INCIDENCE OF AFLATOXIN IN CORN AND A FIELD METHOD FOR AFLATOXIN ANALYSIS

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ABSTRACT

Incidence refers to the proportion of samples with levels of aflatoxin detectable by the test method used. Surveys were made during the 1972 and 1973 crop years to determine the incidence of aflatoxin in commercial lots of marketed corn. Samples being officially graded by several field offices were selected for these surveys. The field offices examined the samples under high intensity black light for evidence of bright greenish-yellow fluorescence (BGYF). Samples with kernels exhibiting BGYF were tested for aflatoxin. During 1972, both the CB thin-layer chromatography and Velasco rapid florisil column methods were used to test for aflatoxin. Only the latter test method was used in the 1973 survey.

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A survey was conducted during the 1972 crop year[3] by the Grain Division, AMS, USDA, for the purpose of determining the extent of aflatoxin contamination in inspected corn. Eighteen field offices of the Grain Division in the major corn markets were requested to examine their corn samples under long-wave ultraviolet (UV) light (black light) for evidence of bright greenish-yellow fluorescence (commonly called "glowers") that indicates possible aflatoxin contamination (1,2). All samples that contained "glowers" were sent to Beltsville for chemical analysis by the CB thin-layer chromatography (TLC) method (3) and by the Velasco rapid florisil column method (4,5).

During the 1973 crop year, another study of aflatoxin in corn was conducted by 16 of the field offices that participated the previous year. Two of the field offices in the 1972 survey were closed before the start of the 1973 survey. Changes in the sampling rate, UV scanning procedure, and implementation of a minicolumn method for field application were incorporated into this study.

A stratified random sampling plan was used to reduce the cost of the 1973 survey. The field offices were assigned to one of three strata based on the previous year's survey. Disproportionate sampling rates of 1/2, 1/5, or 1/10 were established for the different strata. The precision of estimates obtained with this type of sampling can be increased over the precision available when a constant sampling rate for each field office and the same total number of samples are inspected. This increase is achieved by collecting a larger proportion of samples from field offices where the rate of samples contaminated by aflatoxin is high and fewer samples from offices where the proportion affected is low. Unbiased estimates of aflatoxin incidence are obtained by appropriately weighing the data from each stratum.

METHODS

The grain graders were provided with a visual guideline to facilitate the

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[3]A crop year for this purpose was from start of harvest in year given until start of harvest the following year.

identification of "glowers" in corn under black light. One visual guideline consisted of corn kernels cut lengthwise in half and the cut surface streaked with a mixture of UV paint that simulated the "glower" fluorescence. Unfortunately, the fluorescent paint fades under extended exposure to high-intensity (100 watt) UV light and, therefore, should be subjected to the black light for only short periods. Subsequently, another visual guide was obtained from Jack Swarthout of Illinois Cereal Co. It consisted of specific bleaching powder that was dried and sealed in a glass tube. It fluoresces similarly to "glower" corn under UV light. Both guidelines were useful inspection tools for identifying possible aflatoxin contamination in corn.

Since the field offices had no prior experience in examining corn for "glowers," all samples which they thought contained "glowers" were sent to Beltsville for reexamination by black light and for further analysis if positive. Frequently it was noted at Beltsville that certain kernels were of a peculiar color. When these were broken open, they were found to be intensely fluorescent throughout the kernel. A number of samples of whole corn were carefully screened to remove all visible "glowers" and then were cracked by the "Tag" rolls. Some of these samples showed numerous glowing fragments. Studies on the 1972 crop showed that some kernels of whole corn with little evidence of external fluorescence would brightly fluoresce when broken open. Therefore, our 1973 survey included cracking the corn through corrugated steel rolls ("Tag" moisture meter rolls) and examining the corn with the black light as it fell into the collecting pan. Also, it was determined that fluorescence is more readily detected with high-intensity (365 nm) black light than by the smaller portable units used in the 1972 survey. Therefore, all examinations for the 1973 crop were made with the high-intensity lights.

A rapid minicolumn method for determining aflatoxin was developed for field office application. The method, adapted from Velasco's (4) procedure, was modified by Velasco and Hunt to permit analysis to be made in rooms not equipped with water, sinks, or outside vents. A portable refrigeration unit was used to condense solvents. Plastic containers, beakers, bottles, etc., were used for safety purposes. (Details of the revised equipment and method may be obtained from the authors.)

In the 1973 survey, three of the Grain Division field offices (Norfolk, Omaha, and St. Louis) and the Board of Appeals and Review (BAR) were equipped and personnel trained to perform the modified Velasco florisil column method. The three field offices, for purposes of the survey, are designated as "area analytical offices." Participating field offices systematically selected samples which were ground and then examined with UV light. Samples showing the fluorescence characteristic of aflatoxin were forwarded to one of the area analytical offices for florisil testing.

In 1972, a detailed study was made by the Grain Division and the Northern Regional Research Laboratory, USDA, Peoria, Ill., and the Food and Drug Administration, on white corn from the Boothill area of Missouri. Shotwell *et al.* (6) examined numerous samples that contained "glowers," and also those free of "glowers," for aflatoxin. The chance of not detecting "glowers" with the UV test in a sample of corn containing aflatoxin is very small (6), so no subsequent testing was performed on the samples without the characteristic fluorescence.

RESULTS

During the 1972 survey, all 7937 corn samples inspected by the 18 field offices were examined by black light. "Glowers" were found in 250 samples, of which 90 tested positive for aflatoxin by the TLC CB method (3) and 117 were positive by the rapid florisil column method (4,6). Of those samples positive by TLC, 48 contained less than 15 ppb aflatoxin, and 42 were contaminated above this level. The florisil column results showed 73 of the positive "glower" samples contained less than 15 ppb aflatoxin and 44 samples were above this level. The average aflatoxin level of those samples giving positive quantitative results was 44 ppb by the TLC method. The sensitivity of the TLC CB method is expected to be $\geqslant$10 ppb (Table I).

The survey revealed that less than 4% of the 1972 crop examined contained "glowers." Approximately 1.1% of the samples showed evidence of aflatoxin by TLC. If the florisil test results are used, the incidence is about 1.5%. Only 27 samples or 0.3% of all corn samples inspected contained aflatoxin levels exceeding 15 ppb with TLC. Thus, the incidence of seriously contaminated lots, as defined by the FDA guidelines, is not a significant nationwide problem in inspected corn. However, it should be noted, the average level of aflatoxin in affected samples exceeded 20 ppb in four field offices (F, L, M, and Q in Table I).

In the survey of the 1973 crop, 17,245 samples were submitted to the 16 participating field offices for routine inspection between October 1973 and July

TABLE I
Incidence of Aflatoxin in 1972 Corn Survey

Field Office	Number of Samples Collected	% Positive by UV	Number of Samples Analyzed by TLC	Number of Samples Positive by TLC	% of Positive TLC Analyses	Aflatoxin Results Average of positive TLC analysis ppb	Aflatoxin Results Maximum TLC analysis noted ppb
A	49	0	0	...	...	...	...
B	64	2.0	0	...	...	...	...
C	1022	1.5	13	1	8	16	16
D	359	0.2	2	0	0	...	...
E	581	1.0	9	2	22	7.5	11
F	250	9.0	13	4	31	38	107
G	772	1.0	10	3	30	6.3	10
H	137	4.0	3	3	100	6.3	8
I	1473	1.0	45	9	20	11	48
J	50	42.0	18	3	17	13	20
K	314	6.0	9	0	0	...	...
L	109	28.0	52	41	79	71	396
M	456	11.0	36	10	27	42	140
N	1232	1.0	19	3	15	5	12
O	50	0	...	...	...	...	...
P	162	9.0	6	1	17	3	3
Q	211	16.0	15	10	67	23	93
R	646	0.2	0	0	...	...	...
	7913						

TABLE II
Summary of Aflatoxin Survey Data
(October 1973 – July 1974)

Office	Sampling Rate	Samples Tested by UV	Number Positive by UV	% Positive by UV[a]	Florisil Tests	Number Positive Florisil	% of Positive Florisil Tests	Number Positive 15 ppb and Over
Area I								
A	1/2	400	94	23.5	92	38	41.3	14
B	1/10	163	36	22.1	36	13	36.1	3
C	1/5	54	4	7.4	4	3	75	0
D	1/10	139	9	6.5	9	4	44.4	1
E	1/10	131	9	0.8	1	0	0	1
Subtotal	...	887	144	12.4	142	58	...	19
Area II								
A	1/2	249	78	31.3	77	73	94.8	45
B	1/10	150	64	42.7	47	34	72.3	0
C	1/5	28	14	50.0	14	11	78.6	1
D	1/10	97	3	3.1	3	2	66.7	0
E	1/10	75	2	2.7	2	2	100	1
Subtotal	...	599	161	23.8	143	122	...	47
Area III								
A	1/2	426	38	8.9	39	26	66.7	6
B	1/10	110	0	0	0	0	0	0
C	1/10	225	12	5.3	12	4	33.3	1
D	1/5	481	24	5.0	24	18	75.0	0
E	1/10	74	17	23.0	19	4	21.0	0
F	1/10	64	13	20.3	10	3	30.0	0
Subtotal	...	1380	104	7.7	104	55	...	7
Nation (1973–1974)	...	2866	409	12.8	389	235	...	73
Nation (1972)	...	7937	311	4.0	250	90	...	...

[a]Area and national estimates are *weighted percentages*.

1974. A total of 2866 of these were systematically selected for the aflatoxin survey. Of the 409 samples containing BGYF material, 389 were tested for aflatoxin by the modified Velasco florisil method (the sensitivity of the Velasco florisil method is expected to be >5 ppb), and 235 contained detectable levels of aflatoxin.

Nationwide, we estimate 12.8% of all samples submitted for inspection during this time period would have shown the fluorescence characteristic of aflatoxin under the black light. A 95% confidence interval[4] indicates the true value is between 11.6 and 14.0%. The presence of aflatoxin was confirmed by the florisil test in approximately 60% of these samples.

Assuming the UV test will disclose the appropriate fluorescence any time there is aflatoxin in a sample, and if the florisil test can accurately differentiate between contaminated and noncontaminated samples, then an estimate of the percentage

[4]Confidence intervals indicate the amount of error that can reasonably be expected in an estimate.

TABLE III
95% Confidence Intervals for Aflatoxin Survey Data

Region	Positive UV				Confirmed Aflatoxin			
	%		Confidence interval		%		Confidence interval	
Area I	12.4[a]	(18.2)[b]	10.2–14.6[a]	(13.8–22.6)[b]	4.9[a]	(2.6)[b,c]	3.5– 6.3[a]	(0.7– 4.5)[b]
Area II	23.8	(47.0)	20.6–27.0	(41.2–52.8)	18.3	(28.7)	15.3–21.3	(23.3–34.1)
Area III	7.7	(10.6)	6.2– 9.2	(7.7–13.5)	3.2	(6.8)	2.3– 4.1	(4.3– 9.3)
Nation	12.8	(19.3)	11.6–14.0	(16.8–21.8)	7.1	(11.6)	6.2– 8.0	(9.7–13.5)

[a]Data from October 1973–July 1974.
[b]Data in parentheses are estimates for August–October 1974.
[c]Estimates based on all corn samples graded by the respective field offices—not just on those samples showing "glowers."

of submitted samples that contain aflatoxin can be obtained. For the country, this estimate is 7.1% of the 1973 crop. A 95% confidence interval for this estimate indicates the true value is between 6.2 and 8.0%.

Table II provides a summary of the data and analysis for the country and the area field offices. Confidence intervals for the appropriate estimates are provided in Table III.

A comparison between the surveys indicates an increase in the incidence of aflatoxin-contaminated samples from less than 2% of the 1972 crop to over 7% of the inspected 1973 crop. However, changes in UV scanning procedures and the relocation of the florisil testing to the three area analytical offices may account for the observed increased incidence.

ADDENDUM

Additional data collected from August through October 1974 following the presentation of this paper show a significant increase in the proportion of samples containing aflatoxin over the rate reported for the first 9 months of the crop year. Estimates for the August–October period have been added to Table III.

Comparisons between these estimates and those reported for the earlier portion of the 1973 crop can be justified as there were no known changes in the survey procedures. This comparison indicates an increase in estimated incidence from 7.1 to over 11%.

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