interest and laboratories from all over Europe, USA and Singapore indicated by their intention to participate. Finally, analytical samples were distributed to 28 laboratories, from Austria (8), Bulgaria (1), France (1), Germany (9), Greece (1), Hungary (1), Italy (1), the Netherlands (1), Norway (1), Singapore (1), Switzerland (1) and the United States (2). All 28 laboratories took part for ZON, 18 of them took part in the study on DON as well. All participants completed the study and sent in their results. However, one laboratory (No. 17) participated only for the naturally-contaminated samples of both mycotoxins.

Materials

Preparation of calibrant solutions

A 5.122 (± 0.004) mg sample of ZON (Sigma) was weighed into a 500 ml volumetric flask on a microbalance (Micro M 500P, Sartorius). ZON was dissolved in 500.00 (± 0.25) ml methanol (HPLC gradient grade, Merck) to give a final concentration of 10.24 (± 0.01) µg/ml. A 25.00 (± 0.13) ml sample of the 10.24 (± 0.01) µg/ml calibrant solution was diluted with methanol in a 50 (± 0.06) ml volumetric flask. This solution [5.12 (± 0.03) µg/ml] was used to prepare artificially-ZON-contaminated maize by means of spiking 'blank' maize.

Purity and ZON concentration were checked by reversed phase (RP) HPLC (HP 1090 II, Hewlett-Packard) and UV spectroscopy (Perkin Elmer, Lambda 16) at the IFA-Tulln [10.2 (± 0.5) µg/ml] and at the Federal Office of Agrobiology, Linz, Austria [10.0 (± 0.4) µg/ml].

For the preparation of the DON calibrant solution, 4.750 (± 0.004) mg DON (Sigma) was dissolved in 250.00 (± 0.15) ml ethyl acetate (r.a., Baker). This solution [19.00 (± 0.03) µg/ml] was employed for spiking 'blank' wheat.

Owing to the wide range of values for the molar absorptivity that have been published for DON, the concentration and purity of the calibrant solutions were checked by means of RP HPLC analysis with diode array detection (DAD) [19.5 (± 0.8) µg/ml] and GC with electron capture detection (ECD) [19.2 (± 1.0) µg/ml] and not through spectrophotometric UV measurements. Chromatograms and peak areas were compared with a DON standard purchased from Promochem.

It can be concluded that the deviation of the results obtained from the UV absorbance, HPLC and GC measurements for the DON and ZON calibrant solutions was in none of the cases greater than the CV of the employed methods given in parentheses. Accordingly, the assigned values of the calibrants were based on the amounts originally weighed.

Table 1. Stability control mean results of the artificially- and naturally-contaminated samples (n = 8).

Sample	Mean ± 1s [µg/kg] (CV [%])	
	May 1998	August 1998
DON naturally contaminated—level 1	479 ± 48 (10)	484 ± 48 (10)
DON naturally contaminated—level 2	975 ± 98 (10)	1003 ± 100 (10)
DON-spiked wheat	467 ± 47 (10)	450 ± 45 (10)
ZON naturally contaminated—level 1	80 ± 7 (9)	74 ± 6 (8)
ZON naturally contaminated—level 2	398 ± 32 (8)	392 ± 32 (8)
ZON-spiked maize	104 ± 9 (9)	106 ± 9 (9)

Artificially- and naturally-contaminated maize and wheat samples

'Blank' maize and maize naturally contaminated with DON and ZON were collected from different feeding-stuff companies. 'Blank' wheat and naturally-DON-contaminated wheat were provided by Dr. Lew of the Federal Office of Agrobiology, Linz, Austria. All samples were ground (wheat: 0.5 mm and maize: 1.0 mm sieve) and had been pre-analysed. Subsequently, homogenization of the commodities was carried out by use of a worm shaft homogenizer (ACMJ 150, Prodima).

Aliquots (60–65 g) of the 'blank' materials intended as 'blank' control samples for the participants and the same amount of naturally-contaminated samples were weighed into 250 ml polypropylene cans. The sample jars were sealed with screw caps and parafilm.

For the preparation of artificially-contaminated samples 20.00 (± 0.03) g portions of 'blank' materials were weighed into glass vessels. The 'blank' maize samples were spiked with 400 µl ZON calibrant solution which led to a content of 102 (± 1) µg/kg ZON in maize. The 'blank' wheat samples were spiked with 500 µl DON calibrant solution which resulted in a final content of 475 (± 2) µg/kg DON in wheat.

Quality assurance

The dry residue was determined by means of an infrared measuring device for moisture content (Moisture Analyser, Sartorius). The mean relative mass losses were <12%. Since a water content of more than 13% is required for further growth of fungi the samples could be stored and distributed to participants without additional drying (Davis *et al.* 1980, Reiss 1986).

Stability of wheat and maize materials was confirmed by comparison of the analytical results for the respective mycotoxin at the beginning and the end of the interlaboratory study. Eight replicate analyses were carried out for each material in May 1998 and July 1998 when all laboratories had submitted their results. No significant deviation at $p = 0.05$ of the mean values obtained in May and August could be observed for all samples, which indicates that the samples were stable (table 1).

Moreover, the homogeneity of the naturally-contaminated materials was confirmed by additional control analyses of the corresponding batches. Therefore, eight samples of DON and ZON in maize and wheat were taken at regular intervals when the materials were filled into polypropylene cans. These samples were analysed in duplicate for their DON and ZON concentrations in random order. The average mycotoxin concentrations and the corresponding standard deviations (s_1 and s_2) were calculated and the variances obtained were checked by the *F*-test at the 95% level. The results of the *F*-test at the 95% level show that each material could be regarded as homogeneous (table 2). Additionally, no trend could be observed for the samples taken at regular intervals (figure 1).

Additionally, the 'blank' wheat and maize materials intended as control samples and for the preparation of artificially-contaminated samples were checked for undesirable natural DON or ZON contaminations. For all determinations, mycotoxin concentrations were found to be lower than the limits of detection (LODs) of the employed methods. Therefore, the content of ZON in 'blank' maize was lower than

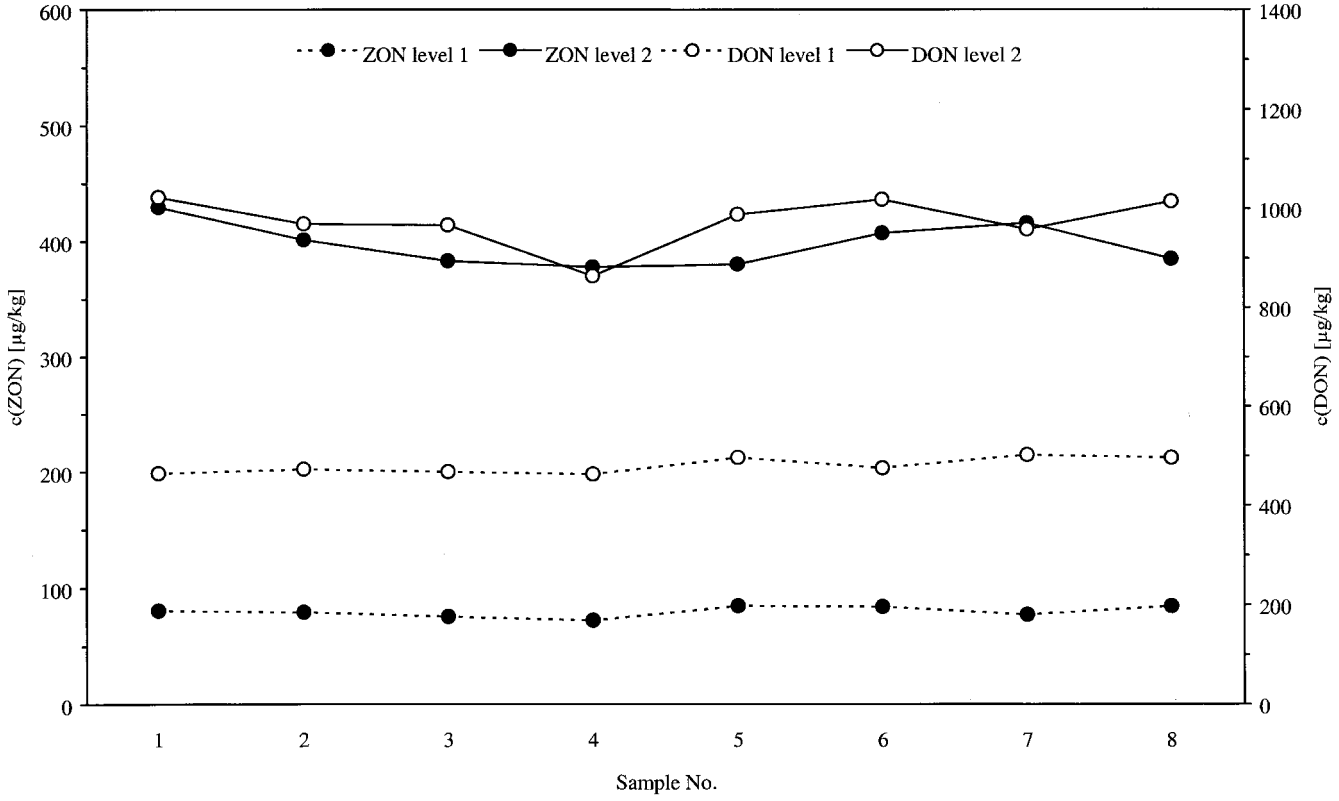

Figure 1. Trend test results for the materials naturally contaminated with DON and ZON. Samples taken at regular intervals in accordance with the sample no.

Table 2. Homogeneity tests of the naturally-contaminated materials (F (f1 = f2 = 7, p = 0.05)).

Sample	s_1 [µg/kg]	s_2 [µg/kg]	s_1^2/s_2^2	*F*-value
DON naturally contaminated—level 1	27	16	2.85	3.79
DON naturally contaminated—level 2	96	52	3.41	3.79
ZON naturally contaminated—level 1	4.61	2.95	2.44	3.79
ZON naturally contaminated—level 2	18.94	10.50	3.25	3.79

about 5 µg/kg and the content of DON in wheat was less than 50 µg/kg.

Analytical methods

Instructions

The participants were asked to perform two independent replicate determinations for each type of sample employing their usual analytical methods for DON and ZON. The first analysis of the DON and ZON samples should be carried out within 1 day. The second analysis of the same samples should be conducted on a separate day (approx. the week after).

In contrast to the interlaboratory study in 1996 (Schuhmacher *et al.* 1997) the participating laboratories had to perform the calibrations with their own calibrants since no common calibrant was provided. For the artificially-contaminated samples, the laboratories were asked to correct their results for matrix

Table 3. Overview of methods for the determination of ZON employed by the participants. The numbers represent the number of laboratories employing the respective approaches.

Extraction	Clean-up	Determination
14 × Acetonitrile/water	10 × Immunoaffinity columns	18 × HPLC-FLD
5 × Methanol/water	7 × l/l-partitioning	5 × ELISA
2 × Acetonitrile/aq. KCl	3 × MycoSep columns	1 × HPLC-DAD
2 × Ethyl acetate/water	2 × SPE C18	1 × HPLC–MS
2 × Chloroform	1 × SPE silica	1 × GC–MS
1 × Ethyl acetate/ aq. acetic acid	1 × Extrelut	2 × TLC
1 × Methanol/methylene chloride	1 × l/l + SPE silica	
1 × Ethyl acetate	3 × None	

interferences (determined by the analysis of 'blank' materials) on their own, if necessary.

Methods for the determination of ZON

Twenty-eight laboratories participated in the inter-laboratory study for ZON and sent in information about the methods employed.

As listed in table 3 mycotoxin extraction was carried out with a mixture of an organic solvent with water or aqueous acidic solutions, with the exception of four participants.

Eight laboratories performed a clean-up using liquid/liquid (l/l)-partitioning by applying an organic solvent, an aqueous alkaline solution or Extrelut® material. In one case l/l-partitioning in combination with solid phase extraction (SPE) was employed. Ten participants used immunoaffinity columns (IAC) for purification of extracts. For the final separation and determination of ZON, 18 laboratories applied HPLC with fluorescence detection (FLD). HPLC with UV/DAD detection and HPLC–MS was chosen by one participant each. Five participants quantified ZON by direct competitive ELISA methods. Two participants used TLC methods and one laboratory applied GC–MS for the determination of ZON.

Due to the information provided by the participants the LODs of the methods employed for the determination of ZON ranged from 0.125 to 72 µg/kg and the individually determined recoveries varied from 60 to 104%. The reported values for the CVs of the methods ranged from 4 to 15%. Accordingly, the LODs of all methods employed were sufficient to detect ZON even in the sample with the lowest level (approx. 75 µg/kg). However, due to different approaches used for the estimation of the uncertainty or CVs a wide range of CVs of methods was reported. Some laboratories provided an in-house CV based on replicate measurements, some provided estimations based on random experience and others simply followed the indications of the test kit manufactures (e.g. ELISA).

Methods for the determination of DON

Eighteen of the 28 participants took part for the determination of DON. As depicted in table 4 extractions were performed with pure water (one laboratory) or with mixtures of water and acetonitrile (15 laboratories), methanol (one laboratory) or PEG 8000 (one laboratory). For purification of raw extracts SPE techniques were employed, either with MycoSep™ columns (nine laboratories) or with florisil/activated charcoal columns. Additionally, two participants used IACs and in one case the Extrelut® technique in combination with SPE florisil was applied for the clean-up.

Determination of DON was carried out by GC-ECD (five laboratories), GC–MS (two laboratories), HPLC-UV/DAD (six laboratories) and HPLC-FLD (one laboratory). Furthermore, four laboratories employed ELISA for the determination of DON. It should be emphasized that the ELISA methods employed for the determination of DON principally cannot distinguish between DON, 3acetylDON, 15acetylDON and 3,15-diacetylDON, since the applied mycotoxin antibodies are designed against 3,7,15-triacetylDON. This evidence was particularly critical in case of the maize sample provided which contained, besides DON, 477 µg/kg 15-acetylDON.

Table 4. Overview of methods for the determination of DON employed by the participants. The numbers represent the number of laboratories employing the respective approaches.

Extraction	Clean-up	Method
15 × Acetonitrile/water	9 × MycoSep columns	6 × HPLC-UV
1 × Methanol/water	2 × Immunoaffinity columns	5 × GC-ECD
1 × Water/PEG 8000[a]	1 × SPE charcoal	4 × ELISA
1 × Water	1 × SPE Florisil	2 × GC–MS
	1 × Extrelut + Florisil	1 × HPLC-FLD
	4 × None	

[a] PEG = polyethylene glycols.

Generally, the LODs of the DON methods employed by the participants ranged from 0.3 to 108 µg/kg. The individually determined recoveries varied from 60 to 100% and the reported CVs of the methods ranged from 3 to 15%.

Data evaluation

Individual laboratory codes were assigned to each of the 28 participants at random. These codes (1–28) were treated as confidential; only participants were informed about their own laboratory code.

Subsequent to outlier elimination by use of the Hampel test (Davies 1988), overall means, corresponding standard deviations and CVs for the intra-laboratory mean results were calculated. The overall mean results have been ranked and are plotted in figures 2–4 and figures 6–8. The whiskers represent the between-day variation of the intra-laboratory mean results. Outlying mean results are marked with an asterisk (*). The assigned between- laboratory means and the between-laboratory standard deviations are plotted as full or dotted lines respectively.

Results and discussion

Zearalenone

The 'blank' maize samples were analysed by all 28 laboratories. The majority of the participants obtained ZON concentrations below the respective LODs. Only seven found ZON concentrations in the range of 1.7–7.45 µg/kg.

It was intended to provide a sample whose ZON content is in the range of the Austrian guideline level of 60 µg/kg (van Egmond and Dekker 1996). Therefore, the chosen ZON spiking level was 102 µg/kg.

All laboratories except No. 17 performed the inter-laboratory study for ZON-spiked maize. Laboratory 3 provided only one value for this type of sample, because of unspecified problems with the repeated measurements.

The mean results of laboratories 6 and 26 (182 and 19 µg/kg respectively) have been identified as outlying results by the Hampel outlier test. After rejection of these two values, the overall mean for ZON-spiked maize (88 µg/kg, CV = 28%, $n = 25$) was significantly lower ($t(f = 24, p = 0.01)$) than the target ZON content of 102 µg/kg (figure 2).

All 28 participants provided results for the maize sample naturally contaminated with ZON—level 1 (low content level). An overall mean of 73 µg/kg and a standard deviation between the laboratories of ± 30 µg/kg (CV = 41%) was calculated since no outlying results could be detected (figure 3). For a comparison of ELISA (laboratories 1, 2, 9, 16, 22) and HPLC methods (laboratories 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15, 17, 18, 20, 21, 23, 24, 25, 27, 28) the results obtained with these techniques were considered as different data sets. However, the mean of the HPLC results (76 ± 28 µg/kg, CV = 37%, no outlier, $n = 20$) was not significantly ($< t(f = 22, p = 0.05)$) different from the ELISA results (53 ± 15 µg/kg, CV = 28%, one outlier, $n = 4$).

Additionally, all 28 laboratories took part in the analysis of maize samples naturally-contaminated with ZON—level 2 (high content level). Likewise, no outlier could be discovered. The overall mean was 291 µg/kg and the standard deviation between

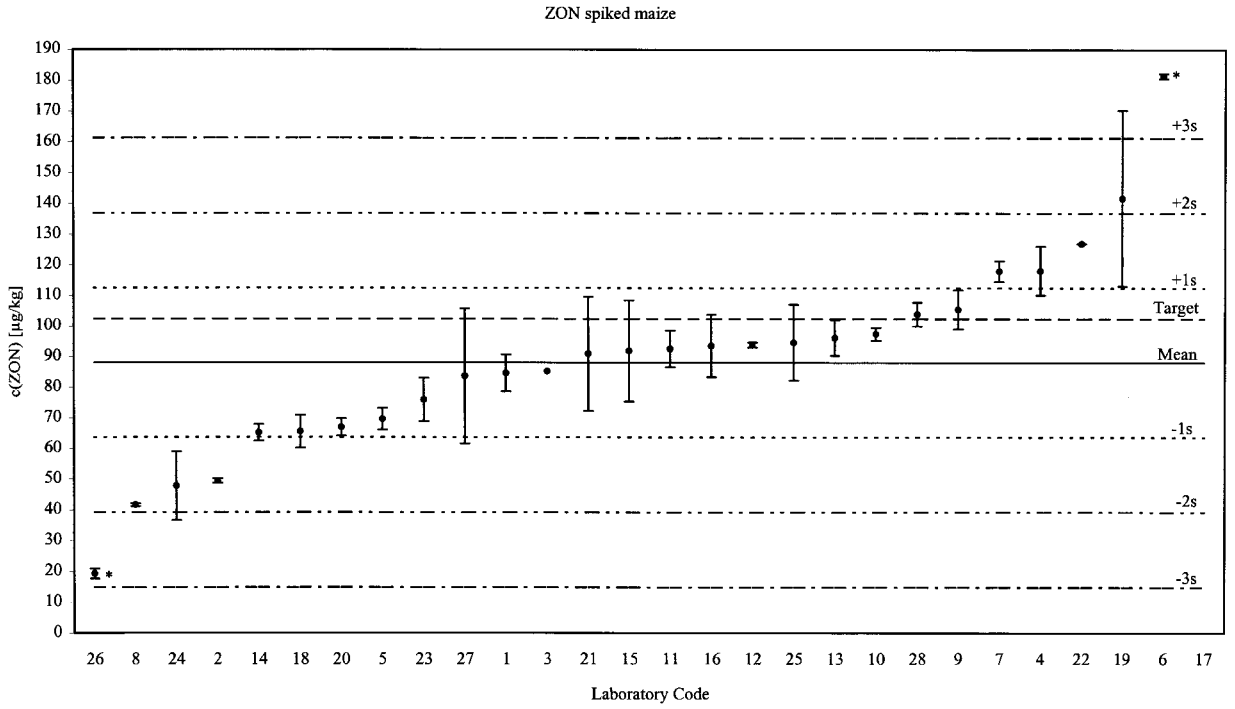

*Figure 2. Results for ZON in the spiked maize sample. Full line: assigned mean concentration of 88 µg/kg, dotted line: target content of 102 µg/kg and *: outlying results.*

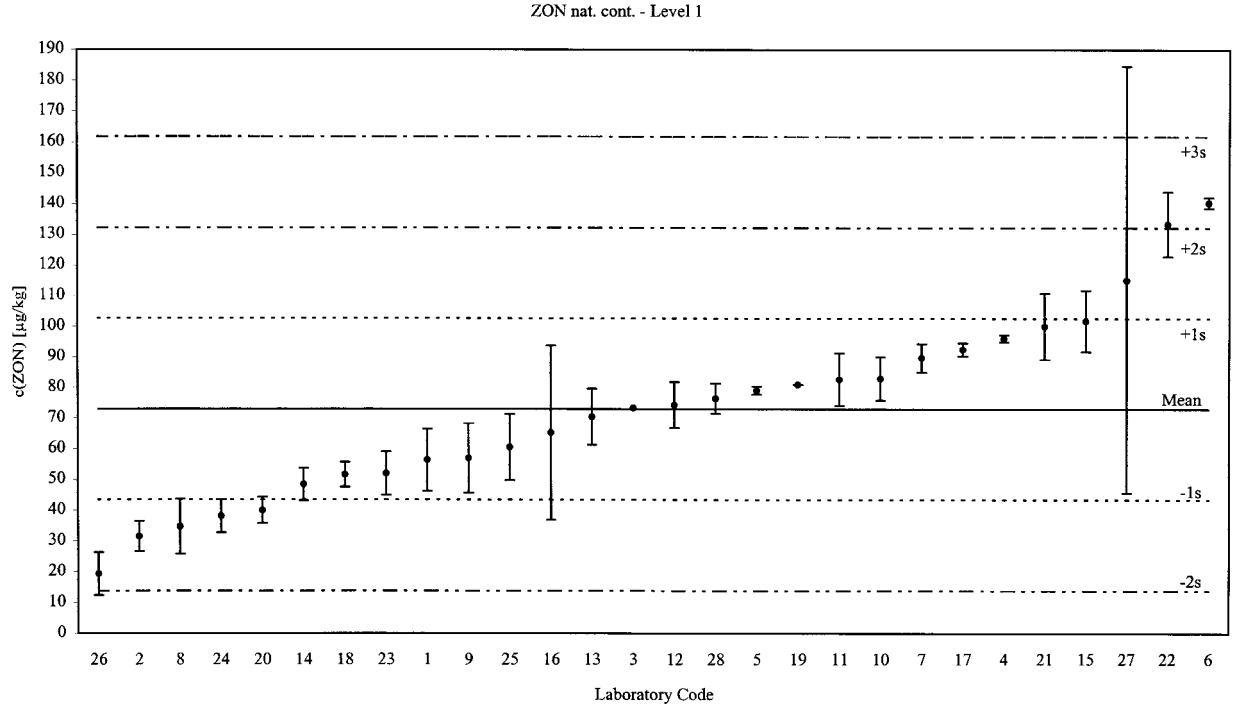

Figure 3. Results for ZON in the low level naturally-contaminated maize sample (level 1). Full line: assigned mean content of 73 µg/kg.

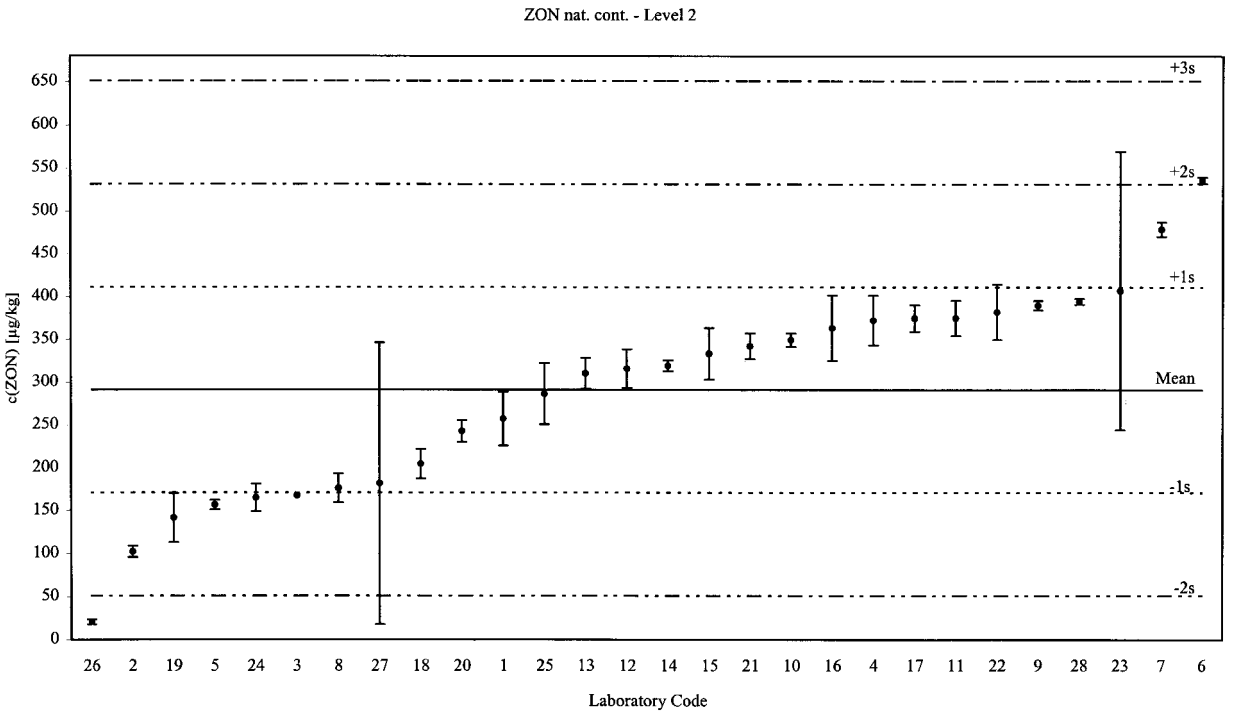

Figure 4. Results for ZON in the high level naturally-contaminated maize sample (level 2). Full line: assigned mean content of 291 µg/kg.

the laboratories was ± 120 µg/kg (CV = 41%) (figure 4). Also in this case the mean of the HPLC results (316 µg/kg ± 104 µg/kg, CV = 33%, no outlier, n = 20) was not significantly ($< t(f = 22, p = 0.05)$ different from the ELISA results (348 ± 62 µg/kg, CV = 18%, one outlier, n = 4) when ELISA and HPLC detection methods are treated separately. But it should be mentioned that the interpretation power is strongly influenced by the low degree of freedom of f = 3 in case of the ELISA results.

Three laboratories (3, 19 and 26) employing methods different to ELISA and HPLC detection (GC and TLC) obtained dramatically lower results ranging from 21 to 168 µg/kg.

A comparison of the mean laboratory results for the low and high level naturally-contaminated maize by means of a standardized plot according to Youden (1972) (figure 5) shows a satisfactory scattering within the 2s circle. Only laboratories 6 and 22 overestimated (quadrant I) and the laboratories 2 and 26 underestimated (quadrant III) the concentrations for both materials. Generally, no concentration dependence could be observed.

Deoxynivalenol

The 'blank' wheat samples were analysed by 18 laboratories. Although, the pre-analytical studies performed at the IFA Tulln indicated that the 'blank' wheat contained less than 50 µg/kg, six participants found DON concentrations up to 107 µg/kg. One laboratory (No. 3) reported unknown problems during the analyses of the repeated measurements. In all other cases the DON concentrations obtained were below the respective LODs.

It was intended to provide a sample whose DON content was close to the Austrian guideline level of 500 µg/kg (van Egmond and Dekker 1996). Therefore, the chosen DON content was 475 µg/kg.

Seventeen laboratories performed the actual interlaboratory study for DON spiked wheat. No outlying result could be determined with the Hampel outlier test (figure 6). Statistically, the overall mean ± 1s for DON-spiked wheat (488 ± 187 µg/kg, CV = 38%, n = 17) could not be distinguished ($< t(f = 16, p = 0.05)$) from the target DON content of 475 µg/kg. However, two outlying results (914 and 882 µg/kg)

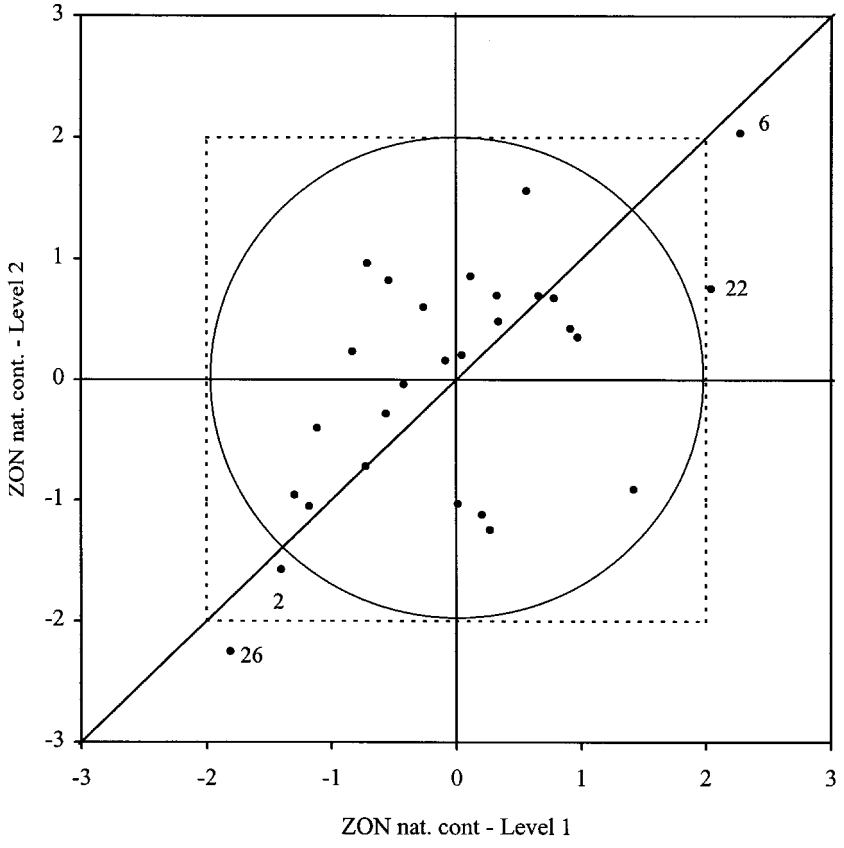

Figure 5. Comparison of the results for the low (level 1) and high (level 2) level naturally-ZON-contaminated maize samples by means of a standardized Youden plot. Circle: ± 2s criterion.

were detected within the 18 provided results for the naturally-DON-contaminated wheat sample level 1 (low content level). After rejection of the outliers, an overall mean ± 1s of 382 ± 121 µg/kg (CV = 32%) was calculated (figure 7).

For a comparison of the results obtained with different analytical methods, the GC results (laboratories 3, 7, 17, 23, 24, 25, 28), the HPLC results (laboratories 5, 10, 14, 15, 18, 20) and the ELISA results (laboratories 1, 9, 16, 22) were treated as different data sets. This led to a mean ± 1s of 370 ± 89 µg/kg for GC (CV = 24%, no outlier, n = 7), 273 ± 41 µg/kg for HPLC (CV = 15%, one outlier, n = 6) and finally a mean ± 1s of 739 ± 184 µg/kg for laboratories applying ELISA methods (CV = 25%, no outlier, n = 4). Compared with the mean of the HPLC results and the mean of the GC results, the mean of ELISA results was in both cases significantly higher ($t(f = 9$, $p = 0.01\%)$, $t(f = 7$, $p = 0.01\%))$. However, despite a difference of about 100 µg/kg the mean of the HPLC results and the mean of the GC results were not significantly ($p = 0.05$) different, which is certainly also a consequence of the standard deviations obtained for both methods.

Likewise, 18 participants took part for the analysis of the maize sample naturally-contaminated with DON, level 2 (high content level). After rejection of two outliers, 16 results led to an overall mean of 806 µg/kg with a standard deviation between the laboratories of ± 263 µg/kg (CV = 33%) (figure 8). A separate treatment of HPLC, GC and ELISA results led to a mean ± 1s of 601 ± 157 µg/kg for HPLC results (CV = 26%, one outlier, n = 5), 834 ± 163 µg/kg for GC results (CV = 20%, no outlier n = 7) and 1702 ± 580 µg/kg for ELISA results (CV = 34%, no outlier n = 4). As already shown for the wheat naturally contaminated with DON—level 1 the mean of the ELISA results was significantly higher than the HPLC and GC results ($t(f = 9$, $p = 0.01)$, $t(f = 7$, $p = 0.01))$ for maize naturally contaminated with DON—level 2. It should

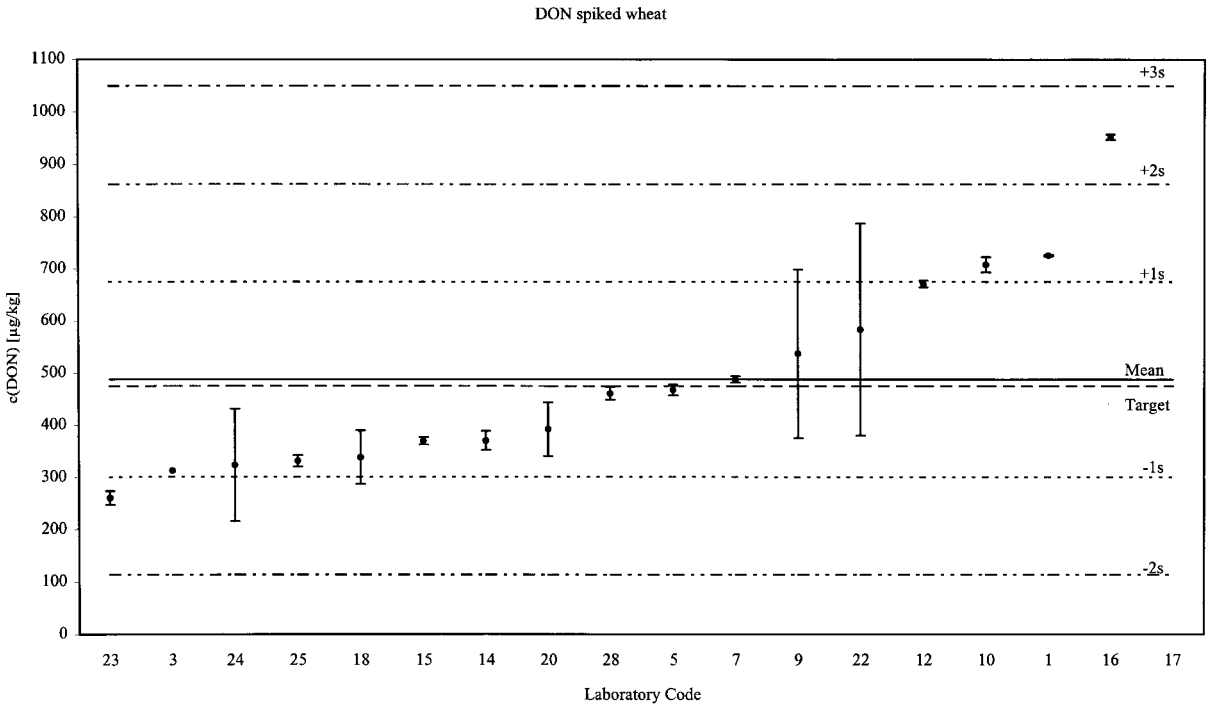

Figure 6. Results for DON in the spiked wheat sample. Full line: assigned mean concentration of 488 µg/kg, dotted line: target content of 475 µg/kg.

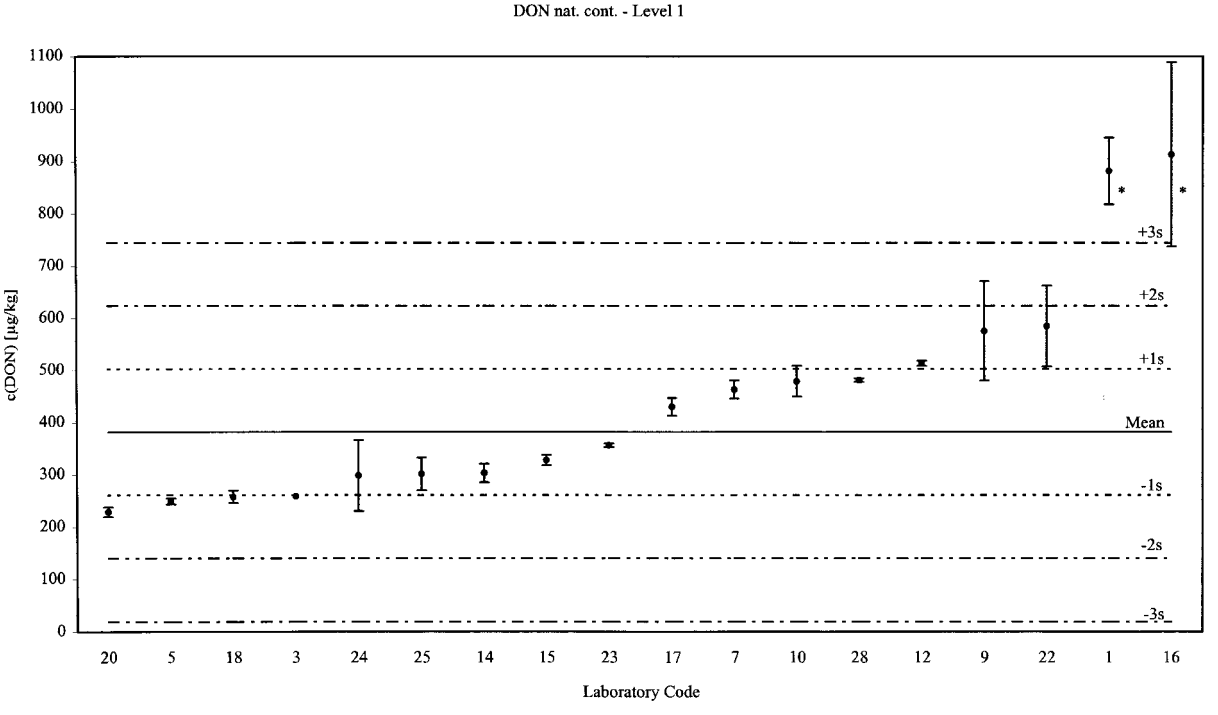

*Figure 7. Results for DON in the low level naturally-contaminated wheat sample (level 1). Full line: assigned mean content of 382 µg/kg and *: outlying results.*

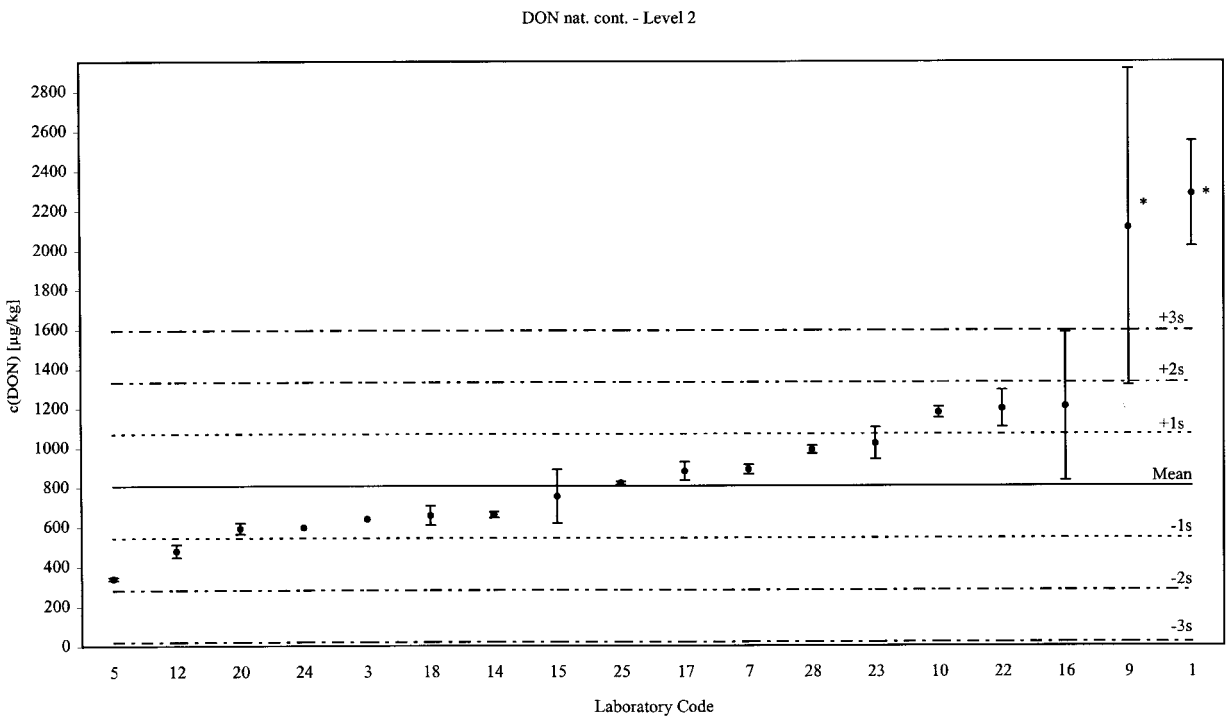

*Figure 8. Results for DON in the high level naturally-contaminated maize sample (level 2). Full line: assigned mean content of 806 µg/kg and *: outlying results.*

be also mentioned that the interpretation power is strongly influenced by the low degree of freedom of $f = 3$ in case of the ELISA results.

A comparison of the mean laboratory results for the naturally contaminated wheat and maize materials by a standardised Youden plot (figure 9) indicates a satisfactory trueness and precision based on the CEN (1999) criteria of 40% for the between-laboratories CV. This demonstrates also that the type of matrix (wheat or maize) has no significant influence on the majority of the employed methods. However, the results of laboratories 1, 9 and 16 plotted in the quadrant I beyond the 2s interval prove a serious method dependence. These are three out of four participants using ELISAs. In general, an overestimation and matrix dependence were observed for the ELISA results.

Conclusions

The number of 28 participating laboratories shows the increasing interest in external quality assurance measures in the field of *Fusarium* mycotoxin analysis. The rather low number of 18 participants for the samples containing the most prevalent *Fusarium* mycotoxin DON compared with the number of 28 laboratories analysing the ZON samples was surprising.

Prior to this interlaboratory study the participants were asked to give an estimation of their own level of accuracy. In summary, the level of accuracy for both mycotoxins was not as estimated by the participants. However, a good trueness was obtained for the DON-spiked wheat. On the contrary, a significant deviation at $p = 0.01$ from the target value was observed for ZON-spiked maize. This phenomenon was also observed in the interlaboratory study organised by the IFA-Tulln in 1996 (Schuhmacher *et al.* 1997).

It also seems that the laboratories had greater problems in analysing maize naturally-contaminated with ZON. In this case, the between-laboratories CV increased from 28% (for spiked samples) to 41%. However, in contrast to 1996 the CV of results obtained for the higher contamination levels was not greater than for the lower contaminated samples. This could be verified through an interpret-

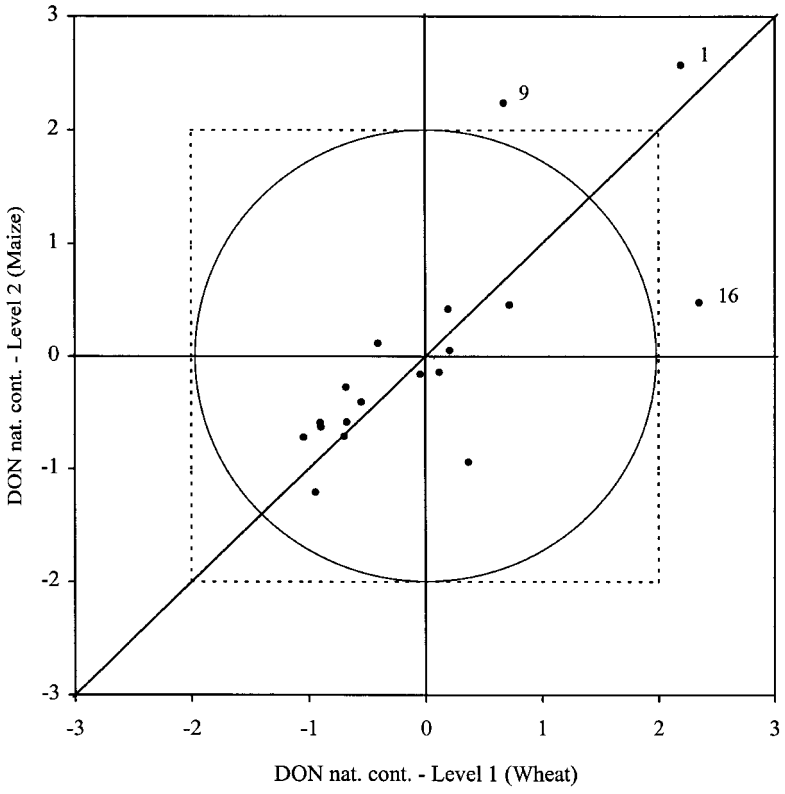

Figure 9. Comparison of the results for the naturally-DON-contaminated wheat (level 1) and maize (level 2) by means of a standardized Youden plot. Circle: ± 2s criterion.

ation of the data by means of a standardized Youden diagram.

Generally, a particularly poor agreement of results was obtained for the naturally-contaminated samples of both mycotoxins with between-laboratories CVs ranging from 32% to 41% based on the CEN (1999) criteria of 40%.

In addition, the CVs for DON and ZON results increased tremendously from 1996 to 1998, which indicates a lower performance of the laboratories for both mycotoxins. We believe that the main reason for this is that no common calibrant solution was provided for calibration in the present study. This assumption is confirmed when comparing the results obtained in two other interlaboratory studies that have been organized recently. On the one hand, in an interlaboratory study of DON methods published by Gilbert *et al.* (1992) in which a common calibrant solution was provided, the agreement of results was similar to that obtained during our interlaboratory study of 1996. On the other hand, the CV for the trichothecene interlaboratory performed within the frame of an EC SMT project organized by Pettersson (1998) in which no common calibrant was provided was 60% for the determination of DON.

In summary, the determination of the concentration of calibrant solutions is still a serious problem for both mycotoxins. Although the spectrophotometric determination of ZON calibrant solutions according to AOAC (1990) is a well described method, there is currently no agreement on a certain international reference wavelength and molar absorptivity for both ZON and DON.

Furthermore, a method dependence of the analytical results was demonstrated in this study. The analytical results obtained for ZON show that HPLC and ELISA methods are not generating significantly ($p = 0.05$) different results keeping in mind the low degree of freedom ($f = 3$) for ELISA methods. However, with methods different to these techniques, in particular TLC, significantly ($p = 0.01$) lower results than the assigned mean value were obtained.

Those laboratories using ELISA methods for DON observed significantly higher results compared with results obtained with HPLC and GC methods. Additionally, this finding could be confirmed employing a Youden plot. In the case of the maize sample one reason for these higher findings is obviously the additional contamination of the maize sample with 15-acetylDON at a concentration of 477 µg/kg determined with GC-ECD, which cannot be distinguished with the ELISA-systems, but in case of the wheat samples the reason for the high ELISA values is still unclear.

Since in a previous EC SMT study for trichothecenes (Pettersson 1996) a response enhancement in the presence of matrix was demonstrated when using GC for the end determination of the silylated derivative of DON, we expected significantly higher results for DON with GC. However, despite a difference of about 100 µg/kg (370 compared with 273 µg/kg) the mean of the GC results was not significantly ($p = 0.05$) higher than the mean of the HPLC results, which is certainly also a consequence of the high standard deviations obtained for both methods. Nevertheless, the matrix induced response enhancement during DON analysis has been clearly demonstrated in this EC SMT project (Pettersson 1998) and is obviously one out of several reasons for the poor agreement of results in DON analysis.

The experience gained during this study clearly shows the need for further improvements in DON and ZON determination to obtain more accurate and comparable results. In the case of DON the regularly use of the available certified reference materials (CRMs) for DON in wheat and maize matrix within the quality control programmes of the laboratories will improve the accuracy, comparability, and traceability of the analytical results. The above findings were a valuable contribution to releasing another project (Krska and Josephs 1998) funded by the EC SMT programme to produce certified reference materials (CRMs) for ZON to ensure comparability and traceability in the field of ZON analysis.

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