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European Intercomparison Study for the Determination of Fumonisins in Maize

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Abstract. Fumonisins are mycotoxins occurring largely in maize and maize products, which cause animal diseases, such as equine leukoencephalomalacia and porcine pulmonary edema, and may also induce liver cancer on experimental rats. The European Commission Standards, Measurements and Testing (SMT, formerly BCR) Programme, has sponsored a project to improve analytical methodologies for the determination of the two major fumonisins (fumonisin B1 and fumonisin B2) in maize materials. The project involved the following steps: i) the preparation of a blank and a maize material contaminated with fumonisins B1 and B2; ii) a preliminary study of the γ-irradiation conditions for sterilization; iii) homogeneity and stability studies of the maize materials; iv) an intercomparison study for fumonisins analysis in the above materials with the involvement of 24 European laboratories, most of which have national or international responsibilities for foodstuff and/or feedstuff quality control. Results of the intercomparison study are presented together with the homogeneity and stability data relevant to the maize materials.

Key words: *Fusarium*, *Fusarium* toxins, mycotoxins, reference materials, maize, food contaminants, feed contaminants.

Fumonisins are a group of mycotoxins produced primarily by the fungus *Fusarium moniliforme* Sheldon, one of the most prevalent seed-borne fungi of maize [1]. At least six fumonisins have been isolated from cultures of *Fusarium moniliforme* and characterized as derivatives of 2-amino-12, 16-dimethylpolyhydroxyeicosane esterified at position 14 and 15 with tricarballilic acid [2]. Fumonisin B1 is the most important of this group of compounds for both the levels of natural occurrence and the biological activity. Fumonisins B2 and B3 have also been frequently found in fungal cultures and naturally contaminated maize [2–4]. Fumonisin B1, in purified form or combined with fumonisin B2 in fungal cultures and in naturally con-

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taminated maize-based feeds, has been shown to cause several animal diseases, such as equine leukoencephalomalacia, porcine pulmonary edema and liver cancer in experimental rats [5–8]. Fumonisins have been shown to occur largely in maize and maize-based foodstuffs at global level. The high levels of fumonisins occurring in maize intended for human consumption have been associated with human esophageal cancer in an endemic region of South Africa [9]. The known naturally occurring levels and toxic effects of fumonisins pose a potential threat to human and animal health [10]. In order to assess realistic tolerance levels of fumonisins in maize products sufficient information on the natural occurrence and human and animal exposure should be available together with an appropriate toxicological evaluation. The European Commission Standards, Measurements and Testing (SMT, formerly BCR) Programme, has sponsored two consecutive projects to improve the quality of the analysis of fumonisins in maize at the European level. The first project consisted in the study of fumonisins stability in different solvents and the interlaboratory evaluation of the final determination of fumonisins in an unknown solution [11, 12]. The second project involved: i) the preparation of a blank and a maize material contaminated with fumonisins B1 and B2; ii) a preliminary study of the γ-irradiation conditions for sterilization; iii) homogeneity and stability studies of the maize materials; iv) an intercomparison study to test the full analytical procedure (extraction, clean-up and determination step) for fumonisins in contaminated and blank maize samples. The results of this second European intercomparison study, together with the relevant data on the homogeneity and stability of fumonisins in maize materials, are reported in this paper.

Experimental

Participating laboratories. A consortium of 24 laboratories (including the coordinating laboratory) from 12 European Countries (including 3 EFTA Countries) was involved in the intercomparison study. Most of the participating laboratories had national or international responsibilities for foodstuff and/or feedstuff quality control and represented industries, research institutions and universities.

Analytical procedures. The analytical method used for the determination of fumonisin B1 and B2 in homogeneity and stability studies has been reported elsewhere [4]. It involves the extraction with methanol: water (3:1), clean-up through a SAX cartridge and determination of fumonisins by reversed phase high performance liquid chromatography (HPLC) with fluorescence detection after derivatization with o-phthaldialdehyde. The method was first developed by Shephard et al. and then tested within an IUPAC collaborative study for fumonisins in maize [13, 14]. Most participants in the European intercomparison study used a similar procedure with some modifications. Details on the methods of analysis applied by each participant will be described in a separate publication (Visconti et al., in preparation).

Preparation of the reference solution and the maize materials. The reference solution, containing 100 µg/ml fumonisin B1 and 50 µg/ml fumonisin B2, was prepared in acetonitrile: water (1:1) and the stability of the solution was checked as described previously [11, 12].

The blank maize material was obtained by mixing batches of different maize genotypes containing traces of or no fumonisins. The contaminated maize material was obtained by mixing part of the blank material with maize containing high levels of fumonisins B1 and B2; fumonisin B2 standard was added

to the mixture in order to obtain an appropriate fumonisin ratio for the intercomparison study. Maize kernels were ground to the size of about 2 mm before mixing for the preparation of the materials. Blank and contaminated maize materials were peaked in 250 and 200 bags of 60 g each, respectively.

A preliminary study of the γ-irradiation conditions effective to sterilize the maize material was performed by separately irradiating at 3 and 15 KGy two sets of 5 bags of ground maize. Sterilization of the two batches of maize was then performed at 15 KGy.

Homogeneity studies. The homogeneity of bags was checked by duplicate determinations of the individual fumonisins (B1 and B2) on each of 5 bags of the blank maize and of 10 bags of the contaminated maize taken at regular intervals during the filling procedure.

Stability studies. A short-term stability study was performed on the contaminated maize material stored at $-20\,^{\circ}C$, $+4\,^{\circ}C$, $+25\,^{\circ}C$ and $+40\,^{\circ}C$, for 2 week and 4 week periods. After each time period bags were kept at $-18\,^{\circ}C$ to stop any further degradation, but not analyzed. After 4 weeks, duplicate determinations of each fumonisin were carried out in the same time interval to minimize analytical variability. A long-term stability study was performed with a similar design with bags of blank and contaminated maize stored at $-18\,^{\circ}C$, $+4\,^{\circ}C$ and $+25\,^{\circ}C$ for 3 and 6 months. Duplicate determinations in each of the two bags stored at the specified conditions were performed after 6 months, again in the same time interval.

Distribution of samples to participants. Four bags of blank maize and 3 bags of contaminated maize materials were distributed to each of the 24 laboratories participating in the intercomparison study. Laboratories were asked to perform recovery experiments on blank maize spiked at levels of 1.5 µg/g fumonisin B1 and 0.75 µg/g fumonisin B2 and to determine the concentration of both fumonisins B1 and B2 in the blank as well as in the contaminated maize material.

Results and Discussion

Effect of γ-irradiation. In order to obtain a satisfactory sterilization of maize materials, γ-irradiation at 15 KGy was required. Such level of irradiation affected considerably the content of fumonisins in the samples generating a decrease of about 20% for both toxins. Irradiation at 3 KGy was not suitable for sterilization, as fungal colonies could still develop from the material after treatment.

Homogeneity and stability studies. No substantial inhomogeneity at the 95% confidence and no gradient due to the filling sequence was detected in the materials used for the homogeneity study. No instability was observed at the different temperatures and storage periods tested in the stability study and the material allowed for normal postal shipment.

Intercomparison study. The results of the intercomparison study after correction for recoveries are shown in Fig. 1. Results of one laboratory were discarded being seriously affected by the application of a wrong clean-up procedure. Two laboratories (nos. 8 and 31) repeated the exercise with an additional method. A total of 25 entries were then considered for the statistical evaluation of the results. The study generated overall data with very high precision (mean $\pm$ 1 SD of 2.29 ± 0.27 µg/g for fumonisin B1 and 1.25 ± 0.17 µg/g for fumonisin B2). However, the recoveries obtained by most participants were quite low and the overall average recoveries

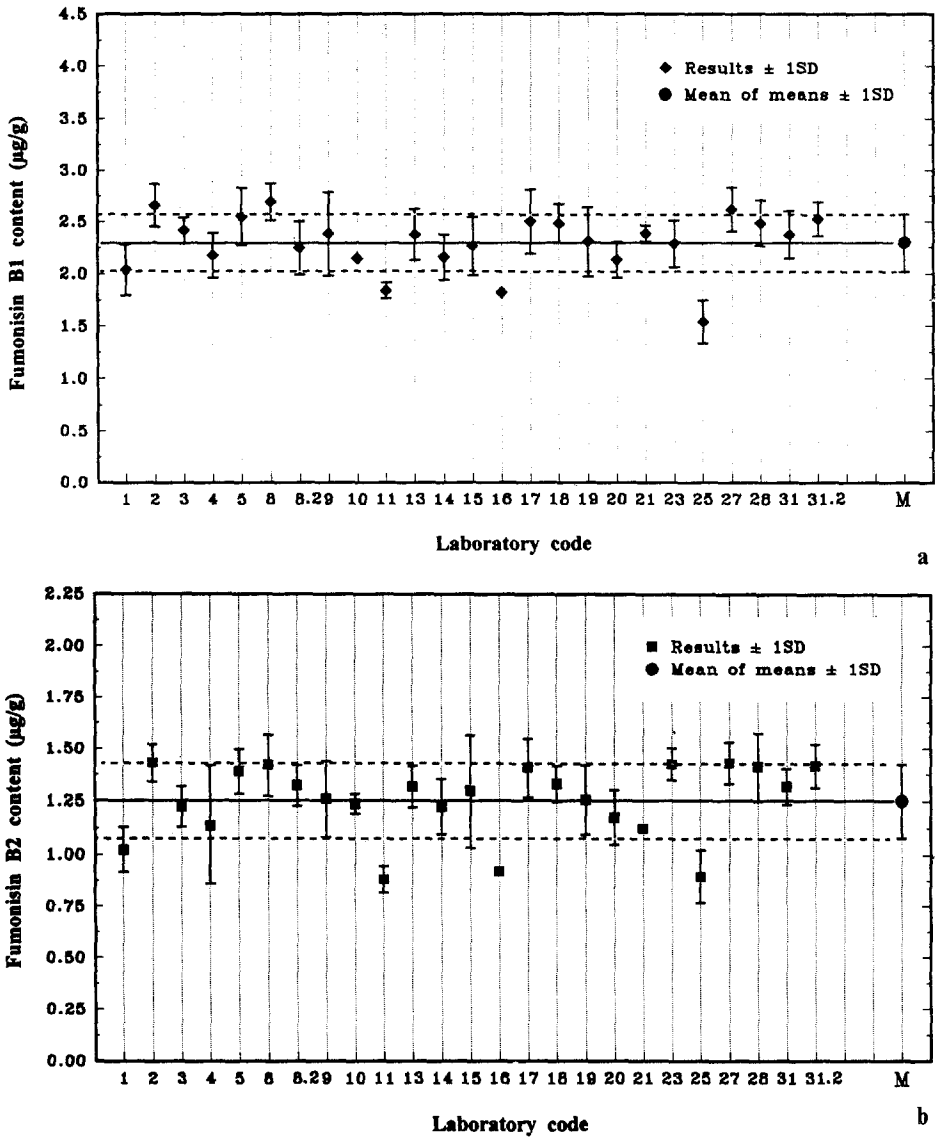

Fig. 1. Bar charts of the results (n = 25, corrected for recoveries) obtained in the intercomparison study for fumonisin B1 (**a**) and fumonisin B2 (**b**) in a maize material

(mean ± 1 SD) were 70 ± 14% and 69 ± 16% for fumonisin B1 and fumonisin B2, respectively (Fig. 2). The examination of data supplied by participants, as well as additional experiments performed in some of the participating laboratories, helped to identify several factors improving recoveries of fumonisins in maize. In particular, when data were separated on the basis of the extraction used by participants, i.e. blending for a few minutes or shaking for 30 min, two major groups with poor and satisfactory recoveries were obtained. Average recoveries for participants using blending were 62 ± 6% and 60 ± 6%, whereas for participants using shaking recoveries these were much better, being i.e. 85 ± 13% and 86 ± 14% for fumonisin B1 and fumonisin B2, respectively. In addition, better recoveries could be obtained

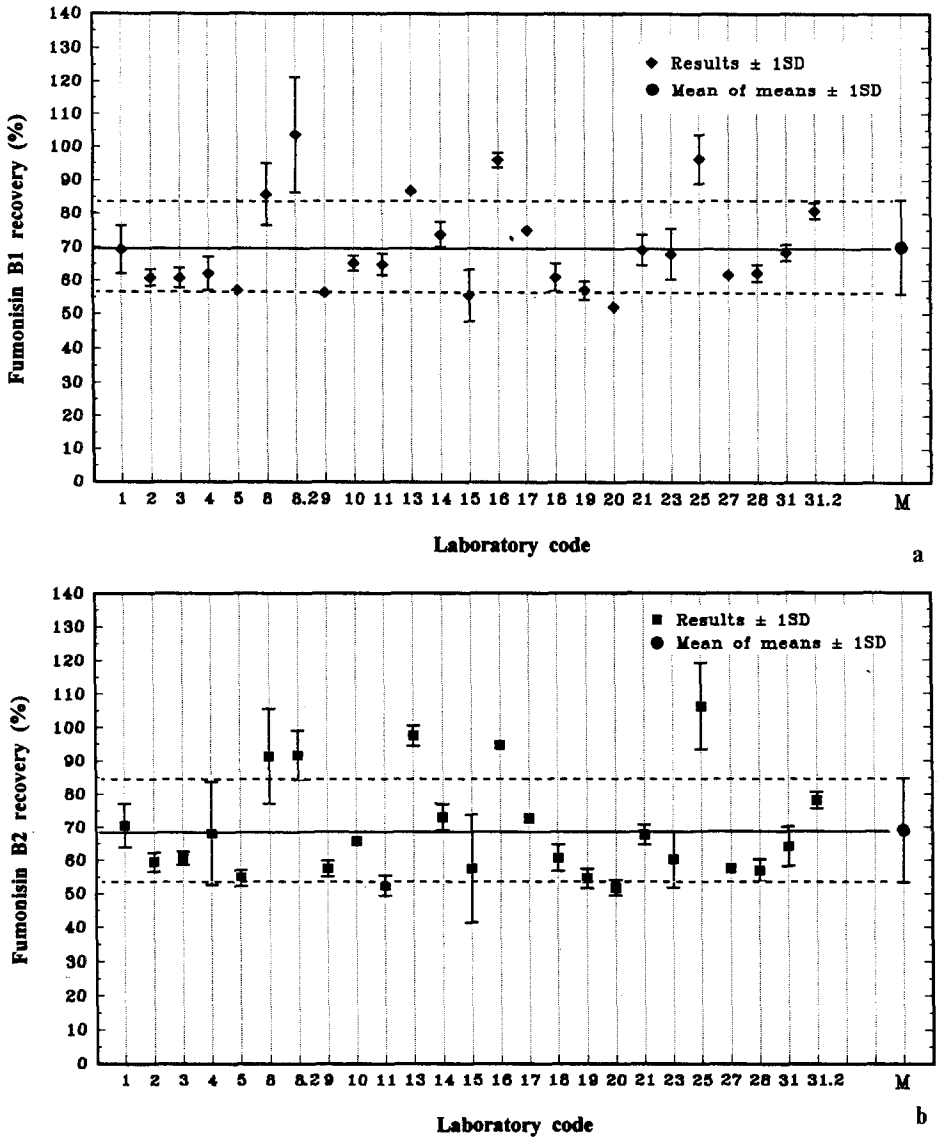

Fig. 2. Bar charts of the recoveries obtained by individual laboratories (n = 25) in the intercomparison study for fumonisin B1 (**a**) and fumonisin B2 (**b**) at spiking levels of 1.50 μg/g and 0.75 μg/g, respectively

by using SAX clean-up instead of C_{18} clean-up, by increasing the solvent-to-maize ratio during the extraction step, or by performing consecutive extractions on the same maize material.

A definitive improvement in the precision of the methods used in this study was observed with respect to previous intercomparison studies. Table 1 shows a comparison of the within-laboratory (RSD_r) and between-laboratory (RSD_R) relative standard deviations obtained in this SMT study, the IUPAC study for fumonisins in maize and the BCR study for fumonisins in acetonitrile-water solution [11, 14]. The improved precision with respect to the IUPAC study is clearly shown by the consistent decrease of both within- and between-laboratory RSD. RSD_R, in particu-

Table 1. Within-laboratory (RSD_R) and between-laboratory (RSD_R) relative standard deviations in international studies for the analysis of fumonisins B1 and B2

Collaborative study	Matrix	N	Mean of means	RSD_r	RSD_R
Fumonisin B1					
SMT[a]	maize	25	2.29 µg/g	10%	11%
IUPAC[b]	maize	10	2.0 µg/g	13%	23%
BCR[c]	solution	24	92 µg/ml	8%	7%
Fumonisin B2					
SMT[a]	maize	25	1.23 µg/g	11%	13%
IUPAC[b]	maize	10	0.7 µg/g	18%	36%
BCR[c]	solution	24	46 µg/ml	9%	7%

[a] This study.
[b] Thiel et al. 1993 [14].
[c] Visconti et al. 1993 [11].

lar, dropped to about 1/2 and 1/3 for fumonisin B1 and fumonisin B2, respectively. Compared to the BCR study, the RSDs of this study were slightly higher. However, considering that the SMT study involved also the extraction and clean-up steps, which are the main cause for the variability of recoveries, these findings clearly indicate a great improvement in both the performance of the laboratories participating in the BCR and SMT studies and the precision of the method used.

Conclusions

The following perspectives should be considered for future investigations of these natural contaminants of foods and feeds: i) there is a need for maize reference materials with certified fumonisin content; ii) more intercomparison studies are necessary before carrying out a certification exercise in order to improve accuracy of measurements; and iii) more methods, based on different procedures of extraction, clean-up and/or determination, should be used in future intercomparison and certification exercises to allow for the elimination of all possible systematic errors.

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References

[1] W. C. A. Gelderblom, K. Jaskiewicz, W. F. O. Marasas, P. G. Thiel, R. M. Horak, R. Vleggar, N. P. J. Kriek, *Appl. Environ. Microbiol.* **1988**, *54*, 1806.

[2] M. E. Cawood, W. C. A. Gelderblom, R. Vleggaar, Y. Behrend, P. G. Thiel, W. F. O. Marasas, *J. Agric. Food Chem.* **1991**, *39*, 1958.
[3] A. Visconti, M. B. Doko, *J. Ass. Off. Anal. Chem. Int.* **1994**, *77*, 546.
[4] M. B. Doko, A. Visconti, *Food Addit. Contam.* **1994**, *11*, 433.
[5] T. S. Kellerman, W. F. O. Marasas, P. G. Thiel, W. C. A. Gelderblom, M. Cawood, J. A. W. Coetzer, *Ondestepoort. J. Vet. Res.* **1990**, *57*, 269.
[6] P. F. Ross, P. E. Nelson, J. L. Richard, G. D. Osweiler, L. G. Rice, R. D. Plattner, T. M. Wilson, *Appl. Environ. Microbiol.* **1990**, *56*, 3225.
[7] W. C. A. Gerderblom, N. P. J. Kriek, W. F. O. Marasas, P. G. Thiel, *Carcinogenesis* **1991**, *12*, 1247.
[8] M. Caramelli, A. Dondo, G. Cantini Cortellazzi, A. Visconti, F. Minervini, M. B. Doko, F. Guarda, *Ippologia* **1993**, *4*, 49.
[9] E. W. Sydenham, P. G. Thiel, W. F. O. Marasas, G. S. Shephard, D. J. Van Scalkwyk, K. R. Kock, *J. Agric. Food Chem.* **1990**, *38*, 1900.
[10] P. G. Thiel, W. F. O. Marasas, E. W. Sydenham, G. S. Shephard, W. C. A. Gelderblom, *Mycopathologia* **1992**, *117*, 3.
[11] A. Visconti, M. B. Doko, B. Schurer, A. Boenke, in: *Proceedings of the UK Workshop on Occurrence and Significance of Mycotoxins* (K. Scudamore, ed.), MAFF, Slough-UK, 1993, pp. 200–202.
[12] A. Visconti, M. B. Doko, C. Bottalico, B. Schurer, A. Boenke, *Food Addit. Contam.* **1994**, *11*, 427.
[13] G. S. Shephard, E. W. Sydenham, P. G. Thiel, W. C. A. Gelderbloom, *J. Liq. Chromatogr.* **1990**, *13*, 2077.
[14] P. G. Thiel, E. C. Sydenham, G. S. Shephard, D. J. Van Schalkwyk, *J. Ass. Off. Anal. Chem. Int.* **1993**, *76*, 361.