Determination of Fumonisins in Corn: Evaluation of Competitive Immunoassay and HPLC Techniques

Eric W. Sydenham,*,† Gordon S. Shephard,† Pieter G. Thiel,† Charles Bird,‡ and Brinton M. Miller‡

Programme on Mycotoxins and Experimental Carcinogenesis, Medical Research Council, P.O. Box 19070, Tygerberg 7505, South Africa, and Neogen Corporation, 620 Lesher Place, Lansing, Michigan 48912

The fumonisins, mycotoxins produced by *Fusarium moniliforme*, are known to occur as natural contaminants of corn worldwide and to be associated with several animal disease syndromes. High-performance liquid chromatography (HPLC) and monoclonal antibody-based (MAb) competitive direct enzyme-linked immunosorbent assay (CD-ELISA) methods, developed for the determination of fumonisins in corn, were compared. CD-ELISA results for naturally contaminated corn were consistently higher than corresponding HPLC results. Quantitative differences were reduced by decreasing the organic phase composition of the extract and by introducing a hexane partitioning step. The results were indicative of a possible lipid-based matrix effect, but when applied to fumonisin-free corn spiked with fumonisin levels ranging from 0.8 to 12.8 μg/g, analyses by both techniques were well correlated ($r = 0.996$). It is concluded that structurally related fumonisin-like compounds, present in naturally contaminated corn, may contribute to the differences recorded between the two methods, although the MAb-based CD-ELISA may still be used as an initial semiquantitative screening technique.

Keywords: *Fumonisins; immunoassay; ELISA; HPLC; corn*

INTRODUCTION

Fumonisins, a group of structurally related mycotoxins, are produced by several fungal species (Chen et al., 1992; Nelson et al., 1992), including *Fusarium moniliforme* Sheldon (Gelderblom et al., 1988), a common contaminant of corn. Of the seven analogues thus far characterized (Bezuidenhout et al., 1988; Branham and Plattner, 1993; Cawood et al., 1991) only three, *viz.* fumonisins B_1 (FB_1), B_2 (FB_2), and B_3 (FB_3), appear to be of major importance, given their worldwide occurrence as natural contaminants of corn-based foods and feeds (Chu and Li, 1994; Murphy et al., 1993; Rheeder et al., 1992; Ross et al., 1992; Thiel et al., 1991). Conversely, production of fumonisins B_4 (FB_4), A_1 (FA_1), A_2 (FA_2), and C_1 (FC_1) appears to occur only under culture conditions (Branham and Plattner, 1993; Cawood et al., 1991). Base hydrolysis of the fumonisins has been shown to yield 1,2,3-propanetricarboxylic acid (tricarballylic acid, TCA) and corresponding aminopolyol moieties (Plattner et al., 1990; Sydenham et al., 1990), while a partially hydrolyzed (PH_1) moiety of FB_1 has also recently been identified (Sydenham et al., 1995).

The most abundant naturally occurring fumonisin, FB_1, has been shown to induce the fatal disease syndromes equine leukoencephalomalacia (Kellerman et al., 1990) and porcine pulmonary edema (Harrison et al., 1990), while in addition it is both hepatotoxic and hepatocarcinogenic to rats (Gelderblom et al., 1991). FB_2 and FB_3 have subsequently also been shown to exhibit cancer-initiating activities similar to those of FB_1 (Gelderblom et al., 1993).

Analytical methods developed for the determination of fumonisins in corn and corn-based foods and feeds include normal phase thin-layer chromatography (TLC) (Gelderblom et al., 1988), reversed-phase TLC (Rottinghaus et al., 1992; Stockenström et al., 1994), capillary gas chromatography (GC) (Plattner et al., 1990, 1992; Sydenham et al., 1990) and various mass spectrometric techniques (Bezuidenhout et al., 1988; Holcomb et al., 1993a; Korfmacher et al., 1991; Plattner et al., 1990). High-performance liquid chromatography (HPLC) has been used extensively for the determination of the fumonisins. To date, most HPLC methods have relied on the purification of crude corn extracts on silica media, chemically modified to incorporate either strong anion exchange (SAX) (Scott and Lawrence, 1992: Shephard et al., 1990) or reversed-phase C_{18} groups (Ross et al., 1991) or a combination of both (Miller et al., 1993). The fumonisins do not exhibit intrinsic ultraviolet (UV), visible, or fluorescence characteristics, necessitating their derivatization for detection by spectrophotometric methods (Sydenham et al., 1990). Gelderblom et al. (1988) described the formation of a maleyl derivative for the UV detection of the fumonsins after separation by reversed-phase HPLC. Improved sensitivity and selectivity have been achieved with the fluorometric detection of several alternative derivatives (Bennett and Richard, 1994; Holcomb et al., 1993b; Scott and Lawrence, 1992; Shephard et al., 1990).

Immunochemical techniques for the determination of mycotoxins are generally rapid, matrix independent, and easy to apply in both the laboratory and field environments, exhibiting comparable sensitivity and higher selectivity characteristics than corresponding chromatographic methods (Chu, 1986; Ward et al., 1993). Immunochemical methods have been developed for the determination of several mycotoxins including the aflatoxins, ochratoxin A, cyclopiazonic acid, rubratoxin, zearalanone, sterigmatocystin, and various trichothecenes (Chu, 1986; Ward et al., 1993). Ascona-Olivera et al. (1992a,b) recently prepared both monoclonal antibodies (MAb) and polyclonal antibodies

* Author to whom correspondence should be addressed [fax (+27)-21-938-0260; e-mail esydenha@eagle.mrc.ac.za].

† Medical Research Council.

‡ Neogen Corp.

0021-8561/96/1444-0159$12.00/0 © 1996 American Chemical Society

(PAb) against the fumonisins from the splenic lyphocytes of mice immunized with a FB_1−cholera toxin conjugate. A MAb-based competitive direct enzyme-linked immunosorbent assay (CD-ELISA) was developed, whereby FB_1−horseradish peroxidase conjugate and free FB_1 competed for antibody binding sites immobilized on microtiter plates (Ascona-Olivera et al., 1992b). Usleber et al. (1994) prepared PAb against FB_1 which were also incorporated into a CD-ELISA system, while Shelby et al. (1994) have described the development of a competitive indirect (CI-) ELISA for the analysis of fumonisins in corn. Antibody technology has also been used for the production of immunoaffinity columns, which have been applied to the analyses of fumonisins in corn (Ware et al., 1994) and milk (Scott et al., 1994). Maragos and Richard (1994) also evaluated the use of a CD-ELISA method for the analysis of fumonisin residues in milk.

Given their potential application as screening techniques, it is important that the performance of these fumonisin immunochemical techniques should, when possible, be evaluated against established methods to assess their reliability. This paper reports a comparison of the performance of the Neogen Fumonisin Agri-Screen CD-ELISA, developed using the MAb prepared by Prof. Pestka and co-workers (Ascona-Olivera et al., 1992b), and an established HPLC procedure (Sydenham et al., 1992). Both techniques were applied to the analyses of corn contaminated (both naturally and artificially) with fumonisins.

EXPERIMENTAL PROCEDURES

Caution: The fumonisins are known carcinogens. Consequently, fungal cultures and solvent extracts should be handled with extreme care.

Corn and Fungal Culture Samples. Corn meals were prepared by grinding naturally contaminated corn kernels in a laboratory mill to pass an 850 μm sieve. Fumonisin-free corn (i.e. <20 ng/g total fumonisins) was similarly treated and spiked with FB_1, FB_2, and FB_3 in a ratio of 5:2:1, at levels of total fumonisins ranging from 0.8 to 12.8 μg/g. Corn-based culture material of *F. moniliforme* strain MRC 826 was prepared as previously described (Cawood et al., 1991).

Analytical Standards. Analytical standards of FB_1, FB_2, FB_3, FB_4, FA_1, and FA_2 were isolated and purified from culture material of *F. moniliforme* strain MRC 826, in accordance with the method of Cawood et al. (1991). The aminopentol (AP_1) and PH_1 moieties of FB_1 were prepared by alkaline hydrolysis, as previously described (Sydenham et al., 1995). TCA was purchased from Fluka AG (Buchs, Switzerland).

Preparation and Purification of Corn Extracts. Subsamples of ground corn (20 g) or culture material (5 g) were extracted with methanol/water (CH_3OH/H_2O 70:30, 100 mL) by blending for 3 min at high speed in a Polytron homogenizer. The crude extracts were centrifuged and filtered, and aliquots were retained for CD-ELISA. The residual filtrates were purified on SAX media, in accordance with the method of Sydenham et al. (1992). The fumonisins were eluted with a CH_3OH/acetic acid (CH_3COOH) mixture (99:1, 10 mL). In selected cases, the SAX media were, in addition, also sequentially washed with 10 mL volumes of CH_3OH/CH_3COOH at ratios of 95:5, 75:25, and 50:50, as well as with CH_3OH/trifluoroacetic acid (CF_3COOH) at ratios of 99.5:0.5 or 90:10, respectively. All eluates were collected separately, evaporated to dryness at 60 °C under a stream of nitrogen, and redissolved in CH_3OH/H_2O (70:30), prior to analyses.

In an additional study, the purified extract of culture material of *F. moniliforme* strain MRC 826 was prepared and the CH_3OH/CH_3COOH (99:1) fraction eluted from the SAX media. The solvent (including the CH_3COOH) was evaporated to dryness under nitrogen gas at 60 °C, and the residue was redissolved in CH_3OH/H_2O (30:70, 10 mL, pH 2.5). This solution was applied to a cartridge packed with reversed-phase Chromabond C_{18} end-capped material (1 g; Macherey-Nagel, Düren, Germany), which was sequentially eluted with 10 mL aliquots of CH_3OH/H_2O (collected as five individual 2 mL fractions), in which the CH_3OH content was increased in 10% increments, from 30% to 100%. All fractions (n = 40) were suitably diluted to yield solutions of CH_3OH/H_2O having a ratio of 70:30, and each was subsequently analyzed by CD-ELISA and HPLC.

Determination of Fumonisins by CD-ELISA. Fumonisin levels were determined by CD-ELISA using Fumonisin Agri-Screen kits (catalog no. 70/8810, Neogen Corp., Lansing, MI), in accordance with the method cited by the manufacturer. Briefly, aliquots of corn extracts initially extracted with CH_3OH/H_2O (70:30) (crude or purified) or fumonisin standard solutions were mixed with equal volumes of a FB_1−horseradish peroxidase conjugate solution. Aliquots of each mixture were then applied to individual MAb-coated microtiter wells which were then incubated for 10 min to allow any free toxin and the toxin−peroxidase conjugate to compete for binding to the available antibody sites. Reagents were washed from the microtiter wells with an excess of distilled water. Bound toxin−conjugate levels were then measured colorimetrically, following the addition of an enzyme substrate, incubation (for 10 min), and addition of a stopping reagent. Fumonisin concentrations were assessed either by visual assessment (against a 5 ppm FB_1 standard) or by recording optical density (OD) readings at 650 nm, using a Bio-Tek EL301 microwell strip reader, against a FB_1 standard curve covering the concentration range (0, 2, 5, and 10 ppm). The fumonisin standards were prepared by spiking filtered CH_3OH/H_2O (70:30) extracts prepared from fumonisin-free corn.

For some analyses, corn extracts were diluted with H_2O to reduce the CH_3OH concentrations. Subsequent CD-ELISA analyses were conducted using fumonisin standards prepared in corresponding CH_3OH/H_2O solutions.

Determination of FB_1, FB_2, and FB_3 by HPLC. Levels of FB_1, FB_2, and FB_3 were determined by reversed-phase HPLC analyses of preformed *o*-phthaldialdehyde (OPA) derivatives as previously described (Sydenham et al., 1992).

Determination of FA_1 and FA_2 by TLC and Capillary GC. Aliquots of selected fractions from SAX media (together with FA_1 and FA_2 standards) were applied to silica gel 60 TLC plates (Merck, Darmstadt, Germany). The plates were developed in chloroform/$CH_3OH/H_2O/CH_3COOH$ (55:36:8:1), dried, and sprayed with a 0.5% *p*-anisaldehyde solution as previously described (Sydenham et al., 1995).

The 5% CH_3COOH eluates from SAX media were taken to dryness, redissolved in 2 N sodium hydroxide (2 mL), and heated at 70 °C for 2 h. Thereafter, 1 N hydrochloric acid (*ca.* 3 mL) was added to yield solutions of *ca.* pH 4. These were applied to short chromatographic columns containing Amberlite XAD-4 resin (2 g prepared in H_2O), which were subsequently washed with H_2O (10 mL). The hydrolysates were eluted with CH_3OH (8 mL), the eluates being collected and the solvent removed at 60 °C under a stream of nitrogen. Trifluoroacetyl (TFA) derivatives of the aminopolyol moieties were prepared according to a procedure similar to that described by Sydenham and Thiel (1987). The resultant derivatives were separated on a fused silica capillary column (12 m × 0.32 mm i.d.) coated with a 0.25 μm film of SE-30. Helium was used as carrier gas at a linear flow rate of 35 cm/s, and chromatographic peaks were detected by flame ionization detection. The column temperature was maintained at 70 °C for 1 min and then increased to 280 °C at a rate of 10 °C/min. Chromatographic peaks were compared with those obtained for authentic FA_1, FA_2, FB_1, and FB_2 standards that had been similarly treated.

RESULTS AND DISCUSSION

Cross-Reactivity of the MAb. Prior to its use in these studies, the cross-reactivity of the MAb used in the Neogen test kits was assessed against various fumonisin analogues (Ascona-Olivera et al., 1992b).

Table 1. Comparison of HPLC and CD-ELISA of a Naturally Contaminated Corn Sample[a]

	fumonisin concn (μg/g)	
fraction in methanol	HPLC[b]	CD-ELISA
crude extract		41.0
1% CH_3COOH[c]	20.0	31.0
5% CH_3COOH[c]	0.7	7.7
0.5% CF_3COOH[c]	0.0	1.5
total (from eluates)	20.7	40.2

[a] Levels are expressed as those calculated to be present in the corn sample. [b] Total fumonisins ($FB_1 + FB_2 + FB_3$). [c] Eluates (10 mL) from SAX media.

Initial studies confirmed that this MAb cross-reacted with FB_2 and FB_3 but showed no cross-reactivity with either the AP_1 or TCA moieties formed by hydrolysis of FB_1. Subsequent studies indicated that the MAb also cross-reacted with FB_4, FA_1, and FA_2 but did not cross-react with the recently described PH_1 moiety (Sydenham et al., 1995).

Comparison of HPLC and CD-ELISA. Comparisons were made between HPLC and CD-ELISA analyses of a series of 26 naturally contaminated corn samples, with the latter results determined by visual assessment. On the basis of the HPLC analyses, 21 of 26 were found to contain combined FB_1, FB_2, and FB_3 levels below 5 μg/g, while CD-ELISA analyses of the same extracts indicated that 21 of 26 samples contained fumonisin levels greater than 5 μg/g. Similar higher values by CD-ELISA than by HPLC were also recently reported by Pestka et al. (1994). In addition, Shelby et al. (1994) also reported that a significant proportion of corn samples were found to contain higher fumonisin levels when analyzed by CI-ELISA than by reversed-phase TLC; however, they considered that the presence of FB_2 and FB_3 (not determined by TLC) may have accounted for the discrepancies.

These observations prompted further evaluation of individual corn samples according to both procedures. Typical results obtained for both crude and purified extracts prepared from one corn sample are presented in Table 1. CD-ELISA of the crude extract indicated that the corn sample contained 41 μg/g fumonisins. However, the contamination level observed in the 1% CH_3COOH fraction, eluted from the SAX media, was only 31 μg/g (Table 1). This latter value was more than 50% higher than the 20 μg/g recorded in the same fraction by HPLC (Table 1). The SAX media were subsequently eluted with stronger solvent blends, with each eliciting a response from the CD-ELISA. Combining the CD-ELISA responses for all eluates gave a result similar to that previously observed for the crude extract (40.2 μg/g; Table 1). However, HPLC analyses of the latter fractions from the SAX media did not indicate the presence of significant levels of detectable fumonisins (Table 1). The CD-ELISA response was therefore approximately twice that determined by HPLC (Table 1), and similar results were observed for other corn samples.

The above study was repeated using an extract prepared from culture material of *F. moniliforme*, and the results are presented in Table 2. As previously observed for naturally contaminated corn (Table 1), the CD-ELISA responses were (for each fraction) higher than the corresponding HPLC analyses, with the overall CD-ELISA result being approximately 3 times that recorded by HPLC (Table 2). Major differences between

Table 2. Comparison of HPLC and CD-ELISA of Corn Culture Material of *F. moniliforme*[a]

		fumonisin concn (μg/g)	
fraction in methanol	vol[b] (mL)	HPLC[c]	CD-ELISA
crude extract			35800
1% CH_3COOH	5	9600	19000
1% CH_3COOH	5	442	1750
1% CH_3COOH	5	52	930
5% CH_3COOH	5	51	4320
5% CH_3COOH	5	34	1960
25% CH_3COOH	10	57	3850
50% CH_3COOH	10	2	350
10% CF_3COOH	10	0	60
total (from eluates)		10298	31850

[a] Levels are expressed as those calculated to be present in the culture sample. [b] Volume of eluate from SAX media. [c] Total fumonisins ($FB_1 + FB_2 + FB_3$).

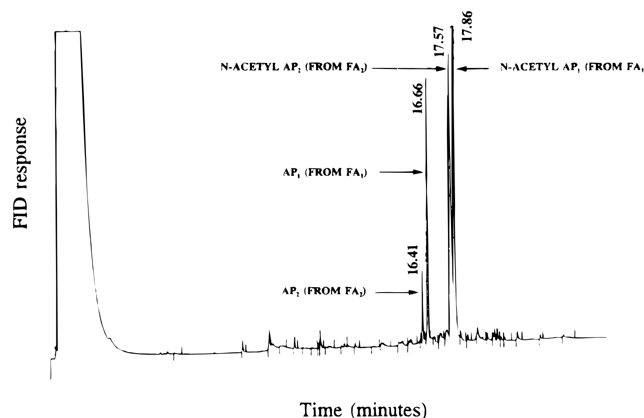

Figure 1. Capillary gas chromatogram of the trifluoroacetate derivative of the hydrolysis product derived from the 5% CH_3COOH fraction (culture material of *F. moniliforme*). The secondary AP_1 and AP_2 chromatographic peaks may also be derived, in part, from the residual FB_1 and FB_2 determined in the purified extract (Table 2, HPLC results).

the two techniques were observed in all fractions collected from the SAX media. The 5% CH_3COOH fractions were combined and analyzed by TLC, by which chromatographic bands corresponding to FA_1 and FA_2 were observed. As the FA analogues do not contain free amino groups (required for derivatization with OPA), they cannot be monitored by HPLC. However, it is possible to hydrolyze the FA analogues and determine their aminopolyol moieties by capillary GC. This technique was applied to the combined 5% CH_3COOH fractions collected from the SAX media (culture material of *F. moniliforme*), and the resultant chromatogram is illustrated in Figure 1.

Four chromatographic peaks were observed, two from each FA analogue (Figure 1), with similar peaks being recorded for authentic FA_1 and FA_2 standards. The major peaks have provisionally been ascribed to the presence of the *N*-acetyl aminopolyol moieties of FA_1 and FA_2, while the secondary peaks are thought to be derived by the hydrolytic conversion of a portion of each FA analogue to its corresponding FB counterpart (Figure 1). The retention times for these secondary peaks were identical to those observed for similarly treated authentic FB_1 and FB_2 standards. The presence of relatively small amounts of FB_1 and FB_2 in the 5% CH_3COOH fraction (as determined by HPLC; Table 2) may also have contributed to these secondary peaks (Figure 1). Given that the MAb cross-reacts with the FA toxins, a significant proportion of the CD-ELISA response

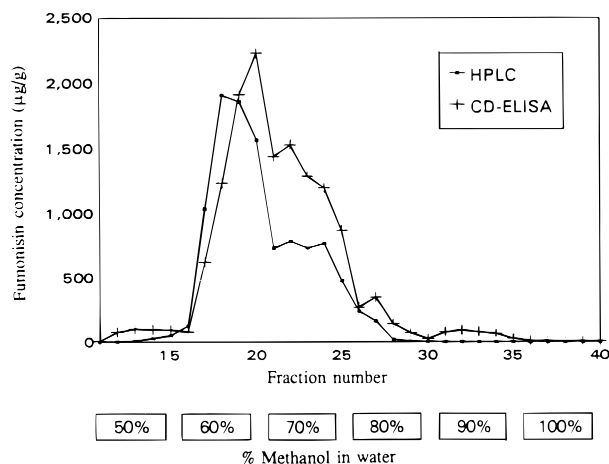

Figure 2. HPLC and CD-ELISA analyses of the 1% CH_3COOH fraction (culture material of *F. moniliforme*) separated on C_{18} media.

Table 3. Comparison of Initial Solvent Extraction Method[a]

	fumonisin concn (µg/g)					
	shaking			blending		
sample	HPLC[b]	ELISA	ratio	HPLC[b]	ELISA	ratio
1	4.0	10.3	1:2.6	3.8	10.9	1:2.9
2	7.8	17.4	1:2.2	8.7	17.9	1:2.1
3	8.5	18.4	1:2.2	8.5	19.2	1:2.3
4	10.9	18.3	1:1.7	10.8	20.6	1:1.9
5	136.1	444.0	1:3.3	142.6	468.0	1:3.3

[a] Levels are expressed as those calculated to be present in the corn samples. [b] Combined fumonisin levels ($FB_1 + FB_2 + FB_3$).

observed in the 5% CH_3COOH fractions (derived from the culture sample; Table 2) may be accounted for by the presence of the FA analogues. Similar GC analysis of the corresponding fraction obtained from the naturally contaminated corn sample (Table 1) failed to result in the observation of either FA_1 or FA_2.

Major differences between the two analytical techniques were observed also in the 1% CH_3COOH fraction eluted from SAX media. Accordingly, these fractions (Table 2) were combined, separated on C_{18} media, and analyzed by HPLC and CD-ELISA, and the results are compared in Figure 2. In fractions 15–17 (60% CH_3OH), the CD-ELISA response was lower than that recorded by HPLC, while in all other cases the CD-ELISA technique recorded higher values than HPLC (Figure 2). The fact that these responses were observed throughout the elution profile indicated that the MAb was probably responding to a series of compounds rather than to a single entity. Dilution of the fractions reduced the overall difference between the results obtained by the two techniques, which was possibly indicative of a matrix effect.

Given that the corn-derived matrix compounds present in crude extracts may be influenced by the initial extraction method, comparisons were made between HPLC and CD-ELISA results derived from extracts prepared by different processes. Five corn samples were extracted by blending (Sydenham et al., 1992) and rapid shaking on a wrist action shaker for 60 min. As previously observed, CD-ELISA assays were between 1.7 and 3.3 times higher than the corresponding results obtained by HPLC (Table 3), but no differences in the results were observed between the different extraction methods (Table 3).

Ascona-Olivera et al. (1992) suggested that the MAb

Table 4. HPLC and CD-ELISA Analyses of Corn Samples Prior to and following Dilution and Hexane Wash[a]

	fumonisin concn (µg/g)			
sample	HPLC[b]	CD-ELISA (initial)	CD-ELISA (25% CH_3OH)	CD-ELISA (hexane wash)
1	3.8	11.6	10.1	5.9
2	8.5	21.2	8.9	7.7
3	8.7	18.0	19.3	7.3
4	10.8	18.0	11.8	11.9

[a] Levels are expressed as those calculated to be present in the corn samples. [b] Total fumonisins ($FB_1 + FB_2 + FB_3$).

exhibited limited tolerance to high organic solvent concentrations. A series of corn extracts, initially used to determine fumonisin levels by HPLC and CD-ELISA (Table 4), were subsequently diluted with water to reduce their CH_3OH content from 70% to 25%, prior to analyses by CD-ELISA (using a range of FB_1 standards prepared in 25% CH_3OH; Table 4). Decreased CD-ELISA responses were observed for three of four samples, with the results of samples 2 and 4 being approximately the same as observed by HPLC (Table 4). The addition of water resulted in the formation of precipitates, which were reduced following liquid partitioning of the diluted samples with hexane (followed by centrifugation). CD-ELISA of the resultant aqueous fractions also resulted in decreases in responses (Table 4), providing additional evidence of a possible lipid-based matrix effect.

Comparisons between HPLC and CD-ELISA were also conducted using fumonisin-free corn spiked with combined fumonisin levels ranging from 0.8 to 12.8 µg/g. An excellent correlation between the results obtained according to both techniques was observed ($r = 0.996$). These results differed considerably from those obtained for the analyses of naturally contaminated corn (Tables 1 and 3), indicating that differences observed between the techniques might not be simple matrix effects but are more likely to be related to the presence of additional compounds (such as fumonisin precursors or unknown structurally related metabolites) produced by fungal species present in food and feed matrices that cross-react with the MAb. Similar conclusions have also been expressed by Pestka et al. (1994) and Shelby et al. (1994) to explain the quantitative differences in fumonsin levels determined between CD-ELISA and HPLC/GC–MS results and between CI-ELISA and TLC results, respectively.

In conclusion, the present data indicate that the MAb incorporated into the commercially available CD-ELISA (marketed by the Neogen Corp.) may be used as a basic screening technique for fumonisin levels in corn, although the method in its present form may be expected to give a high percentage of false positive results. While a decrease in the percentage of the organic phase increased the intensity of the OD readings, dilution of the crude extracts with water prior to analysis was probably responsible for the corresponding decrease in the concentrations of the unknown MAb cross-reactive species. It will, however, be important to isolate and identify these additional compounds, since any structural similarity between these species and the fumonisins will necessitate studies aimed at determining their relative toxicity. The use of the MAb prepared by Ascona-Olivera et al. (1992b) should therefore assist with their isolation. It will also be necessary to more fully evaluate the toxicity status of other known fumonisin analogues.

ACKNOWLEDGMENT

We thank Dr. Denis Lauren (Hort Research, New Zealand) for the provision of fumonisin-free corn.

LITERATURE CITED

Ascona-Olivera, J. I.; Abouzied, M. M.; Plattner, R. D.; Norred, W. P.; Pestka, J. I. Generation of antibodies reactive to fumonisins B_1, B_2, and B_3 by using cholera toxin as the carrier-adjuvant. *Appl. Environ. Microbiol.* **1992a**, *58*, 169–173.

Ascona-Olivera, J. I.; Abouzied, M. M.; Plattner, R. D.; Pestka, J. J. Production of monoclonal antibodies to the mycotoxins fumonisins B_1, B_2, and B_3. *J. Agric. Food Chem.* **1992b**, *40*, 531–534.

Bennett, G. A.; Richard, J. L. Liquid chromatographic method for analyses of the naphthalene dicarboxaldehyde derivative of fumonisins. *J. AOAC Int.* **1994**, *77*, 501–505.

Bezuidenhout, G. C.; Gelderblom, W. C. A.; Gorst-Allman, C. P.; Horak, R. M.; Marasas, W. F. O.; Spiteller, G.; Vleggaar, R. Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*. *J. Chem. Soc., Chem. Commun.* **1988**, 743–745.

Branham, B. E.; Plattner, R. D. Isolation and characterization of a new fumonisin from liquid cultures of *Fusarium moniliforme*. *J. Nat. Prod.* **1993**, *56*, 1630–1633.

Cawood, M. E.; Gelderblom, W. C. A.; Vleggaar, R.; Behrend, Y.; Thiel, P. G.; Marasas, W. F. O. Isolation of the fumonisin mycotoxins: a quantitative approach. *J. Agric. Food Chem.* **1991**, *39*, 1958–1962.

Chen, J.; Mirocha, C. J.; Xie, W.; Hogge, L.; Olson, D. Production of the mycotoxin fumonisin B_1 by *Alternaria alternata* f. sp. *lycopersici*. *Appl. Environ. Microbiol.* **1992**, *58*, 3928–3931.

Chu, F. S. Immunoassays for mycotoxins. In *Modern Methods in the Analysis and Structural Elucidation of Mycotoxins*; Cole, R. J., Ed.; Academic Press: New York, 1986; pp 207–237.

Chu, F. S.; Li, G. Y. Simultaneous occurrence of fumonisin B_1, and other mycotoxins in moldy corn collected from the People's Republic of China in regions with high incidence of esophageal cancer. *Appl. Environ. Microbiol.* **1994**, *60*, 847–852.

Gelderblom, W. C. A.; Jaskiewicz, K.; Marasas, W. F. O.; Thiel, P. G.; Horak, R. M.; Vleggaar, R.; Kriek, N. P. J. Fumonisins—novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Appl. Environ. Microbiol.* **1988**, *54*, 1806–1811.

Gelderblom, W. C. A.; Kriek, N. P. J.; Marasas, W. F. O.; Thiel, P. G. Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisin B_1, in rats. *Carcinogenesis* **1991**, *12*, 1247–1251.

Gelderblom, W. C. A.; Cawood, M. E.; Snyman, S. D.; Vleggaar, R.; Marasas, W. F. O. Structure-activity relationships of fumonisins in short-term carcinogenesis and cytotoxicity assays. *Food Chem. Toxicol.* **1993**, *31*, 407–414.

Harrison, L. R.; Colvin, B. M.; Greene, J. T.; Newman, L. E.; Cole, J. R., Jr. Pulmonary edema and hydrothorax in swine produced by fumonisin B_1, a toxic metabolite of *Fusarium moniliforme*. *J. Vet. Diagn. Invest.* **1990**, *2*, 217–221.

Holcomb, M.; Sutherland, J. B.; Chiarelli, M. P.; Korfmacher, W. A.; Thompson, H. C., Jr.; Lay, J. O., Jr.; Hankins, L. J.; Cerniglia, C. E. HPLC and FAB mass spectrometry analysis of fumonisins B_1 and B_2 produced by *Fusarium moniliforme* on food substrates. *J. Agric. Food Chem.* **1993a**, *41*, 357–360.

Holcomb, M.; Thompson, H. C., Jr.; Hankins, L. J. Analysis of fumonsin B_1 in rodent feed by gradient elution HPLC using precolumn derivatization with FMOC and fluorescence detection. *J. Agric. Food Chem.* **1993b**, *41*, 764–767.

Kellerman, T. S.; Marasas, W. F. O.; Thiel, P. G.; Gelderblom, W. C. A.; Cawood, M. E.; Coetzer, J. A. W. Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B_1. *Onderstepoort J. Vet. Res.* **1990**, *57*, 269–275.

Korfmacher, W. A.; Chiarelli, M. P.; Lay, J. O., Jr.; Bloom, J.; Holcomb, M.; McManus, K. T. Characterization of the mycotoxin fumonisin B_1: comparison of thermospray, fast-atom bombardment and electrospray mass spectrometry. *Rapid Commun. Mass Spectrom.* **1991**, *5*, 463–468.

Maragos, C. M.; Richard, J. L. Quantification and stability of fumonisins B_1 and B_2 in milk. *J. AOAC Int.* **1994**, *77*, 1162–1167.

Miller, J. D.; Savard, M. E.; Sibilia, A.; Rapior, S.; Hocking, A. D.; Pitt, J. I. Production of fumonisins and fusarins by *Fusarium moniliforme* from Southeast Asia. *Mycologia* **1993**, *85*, 385–391.

Murphy, P. A.; Rice, L. G.; Ross, P. F. Fumonisins B_1, B_2, and B_3 content of Iowa, Wisconsin, and Illinois corn and corn screenings. *J. Agric. Food Chem.* **1993**, *41*, 263–266.

Nelson, P. E.; Plattner, R. D.; Shackelford, D. D.; Desjardins, A. E. Fumonisin B_1 production by *Fusarium* species other than *F. moniliforme* in section Liseola and by some related species. *Appl. Environ. Microbiol.* **1992**, *58*, 984–989.

Pestka, J. J.; Azcona-Olivera, J. I.; Plattner, R. D.; Minervini, F.; Doko, M. B.; Visconti, A. Comparative assessment of fumonisin in grain-based foods by ELISA, GC-MS, and HPLC. *J. Food Prot.* **1994**, *57*, 169–172.

Plattner, R. D.; Norred, W. P.; Bacon, C. W.; Voss, K. A.; Peterson, R.; Shackelford, D. D.; Weisleder, D. A method of detection of fumonisins in corn samples associated with field cases of equine leukoencephalomalacia. *Mycologia* **1990**, *82*, 698–702.

Plattner, R. D.; Weisleder, D.; Shackelford, D. D.; Peterson, R.; Powell, R. G. A new fumonisin from solid cultures of *Fusarium moniliforme*. *Mycopathologia* **1992**, *117*, 23–28.

Rheeder, J. P.; Marasas, W. F. O.; Thiel, P. G.; Sydenham, E. W.; Shephard, G. S.; Van Schalkwyk, D. J. *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. *Phytopathology* **1992**, *82*, 353–357.

Ross, P. F.; Rice, L. G.; Plattner, R. D.; Osweiler, G. D.; Wilson, T. M.; Owens, D. L.; Nelson, H. A.; Richard, J. L. Concentrations of fumonisin B_1 in feeds associated with animal health problems. *Mycopathologia* **1991**, *114*, 129–135.

Ross, P. F.; Rice, L. G.; Osweiler, G. D.; Nelson, P. E.; Richard, J. L.; Wilson, T. M. A review and update of animal toxicoses associated with fumonisin-contaminated feeds and production of fumonisins by *Fusarium* isolates. *Mycopathologia* **1992**, *117*, 109–114.

Rottinghaus, G. E.; Coatney, C. E.; Minor, H. C. A rapid, sensitive thin layer chromatography procedure for the detection of fumonisin B_1 and B_2. *J. Vet. Diagn. Invest.* **1992**, *4*, 326–329.

Scott, P. M.; Lawrence, G. A. Liquid chromatographic determination of fumonisins with 4-fluoro-7-nitrobenzofurazan. *J. AOAC Int.* **1992**, *75*, 829–834.

Scott, P. M.; Delgardo, T.; Prelusky, D. B.; Trenholm, H. L.; Miller, J. D. Determination of fumonisins in milk. *J. Environ. Sci. Health* **1994**, *29*, 989–998.

Shelby, R. A.; Rottinghaus, G. E.; Minor, H. C. Comparison of thin-layer chromatography and competitive immunoassay methods for detecting fumonisin on maize. *J. Agric. Food Chem.* **1994**, *42*, 2064–2067.

Shephard, G. S.; Sydenham, E. W.; Thiel, P. G.; Gelderblom, W. C. A. Quantitative determination of fumonisins B_1 and B_2 by high-performance liquid chromatography with fluorescence detection. *J. Liq. Chromatogr.* **1990**, *13*, 2077–2087.

Stockenström, S.; Sydenham, E. W.; Thiel, P. G. Determination of fumonisins in corn: evaluation of two purification procedures. *Mycotoxin Res.* **1994**, *10*, 9–14.

Sydenham, E. W.; Thiel, P. G. The simultaneous determination of diacetoxyscirpenol and T-2 toxin in fungal cultures and grain samples by capillary gas chromatography. *Food Addit. Contam.* **1987**, *4*, 277–284.

Sydenham, E. W.; Gelderblom, W. C. A.; Thiel, P. G.; Marasas, W. F. O. Evidence for the natural occurrence of fumonisin B_1, a mycotoxin produced by *Fusarium moniliforme*, in corn. *J. Agric. Food Chem.* **1990**, *38*, 285–290.

Sydenham, E. W.; Shephard, G. S.; Thiel, P. G. Liquid chromatographic determination of fumonisins B_1, B_2, and B_3 in foods and feeds. *J. AOAC Int.* **1992**, *75*, 313−318.

Sydenham, E. W.; Thiel, P. G.; Shephard, G. S.; Koch, K. R.; Hutton, T. Preparation and isolation of the partially hydrolyzed moiety of fumonisin B_1. *J. Agric. Food Chem.* **1995**, *43*, 2400−2405.

Thiel, P. G.; Shephard, G. S.; Sydenham, E. W.; Marasas, W. F. O.; Nelson, P. E.; Wilson, T. M. Levels of fumonisins B_1 and B_2 in feeds associated with confirmed cases of equine leukoencephalomalacia. *J. Agric. Food Chem.* **1991**, *39*, 109−111.

Usleber, E.; Straka, M.; Terplan, G. Enzyme immunoassay for fumonisin B_1 applied to corn-based food. *J. Agric. Food Chem.* **1994**, *42*, 1392−1396.

Ward, C. M.; Wilkinson, A. P.; Morgan, M. R. A. Emerging techniques: enzyme-linked immunosorbent assay (ELISA) as alternatives to chromatographic methods. In *Chromatography of Mycotoxins*; Betina, V., Ed.; Elsevier: New York, 1993; pp 124−138.

Ware, G. M.; Umrigar, P. P.; Carman, A. S., Jr.; Kuan, S. S. Evaluation of fumonitest immunoaffinity columns. *Anal. Lett.* **1994**, *27*, 693−715.

Received for review April 5, 1995. Revised manuscript received August 17, 1995. Accepted September 15, 1995.[⊗]

JF950203K

[⊗] Abstract published in *Advance ACS Abstracts,* October 15, 1995.