MYCOTOXINS

Thin Layer Chromatographic Determination of Aflatoxin in Corn Dust

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Methods adopted by the AOAC and the American Association of Cereal Chemists for determining aflatoxin in corn were modified, and techniques were developed for application to samples of <1 to 10 g instead of the specified 50 g samples. Analysis included chloroform extraction of dust samples or dust collected from glass fiber filters, purification of extracts on a silica gel column of appropriate size, and measurement of aflatoxin by either 1- or 2-dimensional thin layer chromatography (TLC). The solvent for 1-dimensional TLC was chloroform-acetone-water (91 + 9 + 1). Solvents for 2-dimensional TLC were, first direction, ether-methanol-water (95 + 4 + 1, lined tank) and second direction, chloroform-acetone-water (91 + 9 + 1, unlined tank), or first direction, chloroform-acetone-water (91 + 9 + 1, unlined tank) and second direction, toluene-ethyl acetate-formic acid (60 + 30 + 10, unlined tank). When samples weighed ≤0.1 g, the entire concentrated extract was applied to the TLC plate. About 0.5-1.0 ng aflatoxin B_1 could be detected on the plate, making the limit of detection about 9 ng/g for 0.1 g samples.

Two reports have discussed possible effects on humans from inhaling dusts that contained aflatoxin, but the degree of exposure was not determined nor was a cause-effect relationship established. One report was a study of workers' health in a peanut processing plant in the Netherlands. Exposures to aflatoxin from contaminated dust over a period of 13 years in different parts of the plant were estimated. The exposed group had a rate of multiple cancers and liver cancer more than 3 times that in the matched control group. The authors concluded that there was a strong indication of carcinogenic factors in the dust, but the number of workers exposed was too small to provide proof (1). The other report speculated that possible exposure of 2 scientists to aflatoxin-containing dust during preparatory thin layer chromatography (TLC) in an unventilated room could have been responsible for the carcinomas of the colon that they developed (2).

There is currently no basis for determining whether agricultural workers exposed to airborne dust during the handling of aflatoxin-containing corn are at risk. Aflatoxin contamination of corn is a recurring problem in certain regions of the United States; the severity varies from year to year (3). In the summer of 1977, conditions in the Southeast were favorable for aflatoxin formation in corn, and in one study, 16% of the samples collected had levels above 1000 ng/g (4). The incidence and levels of aflatoxin were not as high in 1978 corn as in 1977 corn (5). Because aflatoxin forms in the field as well as in storage (6), workers could be exposed to dust-borne toxin at the point of harvest and through any other operation up to the time the corn is destroyed or detoxified by ammoniation (7). There was, therefore, a need to develop analytical methods to determine aflatoxin levels in dust samples.

This paper reports on modifications of methods adopted by the AOAC and the American Association of Cereal Chemists (AACC) for aflatoxin in corn (8, 9) and the development of procedures to determine toxin in airborne corn dust samples.

Experimental

Preparation of Ground Corn Samples to Test Analytical Procedures

Three samples of ground corn containing different levels of aflatoxin were prepared by combining available naturally contaminated samples to obtain approximately the levels desired. Each of the 3 samples was partitioned on a U.S. standard No. 20 sieve. The material that did not pass through the sieve (11%) was re-

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ground in a 6 in. Raymond hammer mill equipped with a screen with 1/8 in. perforations, and resieved. Reground corn that did pass through the No. 20 sieve was combined with original sample material that passed through the sieve, and blended in a Hobart planetary mixer. Material that did not pass through the No. 20 sieve was discarded.

Collection of Dust Samples

Samples of airborne dust were collected with a high volume, total air sampler fitted with 8 × 10 in. Type A glass fiber filters, and a high volume, 4-stage Anderson sampler (Model 65-000) with perforated glass fiber type "AE" filters to sequentially retain particles. The effective cutoff diameters for the stages are 7, 3.3, 2.0, and 1.1 μm; a final backup filter collects particles in the submicron range. Samplers were positioned to collect airborne dust generated during handling of contaminated corn. The glass fiber filters were initially equilibrated in a constant temperature and humidity room and weighed before installation in the air samplers. After sample collection, the control filter and sample filters containing dusts were equilibrated in the same room 24 h before weighing to correct for moisture adsorbed on the filter. Dust samples ranged in weight from 7 mg to 11.8 g.

Extraction

Collected dust samples and corn samples prepared as described above were extracted 30 min on a wrist-action shaker. Glass fiber filters containing dust samples were extracted 3–4 min in a Waring blender by a slight modification of the AOAC–AACC method for corn (8, 9). The action of the blender shredded the glass fiber filters, ensuring contact of the dust with the solvent. Dust samples or glass fiber filter papers containing dust were extracted with 150 mL $CHCl_3$, 15 mL water, and 15 g Celite. The entire filtrate was collected from dust or glass fiber filter papers containing dust, and extracted dust and residues were thoroughly washed with $CHCl_3$. The combined extracts and washes from large samples (>1 g) were concentrated under vacuum to ca 35 mL. The concentrate was transferred quantitatively to a graduated cylinder, and diluted to 50 mL with $CHCl_3$ for transfer to the specified silica gel column. The graduated cylinder was rinsed with the hexane wash used on the column. Extracts and washes from smaller samples (<1 g) were concentrated to 2–3 mL, transferred quantitatively (Pasteur pipet) to a vial with $CHCl_3$, and dried under nitrogen for chromatography on a smaller column.

Column Chromatography

The silica gel (E. Merck, Darmstadt) silica gel 60, 0.063–0.2 mm) column (22 × 300 mm) and solvents (washes 150 mL hexane and 150 mL anhydrous ether, and elution solvent 150 mL methanol-$CHCl_3$ (3 + 97)) for samples >1 g were those specified in the AOAC-AACC method (8, 9). For samples <1 g, a 6 mm id × 21 cm column was used, with the top flared to a funnel shape to facilitate solvent addition and with a stockcock at the bottom to control solvent flow. The column was packed as a $CHCl_3$ slurry. The following were added to the column in order: glass wool plug, 1 cm anhydrous granular Na_2SO_4, 5.5–6 cm silica gel (8, 9), and 1–2 cm anhydrous granular Na_2SO_4. Residues from extraction of samples weighing ≤1 g were dissolved in 2 mL $CHCl_3$ and transferred with a Pasteur pipet to the column; vials were rinsed 3 times with 1–2 mL $CHCl_3$, and rinses were placed on the column. The column was washed with 5 mL hexane followed by 5 mL ether and then eluted with 10 mL ethanol–$CHCl_3$ (5 + 95). The entire eluates were dried under nitrogen and retained for TLC.

Thin Layer Chromatography (8, 9)

TLC plates (20 × 20 cm) were coated with 0.25 mm Adsorbosil-1 silica gel (Applied Science). For 1-dimensional preliminary plates and quantitative TLC, plates were developed with $CHCl_3$-acetone-water (91 + 9 + 1) in an unlined tank. The reference standard solution was 0.5 μg aflatoxin B_1/mL, 0.1 μg B_2/mL, 0.5 μg G_1/mL, and 0.1 μg G_2/mL in acetonitrile-benzene (98 + 2). Residues from smaller samples (0.1 to ≤1 g) from extraction and column chromatography were dissolved in 0.100–0.250 mL acetonitrile–benzene (98 + 2). If observations from the preliminary TLC plate were negative or indicated very little aflatoxin B_1 in an extract, the entire remaining extract, including washes of the vial in which it was contained, was applied to the TLC plate for 2-dimensional development.

For 2-dimensional TLC, solutions of unknowns and the diluted reference standard solution were applied to TLC plates and developed according to a spotting and development pattern similar to that shown in *Official Methods of Analysis* (10). Optional development solvents for 2-dimensional TLC were, first direction, $CHCl_3$–acetone–water (91 + 9 + 1, unlined tank) and second direction, toluene–ethyl acetate–formic acid (60 + 30 + 10, unlined tank). Afla-

Table 1. Comparison of analyses [a] of 3 corn lots for aflatoxins using 50, 10, and 1 g subsamples

Subsample size, g [b]	Aflatoxin	Lot 1 Mean, ng/g	Lot 1 Std dev.	Lot 1 Coeff. of var., %	Lot 2 Mean, ng/g	Lot 2 Std dev.	Lot 2 Coeff. of var., %	Lot 3 Mean, ng/g	Lot 3 Std dev.	Lot 3 Coeff. of var., %
50	B_1	2380	497	20.7	89.2	3.59	4.42	33.7	1.71	5.06
	B_2	97	24	24.5	7.5	0.58	7.70	3.75	0.96	25.6
	G_1	145	32	21.9						
	G_2	17 [c]								
10 [d]	B_1	2406	126	5.3	99.5	11.09	11.14	32.7	3.30	10.08
	B_2	102	13	12.7	7.25	2.50	34.48	3.75	0.50	13.31
	G_1	142	41	29.2						
	G_2	21	18	88.5						
1 [e]	B_1	2000	117	5.6	104	20.12	19.30	39.2	4.72	12.02
	B_2	103	20	19.1	15.2	6.60	43.42	7.75	0.96	12.34
	G_1	166	50	28.1						
	G_2	31	28	90.6						

[a] Analyzed by CB method approved for corn (8, 9).
[b] Four samples of each weight from all lots were analyzed.
[c] Only one value was obtained for G_2.
[d] No aliquot was taken of extract. Extract of entire sample was placed on standard CB column.
[e] Silica gel chromatography was carried out on 6 mm id columns

toxins were measured densitometrically on TLC plates unless extracts applied to the plate contained 0.5–2.0 ng aflatoxin B_1; then amounts were estimated by visual comparisons with standards.

Recoveries of Standard Aflatoxin B_1 and Aflatoxin B_1 in Naturally Contaminated Corn from Glass Filters

Standard aflatoxin B_1 (1.5 and 3.0 μg in 1 and 2 mL, respectively, of acetonitrile–benzene (2 + 98)) was incorporated into the 8 × 10 in. glass filters used in the high volume total sampler. Aflatoxin B_1 was extracted by blending 3–4 min with 150 mL $CHCl_3$, 15 mL water, and 15 g Celite. Extracts were dried and retained for TLC. Four portions (0.1, 0.25, 0.5, and 1.0 g) of naturally contaminated corn (2100 ng B_1/g) were weighed and spread onto 4 glass filters. Filters were extracted in a blender as above; extracts were purified for TLC on a small silica gel column (6 mm id) designed for ≤1 g samples.

Confirmatory Tests

Identity of either aflatoxin B_1 or G_1 was confirmed by the formation of water adducts with trifluoroacetic acid (TFA) (11, 12). On 2-dimensional plates to which the entire extract had been applied, TFA was applied to the developed unknown spot, and to a spot of standard aflatoxin solution applied next to the unknown spot. The plate was then developed in a third direction.

Identity of aflatoxins in one sample was confirmed by high pressure liquid chromatography (HPLC) of the toxins, using a Waters Model ALC-202 chromatograph equipped with 2 M-6000 pumps, a reverse phase column, and a fluorescence detector. Operating conditions were: column, Waters μBondapak C_{18}; flow rate, 1.5 mL/min; ambient temperature; solvent, water–methanol–acetonitrile (55 + 25 + 20); primary filter, 360 nm; secondary filter, 440 nm.

Results and Discussion

Results of analyzing 50, 10, and 1 g subsamples of the 3 samples of naturally contaminated ground corn are summarized in Table 1. The mean aflatoxin B_1 levels in the 3 samples were 2380, 89, and 34 ng/g. Extracts of 1 g portions were cleaned up on the smaller silica gel columns (6 mm id) to avoid losses encountered when the column specified in the AOAC–AACC method was used. The pooled repeatabilities (n = 3) for the determination of aflatoxin B_1 were 10, 9, and 12% for the 50, 10, and 1 g sample sizes, respectively, which were considerably better than the 30% anticipated from the results of collaborative studies (13). More recently, a study on the variability associated with testing corn for aflatoxin revealed that the coefficient of variation for one analysis of a subsample from finely ground, well blended meal is 26% (14).

Recoveries of 1.5 and 3.0 μg standard aflatoxin B_1 from glass filters (8 × 10 in.) were 98 and 67%, respectively. Recoveries of B_1 from naturally contaminated corn (2400 ng/g) on glass filters were 107% for the 0.1 g portion; 62%, 0.25 g; 75%, 0.5 g; and 87%, 1.0 g. Recoveries were variable,

probably because of the difficulty in obtaining a homogeneous naturally contaminated corn sample for subsampling in quantities of 0.25–1.0 g. Recoveries >98% have been reported from dry films of 25 μg aflatoxin B_1 in glass vials (15).

On the 2-dimensional TLC plate, 0.5–1.0 ng aflatoxin B_1 could be detected. When 90% of the weight of original sample was applied to the TLC plate for 2-dimensional development, the limit of detection was approximately 9.5 ng aflatoxin B_1/g for a 0.1 g sample. The limit of detection was determined by applying known amounts of B_1 in a corn extract to a TLC plate and developing the plate. The detection limit can vary depending on interferences in a given sample. The water adduct of the separated spot of aflatoxin B_1 could be prepared by treatment with TFA on the same plate, and development in a third direction to confirm its identity (11, 12). All results were confirmed by the TFA test on 1- or 2-dimensional TLC plates with one exception: It was impossible to confirm aflatoxin G_1 in one sample because of the presence of extract impurities with low R_f values. G_1 identity was confirmed by HPLC. These methods were satisfactorily applied to dust samples scraped from farm equipment and surfaces of an elevator and to airborne corn dust collected with a high volume total sampler and with Anderson 4-stage samplers. Analytical results are reported in another paper (16).

References

(1) Van Nieuwenhuize, J. P., Herber, R. F. M., DeBruin, A., Meyer, P. B., & Duba, W. C. (1973) *T. Soc. Geneesk.* **51**, 754–760

(2) Deger, G. E. (1976) *Ann. Intern. Med.* **85**, 204–205

(3) Shotwell, O. L. (1977) *J. Am. Oil Chem. Soc.* **54**, 216A–224A

(4) McMillian, W. W., Wilson, D. M., & Widstrom, N. W. (1978) *J. Environ. Qual.* **7**, 564–566

(5) McMillian, W. W., Wilson, D. M., Widstrom, N. W., & Gueldner, R. C. (1980) *Cereal Chem.* **57**, 83–84

(6) Lillehoj, E. B., Kwolek, W. R., Shannon, G. M., Shotwell, O. L., & Hesseltine, C. W. (1975) *Cereal Chem.* **52**, 603–611

(7) Bagley, E. B. (1979) *J. Am. Oil Chem. Soc.* **56**, 808–811

(8) *Official Methods of Analysis* (1980) 13th Ed., AOAC, Arlington, VA, secs **26.049, 26.026-26.031**

(9) 1972 Revision to *AACC Approved Methods* (1972) American Association of Cereal Chemists, St. Paul, MN, Method 45-05

(10) *Official Methods of Analysis* (1980) 13th Ed., AOAC, Arlington, VA, Fig. **26:01**

(11) *Official Methods of Analysis* (1980) 13th Ed., AOAC, Arlington, VA, sec. **26.083**

(12) 1977 Revisions to *AACC Approved Methods* (1977) American Association of Cereal Chemists, St. Paul, MN, Method 45-25

(13) Shotwell, O. L., & Stubblefield, R. D. (1972) *J. Assoc. Off. Anal. Chem.* **55**, 781–788

(14) Whitaker, T. B., & Dickens, J. W. (1979) *J. Am. Oil Chem. Soc.* **56**, 789–794

(15) Rodericks, J. V. (1969) *J. Assoc. Off. Anal. Chem.* **52**, 979–980

(16) Burg, W. R., Shotwell, O. L., & Saltzman, B. E. (1981) *Am. Ind. Hyg. Assoc. J.* **42**, 1–11