# Survey of Cereal Grains and Soybeans for the Presence of Aflatoxin: I. Wheat, Grain Sorghum, and Oats[1]

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## ABSTRACT

A total of 531 wheat samples, 533 grain sorghum samples, and 304 oat samples, representing all marketing grades, were analyzed for the presence of aflatoxin. Samples were extracted by the procedure, slightly modified, developed by the Food and Drug Administration for the analysis of peanut and peanut products. Extracts were assayed for the presence of aflatoxins by thin-layer chromatography (TLC). The sensitivity limit of the analysis as carried out was 2 to 5 p.p.b. of the metabolite. According to results of TLC, two wheat samples, six grain sorghum samples, and three oat samples, all in poorer

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grades, appeared to contain low levels of aflatoxin (2 to 19 p.p.b.). Samples that were positive by TLC were tested in ducklings. No aflatoxinlike activity was detected in grain sorghum samples. Although the duckling test indicated that traces of toxins might be present in wheat and oat samples, more likely the test was negative. The sensitivity of the test as conducted was 1 to 2 p.p.b.

Surveys have been made to determine the occurrence of aflatoxins in peanuts (1) and in cottonseeds (2-3). This paper describes the analysis of wheat, grain sorghum, and oat samples from commercial markets for the presence of aflatoxin. All these cereal grains are suitable substrates for aflatoxin production under laboratory conditions (4,5), so it is possible that the toxins could occur as natural contaminants.

## MATERIALS AND METHODS

### Collection and Preparation of Samples

Samples (1 kg.) from all grades of grain were collected by the Grain Division of Consumer and Marketing Service, USDA. Some of the factors considered in specifying the distribution of samples taken in the various grades were monthly volume of each grade received at the markets, distribution of grades by commodity, likelihood of higher incidence of aflatoxin in the poorer grades, extent of grain mixing, and locations of markets from which to obtain samples. Seasonal changes in the proportion of commodity in different grades would be the deciding factor for the number of samples actually obtained. The number of samples desired from each location was set at 300, since this number is associated with a 0.95 chance of detecting at least one positive sample if the aflatoxin incidence at a detectable level is 1%. The proposed and actual distribution of samples is shown in Tables I and II.

Samples (1 kg.) were ground in a Raymond 6-in. stainless-steel laboratory mill equipped with a screen having 1/8-in. round-hole perforations.

### Extractions

Grain samples were extracted by the procedure, slightly modified, recommended for determining aflatoxins in peanuts and peanut products (6). The sample (50 g.) was suspended in 250 ml. of methanol:water (55:45 v./v.) and extracted in a Waring Blendor for 3 min. Aliquots (50 ml.) of the extract were mixed with acid-washed Celite 545 (55 g.) and water (5 ml.). The mixture was packed into a column and washed with hexane and eluted with hexane:chloroform

TABLE I. WHEAT SAMPLES FROM 1964 CROP EXAMINED FOR PRESENCE OF AFLATOXIN

| Grade | Samples Requested from Each Location | Source of Samples | | | Total Samples Received | Positive Samples According to TLC |
|---|---|---|---|---|---|---|
| | | Fort Worth | Omaha | Kansas City | | |
| 1 | 100 | 76 | 33 | 59 | 168 | 0 |
| 2 | | 23 | 39 | 37 | 99 | 0 |
| 3 | 26 | 10 | 9 | 28 | 47 | 0 |
| 4 | 26 | 15 | 7 | 26 | 48 | 0 |
| 5 | 48 | 16 | 10 | 18 | 44 | 0 |
| SG | 100 | 41 | 9 | 75 | 125 | 2 |
| Total | 300 | 181 | 107 | 243 | 531 | 2[a] |

[a]Thin-layer chromatography indicated 7 p.p.b. B-1 and 2 p.p.b. G-1; duckling test was inconclusive, but probably negative (limit of assay is 1 to 2 p.p.b.).

TABLE II. GRAIN SORGHUM AND OAT SAMPLES IN THE SURVEY FOR THE PRESENCE OF AFLATOXIN

| | | Grain Sorghum Samples (1964) | | | | Oats | |
|---|---|---|---|---|---|---|---|
| | | Source of Samples | | Total | Positive | Received | Positive |
| Grade | Requested[a] | Fort Worth | Kansas City | Received | by TLC | (1966) | by TLC |
| 1 | 36 | 42 | 7 | 49 | 0 | 37 | 0 |
| 2 | 60 | 56 | 180 | 236 | 0 | 59 | 0 |
| 3 | 26 | 24 | 37 | 61 | 0 | 25 | 0 |
| 4 | 100 | 31 | 29 | 60 | 2 | 103 | 2 |
| SG | 80 | 82 | 45 | 127 | 4 | 80 | 1 |
| Total | 302 | 235 | 298 | 533 | 6[b] | 304 | 3[c] |

[a]No. of samples requested from each location.

[b]Not positive by the duckling test.

[c]Duckling test was inconclusive, but probably negative.

(1:1 v./v.). Residues from eluates were dissolved in chloroform (1 ml.) and subjected to thin-layer chromatography (TLC).

Three other methods were tried for the extraction of aflatoxin, if present, from grains. One method was the chloroform extraction developed by Lee (7) for defatted peanuts, but too many impurities that interfered with TLC were removed, particularly when the grain was sorghum. Other solvent systems investigated were 70% acetone (8) and acetone:hexane:water (5:48.5:1.5 v./v.) (9).

**Partial Purification of Oat Extracts**

Of 304 oat sample extracts, 28 contained fluorescing substances that interfered with TLC and were further purified when placed on silica gel (0.05 to 0.20 mm.) columns (35 by 60 mm.) (8), washed with absolute diethyl ether (100 ml.), and eluted with methanol:chloroform (3:97 v./v.) (150 ml.).

**Thin-Layer Chromatography**

Chromatoplates (20 by 20 cm.) were prepared for TLC by mixing 30 g. of Silica Gel G-HR with 64 ml. of distilled water in a Waring Blendor for 30 sec. and spreading a layer 0.250 mm. thick on the plates. Plates were dried at 105°C. for 2 hr. and stored in a desiccating cabinet. Extracts (20 microliters) were spotted on the plates alone and in admixture with a standard solution in chloroform of 0.0029 $\gamma$ aflatoxin B-1 per microliter, 0.00075 $\gamma$ B-2 per microliter, 0.0024 $\gamma$ G-1 per microliter, and 0.00050 $\gamma$ G-2 per microliter. On each thin-layer plate, two spots containing 0.5 and 1 microliter of the standard solution were used for visual estimation of the amount of aflatoxin present in the unknown. The developing solvent for wheat and grain sorghum extracts was 3% methanol in chloroform (8), and for oat extracts, 7% methanol in chloroform (6). Plates were developed 15 cm., dried, and inspected with a high-intensity long-wave ultraviolet Blak-Ray lamp or Chromato-Viewer to locate zones of fluorescence caused by aflatoxins B-1, B-2, G-1, and G-2.

**Samples for Duckling Tests**

The two wheat samples (SG) that were positive by TLC were combined for the duckling test to make possible a significant response at the low levels detected. The four SG and two grade 4 grain sorghum samples positive by TLC were combined for the test. Likewise, the three positive oat samples were combined. The sensitivity of

the test is 1 to 2 p.p.b. when samples of this size (2 to 4 kg.) were extracted.

Extracts dissolved in propylene glycol were administered to day old ducklings via stomach tube over a period of 4 days. Initial dosing preceded any food intake. Ducklings were sacrificed 6 days after first dose, and a sample of liver tissue was examined histologically.

#### Examination of Samples for Fungi

Small amounts of the ground grain samples were added to Petri dishes containing Czapek's solution agar with tetracycline (15 $\gamma$ per ml.) added to inhibit bacterial growth. Inoculated plates were incubated at 25°C. for a week or more and then read for occurrence of any colonies of the *Aspergillus flavus* series, the group of organisms that produce aflatoxin. No attempt was made to isolate individual colonies. Not all grain samples were examined, but those that were included both positive and negative aflatoxin samples. However, in most wheat, sorghum, and oat samples positive for aflatoxin, the whole sample had to be used for extraction for the duckling test and no examination could be made for the presence of *A. flavus*.

## RESULTS AND DISCUSSION

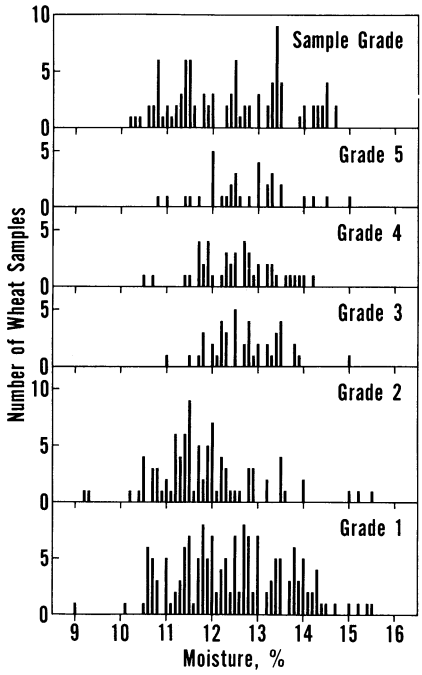
Extracts of wheat samples contained few extraneous fluorescing substances that interfered with TLC. Of the 531 wheat samples assayed, two appeared to contain 7 p.p.b. aflatoxin B-1 and 2 p.p.b. G-1 by TLC as shown in Table I. The biological test in ducklings, performed by the Wisconsin Alumni Research Foundation (WARF), indicated that trace amounts of aflatoxin could be present but that the test was probably negative. Grading information on these samples, both of which were in sample grade, is given in Table III. The percentages of moisture and damage of the wheat samples assayed are shown in Figs. 1 and 2, respectively. Comparisons of Table III and Figs. 1 and 2 reveal that the two wheat samples positive by TLC do not have high moisture content, but they do have a relatively high percentage of damage. Of course, the moisture level was determined at the time of grading and does not preclude a higher moisture level before grading, which would permit mold growth. Most grains are placed in SG on the basis of factors connected with mold growth, such as odor and heat-damage. Of the 125 SG samples tested, 66 had a musty odor and 16 a sour odor. Both samples that appeared to contain aflatoxin had odors.

Extracts of grain sorghum samples contained a fluorescing substance that tended not to separate from aflatoxins G-1 and G-2 unless thin-layer plates were developed long enough. Of the 533 grain sorghum samples tested, two in grade 4 and four in SG appeared by TLC to contain aflatoxin B-1 (3 to 19 p.p.b.) (Table II). Three of the apparently positive samples also appeared to contain G-1 (3 to 19 p.p.b.). The

TABLE III. WHEAT SAMPLES (SG) APPEARING TO CONTAIN AFLATOXIN BY TLC[a] (TOTAL NUMBER ASSAYED = 531)

| Sample Number | Moisture % | Total Damage % | Odor |
|---|---|---|---|
| F-1097 | 12 | 14 | Musty |
| F-1157 | 12 | 17 | Sour |

[a]TCL indicated 7 p.p.b. B-1 and 2 p.p.b. G-1; duckling test was inconclusive but probably negative on a composite of the two samples.



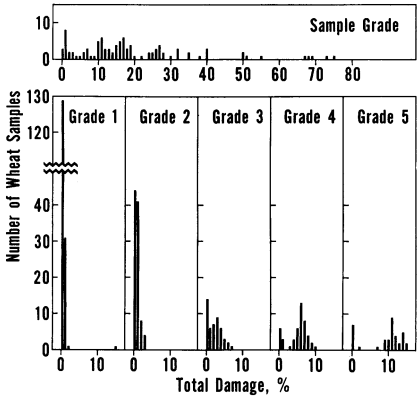




**Fig. 1(left). Moisture in wheat samples assayed for the presence of aflatoxin.**

**Fig. 2(right). Damage in wheat samples assayed for the presence of aflatoxin.**

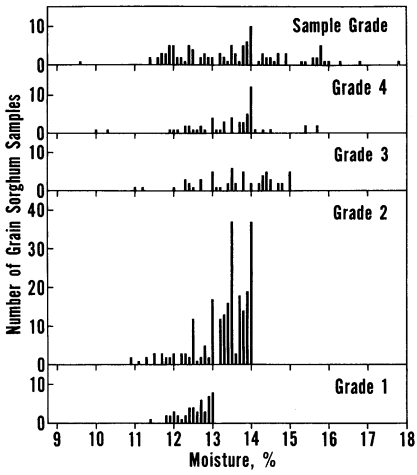
biological test in ducklings done at WARF did not show any evidence of aflatoxinlike activity in these six samples. Grading information on these is shown in Table IV. The percent moisture and percent damage of grain sorghum samples in various grades assayed are depicted in Figs. 3 and 4, respectively. The six samples that were positive by TLC did not have unusually high or low moisture contents, but tended to have relatively high percent damage. Extracts of highly damaged samples contained substances that made interpretation of TLC results more difficult. Of the 127 samples tested in SG, 78 had a musty odor and 31 a sour odor. The four samples that appeared to contain aflatoxin in SG had either a musty or sour odor.

TABLE IV. GRAIN SORGHUM SAMPLES APPEARING TO CONTAIN AFLATOXIN BY TLC[a] (TOTAL NUMBER ASSAYED = 533)

| Sample Number | Grade | Aflatoxin by TLC | | Grading Information | | |
|---|---|---|---|---|---|---|
| | | B-1 p.p.b. | G-1 p.p.b. | Moisture % | Total Damage % | Odor |
| F-988 | 4 | 3-6 | ND[b] | 14 | 4 | ... |
| F-1021 | 4 | 3-6 | ND | 13 | 12 | ... |
| F-4 | SG | 7-19 | 7-19 | 11 | 9 | Musty |
| F-1473 | SG | 3-6 | 3-6 | 12 | 7 | Musty |
| F-1474 | SG | 3-6 | 3-6 | 12 | 20 | Musty |
| F-2551 | SG | 7-19 | ND | 14 | 5 | Sour |

[a]Not positive by duckling test on composite of the six samples.

[b]Not detected.



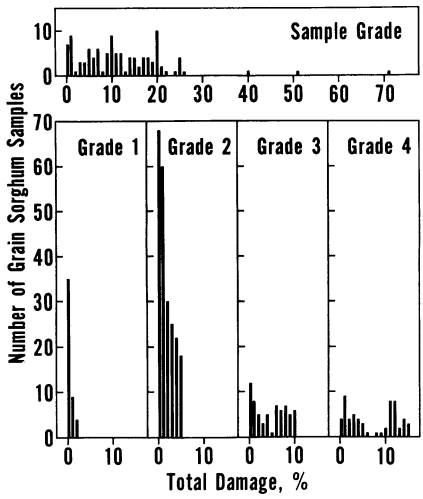




**Fig. 3(left). Moisture in grain sorghum samples assayed for the presence of aflatoxin.**

**Fig. 4(right). Damage in grain sorghum samples assayed for the presence of aflatoxin.**

About one-third of the oat samples analyzed had fluorescing substances that tended to behave like aflatoxins B-1 and G-1 in TLC. As discussed in a previous paper (10), the interfering fluorescing substances necessitated the use of 5 or 7% methanol in chloroform to develop thin-layer plates. Of the 304 samples assayed, two in grade 4 and one in SG appeared by TLC to contain 6 p.p.b. aflatoxin B-1 (Table II). These results were not completely confirmed biologically at WARF. The duckling test indicated that trace amounts of aflatoxin could be present, but it was more likely that the test was negative. Grading information on the three samples is shown in Table V. The percentages of moisture, foreign material, and sound cultivated oats in the different grades of oat samples analyzed are shown in Figs. 5, 6, and 7, respectively. Comparisons of Table V and Figs. 5, 6, and 7 indicate that the apparently positive oat samples did not have unusual moisture content, but they did have a relatively high percentage of total damage and a low percentage of sound cultivated oats.

Twenty-seven samples of sorghum, including one sample that appeared to contain aflatoxin by TLC, were examined for *A. flavus*. Some strains of the *A.*

TABLE V. OAT SAMPLES APPEARING TO CONTAIN AFLATOXIN (6 p.p.b. B-1) by TLC[a] (TOTAL NUMBER ASSAYED = 304)

| Sample Number | Grade | Grading Information | | |
|---|---|---|---|---|
| | | Moisture % | Sound Cultivated Oats % | Foreign Material % |
| F-3697 | 4 | 12 | 92 | 5.0 |
| F-3699 | 4 | 12 | 94 | 4.7 |
| F-3702 | SG | 12 | 89 | 7.0 |

[a]Duckling test was inconclusive, but probably negative on composite of the three samples.

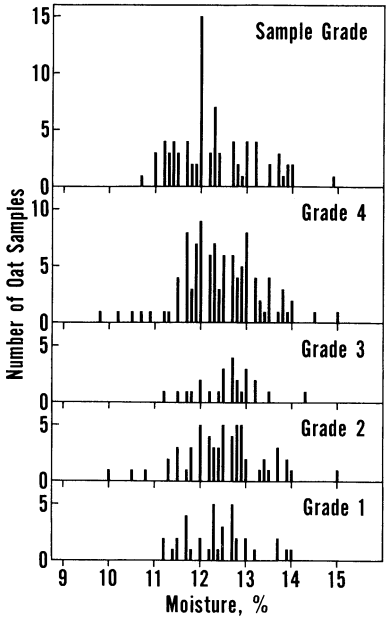



Fig. 5. Moisture in oat samples assayed for the presence of aflatoxin.

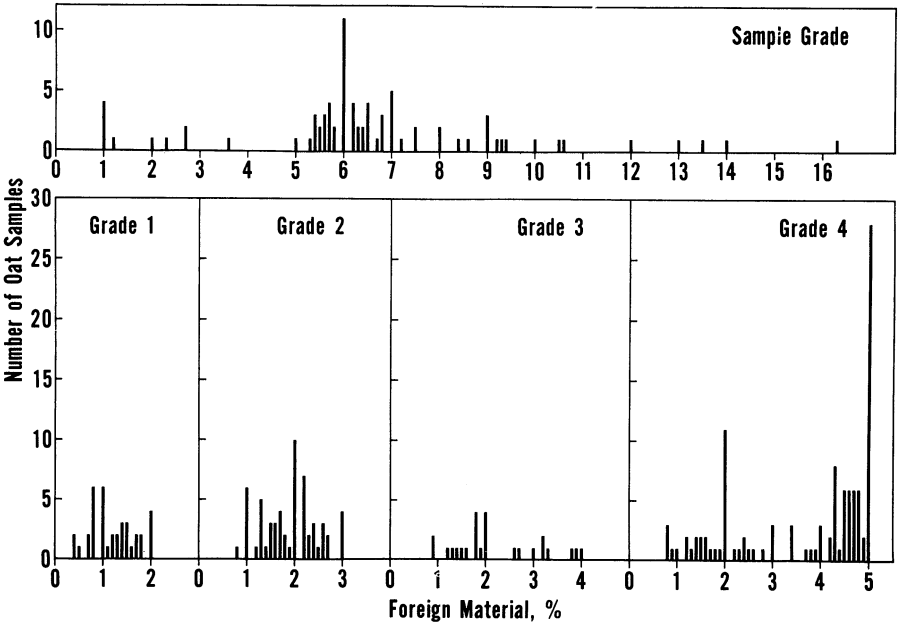



Fig. 6. Foreign material in oat samples assayed for the presence of aflatoxin.

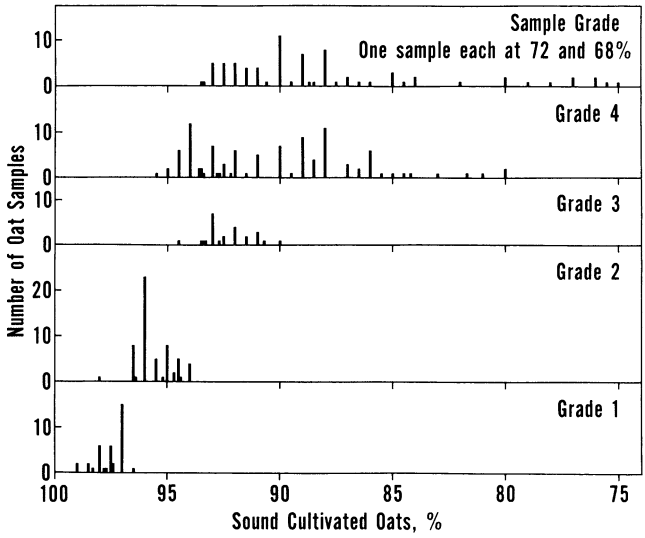



**Fig. 7. Percentage of sound cultivated oats in oat samples assayed for the presence of aflatoxin.**

*flavus* series produce aflatoxin. This mold was found in 11 of these, including the apparently positive sample. Forty-three samples of wheat and seven samples of oats were examined for the presence of *A. flavus*. Although none of the oat or wheat samples examined contained aflatoxin, seven samples of wheat and one of oats showed the occurrence of the mold. In wheat, several other groups of fungi were more common. *Fusarium* was present in 34 samples, yeasts in 16, and *Pencillium* in 18 of the 43 samples examined for *A. flavus*.

## CONCLUSION

Very low levels of what appeared to be aflatoxin (2 to 19 p.p.b.) were detected by TLC in a total of 9 out of 1,368 assayed samples of wheat, grain sorghum, and oats. Samples that were positive by TLC were in the poorest grades. None of the results obtained by TLC was definitely confirmed by the duckling test, the sensitivity of which was 1 to 2 p.p.b. In fact, according to the latter test, although it is possible that trace amounts could be present in wheat and oats, the samples were probably negative. No aflatoxin response was detected by the duckling test even in the grain sorghum samples that appeared to contain aflatoxin by TLC.

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