Aflatoxin in Corn

O.L. SHOTWELL, Northern Regional Research Center, ARS, USDA, Peoria, Illinois 61604

ABSTRACT AND SUMMARY

Low incidence and levels of aflatoxin were identified in corn of all grades grown in the Midwest in 1964, 1965, and 1967. Later surveys indicate that corn grown in southern regions is subject to invasion by *Aspergillus flavus* and subsequent aflatoxin formation. This mycotoxin is formed either in the field or in storage. In the field, such factors as insect damage and weather conditions probably influence aflatoxin formation. In storage, temperatures must be above 25 C and moisture levels above 16% if toxin is to form. Aflatoxin formed in a hot spot in stored corn in the Midwest when temperatures rose early in the summer and when the grain became wet because of leaks in the storage building. Analytical methods to detect and determine aflatoxin fall into three categories: presumptive tests indicating the presence of *A. flavus* and the possible occurrence of aflatoxin, rapid screening tests establishing the presence or absence of the toxin, and quantitative procedures determining toxin levels. Detoxification methods being studied include ammoniation and roasting. Ammoniated corn is being fed domestic animals to determine whether it has adverse effects and whether toxic compounds are transmitted in animal tissues.

The results of several surveys (1-3) have indicated a significant occurrence of aflatoxin in corn grown in various regions of the country. Actions by the Food and Drug Administration (4,5) in recalling corn meal allegedly tainted by aflatoxin and seizing corn caused concern in the corn industry. Extensive programs to monitor corn for aflatoxin have been initiated by both industry and governmental agencies.

Some of the first studies of aflatoxin naturally occurring in corn were made on 1964, 1965, and 1967 corn of all grades moving through commercial channels in the Midwest (6,7) (Table I). The results of these studies did not appear to be alarming because of the low incidence (2.1-2.3%) and low levels (3-37 ppb total) of toxin detected, mostly samples of poorest grades. The ratio of the samples in the poorest grades assayed to those in the better grades assayed was higher than the ratio of these moving through the market.

In 1965, analyses for aflatoxin B_1 were performed on official grain inspection samples taken from 230 cars of corn purchased for delivery to six different wet milling plants (8). Only four of the 230 examined contained aflatoxin in levels of 3 to 5 ppb (Table I). In the same study, lots of corn in three processing plants were sampled daily for a year and composited into 142 weekly samples. Aflatoxin B_1 was detected in six of the weekly samples at levels of 3 to 5 ppb. Persons in the corn wet-milling industry concluded that corn arriving at major markets is largely free of aflatoxin at the time sampled. Aflatoxin B_1 was found in spot samples from eight of 500 carloads of corn visually inspected by the corn wet millers. All of the eight carloads had visible mold damage, and two had already been rejected on this basis. The potential for aflatoxin contamination does exist in improperly handled corn.

A survey for aflatoxin in export corn in all grades except U.S. No. 1 collected from ten ports was made in 1968 and 1969 (9) (Table I). The incidence was 2.7% and levels of aflatoxin B_1 were from <6 to 25 ppb. Positive samples occurred in the better grades, but relatively few of the total samples assayed were in the poorest grades.

In a study of 1971 and 1972 preharvest corn in Indiana, no aflatoxin was detected in the 525 samples collected (10). However, eight of 163 combine-harvested corn samples obtained in 1972 from yield plots near Evansville, Indiana, were aflatoxin-positive.

Higher incidences and levels of aflatoxin have been observed in corn grown in the South. In 1969 and 1970, 60 corn samples from Alabama, North Carolina, South Carolina, Tennessee, and Virginia were analyzed for aflatoxin (1). There were 21 positive samples (Table I) and levels of the toxin were higher than those observed in previous surveys (6-9). Aflatoxin was detected in 31% of 1283 truckloads of white corn delivered from 77 loans in seven counties in southeastern Missouri (2) (Table I). Usually the corn from one loan came from one farm. All of the white corn was from the 1971 crop year and had been stored on the farm for 1 yr. Only 13% of the samples contained more than 20 ppb, the Food and Drug Administration (FDA) guideline. All of the truckloads of corn from one farm contained more than 100 ppb total aflatoxin. However, 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. In a survey of 1973 corn freshly harvested in northeastern South Carolina, 51% of 297 samples examined contained detectable aflatoxin (Table I) and 32% contained aflatoxin B_1 above 20 ppb (3).

The Grain Division, Agricultural Marketing Service, USDA, tested commercial lots of marketed corn for aflatoxin in 1972, 1973, and 1974 (11). In 1972, all samples (7913) submitted for grading to 18 field offices were inspected for the bright greenish-yellow (BGY) fluorescence associated with *Aspergillus flavus* and possibly aflatoxin to determine which samples to assay for aflatoxin. Samples were tested by the CB method approved in Official First Action by the Association of Official Analytical Chemists and the American Association of Cereal Chemists (12,13) and by a screening method using a Florisil minicolumn (14). Approximately 1.1% of the samples had detectable aflatoxin by the CB method, and 1.5% were positive by the screening method (minicolumn). Only 0.3% of the corn samples had levels of aflatoxin higher than 15 ppb. In 1973, the survey was limited to 2866 systematically selected from 17,245 samples submitted to 16 field offices. The samples were inspected for BGY, and BGY-positive samples were tested using the Florisil minicolumn technique. The same approach was used in 1974. Larger numbers of samples were collected from field offices where the incidence of aflatoxin is expected to be high. Taking into account the disproportionate sampling rates, the estimated incidence of aflatoxin in corn in the early part of 1973 was 7.1%. During the same period in 1974, the estimated incidence was 11%. The screening methods used did not differentiate among the different aflatoxins but measured the total aflatoxin contamination. At present, the Grain Division is screening for aflatoxin in three inspection offices: Omaha, NE; St. Louis, MO; and Norfolk, VA.

Samples of 1972 crop corn were collected by FDA (15) in the spring of 1973 in areas known to have problems with *Fusarium* contamination and damage. Samples were obtained from terminal elevators and stocks on hand at food processing plants. The corn was analyzed for aflatoxin as

well as for zearalenone and for rabbit skin irritants. Only five of 223 samples were found to contain aflatoxin and in low levels—trace to 10 ppb, but zearalenone was detected in 17% of the samples. These samples originated in the area known as the Corn Belt. In another FDA survey designed to determine the incidence of aflatoxin in corn from different geographical areas, samples were collected from farms and country elevators during April, May, and June 1974 (16) so that there would be a maximum storage period for the 1973 crop. The farms and elevators were located in counties producing more than 1 million bushels of corn. Aflatoxin contamination was most frequently encountered in the areas designated as Southeast-Appalachia (Table I) with an incidence of 34% in those areas.

In 1975, freshly harvested corn from a region of West Central and Central Iowa was examined for the field occurrence of aflatoxin (17). During the latter part of October, 214 samples of shelled corn were collected and dried to 13% moisture within 4 to 156 hr after harvest. Aflatoxin B_1 was detected in 16% of the 214 lots of corn and about 1% of all samples had over 20 ppb toxin. The highest level of toxin was 56 ppb B_1.

Aflatoxin G_1 does not occur naturally in corn as often as B_1 and has not been reported in the absence of B_1. In studies reporting the presence of individual aflatoxins (1,2,6,7,9), G_1 occurred in 7% of the aflatoxin-positive samples. Aflatoxin B_2 was identified in most of the samples containing higher levels of B_1 (>40 ppb) and in many of the other samples. Aflatoxin M_1 has been reported in stored white and yellow corn, freshly harvested yellow corn, and acid-treated stored yellow corn (18). The identity of M_1 was confirmed in highly contaminated kernels from the lots listed. Analysis of blended ground samples from eight lots of corn containing high levels of B_1 (210-3200 ppb) revealed low levels of M_1 (1-35 ppb). The levels of B_1 were so high that M_1 would contribute relatively little to the overall toxicity of a given lot of contaminated corn.

Results of one or two studies on the incidence of aflatoxin in corn in the United States cannot be used to evaluate the entire problem. A number of studies over an 11-yr period indicate that corn grown in certain regions is subject to aflatoxin contamination (Table I). However, the incidence of aflatoxin in corn grown in the Corn Belt or Midwest has been found to be 2-3% with very few lots of corn containing more than 20 ppb. Studies in 1969, 1970, 1971, and 1973 indicate that 13-32% of the analyzed samples of corn grown in the South contained 20 or more ppb aflatoxin. The sources and methods of collecting samples varied greatly between studies.

It would be impossible to monitor the entire United States corn crop for aflatoxin, because corn is marketed and used in many different ways. Some corn is fed on the farm where it is grown and would not come under state or federal regulations. Some is sold to elevators to be converted to feed or sold to larger feed companies. Feedlot operators may also contract with farmers for their corn crop. The corn milling and brewing industries are more likely to purchase corn grown under contract to ensure quality products. Much corn does not move in interstate commerce and would not be subject to federal regulations. Export corn does move through terminal elevators and must be graded by licensed inspectors, but the grading factors presently used are no indication of possible aflatoxin contamination (1,2,6,7,9).

Although the emphasis in this review is on corn grown in the United States, aflatoxin occurrence in corn is worldwide. Aflatoxin has been reported as a natural contaminant of corn sampled in Australia, France, Mozambique, Hong Kong, Phillipines, Thailand, and Uganda (19). The corn did not, however, necessarily originate in the country in which it was tested.

A number of studies have been made on the aflatoxin-producing capabilities of strains of *Aspergillus flavus*. One

TABLE I

Aflatoxin Incidence in Corn

Year	Agency surveying	Origin samples	Type samples and source	Number of samples assayed	Percent samples with indicated level aflatoxin (ppb)			
					ND[a]	<20	20-49	50-100	>100
1964-1965	NRRC	Corn Belt	Grain inspection AMS	1311	98	2	<0.1		
1965	Wet milling industry	Corn Belt	Corn received by industry	372	97	3			
1967	NRRC	Corn Belt	Grain inspection AMS	283	98	1	1		
1968-1969	NRRC	Export cargo	Grain inspection AMS	293	97	2	1		
1969-1970	NRRC	South	Grain inspection AMS	60	65	13	5	8	8
1971	NRRC	Southeast Missouri	Stored ASCS white corn	1283	68	18	7	4	2
1972	FDA	Corn Belt	Elevator and food processing plants	223	98	2			
1973	NRRC	South Carolina	Field–freshly harvested	297	49	19	17	8	7
1973	FDA	Corn Belt	Farm and country elevator	169	98	2			
1973	FDA	South[b]	Farm and country elevator	146	64	22	7	4	2
1975	NRRC	Iowa	Field–freshly harvested	214	83	15	1	<1	

[a]ND = not detected.

[b]Includes Southeast, Appalachia, Southeast MO, KY, TN, OK, TX, and CA.

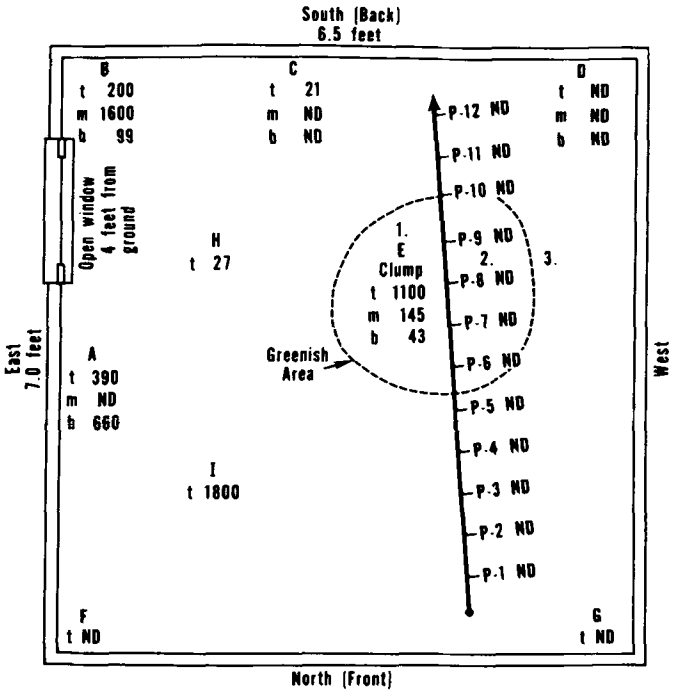

FIG. 1. Diagram of bin showing aflatoxin levels (ppb $B_1 + B_2$) found in samples taken at different locations on August 27, 1973. East and south were outside walls of building. t = top, m = middle, b = bottom, p = probe.

of the first, by Hesseltine et al. (20), compared the morphology of 67 strains with the aflatoxin and types of aflatoxin produced. Consistently, 11 of 14 strains of the *A. parasiticus* group formed aflatoxin G_1 as well as B_1, and M was identified in many extracts of the molded substrates. In the remaining three strains, very little B_1 was produced. A second group of five strains produced all the aflatoxins; these strains formed sclerotia on Czapek's agar. The third and largest group represented by *A. flavus* produced only aflatoxins B_1 and M—no G. Six *A. flavus* strains did not produce aflatoxins. One unusual strain isolated originally from walnuts sporulates poorly and produces many small sclerotia; it formed high levels of B_1, some M, and no G_1. The sources of these strains was the author identity (NRRL) Culture Collection.

In 1971, Richard and Cysewski reported the occurrence of aflatoxin-producing strains of *A. flavus* in stored corn (21). They isolated 15 strains of *A. flavus* from 12 of 25 samples of stored, moldy, shelled corn. Seven of 15 isolates produced aflatoxin on a rice substrate. The presence of aflatoxin was not confirmed in any of the original 25 samples. In a study of naturally contaminated stored white corn, four *A. flavus* isolates were selected from four different groups based on cultural differences (22). Two of the isolates produced aflatoxin.

In an intensive study of white corn stored under government loan and delivered in Southeastern Missouri in 1972, mold profiles were run on each of 1283 truckloads from 77 loans (23). Aflatoxin had been detected in 394 loads (2). All but seven of the aflatoxin-positive samples had strains of the *A. flavus* group. *A. parasiticus* was isolated only 15 times which probably explains why aflatoxin G_1 was identified in so few samples. No correlation seemed to exist between grade of corn and presence of *A. flavus*. The occurrence of *A. flavus* closely paralleled the aflatoxin content of corn samples when actual counts of *A. flavus* were made on corn from three loans. The study of the aflatoxin occurrence in 1973 corn at harvest in South Carolina included mold profiles of the samples collected and assayed (24). *A. flavus* was detected in 120 of the 152 aflatoxin-contaminated samples and in 59 of the 149 negative samples.

Samples of white corn were taken from discolored spots on the surface of stored grain in a southeast Missouri bin and examined for molds and aflatoxin (25). The percent incidence *A. flavus* on the six samples taken ranged from 23 to 80% compared to 10% incidence on a sample of non-discolored corn. The sample with 80% *A. flavus* infection contained 400 ppb aflatoxin. Aflatoxin was not detected in the other five samples of discolored corn (detection limit = 20 ppb).

A hot spot that developed *A. flavus* growth in a bin of 1972 yellow corn in Central Illinois during warm weather in June 1973 contained aflatoxin (26). Samples collected near the center of the spot that was defined by visible *A. flavus* sporulation contained 1000-1700 ppb aflatoxin. The location of the spot relative to an open widow indicated the moisture necessary for mold growth and aflatoxin formation could have come from rains blown through the window (Fig. 1). Corn collected at all depths from seven locations and probe samples taken under the spot were assayed for aflatoxin and zearalenone. Aflatoxin was not detected in samples collected farthest from the window. Zearalenone was detected in some of the samples collected, but it was not confined to any one part of the bin.

In an attempt to inhibit the growth of molds in stored wet grain, four lots of freshly harvested yellow dent corn with a moisture content of 27% were treated with ammonia (0.5%), ammonium isobutyrate (1.75%), isobutyric acid (1.5%), or propionic-acetic acid (1.2%) (27). The treated corn was stored in partially open wooden bins in Central Illinois. After ca. 30 days of storage, all treatments had secondary fungal growth. *Scopulariopsis brevicaulis* predominated on ammonia treated corn; species of *A. flavus, Monascus, Penicillium, Fusarium,* and *A. fumigatus* were in ammonium isobutyrate treated corn. *A. flavus* was the predominant mold infecting isobutyric acid and propionic-acetic acid treated corn late in storage. Low levels of aflatoxin were detected in samples taken from the adjacent isobutyric and propionic-acetic acid treated bins after 6 months (28). None of the samples taken a month later from moldy spots in the propionic-acetic acid bin had aflatoxin, although *A. flavus* was detected in 40% of them. Similar samples from the isobutyric acid bin had an incidence of 79% *A. flavus* and 57% aflatoxin (2-857 ppb). One kernel can be responsible for contaminating a bulk sample (29), so adjacent samples in a bin of corn can vary tremendously in aflatoxin content.

Because surveys of corn for aflatoxin before 1971 were made on corn moving in commercial channels, the time at which the mold invaded the grain and toxin production was initiated was not known. If aflatoxin forms only in storage, its formation could be prevented by proper handling—for example, by maintaining moisture levels below 15% and avoiding excessive breakage. There is evidence of aflatoxin formation in storage, but the possibility of toxin formation in the field could not be ignored because of a 1920 report by Taubenhaus of an invasion of field corn by *A. flavus* in Texas (30). The presence of *A. flavus* was reported in 1971 and 1972 preharvest corn in counties in southern Indiana (10). The incidence was higher in physically damaged corn. *A. flavus* was also identified in corn freshly harvested in 1972 in Missouri (31). There were significant differences between counties, and insect-damaged ears were more subject to *A. flavus* invasion. In South Carolina, ear corn before harvest (32) and freshly harvested corn (24) were found to contain *A. flavus*.

Studies conducted in 1971, 1972, and 1973 in essentially all of the corn-producing areas of the United States indicated that aflatoxin was formed in the field (33). Aflatoxin was found as a natural contaminant in corn samplings at all stages of development and maturity from the late milk stage until harvest. The highest incidence of aflatoxin was found in the warmer, more humid growing regions of the country. By a series of experiments using a

direct inoculation of *A. flavus* spores, the corn kernel was found to be most susceptible to contamination by aflatoxin during a 6-8 wk period during growth and maturity.

Freshly harvested corn (40-45% moisture) from the 1972 crop in southeastern Missouri was found to contain aflatoxin (34). The ears of corn had been taken 4-6 wk before the usual harvest to be examined for *A. flavus*-induced bright greenish yellow (BGY) fluorescence and for aflatoxin. The toxin was detected in about one-third of the Missouri fields. Aflatoxin contamination could have occurred in the field, but there was a possibility it could have occurred in the time between sample collection and drying of the corn to 12-14% moisture. In the following year, a study on field corn was planned to eliminate the possibility of aflatoxin formation after the samples were taken (3). Samples of freshly harvested corn (18% moisture average) were acquired in northeastern South Carolina and dried to 13% moisture at 90 C within 6 hr (mean time) after collection. Aflatoxin was detected in 51% of the samples. Levels of toxin were as high or higher than any encountered in previous surveys in southern regions (1,2)—32% of the samples contained 20 or more ppb aflatoxin and 7% more than 100 ppb. It was concluded that aflatoxin was formed in the field.

Although an association has been postulated between insect damage and *A. flavus* infection of corn and subsequent aflatoxin formation in the field, a definite cause-effect relationship has not been established. Insects that have been implicated with *A. flavus* invasion are rice weevils, corn earworms, corn borers, stink bugs, and mites. Taubenhaus (30) first reported that *A. flavus* infection of field corn was frequently associated with corn earworm damage. The incidence of *A. flavus* on kernels from ears damaged by earworms, borers, mites, and stinkbugs was found to be significantly higher (5.1-7.2%) than on those from undamaged ears (2.5%) (31). There has been some evidence that aflatoxin incidence could be related to earworm damage (34) or to European corn borer infestation (35). In the 1973 study in South Carolina on aflatoxin contamination in the field, insect damage was observed on 90% of the samples that had the BGY fluorescence associated with presence of *A. flavus* and possibly aflatoxin in corn (33). All aflatoxin-contaminated samples of freshly harvested South Carolina corn found by Lillehoj et al. (32) came from fields with preharvest ears damaged by insects. However, in samples of corn from one field, no correlation was found between percent insect damage on an ear basis and aflatoxin occurrence. A more extensive study by Hesseltine et al. (24) on the same samples indicated a relationship between rice weevil (*Sitophilus oryza* [L]) damage and *A. flavus* infection. Of 85 rice weevils collected from this corn, 78 were carrying *A. flavus* spores.

Individual kernels from six lots of the 1973 freshly harvested corn from South Carolina were inspected to find whether insect damage on a particular kernel or group of kernels could be associated with aflatoxin contamination (36). All six lots contained more than 20 ppb aflatoxin. The broken corn-foreign material (BCFM) was removed and kernels were separated into BGY fluorescing kernels; kernels with BGY fluorescence under the seed coat; insect-damaged kernels; damaged, cracked or discolored kernels; and outwardly sound kernels. Insect damage was that typical of the rice weevil, small round entry holes visible under 3X magnification and larger exit holes. The fractions were analyzed for their aflatoxin content.

The BGY fluorescing kernels and kernels with BGY fluorescence under the seed coat had insect damage typical of the rice weevil. More exit holes that are formed as the weevil emerges from the kernel were observed in the kernels with obvious fluorescence than in those with fluorescence under the seed coat. The BGY material contained the highest levels of aflatoxin B_1 (9000-27,000 μg/kg) and ac-

TABLE II

Distribution of Aflatoxin B_1 in Corn Fractions (% of Total)

Total aflatoxin B_1 in sample (ppb)	BCFM[a]		Fluorescent[b] material		Insect-damaged kernels[c]		Damaged, cracked, and discolored kernels		Outwardly sound kernels	
	Weight (%)	B_1 (%)	Weight (%)	B_1 (%)	Weight (%)	B_1 (%)	Weight (%)	B_1 (%)	Weight (%)	B_1 (%)
24	0.2	0.1	0.5	90	1.4	1.3	18	9	80	0
24	0.7	1.0	0.1	69	5.0	3.1	21	26	73	0
25	2.0	9.0	<0.1	41	6.0	10.0	18	39	74	0
38	2.0	8.0	0.1	54	2.0	14.0	13	16	82	9
47	2.0	24.0	0.2	35	1.0	15.0	12	25	85	0
209	0.5	3.0	0.3	54	28.0	27.0	5	3	67	13

[a]Broken corn-foreign material.
[b]Bright greenish-yellow (BGY) fluorescence under 365 nm ultraviolet light including BGY fluorescence under seed coat.
[c]Round cavities and small holes indicating cavities (visible under 3X magnification) typical of rice weevil damage.

counted for much of the contamination in the six lots of corn (Table II). Outwardly sound kernels accounted for most of the weight and very little of the contamination. In fact, aflatoxin could not be detected in outwardly sound fractions from four of the six lots. The aflatoxin was found for the most part in fractions definitely having rice weevil damage. Part of the broken corn-foreign material (BCFM) could come from insect-damaged kernels. Also insect damage could be obscured in the damaged, cracked, and discolored kernels.

The distribution of aflatoxin in the separated fractions from the six lots of corn indicates a relationship between rice weevil damage and aflatoxin contamination in freshly harvested corn from South Carolina (Table II). The variation in aflatoxin levels between fields in South Carolina was found to be significant (3). Studies of rice weevil infestations also reveal a definite relationship between fields in percentage of weevil-damaged kernels (37). The *A. flavus* inoculum for producing aflatoxin may be carried by the rice weevil coming from stored corn. If the rice weevil is one of the vectors in aflatoxin contamination in the field, methods of prevention of toxin formation are available. There are significant differences between corn hybrids in susceptibility to rice weevils. Also, insecticides might be effective in controlling aflatoxin formation.

Weather conditions that cause stress or damage to the maturing corn could cause it to be vulnerable to *A. flavus* invasion. In southwestern Iowa, some 1975 corn was found to contain aflatoxin; adverse weather conditions in August might have been responsible (38).

At this time, the presence of aflatoxin in some corn is unavoidable because of mycotoxin formation in the field. Therefore, it is even more important to have available rapid reliable procedures for determining its occurrence and contamination levels. A number of analytical methods have been devised to detect and determine aflatoxin in corn. There is a need for improvement in existing methods and development of more rapid methods.

The reliability of any analysis depends on the sample analyzed. The difficulty in obtaining a sample for analysis begins when the original lot of corn is sampled. Aflatoxin does not occur uniformly throughout contaminated corn. An early study on the distribution of aflatoxin in two bins of contaminated corn indicated that toxin levels could vary from 0 to 376 ppb in 200-g samples depending on location of the corn in a bin in which the mean toxin level was 21 ppb (39). The mean level of aflatoxin in the second bin was 15 ppb and toxin levels varied from 0 to 332 ppb in 200-g samples depending on place in the bin where the sample was taken. In a lot of corn, mold growth and aflatoxin formation can be localized in a small area (26). Highly contaminated kernels can even be adjacent to kernels in which aflatoxin is not detected. In a clump of kernels held together by *A. flavus* mycelia, two adjacent kernels had 22,000 ppb and nondetectable aflatoxin. In two samples (4.8 kg) containing 24 ppb aflatoxin B_1, 1 kernel in 2000 kernels (500-600 g) accounted for over two-thirds of the B_1. It has been suggested that a 10-lb sample (containing about 16,000 kernels) should be taken either with a continuous sampler or with a probe from all parts of a lot of corn.

After a lot of corn is sampled, a subsample suitable for analysis must be prepared. The usual practice has been to grind the entire lot sample to pass a No. 14 sieve and split the sample sequentially to obtain a 1-2 kg portion. The 1-2 kg portion is ground to completely pass a No. 20 sieve and then blended thoroughly in a planetary mixer or twin-shell blender. Analytical subsamples (50 g) are taken from the finely ground corn. The larger the sample that is extracted, the more reliable the results. One approach to analyzing larger samples has been to prepare water slurries of kilogram-size samples in a 1-gal blender and extract 100 g aliquots of the slurries (40).

Methods for aflatoxin analysis of corn can be divided into three categories: (a) rapid presumptive test by visual inspection under ultraviolet light (365 nm) to locate lots of corn that may contaminated with aflatoxin; (b) rapid screening procedures to determine the presence or absence of the toxin in a lot, but not the level; (c) finally, quantitative methods that are rather lengthy to measure actual amounts in corn.

The BGY fluorescence under ultraviolet light (365 nm) associated with the presence of *A. flavus* or possibly aflatoxin is the basis of the presumptive test for aflatoxin in corn (22,41,42). The same type of fluorescence is observed in cottonseed and may result from the action of plant enzymes on kojic acid formed by *A. flavus* concurrently with aflatoxin (43). A number of individual kernels showing BGY fluorescence either externally or after crushing and kernels showing no fluorescence from contaminated lots of corn have been analyzed for aflatoxin (26,29,36). Practically all of the kernels with BGY fluorescence examined so far have contained aflatoxin; kernels without BGY fluorescence did not. However, in a lot containing kernels or fragments with BGY fluorescence, fluorescing kernels may contribute so little aflatoxin that when the entire lot is ground and blended it would not contain detectable or appreciable levels of toxin. Therefore, the presence of BGY-fluorescent material is only a presumptive indication of toxin. Because some kernels contain BGY fluorescence under the seedcoat, corn should be cracked before inspection under ultraviolet light.

Inoculation of harvested corn with *A. parasiticus* and *A. flavus* led to the production of BGY fluorescence and aflatoxin in the laboratory (44,45). Others have observed the formation of BGY fluorescence and sometimes aflatoxin after inoculation of ears of corn in the field with *A. parasiticus* or *A. flavus* strains (33,46-48).

Inspection of stored white corn under loan for BGY fluorescence led to ca. 2-2.5 times as many BGY positive samples for analysis as were actually found to contain measureable amounts (over 1-3 ppb) of aflatoxin (42). These results are similar to results obtained in other studies (Table III). Use of the fluorescence test to minimize the analytical workload by assaying only lots with BGY fluorescence would have more value at low levels of incidence encountered in the Corn Belt rather than at the high level observed in other areas. Informal reports from corn mills using both the fluorescence test and quantitative analysis indicate that at low levels of incidence the relation between fluorescence and measurable aflatoxin is about the same as that observed in corn with higher levels. Therefore, the 2.1-2.7% incidence observed in surveys of market corn (6-9) would give rise to about 4-5% of samples to be analyzed if samples were selected on basis of BGY fluorescence.

Although inspection for BGY fluorescence in corn is useful for identifying corn that should be tested further for toxin, the actual presence or absence of aflatoxin can be determined by several screening procedures. The ideal screening procedure would take a minimum of time and equipment and give reliable results in the assay of a number of commodities. A number of methods have been developed in efforts to devise the ideal screening procedure. One of the more useful approaches has utilized minicolumns to detect aflatoxin in partially purified extracts of corn (Table IV). These screening methods all include extraction, precipitation, concentration of the filtrate from the precipitation, and chromatography on minicolumns.

Holaday first developed the minicolumn screening procedure for peanuts (49) and has recently published an improved procedure (50) that is applicable to number of commodities including corn. A method that was developed originally by Pons for cottonseeds has been changed somewhat for application to corn and a variety of commodities (51). A very sensitive method designed by Velasco (52) for detecting aflatoxin in cottonseed utilizing Florisil mini-

TABLE III

Relation Between BGY[a] Fluorescence and Measurable Aflatoxin in Corn

Source of corn	Percentage of total lots of corn samples			
	BGY positive	Detectable[b] aflatoxin	Aflatoxin >15-20 ppb	Reference
Stored white corn, Southeastern Missouri	75 (cracked)[c]	31 (CB)[d]	13	42
Yellow field corn, South Carolina	73 (cracked)	51 (CB)	32	3
Corn in U.S. commercial markets	3.2 (whole)	1.5 (minicolumn)	0.5	11
Corn in U.S. commercial markets	13 (cracked)	8 (minicolumn)	2.5	11

[a]Bright greenish-yellow fluorescence under ultraviolet light (365 nm).

[b]Detection limit is 1-5 ppb by either method.

[c]Corn kernels were cracked before inspection.

[d]AOAC-AACC Official First Action Method.

columns has been applied to corn (53) and was slightly modified for grain inspection offices (11). A fluorometer has been used to measure amounts of aflatoxin in Florisil columns (54). Results obtained by use of the fluorometer to measure aflatoxin on minicolumns are being compared to those obtained by the CB method (12,13).

Modifications in methods have been made for specific purposes. One procedure was developed by Shannon et al. to be used in an elevator where rapid analysis using nontoxic reagents was needed (55). It was used as 1283 truckloads of white corn were delivered at an elevator to segregate aflatoxin-contaminated corn from good corn. Corn was analyzed as the trucks were unloaded to determine where to place it. The method was found to be effective in segregating toxin-containing corn (56). A purification step was added by Romer to the Velasco method for application to mixed feeds (57). A procedure incorporating steps from several methods is used to detect very low levels of aflatoxin in corn and corn-derived products (58). The procedure was used at an elevator in southeastern Missouri as bins of corn were unloaded to decide which lots contained less than 20 ppb aflatoxin and could be sold for animal feed (59).

Detection limits of minicolumns range from 1 to 10 ppb aflatoxin. The analysis takes from 15 to 90 min excluding sample preparation. In fact, it can take as long to prepare the sample as it does to do the analysis. Three minicolumn methods have been approved in official first action by the Association of Official Analytical Chemists and the American Association of Cereal Chemists: the Velasco method (60,61), the Shannon method (62,63), and the Romer method (64,65).

Screening methods to detect aflatoxin in corn using thin layer chromatography (TLC) have been used with success in laboratories equipped for TLC. An abbreviation of the approved quantitative method (12,13) has been developed in which the column chromatography step is elininated (66). The chloroform extract of corn is evaporated and the residual oil is used to spot the TLC plate, which is first developed with ether to remove oily interferences to the solvent front. After drying, the plate is developed a second time with acetone:chloroform (1:9 v/v). If the proper combination of a specified type of silica gel for TLC plates and grade of ether is used, aflatoxins can be separated and identified from interfering lipids in one rapid development with ether (67). In one screening method, extracts of corn are purified by a series of liquid-liquid transfers in separatory funnels before obtaining a residue to be dissolved for TLC (68).

Because more than one mycotoxin can occur in a lot of corn, it is advantageous to have multitoxin screening methods. Two methods have been developed for the simultaneous detection of aflatoxin and zearalenone in corn (69,70). Methods have been developed for the simultaneous detection of the following groups of mycotoxins: zearalenone, aflatoxin, and ochratoxin (71); aflatoxin, ochratoxin, and sterigmatocystin (72); zearalenone, aflatoxins, ochratoxin, sterigmatocystin, and patulin (73); aflatoxins, ochratoxins, zearalenone, penicillic acid, and citrinin (74); and aflatoxins, ochratoxins, zearalenone, and sterigmatocystin (75).

The method commonly designated as the CB (Contaminants Branch) method has been approved in official action by the AOAC and AACC (12,13) for the determination of actual levels of aflatoxin in corn. The collaborative study (76) that led to approval established that the CB method could be applied to corn and soybeans as well as to the peanut products for which it was developed. Three different methods of aflatoxin analysis were used on spiked and naturally contaminated corn, and the results were compared (77) (Tables V and VI). The methods were the CB method, the Pons method for cottonseed (78), and the BF (Best Foods) method for peanuts (79). The Pons method gave good recoveries and results at levels of 50 ppb aflatoxin and below. Recoveries obtained by the BF method were very low at all levels of contamination.

Some of the first studies on detoxification of aflatoxin in corn included methods already in use by agriculture and industry. High-moisture corn can be preserved by ensiling, and it is known that aflatoxin B_1 can be converted by acid catalysis to B_2a, the water adduct that has 1/200 the toxicity of B_1. It was also possible that microflora in the silage fermentation would degrade the mycotoxin. Aflatoxin-contaminated, high-moisture corn was ensiled in detoxification attempts (80). The moldy corn did undergo a lactic fermentation, but insufficient acid was produced to form aflatoxin B_2a from the B_1 present. The silage fermentation was not effective in removing or degrading aflatoxin B_1.

Although the corn the wet milling industry was purchasing was almost entirely free of aflatoxin, they decided a study should be made on the fate of aflatoxin during the milling of contaminated corn (81). The study showed that aflatoxin was found primarily in the steepwater (39-42%) and fiber (30-38%) with the remainder in gluten (14-17%) and germ (6-10%). Concentrations of aflatoxin increased four- to five-fold in steepwater, 2.5- to 3-fold in fiber, 1- to 1.5-fold in gluten, and onefold in germ. The starch fraction used for food contains only ca. 1% of the toxin originally in corn.

Because of early reports that aflatoxin might be concentrated in the BCFM, studies were made on the distribution of aflatoxin in ten naturally contaminated lots of corn (29). It was found that aflatoxin was predominate in the BCFM of only one of the ten lots. Although levels of aflatoxin were high, the BCFM accounted for very little of the weight. Levels of aflatoxin in fractions of kernels outwardly sound by hand selection from the ten lots varied from 3 to 250 ppb. The average level of contamination in the ten outwardly sound kernel fractions was 56 ppb aflatoxin. This study indicated that, in general, aflatoxin contamination in corn could not be lowered to safe levels by physical separations. Because BGY fluorescence was found to be fully hidden under the seed coat of some kernels, electronic

TABLE IV

Screening Method Using Minicolumns

Step	Holaday (50)[a]	Pons (51)	Velasco (52)	Shannon (55)	Barabolek (58)
Extraction	Methanol:water (80:20 v/v)	Acetonitrile:water (80:20 v/v)	Acetone:water (85:15 (v/v)	Acetone:water (85:15 v/v)	Acetone:water (85:15 v/v)
Precipitation	Zinc acetate	Lead acetate	Ferric gel	Ammonium sulfate	Ammonium sulfate
Concentration	Liquid-liquid transfer	Liquid-liquid transfer	Evaporation	Liquid-liquid transfer	Evaporation
Minicolumn adsorbant	Florisil	Silica gel	Florisil	Silica gel	Florisil
Minicolumn development	Descending	Ascending	Descending	Ascending	Descending
Detection limit	2	10	5	10	1

[a]Reference number given in parentheses.

TABLE V

Comparison of Aflatoxin Analysis by Various Methods

Type of corn	Percent of added aflatoxin B_1 found by				Percent of added G_1 found by				Percent of added total aflatoxin found by			
	B_1 added, μg/kg	CB[a]	BF[a]	Pons[a]	G_1 added, μg/kg	CB	BF	Pons	Aflatoxin added, μg/kg	CB	BF	Pons
White	5	120[b]	20	100	4	150	50	92	11	142	43	100
Yellow	10	113	27	87	8	109	62	92	22	117	48	88
White	15	130	27	112	12	142	55	92	33	118	46	105
Yellow	20	107	22	84	16	94	67	65	44	104	48	78
Yellow	25		28		20		70		55		53	
White	30	104		92	24	112		71	66	113		82
Yellow	50	93	30	53	40	95	52	40	110	89	44	50
Yellow	100	84		45	80	65		57	220	74		46
Average recovery, %		107	26	82		109	59	73		108	47	78
Coefficient variation, %		17.1	27.2	31.1		28.9	21.4	32.0		25.3	19.2	31.0

[a]Method used. CB = Contaminants Branch, BF = Best Foods

[b]Each recovery run in triplicate.

TABLE VI

Comparisons of Results from Determination of Aflatoxins (μg/kg) in Naturally Contaminated Corn

Corn	CB method[a]					BF method[b]				Pons method[c]		
	B_1	B_2	G_1	G_2	Total	B_1	B_2	G_1	Total	B_1	B_2	Total
White	14[d]	1	2	1	18	6	<1	1	<8	8	<1	<9
Yellow	8	1	5	1	15	ND[e]	ND	ND	ND	3	<1	<4
White	9	1	3	1	14	2	<1	1	<4	11	1	12
Yellow	6	1	2	<1	<10	1	<1	1	<3	8	1	9
White	71	10	ND	ND	81	16	2	2	20	52	8	60
Yellow	21	2	ND	ND	23	6	1	1	8	24	2	26
Yellow	4	ND	ND	ND	4	1	ND	ND	1	5	1	6

[a]CB = Contaminants Branch

[b]Aflatoxin G_2 was not detected in any sample using BF (Best Foods) method.

[c]Aflatoxins G_1 and G_2 were not detected in any sample using Pons method.

[d]All values given in Table are average of triplicate assays.

[e]ND = not detected.

devices could not be used for decontamination.

An intensive study was made on aflatoxin-contaminated corn of physical separation methods ordinarily used to clean corn (82). It was found that dry cleaning, wet cleaning, density separation, and preferential fragmentation could not be used to decontaminate corn satisfactorily.

The dry milling of three lots of naturally contaminated corn—one yellow and two white—was studied to determine the distribution of aflatoxin in product fractions (83). Aflatoxin level was always lowest in the grits. Concentrations of B_1 in the grits from the three lots of corn containing 13, 160, and 510 ppb were trace, 12, and 50 ppb, respectively. Proportion of aflatoxin in the prime product mix (i.e., grits, low-fat meal, and low-fat flour) amounted to only 7 to 10% of total quantity of B_1 in all products. Aflatoxin level in the germ, hull, or degermer fines was always highest, with concentrations exceeding that of the corn milled.

After an aflatoxin-contaminated lot of corn has been identified, it must be destroyed. Methods of detoxification are being developed. A great deal of successful effort has been placed on the process of ammoniating cottonseed to remove aflatoxin (84,85). Levels of aflatoxin (316-545 ppb) in contaminated cottonseed meal were reduced to nondetected to 4 ppb by ammoniation under pressures of 45-50 psig and at 235-250 F.

Ammoniation under atmospheric pressure has resulted in detoxification of aflatoxin-containing corn. The ammoniation of 11 different lots of corn containing 30-1200 ppb aflatoxin has been studied in the laboratory (O.L. Brekke, A.J. Peplinski, and E.B. Lancaster, unpublished information). Conditions such as moisture, temperature, and time were investigated. Levels could be reduced to nondetectable aflatoxin (detection limit of assay was 1-3 ppb). Tests on ducklings, broiler chicks, and trout indicated that ammoniation could be used to detoxify contaminated corn. Because transfer of freshly ammoniated corn into storage bins resulted in unacceptably high losses of ammonia, a recycle gas method was investigated in the laboratory (O.L. Brekke and A.C. Stringfellow, unpublished information). Variabilities studied included ammonia addition level, moisture content of corn, gas velocity, recycle time, and initial aflatoxin level in the corn. Based on these laboratory studies, conditions were selected to be used in a field study in which gaseous ammonia treatment of a 1100-bushel lot of aflatoxin-contaminated corn reduced the aflatoxin level from 750 ppb to less than 20 ppb (O.L. Brekke, unpublished information). The corn was ammoniated outdoors in a sealed 18-ft diameter metal bin of the type used for drying and storing corn on the farm. The detoxified corn is to be used in feeding tests on swine and laying hens to obtain FDA clearance for the gaseous ammonia decontamination process. Ammoniation under different conditions has resulted in the detoxification of aflatoxin-containing corn. Animal tests are being conducted to determine the effect of feeding corn that has been ammoniated to remove aflatoxin.

Another approach that may be successful in detoxifying contaminated corn is roasting—a process that has been studied on peanuts and pecans (86-88). Commercial roasters are available to improve the nutritive value of grains such as corn. Roasting aflatoxin-contaminated corn is being studied using an analytical method developed for determining the toxin in roasted corn (89).

Storage of high-moisture corn pressents problems with mold growth and possible aflatoxin formation. A study of preservatives used on high-moisture corn revealed that either 2% ammonia or 1% propionic acid was effective in inhibiting *A. parasiticus* or *A. flavus* growth and aflatoxin production (90).

The best solution to the aflatoxin problem in corn would be prevention by the use of resistant hybrids, if such exist or could be developed. A study of aflatoxin production in *A. flavus*-inoculated ears of corn grown at several locations revealed differences in suceptibility to toxin formation in corn types (47). Fewer toxin-contaminated ears were found in the double cross hybrid adapted to the southern United States than in the single cross that is widely grown throughout the country. A second study on aflatoxin production in several corn hybrids grown in South Carolina and Florida further indicated differences in susceptibility to aflatoxin formation (48). Five single cross hybrids grown in the South exhibited lower levels of aflatoxin than a single cross hybrid adapted to the Corn Belt. Both the *A. flavus* inoculated and uninoculated corn was extensively contaminated with aflatoxin. These studies are being continued.

REFERENCES

1. Shotwell, O.L., C.W. Hesseltine, and M.L. Goulden, Cereal Sci. Today 18:192 (1973).
2. Shotwell, O.L., W.F. Kwolek, M.L. Goulden, L.K. Jackson, and C.W. Hesseltine, Cereal Chem. 52:373 (1975).
3. Lillehoj, E.B., W.F. Kwolek, G.M. Shannon, O.L. Shotwell, and C.W. Hesseltine, Ibid. 52:603 (1975).
4. Anon., Southwest Miller 50:26 (1971).
5. Anon., FDA Consumer 7:32 (October 1973).
6. Shotwell, O.L., C.W. Hesseltine, H.R. Burmeister, W.F. Kwolek, G.M. Shannon, and H.H. Hall, Cereal Chem. 46:454 (1969).
7. Shotwell, O.L., C.W. Hesseltine, M.L. Goulden, and E.E. Vandegraft, Ibid. 47:700 (1970).
8. Watson, S.A., and K.R. Yahl, Cereal Sci. Today 16:153 (1971).
9. Shotwell, O.L., C.W. Hesseltine, E.E. Vandegraft, and M.L. Goulden, Ibid. 16:266 (1971).
10. Rambo, G.W., J. Tuite, and R.W. Caldwell, Cereal Chem. 51:848 (1974).
11. Hunt, W. Haward, R.C. Semper, and E.B. Liebe, Ibid. 53:227 (1976).
12. Ass. Offic. Anal. Chem., Washington, D.C., Official Methods of Analysis, 12th ed., Sec. 26.037, 26.014-26.019 (1975).
13. Methods of the Am. Assoc. Cereal Chem.: 1972 Revisions to AACC Approved Methods 45-05:1-9.
14. Ass. Offic. Anal. Chem., Washington, D.C., Official Methods of Analysis, 12th Ed., Sec. 26.044-26.047 (1975).
15. Eppley, R.M., L. Stoloff, M.W. Trucksess, and C.W. Chung, J. Ass. Offic. Anal. Chem. 56:632 (1974).
16. Stoloff, L., S. Henry, and O.J. Frances, Jr., Ibid. 59:118 (1976).
17. Lillehoj, E.B., D.I. Fennell, and W.F. Kwolek, Aflatoxin and *Aspergillus flavus* in 1975 Corn at Harvest from Specific Regions of Iowa, Abstract ASM meeting, Atlantic City, NJ, May 2-7, 1976.
18. Shotwell, O.L., M.L. Goulden, and C.W. Hesseltine, J. Agr. Food Chem. 24:683 (1976).
19. Hesseltine, C.W., Mycopathol. Mycol. Appl. 53:141 (1974).
20. Hesseltine, C.W., O.L. Shotwell, M.L. Smith, J.J. Ellis, E.E. Vandegraft, and G.M. Shannon. Proc. U.S.-Jpn. Conf. Toxic Micro-Org., 1968, U.S. Gov. Printing Office, Washington, DC, pp. 202-210 (Conf. Proc.) 1970.
21. Richard, J.L., and S.J. Cysewski, Mycopathol. Mycol. Appl. 44:221 (1971).
22. Fennell, D.I., R.J. Bothast, E.B. Lillehoj, and R.E. Peterson, Cereal Chem. 50:404 (1973).
23. Hesseltine, C.W., R.J. Bothast, and O.L. Shotwell, Mycologia 47:392 (1975).
24. Hesseltine, C.W., O.L. Shotwell, W.F. Kwolek, E.B. Lillehoj, W.K. Jackson, and R.J. Bothast, Mycologia. 68:341 (1976).
25. Lillehoj, E.B., and D.I. Fennell, J. Stored Prod. Res. 11:47 (1975).
26. Shotwell, O.L., M.L. Goulden, R.J. Bothast, and C.W. Hesseltine, Cereal Chem. 52:687 (1975).
27. Bothast, R.J., G.E. Adams, E.E. Hatfield, and E.B. Lancaster, J. Dairy Sci. 58:386 (1975).
28. Bothast, R.J., M.L. Goulden, O.L. Shotwell, and C.W. Hesseltine, J. Stored Prod. Res. 12:177 (1976)
29. Shotwell, O.L., M.L. Goulden, and C.W. Hesseltine, Cereal Chem. 51:492 (1974).
30. Taubenhaus, J.J., Texas Agr. Exp. Sta. Bull. 270 (1920).
31. Fennell, D.I., E.B. Lillehoj, and W.F. Kwolek, Cereal Chem. 52:314 (1975).
32. Lillehoj, E.B., W.F. Kwolek, R.E. Peterson, O.L. Shotwell, and C.W. Hesseltine, Ibid. 53:505 (1976).
33. Anderson, H.A., E.W. Nehring, and W.R. Wichser, J. Agr. Food Chem. 23:775 (1975).
34. Lillehoj, E.B., W.F. Kwolek, D.I. Fennell, and M.S. Milburn, Cereal Chem. 52:403 (1975).
35. Widstrom, N.W., A.N. Sparks, E.B. Lillehoj, and W.F. Kwolek, J. Econ. Entomol. 68:855 (1975).
36. Shotwell, O.L., M.L. Goulden, E.B. Lillehoj, W.F. Kwolek, and

C.W. Hesseltine, Abstract 60th Annual meeting, American Association of Cereal Chemists, Kansas City, MO, October 26-30, 1975.
37. Blickenstaff, C.C., J. Econ. Entomol. 53:745 (1960).
38. Muhn, D., Des Moines Register, October 24, 1975.
39. Johnson, R.M., W.T. Greenaway, and C. Golumbic, Cereal Sci. Today 14:26 (1969).
40. Velasco, J., and S.L. Morris, J. Agr. Food Chem. 24:86 (1976).
41. Shotwell, O.L., M.L. Goulden, and C.W. Hesseltine, Cereal Chem. 49:458 (1972).
42. Shotwell, O.L., M.L. Goulden, A.M. Jepson, W.F. Kwolek, and C.W. Hesseltine, Ibid. 52:670 (1975).
43. Marsh, P.B., M.E. Simpson, R.J. Ferretti, G.V. Merola, J. Donoso, G.O. Craig, M.W. Trucksess, and P.S. Work, J. Agr. Food Chem. 17:468 (1969).
44. Bothast, R.J., and C.W. Hesseltine, Appl. Microbiol. 30:337 (1975).
45. Rambo, G.W., J. Tuite, and G.L. Zachariah, Cereal Chem. 52:757 (1975).
46. Rambo, G.W., J. Tuite, and P. Crane, Phytopathology 64:797 (1974).
47. Lillehoj, E.B., W.F. Kwolek, E.E. Vandegraft, M.S. Zuber, O.H. Calvert, N. Widstrom, M.C. Futrell, and A.J. Bockholt, Crop Sci. 15:267 (1975).
48. Lillehoj, E.B., W.F. Kwolek, A. Manviller, J.A. DuRant, J.C. LaPrade, E.S. Horner, J. Reid, and M.S. Zuber, Crop Sci. 16: 483 (1976).
49. Holaday, C.E., JAOCS 45:680 (1968).
50. Holaday, C.E., J. Agr. Food Chem. 23:1134 (1975).
51. Pons, Jr., W.A., A.F. Cucullu, A.O. Franz, Jr., L.S. Lee, and L.A. Goldblatt, J. Ass. Offic. Anal. Chem. 56:803 (1973).
52. Velasco, J., JAOCS 49:141 (1972).
53. Shotwell, O.L., and R.D. Stubblefield, J. Ass. Offic. Anal. Chem. 56:808 (1973).
54. Velasco, J., Ibid. 58:757 (1975).
55. Shannon, G.M., R.D. Stubblefield, and O.L. Shotwell, Ibid. 56:1024 (1973).
56. Shotwell, O.L., G.M. Shannon, and C.W. Hesseltine, Cereal Chem. 52:381 (1975).
57. Romer, T.R., J. Ass. Offic. Anal. Chem. 58:500 (1975).
58. Barabolak, R., C.R. Colburn, and R.J. Smith, Ibid. 57:764 (1974).
59. Shotwell, O.L., M.L. Goulden, and G.M. Shannon. J. Ass. Offic. Anal. Chem. In press.
60. Ass. Offic. Anal. Chem., Washington, DC, Official Methods of Analysis, 12th Ed., Sec. 26.044-26.047 (1975).
61. Methods of Am. Assoc. Cereal Chem.: 1975 Revisions to AACC Approved Methods 45-15:1-3 (1975).
62. Ass. Offic. Anal. Chem., Washington, DC, Official Methods of Analysis, 12th Ed., Sec. 26.040-26.043 (1975).
63. Methods of Am. Assoc. Cereal Chem: 1975 Revisions to AACC Approved Methods 45—16:1-3 (1975).
64. Ass. Offic. Anal. Chem., Washington, DC, Official Methods of Analysis, 12th Ed., Chapter 26, Natural Poisons Handbook, Sec. 26A01-26A08 (1975).
65. Methods Am. Assoc. Cereal Chem: 1975 Revisions to AACC Approved Methods 45-10:1-4 (1975).
66. Dantzman, J., and L. Stoloff, J. Ass. Offic. Anal. Chem. 55:139 (1972).
67. Trucksess, M., Ibid. 57:1220 (1974).
68. Seitz, L.M., and H.E. Mohr, Cereal Chem. 51:487 (1974).
69. Thomas, F., R.M. Eppley, and M.W. Trucksess, J. Ass. Offic. Anal. Chem. 58:114 (1975).
70. Seitz, L.M., and H.E. Mohr, Ibid. 59:106 (1976).
71. Eppley, R.M., Ibid. 51:74 (1968).
72. Vorster, L. J., Analyst 94:136 (1969).
73. Stoloff, L., S. Nesheim, L. Yin, J.V. Rodricks, M. Stack, and A.D. Campbell, J. Ass. Offic. Anal. Chem. 54:91 (1971).
74. Wilson, D.M., W.H. Tabor, and M.W. Trucksess, Ibid. 59:125 (1976).
75. Hagan, S.N., and W.H. Tietjen, Ibid. 58:620 (1975).
76. Shotwell, O.L., and R.D. Stubblefield, Ibid. 55:781 (1972).
77. Shotwell, O.L., and M.L. Goulden, J. Ass. Offic. Anal. Chem. (In press).
78. Ass. Offic. Anal. Chem., Washington, DC, Official Methods of Analysis, 12th Ed., Sec. 26.048-26.056 (1975).
79. Ass. Offic. Anal. Chem., Washington, DC, Ibid. Sec. 26.020-26.024 (1975).
80. Lindenfelser, L.A., and A. Ciegler, J. Agr. Food Chem. 18:640 (1970).
81. Yahl, K.R., S.A. Watson, R.J. Smith, and R. Barabolok, Cereal Chem. 48:385 (1971).
82. Brekke, O.L., A.J. Peplinski, and E.L. Griffin, Ibid. 52:198 (1975).
83. Brekke, O.L., A.J. Peplinski, G.E.N. Nelson, and E.L. Griffin, Jr., Ibid. 205 (1975).
84. Gardner, Jr., H.K., S.P. Koltun, F.G. Dollear, and E.T. Rayner, JAOCS 48:70 (1971).
85. McKinney, J.D., G.C. Cavanaugh, J.T. Bell, A.S. Hoversland, D.M. Nelson, J. Pearson, and R.J. Selkirk, Ibid. 50:79 (1973).
86. Lee, L.S., A.F. Cucullu, A.O. Frantz, Jr., and W.A. Pons, Jr., J. Agr. Food Chem. 17:451 (1969).
87. Waltking, A.E., J. Ass. Offic. Anal. Chem. 54:533 (1971).
88. Escher, F.E., P.E. Kochler, and J.C. Ayres, J. Food Sci. 38:889 (1973).
89. Shannon, G.M., and O.L. Shotwell, J. Ass. Offic. Anal. Chem. 58:743 (1975).
90. Vandegraft, E.E., C.W. Hesseltine, and O.L. Shotwell, Cereal Chem. 52:79 (1975).

[Received July 6, 1976]