AFLATOXIN OCCURRENCE IN SOME WHITE CORN UNDER LOAN, 1971. I. INCIDENCE AND LEVEL

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ABSTRACT

Aflatoxin was detected in 30% of 1,283 truckloads of Commodity Credit Corporation white corn (1971) delivered to a designated elevator from 77 loans in seven counties in southeastern Missouri (detection limit 1–3 ppb). Only 14% of the truckloads of corn contained more than 20 ppb aflatoxin. The toxin was not detected in any corn delivered from 20 of the farmers' loans. The geometric means of aflatoxin levels in corn from seven loans were higher than 20 ppb. Very few truckloads of corn contained aflatoxin G-1. Based on geometric means of aflatoxin level of loans, toxin content was related to grade although with a low correlation coefficient of 0.29 for aflatoxin B-1 and 0.27 for B-2.

Initial surveys of corn in 1964-1969 from the Midwest and seaports revealed low incidence of aflatoxin (1.7–2.7%) and levels (3–37 ppb total aflatoxin) (1–4). However, a survey of white and yellow corn in crop years 1969–1970 from the South indicated higher incidence (35%) of the toxins and higher levels (4–350 ppb total aflatoxin) (5). The more highly contaminated samples tended to be in the

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poorer grades. In 1971, the Food and Drug Administration (FDA) seized corn meal prepared from white corn originating in southeastern Missouri (6). The Commodity Credit Corporation (CCC) accepted delivery of 1971 crop year white corn under loan from seven counties in that region during August and September 1972. Yellow corn under loan from the same region was not studied. White corn was considered to be more important because it is used in food products.

A rapid screening procedure was used on delivery of corn at the designated elevator to segregate any corn containing aflatoxin from uncontaminated corn. The entire delivery was arranged in such a manner that several aspects of the problem of aflatoxin contamination in corn could be studied. The Agricultural Marketing Service conducted studies on sampling. Investigated were rapid and quantitative methods of aflatoxin detection and determination, molds occurring in white corn, factors such as storage, weather, and other conditions possibly leading to aflatoxin contamination, and occurrence of aflatoxin in 1971 white corn delivered at the elevator designated by the CCC. We are now reporting the incidence and levels of aflatoxin in more than 1,000 truckloads of CCC 1971 white corn grown, stored, and delivered in southeastern Missouri. Results of rapid testing at the elevator and studies of the bright greenish-yellow (BGY) fluorescence associated with the presence of aflatoxin (7) in corn will be reported separately.

MATERIALS AND METHODS

Sample Collection

Truckloads of corn were sampled and tested by a rapid screening test as trucks arrived at the elevator to deliver the white corn. On the basis of the rapid screening procedure, a decision was made as to which storage bin to deliver a truckload (capacity 200–400 bu) of corn. A 50-lb continuous sample from every truckload was taken with a primary mechanical sampler (Dean Gomet Series 6800, vane type) during unloading. From the 50 lb of corn, a rotary divider (Dean Gomet 4500) split out a 10-lb sample to be inspected for BGY fluorescence and analyzed for aflatoxin content at the Northern Regional Research Laboratory (NRRL).

Inspection for BGY Fluorescence at NRRL

The 10-lb corn samples were inspected under high-intensity ultraviolet (365 nm) with a Blak ray lamp (Model B, 100-A) for BGY fluorescence. Numbers of fluorescent kernels and particles were counted. If no BGY particles or kernels were observed, kernels were cracked in a Straub disc mill (Model 4-E). As the cracked corn came from the mill, the stream was inspected for BGY fluorescence under ultraviolet light.

Sample Preparation

After inspection for fluorescence was completed, 10-lb samples were ground in a 12-in. Raymond hammer mill with screens containing 1/8-in. round-hole perforations. Ground samples were blended 15–30 min in a Hobart planetary mixer, A200 (12-qt capacity) or in a Twin Shell Blender (PK-LB-6948).

Aflatoxin Determination

Corn that contained BGY fluorescing particles and kernels in the whole kernel sample was assayed directly by the quantitative procedure approved in Official First Action by the Association of Official Analytical Chemists (8). Because in some extracts of white corn a fluorescing impurity interferred with the identification of aflatoxin G-1, preliminary thin-layer chromatographic (TLC) plates were developed initially with anhydrous ether before the development with the usual aflatoxin solvent. Samples that did not have BGY fluorescing material when uncracked kernels were examined under ultraviolet light were finely ground, extracted, and assayed by the rapid TLC technique for corn (8). If the rapid TLC screening method showed that aflatoxin was present, the extract was placed on a silica gel column for the usual quantitative determination. Amounts of aflatoxin in partially purified extracts were determined on TLC plates coated with 0.5 mm Absorbosil-1. Plates were developed with water:acetone:chloroform (1.5:12:88 v/v/v) (9), and fluorescent zones were measured densitometrically.

When the analyses of all corn samples were completed, 52 samples were reassayed to check the variability of the assay within the laboratory. Three technicians were involved in the analytical work and distribution of samples among them was random.

Statistical evaluation was made on the 53 loans out of 77 with four or more truckloads of corn delivered and at least one of these contained aflatoxin. A total of 1,056 loads were involved and two correlations of grade factors with aflatoxin content were investigated: a) an overall correlation ignoring loan; and b) correlations within loans.

Confirmatory Tests

The identity of aflatoxin B-1 was confirmed in representative samples from groups of positive corn samples by the formation of the water adduct. Extracts were applied to TLC plates and trifluoroacetic acid (1 μl) was superimposed on spots (10). After drying, TLC plates were developed with the water:acetone:chloroform solvent.

Farm Loan Histories

Farmers who delivered CCC white corn (1971) in southeastern Missouri were asked to fill out a questionnaire. They were asked what crops had been planted the previous year and if fields had been plowed to bury all plant material. Other questions concerned seed, herbicide, and pesticide applications; weather conditions during the growing period and immediately before harvest, as well as during harvest and storage; possible storm damage; and irrigation practices. Farmers also supplied information on the type of harvester used, moisture content at harvest, drying conditions, type of storage bin, and unloading equipment.

RESULTS AND DISCUSSION

There were 1,283 truckloads of white corn delivered from 77 CCC loans in southeastern Missouri. In most cases, a farmer's loan involved corn from one farm, but a few loans included corn from several farms. All truckloads were sampled and analyzed as described. The number of truckloads of corn from a loan varied from 1 to 73. When the corn was graded by the Missouri State Inspection Service, two-thirds of the loads was placed in Grades U.S. No. 1 and 2.

Analyses for aflatoxin in 52 corn samples, each analyzed at two different times, were evaluated to determine what the variation would be within NRRL. The 52 corn samples were randomly selected for duplicate analyses. No significant differences were reflected between the three analysts involved. The relative standard deviation per analysis was 37% based on an analysis of log aflatoxin; with a single analysis on each sample, a ratio of two aflatoxin values exceeding 2.44 would indicate a statistically significant (0.05 level) difference in aflatoxin. Duplicate analyses on each sample lowers this ratio to 1.88 (relative standard error is 25%). For example, the relative standard error per geometric mean if five assays are run on a truckload of corn is 15%. If two loads of corn are compared, based on five assays for each 10-lb sample, the ratio of means for the two loads would have to exceed 1.49 to conclude that the loads differ significantly in aflatoxin content. To attain a relative error of less than 10% requires a prohibitive number of analyses.

The overall incidence of aflatoxin in 1,283 truckloads of 1971 white corn delivered in southeastern Missouri was 30% (Table I). The incidence of toxin in this 1971 corn was not so high as rumored in unsubstantiated reports after harvest. Aflatoxin was present at levels higher than the current FDA guideline of 20 ppb (11) in 14% of the truckloads and 11% contained less than 10 ppb aflatoxin.

The aflatoxins detected in 1971 white corn were those that might be expected from previous studies (1,2,4,5,12). Because more samples containing aflatoxin were available, we were able to make a statistical evaluation of the relation between aflatoxin B-1 and B-2 in the white corn. The amount of aflatoxin B-2 in a positive sample was approximately 0.18 that of B-1. No B-2 was detected in samples that did not contain B-1; to date, there is only one confirmed report that

TABLE I
Total Aflatoxin Incidence in White Corn, 1971
(Number of Truckloads with Indicated Level)

Grade U.S. No.	Total	Aflatoxin (ppb)[a]					
		ND[b]	<10	10–19	20–29	30–100	>100
None	1					1	
1	371	280	45	16	11	16	3
2	477	354	40	42	16	23	2
3	107	73	14	8	4	5	3
4	72	32	7	9	1	14	9
5	80	38	12	8	6	13	3
SG[c]	175	112	18	10	7	19	9
Total	1,283	889	136	93	45	91	29

[a]Total number of samples containing detectable (at least 1–3 ppb toxin by method used) aflatoxin is 394.
[b]ND = not detected.
[c]SG = Sample Grade.

a strain of *Aspergillus flavus* accumulated only B-2 (13). As expected, detection of B-2 depends on the level of B-1. The sensitivity of the analytical method is 1–3 ppb toxin. Roughly, aflatoxin B-2 could be detected in 1 out of 10 samples containing 5 ppb B-1, in 5 out of 10 samples containing 15 ppb B-1, and in 9 out of 10 samples containing 40 ppb B-1.

Out of 394 truckloads of corn delivered that contained aflatoxin, only 24 contained aflatoxin G-1. The B-1 to G-1 ratio varied between truckloads particularly between truckloads delivered by different farmers.

In previous surveys (1,2,4,5), we attempted to correlate the presence of aflatoxin with factors used by USDA Grain Inspection Service to grade corn. We were, however, handicapped by not having many aflatoxin-positive samples. With the positive samples available in this study because of the large number of samples assayed and incidence of toxin, we hoped to be able to make such correlations.

Moisture determinations made at the time of grading are not considered a significant measurement since the corn usually has been artificially dried prior to, or during storage, or has dried out naturally. Moisture levels at the time of invasion of corn by *A. flavus* would be significant. When corn was delivered from storage bins and graded in southeastern Missouri, moisture levels were 11–14%; only 49 of 1,283 samples had more than 15% moisture and 12 of these contained aflatoxin. As expected, moisture levels of corn as delivered did not correlate with the presence and levels of aflatoxin.

There were no overall correlations, ignoring loans, between aflatoxin content and any of the grading factors: test weight per bushel, moisture, total damaged kernels, and broken corn-foreign material. Present grading factors could not be used to identify aflatoxin-suspect samples. However, when correlations were considered on truckloads of corn within a given loan, significant positive correlations (coefficient = 0.20) between aflatoxin B-1 and damaged kernels were found. That is, the higher the amount of total damaged kernels in a load of corn from the same farm the higher the level of aflatoxin.

When correlations were studied between means of all 77 loans, there were no correlations between aflatoxin contamination and grading factors considered individually. However, based on means of aflatoxin levels in truckloads from individual loans, aflatoxin was related to grade although with a low correlation coefficient of 0.29 for B-1 and 0.27 for B-2.

The degree of aflatoxin contamination on the basis of loan is given in Table II. No aflatoxin was detected in corn from 21 of the loans. There was more than 20 ppb total aflatoxin in a few truckloads of corn delivered from 22 loans. Corn from 11 loans was identified as having an aflatoxin problem based on inspection of results of quantitative determinations. The number of truckloads of corn with aflatoxin levels above 20 ppb was considered in identifying the 11 loans. The geometric means of the aflatoxin levels in truckloads of corn delivered from these loans were also the highest (15–260 ppb total aflatoxin). Four loans accounted for most of the corn containing more than 100 ppb aflatoxin (Table III). All 12 loads from one farm had levels of toxin above 100 ppb.

The geometric mean of the aflatoxin level in truckloads from loans representing four or more loads was determined on the assumption that every load contained the same amount of corn (Table IV). The geometric means of aflatoxin level in corn from only seven loans were more than 20 ppb. There was no significant variation in geometric means of aflatoxin levels between counties.

Aflatoxin contamination in corn from some loans was in the first truckload delivered; from other loans it was in the last truck. From some loans the contamination was throughout all loads delivered. If corn had been stored in several bins as it was on some farms, all bins would not necessarily have been

TABLE II
Contamination in Corn from Loans[a]

Aflatoxin contamination	Number of Loans
None	21
No truckloads with levels above guidelines (20 ppb)	23
Some truckloads with levels above guidelines	22
Higher levels, more truckloads with levels above guidelines	11
Total	77

[a]A loan includes all of the corn delivered by one farmer and usually was from one farm.

TABLE III
Loans with Truckloads of Corn Containing More Than 100 ppb Aflatoxin

Loan Number	Total number of truckloads delivered	Truckloads Containing Aflatoxin:			Truckloads Containing >100 ppb Aflatoxin	
		Nondetectable	<20 ppb	20–100 ppb	Number	Levels (ppb)
29-069-8	34	5	5	22	2	101, 106
29-133-17	12	0	0	0	12	145–306
29-143-29A	11	5	3	2	1	190
29-143-99A	15	9	1	0	5	179–245
29-201-6A	23	14	3	5	1	121
29-201-37A	11	1	1	5	4	125–236
29-207-29	19	1	7	7	4	109–124

TABLE IV
Geometric Mean of Aflatoxin in White Corn from Loans Delivering Four or More Truckloads

Aflatoxin ppb Mean	Number of Loans
<1	18
1–5	23
6–10	2
10–20	3
20–30	3
30–40	2
40–50	1
210	1

contaminated with aflatoxin. Results of aflatoxin assays on truckloads delivered from farms with one bin indicate that one location in a bin could have all of the toxin-containing corn—top, bottom, sides, or center. From four loans, there was only one truckload of corn per loan containing detectable toxin. In only one portion of corn from these loans had there been mold growth with aflatoxin formation.

Comparison of farm loan histories with aflatoxin contamination did not yield as much useful information as we hoped. Perhaps not enough loans were involved to reach meaningful conclusions. Soybeans had been planted in most of the fields the previous year, but in a few, cotton, corn, or wheat had been planted. The presence of aflatoxin did not appear to be associated with the previous crop. Almost all the farmers said that when fields were plowed all plant material had been buried, but there was no apparent relationship to aflatoxin contamination. Not enough farmers listed specific variety of seed planted to reach any conclusion as to the relation of the seed planted to toxin occurrence. Weather conditions during the growing season and immediately before harvest, and during harvest, were insufficiently described for the study, perhaps because a year had elapsed.

Questions on whether storm damage occurred, pesticides were used, or fields were irrigated yielded rather definite answers. There were, however, no correlations between answers and levels of aflatoxins detected in loans. More information on when storm damage occurred, what stage irrigation was necessary, and when and how often pesticides were applied might show definite relations. The type of equipment used to harvest, and moisture content determined at harvest, did not appear to be related to toxin occurrence or level in this study. Drying conditions should be a critical factor in formation of aflatoxin by *A. flavus* group. Description of drying conditions varied a great deal. Six farmers said they did not artificially dry corn, and those who reported highest moisture levels at harvest time delivered some corn containing aflatoxin. Ten farmers reported that they had dried corn in the bins using fans, but no heat; the incidence of aflatoxin in this corn was slightly higher than the overall incidence. No conclusions could be reached from information provided on storage conditions and unloading procedures. An attempt will be made to obtain additional information from farms where corn in loans had been aflatoxin-free and where corn had highest levels of toxin.

CONCLUSION

Aflatoxin was detected in 30% of 1,283 truckloads of white corn delivered from 77 loans in seven counties in southeastern Missouri by quantitative analysis sensitive to 1–3 ppb. Only 14% of the corn samples contained more than 20 ppb aflatoxin. Since aflatoxin was not detected in any corn from 20 of the loans, it is possible to grow, harvest, and store corn without toxin formation in an area where conditions are favorable for its formation. It is important in future studies to determine when and how *A. flavus* invades corn and what factors are involved in aflatoxin formation by the mold. Eleven loans had aflatoxin in levels and amounts that would definitely cause concern. The geometric mean of aflatoxin in corn from seven loans was higher than 20 ppb (30–260 ppb total aflatoxin). The ratio of aflatoxin B-2 and B-1 in positive corn samples was about 0.18. Very few truckloads of corn contained aflatoxin G-1. On the basis of geometric mean of aflatoxin level for loans, toxin content was related to grade, but the correlation coefficients were low (0.29 for B-1, 0.27 for B-2). If truckloads of corn from one farm are compared, there was a correlation (coefficient = 0.20) between total damaged kernels and aflatoxin content. Aflatoxin content in truckloads of corn from the same bin indicated that one section of a bin could contain highly contaminated corn while corn from other parts could be toxin-free.

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