Control and Removal of Aflatoxin[1]

LEO A. GOLDBLATT, Southern Regional Research Laboratory,[2]
New Orleans, Louisiana 70119

ABSTRACT

The best approach to contain the problem of aflatoxin is prevention and enough is now known about prevention to reduce contamination drastically. Guidelines for preventing mycotoxins in farm commodities have been suggested by the U.S. Department of Agriculture. Moisture is the single most important parameter and prompt drying to safe levels is essential for control of toxigenic molds. Foreign matter and damaged seed should be removed. Provision of clean, dry, adequately cooled and ventilated storage is important and good sanitation is essential to minimize mold contamination during storage and processing. Genetic approaches which may result in resistance to elaboration of aflatoxins are under investigation. When aflatoxin is found in a sample of oilseeds the contamination generally resides in only a small proportion of the kernels, commonly less than 1%. Sorting or separation can concentrate the vast majority of aflatoxin-contaminated kernels into relatively small fractions and only a small loss is incurred as a result of their removal. Aflatoxin is frequently found deeply imbedded within individual kernels so removal by simple washing does not seem feasible. However, extraction with polar solvents such as alcohols and ketones to achieve essentially complete removal of aflatoxins appears technically feasible. Heat is relatively ineffective for destruction of aflatoxin although normal roasting, as of peanuts for the preparation of peanut butter, results in considerable reduction in aflatoxin content. Treatment with *Flavobacterium aurantiacum* removes aflatoxin and may be useful for beverages. Oxidizing agents readily destroy aflatoxin, and treatment with hydrogen peroxide may be useful. Treatment of defatted oilseed meals with ammonia can reduce aflatoxin content to very low or undetectable levels with only moderate damage to protein quality.

INTRODUCTION

The purpose of this paper is to discuss methods for prevention of the development of aflatoxin and for the removal or destruction of aflatoxin from contaminated materials when efforts at prevention fail.

Aflatoxins are metabolites of several widely distributed toxin producing strains of fungi (molds), especially of *Aspergillus flavus* or *Aspergillus parasiticus*. *A. flavus* is probably about as ubiquitous an organism as can be encountered and production of aflatoxin is not characteristic of just a few uncommon strains of *A. flavus*; many strains of the fungus produce aflatoxin, so the potential for production of aflatoxins is worldwide. Aflatoxins may be found in agricultural commodities before and at harvest or may be produced during storage after harvest. Although most attention has been given to the occurrence of aflatoxins in oilseeds, especially peanuts, cottonseed and coconut (copra), the presence of aflatoxin has been detected at biologically significant levels in a wide spectrum of agricultural commodities and probably none should be considered immune. As of now at least 10 different mold metabolites have been designated aflatoxins. These are all closely related chemical compounds which are derivatives of furofuromethoxycoumarin. The different aflatoxins may be produced in widely varying amounts and proportions depending upon the genetic capabilities of the fungus, the substrate and the environmental conditions. Two aflatoxins, B_1 and G_1, are the ones most commonly found in agricultural products. When any of the others are found they are accompanied by much larger proportions of aflatoxin B_1 or G_1, or both. Accordingly, research efforts have been centered very largely on these two toxins but, as indicated by Keyl and Booth (1), aflatoxin M_1 may be found in the milk of lactating animals ingesting relatively large amounts of aflatoxin B_1 in their diet.

What can be done in the way of control? Unquestionably the best approach is prevention, and the first step is recognition and awareness that the threat exists. A major problem is motivation of untrained personnel at all stages of culture, harvest, transportation, storage and processing. The Agricultural Research Service of the U.S. Department of Agriculture has issued a special report entitled "Preventing Mold-Caused Toxins in Farm Commodities" (2) which should be very helpful. The importance of good farm management practices is emphasized. The report notes that mold prevention should begin with proper planting and growing of the crop. This includes use of just enough fertilizer and irrigation water for optimum growth and may call for special procedures such as, in the case of cotton, skip row planting and bottom defoliation when the lower bolls are mature. Harvesting at maturity is generally recommended and equipment should be properly adjusted and operated to avoid damaging the crop and picking up large amounts of leaves and dirt along with the crop. The farmers' responsibility is to take the proper measures so that commodities are neither damaged by mold in the field nor harvested and stored in a condition favorable to molding before they reach marketing channels. Special attention should be given to detecting and diverting from food and feed channels any aflatoxin containing lots as early as possible in the marketing process. The Department has issued a separate Bulletin directed at mold control in high moisture corn (3) and a Bulletin directed toward mold control in peanuts is planned.

High moisture is the single most important condition contributing to mold. Although species of the *A. flavus* series have been characterized as storage molds, this can be misleading as they may occur prior to harvest. Prompt drying to the level recommended for safe storage as well as maintenance at that level is essential. Recommended safe moisture levels will vary with the crop and with other conditions of storage but it should be emphasized that they refer to all of the seeds in a lot and not just the average moisture content. Consequently adequate aeration is also essential as otherwise significant differences in temperature may build up, causing moisture to concentrate to damaging levels in colder spots. Mold produces moisture as it grows; so once fungal growth has started in one excessively wet kernel, the moisture content of the immediately adjacent kernels also increases and fungal proliferation may proceed, regardless of average moisture content. Warm temperature also promotes molding. Other conditions favorable to development of mold, and thus to be avoided, are damage from insects and presence of foreign matter. Provision of clean,

[1]One of 21 papers presented at the Symposium, "Oilseed Processors Challenged by World Protein Need," ISF-AOCS World Congress, Chicago, September 1970.

[2]So. Market. Nutr. Res. Div., ARS, USDA.

TABLE I

Removal of Aflatoxin-Contaminated Peanuts by Sequential Sorting

Fractions	% of Sample	Aflatoxin content, ppb (μg/kg)
Whole sample	100	150
Rejected by mechanical screening	0.8	2,500
Rejected by electric eye	14.9	30
Rejected by manual sorting	0.7	150-375
Final product	83.7	Neg. (<3)

dry, adequately cooled and ventilated storage is important, and good sanitation is essential to minimize mold contamination during storage and processing.

Genetic approaches which may result in resistance to elaboration of aflatoxin are under investigation. Development of commercially acceptable varieties that would resist toxin producing molds or that would completely inhibit production of aflatoxin by them would be an ideal solution. This is a long term approach and no lines have yet been released. The report (4) that impermeable seed coat cottonseed, so-called "hard seed," have less tendency to allow *A. flavus* to grow and produce aflatoxins than do seed without this "hard coat" trait indicates that the possibility exists for control of mold invasion, and hence of production of aflatoxin in cottonseed by genetic means. A varietal difference in the production of aflatoxins in peanuts inoculated with a toxigenic strain of *A. flavus* has been reported (5). Of sixty different varieties of peanuts screened, aflatoxin was produced in all but one, designated as U.S. No. 26, a variety with white testa. Kulkarni et al. (6) reported that the red-seeded Asiriya Mwitunde was "tolerant to aflatoxin." However, Doupnik (7) recently reported that of two aflatoxigenic isolates of *A. flavus* he used to test for inhibition of aflatoxin production on two cultivars of Asiriya Mwitunde (P.I. 268893 and 295170) and a U.S. 26 cultivar (P.I. 246388) both produced substantial levels of aflatoxin. The differences in results were ascribed as possibly owing to certain cultural practices or some unique characteristic(s) of the *A. flavus* isolates used, or both. A striking difference was reported (8) among three varieties (Starr Spanish, Early Runner and Florigiant) in susceptibility to aflatoxin contamination. Research directed toward identifying peanut lines with resistance to toxin producing molds is continuing.

The use of antifungal agents to control fungal infestation has been the subject of much study but according to Golumbic and Kulik (9) "Thus far, there appears to be no fungicidal treatment that has been successful for large-scale application despite the considerable effort that has been exerted in this area." Quite recently it was reported (10) that a mixture containing propionic acid as the active ingredient, added to mixed feeds at 1 and 2 lb. per ton, resulted in a significant reduction in mold counts.

Damage and contamination with aflatoxin may occur despite the most strenuous efforts directed at prevention. Then other approaches must be considered, fully recognizing that they are to be applied only if preventive measures have failed and not as an alternative to good cultural and storage practice. Detoxification of aflatoxins in foods and feeds is the subject of a recent review (11). Two approaches will be discussed here: (1) removal, and that includes removal by mechanical means and by use of solvents, and (2) destruction.

It has been the experience of workers at the Southern Marketing and Nutrition Research Division of USDA, as well as other investigators, that the vast majority of the aflatoxin in contaminated cottonseed and peanuts generally resides in a relatively small number of seeds. This affords an exceptional opportunity for effectively yet economically reducing the aflatoxin content by mechanical removal of those few seeds or kernels that may have become contaminated. Physical separation methods are being used successfully in the peanut industry. The peanut industry practiced culling to select only high quality peanuts for food products long before the discovery of aflatoxin. This culling is typically accomplished by screening at shelling plants, by removing discolored kernels by hand sorting on picking tables, by various mechanical sorters or by electronic sorting devices which examine each kernel separately and either pass or reject it on the basis of color when scanned by a photoelectric cell. With the recognition of the aflatoxin problem this culling of peanuts for food products has been intensified. Segregation after splitting peanut kernels may be necessary to completely eliminate aflatoxin resulting from mold invasion of the interior not manifest on the outer surface.

Data for removal of aflatoxin-contaminated peanuts by sequential sorting were reported by Wogan (12) (Table I). Pattinson et al. (13) reported results on color sorting sized, raw, Natal and Virginia type peanuts from Tanzania. Levels of 5 ppb or less of aflatoxin were achieved in nearly 90% of the accept fractions. Kensler and Natoli (14) cited two examples illustrating the effectiveness of high speed photoelectric sorting. In each case a single pass and a single setting of the sorter was used. The hot air roasted peanuts were sampled as the stream entered the sorter and as the two streams emerged. In one example the input stream contained 25 ppb aflatoxin while the accept stream had less than 5 ppb and the reject stream contained 350 ppb. In the other example cited the input stream contained 20 ppb; the accept stream contained 5 ppb and the reject stream 100 ppb. A subsequent hand sorting of the accept stream would further reduce the aflatoxin content. A final hand sorting procedure is recommended in the Voluntary Code of Good Practices for Purchasing, Handling, Storage, Processing and Testing of Peanuts (15), originally adopted in 1964 and revised and updated at least once each year since then. The peanut industry has done an outstandingly effective job in safeguarding from the danger of aflatoxin, and this has had the salutary effect of providing the American consumer with the highest quality peanuts and peanut products in history.

Similar improvements have been accomplished for brazil nuts. Duggan (16) reported that "during the past season, less than 1% of the tonnage exceeded FDA guidelines on the original analysis, which is substantially below that in previous years." Lots exceeding current guidelines on the original analysis are detained and are reconditioned by the importer under FDA supervision, "by removal of the objectionable nuts."

Aflatoxin-contaminated cottonseed cannot be distinguished from uncontaminated cottonseed in ordinary light, but several laboratories have reported a high correlation between a greenish yellow fluorescence in fuzzy seed and aflatoxin content. Ashworth et al. (17) proposed using this property to separate contaminated cottonseed. They reported on results obtained with a machine used in the almond industry to separate nicked almonds from sound seeds. When applied to cottonseed, individual seeds are held by vacuum to finger-like holding ferrules on a revolving presentation wheel. Each seed passes separately through the

ultraviolet lamp house. If it fluoresces it is deflected into the reject product compartment by an air jet that is activated by the emitted fluorescent light; if it does not fluoresce it is released to fall into the accepted product compartment by automatic release, at the appropriate point, of the vacuum that holds the seed to the ferrule. The data of Ashworth et al. show that using this equipment it is feasible to sort out from seed lots fluorescent gin run seeds but not mechanically delinted seed. The fluorescent seeds accounted for only a small proportion of the total seeds in any seed lot tested, about 0.3% on the average, but the machine was never less than 90% effective in removing the fluorescent seed in gin run fuzzy seed. Although electric eye sorting probably does not offer a practical solution to the problem of removing aflatoxin contaminated seeds, it may be useful in identifying contaminated lots of cottonseed and permit early segregation.

A projection device to separate infested from noninfested grain kernels has been described by Katz et al. (18). Holzenthal et al. (19) showed that cottonseed can be separated by projection devices into fractions of different quality, e.g., different in content of free fatty acids, light immature and decayed seeds, or foreign matter. The highest quality seed are projected farthest because of their ballistic characteristics. Dollear and Gardner (20) reported the results of two tests to separate aflatoxin-contaminated cottonseed by projection. In one trial a lot of delinted cottonseed was used that contained 40-80 ppb of aflatoxin. More than 63% of the aflatoxin was concentrated in about 6% of the seed, and about 85% was concentrated in 25% of the seed. Although the separation was not as good as might be desired, it did indicate potential for separation of aflatoxin-contaminated cottonseed by this procedure. Unfortunately in another test with another lot of seed that contained about 750 ppb of aflatoxin, little or no segregation was achieved. A suggested explanation for the difference in results is that two different types of aflatoxin contamination were involved. In the first lot the contamination probably occurred before harvest; in the second lot the seed may have been subjected to biological heating in the storage pile, and thus the mold and the resulting aflatoxin had spread throughout the whole mass of seed.

As far as is known, reconditioning of other agricultural commodities by removal of aflatoxin-contaminated kernels has not been attempted.

REMOVAL BY EXTRACTION

The feasibility of removing aflatoxin by a simple washing or "laundering" operation, i.e., washing whole peanut kernels with water or dilute alkali, has been the object of much discussion. The aflatoxin contents of different parts of peanut kernels containing large amounts of aflatoxin have been determined (21) and high concentrations of aflatoxin were found deeply embedded in individual peanut kernels. Accordingly even if simple laundering of whole or split peanuts removed superficial aflatoxin, effective removal would not be realized.

On the other hand good potential for removing aflatoxin is offered by extraction with solvents during the processing of various oilseeds, such as cottonseed and peanuts, to oil and meal. Current processing practices, either mechanical expression or extraction with commercial hexane, leave in the defatted meal the vast majority of any aflatoxin that may be present in the seed. The crude oils obtained may contain various amounts of aflatoxin depending upon the raw material used and the conditions used in processing. However Parker and Melnick (22) established quite conclusively that conventional processing of cottonseed and corn oil, deliberately prepared to contain high levels of aflatoxin (more than 100 ppb of B_1), removes essentially all of the aflatoxin. Refining with aqueous sodium hydroxide removed the greatest part of the aflatoxin and, after bleaching with AOCS Official Bleaching Earth, the oils contained less than 1 ppb of aflatoxin. It would be reasonable to infer that aflatoxin would also be removed from other oils by conventional refining, water washing and bleaching. Accordingly there is no real problem with aflatoxin contamination in edible oils in the U.S., because we do not use crude oils in edible products or in the home except for olive oil. However that is not the case in some foreign countries where much peanut oil and other oils are used in the crude state. In fact the crude oil may be preferred not only because it is cheaper but also because of its flavor.

Several possibilities exist for removal of aflatoxins from oilseed meals. These include (1) extraction of aflatoxin from meals with appropriate solvents, (2) simultaneous solvent extraction of oil and aflatoxin from flaked meats or prepress cake, and (3) selective extraction of essentially all of the aflatoxin and free fatty acids and some of the water soluble components but negligible quantities of neutral oil and protein leaving an essentially full fat product, free of aflatoxin, available for conventional oil extraction.

Sreenivasamurthy et al. (23) reported that an aqueous solution of calcium chloride extracted 80% of the toxin but only 6% of the protein in three extractions from a standard test peanut meal. They found also that, in the preparation of protein isolates, addition of calcium chloride at neutral pH instead of acid precipitation at isoelectric pH prevented nearly 80% of the toxin from going with the protein fraction.

A solvent system of acetone, hexane and water was found to remove aflatoxin readily and quantitatively from ground peanuts or peanut meal while removing relatively little extraneous material other than oil (24). This solvent mixture has been proposed as a practical system in processing peanuts to oil and aflatoxin free meal (25). Aqueous acetone may also be used as a selective solvent. Gardner et al. (26) recently reported on the use of these solvents for extraction of aflatoxin. They concluded: "Aflatoxin can be removed or significantly reduced in cottonseed and peanut meals by extracting with a tertiary solvent system of 54% acetone, 44% hexane and 2% water (w/w) or a binary solvent system of 90% acetone and 10% water (w/w). The tertiary solvent system simultaneously removes oil and aflatoxin from prepressed cake containing 12-15% oil, resulting in residual lipids content of approximately 1%." Both solvent systems offer feasible methods for reducing the aflatoxin in cottonseed and peanuts to a level of 30 ppb (μg/kg) or below. The presence of some water appears to facilitate removal of aflatoxin or the release of aflatoxin into the extracting solvent.

Mixtures of hexane-methanol, hexane-ethanol, hexane-ethanol-water and hexane-acetone-water were evaluated by Vorster (27). Promising results were reported to have been obtained with all of these solvents using a Soxhlet apparatus on a laboratory scale, as in each case the percentage of aflatoxin in the meal was reduced considerably. Greatest reduction was obtained with hexane-acetone-water and hexane-methanol.

Extraction of cottonseed flakes with acetone containing 25-30% water has been reported to remove essentially all the gossypol, most of the free fatty acids, half the raffinose and negligible quantities of neutral oil and protein yet to be effective for the removal of aflatoxin (28). Reduction of aflatoxin by 96-98% was reported. The residual, full fat product, now essentially free of any aflatoxins that may have been present, can then be processed for oil removal by any conventional means. This solvent system is also potentially applicable to peanuts and other oilseeds.

Removal of aflatoxin from oilseed meals by aqueous alcohols has been studied, and Rayner and Dollear (29) reported that extraction with 80% aqueous isopropanol at 60 C resulted in complete removal of aflatoxin from cot-

tonseed and peanut meals in six passes. Extraction with the isopropanol-water azeotrope (87.7% isopropanol, w/w) was less effective, resulting in only about 80% reduction of aflatoxin content. Good reduction in aflatoxin content of contaminated cottonseed and peanut meals was also obtained by extraction with 95% ethanol (30).

Thus a variety of polar solvents are effective for the removal of aflatoxins. Several solvent systems may be quite suitable for use in the preparation of meals or flours, especially solvent systems based on alcohols such as isopropanol or ethanol, or on acetone. Such solvent systems have the advantage that under suitable conditions they can remove essentially all the aflatoxins with little likelihood of forming from the aflatoxins products having adverse physiological activity and without appreciable reduction of protein content or of its nutritional quality. On the other hand there is the cost of additional processing, the need for special extraction and solvent recovery equipment, the loss of some water soluble components of the residual meals (chiefly carbohydrates) and provision for their disposal. Also in the case of acetone containing solvents, adverse effects on flavor have sometimes been noted, presumably as a result of reaction with acetone condensation products such as diacetone alcohol and mesityl oxide.

DESTRUCTION OF AFLATOXIN

Finally there remains the possibility of degrading, destroying or otherwise inactivating the aflatoxins, i.e., by heat, or by chemical or biological methods. Any such treatment must, of course, not only inactivate the aflatoxins but also retain the nutritive value of the material processed and leave no deleterious residues.

The possibility of destroying aflatoxin by radiation is a subject of frequent speculation. Feuell (31) reported that peanut meal contained in a thin polyethylene bag exposed to gamma rays at a dosage of 2.5 megarads showed no apparent difference from an unirradiated control meal when examined by a fluorescence test. In feeding trials with ducklings, birds ingesting either the irradiated or control meals died within a few days and showed severe liver lesions, both meals giving indistinguishable results. Instability of aflatoxins on exposure to ultraviolet has been reported by Pons et al. (32) and more recently by Andrellos et al. (33). The latter workers reported that the principal photoproduct developed from aflatoxin B_1 is significantly less toxic than the parent aflatoxin. Ultraviolet irradiation of oilseed meals to destroy aflatoxin has been patented (34). On the other hand Feuell (31) reported no apparent change, as judged by the fluorescence test, when peanut meal was exposed in a thin layer 10 cm beneath an ultraviolet lamp for eight hours. When suitable extracts were dosed to ducklings they died in a few days, severe liver lesions being present.

Aflatoxin is very stable to heat. A detailed study of the effect of heat and moisture on aflatoxins in oilseed meals was made by Mann et al. (35). Treatments at 60 C and 80 C resulted in very little reduction of aflatoxin but definite reduction was obtained at 100 C. The effect was enhanced by increasing times of heating and by increasing moisture contents. About 80% reduction in aflatoxin was achieved by heating for 2 hr at 100 C at 20% moisture. They concluded that, although increased moisture content results in increased destruction of aflatoxin, heat and moisture alone do not supply a very satisfactory method to inactivate or remove aflatoxin from oilseed meals.

Comparison of the aflatoxin contents of individual raw half peanut kernels and of the corresponding half kernels after dry roasting under conditions simulating those that might be used for the production of peanut butter indicated an average reduction after roasting of about 70% for aflatoxin B_1 and 45% for aflatoxin B_2 (36). In a subsequent study of the effect of dry and oil roasting of small batches of peanuts containing graded levels of aflatoxin ranging from 130-6,300 ppb total aflatoxin, the average reduction in aflatoxin content ranged from 45-83% depending on roasting conditions and the level of toxin in the raw kernels. An over-all reduction of 65% in B_1 and 62% in G_1 for oil roasting, and 69% in B_1 and 67% in G_1 for dry roasting was reported (37). Thus an additional margin of safety is afforded for such roasted products.

Ciegler et al. (38) investigated microbial detoxifications of aflatoxin. Approximately 1000 organisms representing yeasts, molds, mold spores, bacteria, actinomycetes and algae were screened for their ability to destroy or transform aflatoxins B_1 and G_1. Of the organisms tested only one of the bacteria, a flavobacterium, *Flavobacterium aurantiacum* (NRRL B-184), removed aflatoxin from solution. Aflatoxin-contaminated milk, corn oil, peanut butter, peanuts and corn were completely detoxified, and contaminated soybean was partially detoxified by cells of *F. aurantiacum*. Duckling assays showed that detoxification of aflatoxin solutions by B-184 was complete with no new toxic products being formed. A process for microbiological decontamination of aflatoxin-contaminated edibles has been patented by Ciegler and Lillehoj (39).

A host of chemicals have been screened as reagents for the destruction of aflatoxin (20,31,40,41,42). Trager and Stoloff (41) reported on a number of reactions, chiefly with oxidizing agents, of possible utility in detoxification procedures. The reactions appear to be primarily addition and oxidation involving the olefinic double bond of the terminal furan ring, and oxidation involving the phenol formed on opening of the lactone ring. Benzoyl peroxide and osmium tetroxide reacted with aflatoxins B_1 and G_1 but not with B_2 and G_2, but NaOCl, $KMnO_4$, $NaBO_3$, $Ce(NH_4)_2(SO_4)_3$ and 3% H_2O_2 + $NaBO_2$ (1 + 1) reacted with B_1, B_2, G_1 and G_2. Detoxification after contact with gaseous chlorine, chlorine dioxide and nitrogen dioxide, and after treatment with 5% NaOCl solution was confirmed by bioassay.

Feuell (31) investigated the effect, on a highly contaminated peanut meal, of various chemicals including aqueous sodium hydroxide, hydrochloric acid, gaseous propylene oxide, sulfur dioxide and chlorine. For preliminary tests suitable extracts from a highly contaminated peanut meal were treated in dilute ethanol and the treatments evaluated by the duckling test. Under the conditions used the effective treatments were hydrochloric acid, chlorine and, with reservations, sulfur dioxide as judged by the absence of liver lesions; the ducklings receiving the extracts from the treatment with sulfur dioxide did not develop liver lesions but they all died. The result with alkali was doubtful, as severe liver lesions were present although the treatment resulted in an increased equivalent mean lethal dose. Propylene oxide was apparently without effect. Treatments with chlorine and sulfur dioxide were extended to peanut meal and these treatments reduced the toxicity of the meal to ducklings but did not prevent liver lesions. Feuell has warned that chlorinated fats and proteins can be highly toxic.

A systematic study of detoxification of peanut meal by hydrogen peroxide was reported by Sreenivasamurthy et al. (42). Suspension of a highly contaminated, defatted peanut meal in water to give 10% solids, adjustment to a pH of 9.5 with strong alkali, and treatment with an equal weight of 6% hydrogen peroxide at 80 C for 30 min resulted in destruction of 97% of the aflatoxin present (90 ppm). Duckling tests indicated that the hydrogen peroxide treatment effectively destroys the toxicity. It was concluded that since the treated and untreated meals had essentially the same protein efficiency ratio (PER), 2.52 compared to 2.42, and as the treated product does not have a residual smell or taste, the treatment can be applied to edible pea-

nut meal. Because of the high dilutions (10% solids) used, such treatments with hydrogen peroxide might be attractive for detoxification of protein beverages, isolates, and milk.

Results of an extensive screening of various chemicals applied to a contaminated peanut meal were reported by Dollear and Gardner (20). More detailed studies were conducted with the most promising reagents: ammonia, methylamine, sodium hydroxide and ozone. Some typical results obtained with a peanut meal containing 70 ppb of aflatoxin B_1, 30 ppb of aflatoxin B_2 and 11 ppb of aflatoxin G_1, or a total of 111 ppb, may be summarized as follows: when this meal was cooked with sodium hydroxide (2% of the weight of the meal) and 30% moisture for 2 hr at 100 C, only a trace of B_1 remained, no B_2 was discernible, and four ppb of G_1 was detected. Under the same conditions but using methylamine (1.25% of the weight of the meal) resulted in destruction of all but barely detectable traces of each of the aflatoxins. Treatment of the meal with ammonia gas was also effective in reducing the aflatoxin content to barely detectable traces. The conditions used were 15 min, 15% moisture, 163 F, 43 pounds per square inch gage (psig) and 6.7% concentration of ammonia. The nitrogen content of the meal was increased by 0.46% as a result of the treatment. Detoxification with ammonia has been patented by Masri et al. (43). Treatment of the meal with ozone was somewhat less effective, the ozone being more effective for inactivating aflatoxin B_1 than the more saturated aflatoxin B_2. After treatment of 800 g of the peanut meal containing 30% moisture for 2 hr at 100 C with a stream of ozone gas at the rate of 1.5 g/hr, 5 ppb aflatoxin B_1 10 ppb aflatoxin B_2 and 3 ppb aflatoxin G_1 remained (44).

The peanut meals that originally contained 111 ppb total aflatoxin and had been treated with sodium hydroxide, ammonia, methylamine and ozone, and a meal which was extracted with aqueous acetone were subjected to various chemical analyses and to biological evaluation (45). None of the treated peanut meals nor the 90% acetone-extracted peanut meal produced any observable liver damage in the duckling test but some of the treatments appeared to result in some reduction in protein quality as judged both by physico-chemical characteristics and PER. The nitrogen solubility and available lysine content were reduced by some of the treatments. The greatest changes resulted from treatments with sodium hydroxide and ozone—for lysine from 2.8 g/16 g N to 2.4 and 2.5, respectively, and for nitrogen solubility in 0.02 N NaOH from 82.4% to 55.9 and 59.2%, respectively. Acetone extraction produced no change in available lysine and only a small reduction, to 79.6%, in nitrogen solubility. The best weight gains and PER were observed with the 90% acetone-extracted peanut meal and the lowest with the ozone-treated meal (45).

The effect of ammoniation on the destruction of aflatoxin in a cottonseed meal was also reported by Dollear and Gardner (20). This cottonseed meal originally contained 187 ppb total aflatoxins and had 6.6% moisture. It was treated with anhydrous ammonia gas at a pressure of 40 psig for one hour at a temperature of 178 F (81 C) in a 25 gal black iron steam jacketed pressure reaction vessel. This treatment appeared to be 100% effective in destroying the aflatoxin as none could be detected in the treated meal. The initial nitrogen content of the meal was 6.56%, corresponding to 41.1% protein. After ammoniation the meal contained 7.13% nitrogen indicating addition of 0.57% nitrogen from the ammonia. Chemical indices indicated that the nutritive quality of the ammoniated cottonseed meal had not been appreciably degraded by the treatment. Thus the initial nitrogen solubility of 71.80% was reduced to a final value of 58.06% and the available lysine content was reduced from 2.74 to 2.57 g/16 g nitrogen.

A series of large scale ammoniations using 2000 and 2500 pound batches of cottonseed meals containing about 500 ppb aflatoxin was recently completed successfully (46). Animal feeding studies are currently in progress to evaluate the physiological properties of the ammoniated meals prepared in these tests.

So—where do we stand? Unquestionably, prevention of contamination is the best approach and appropriate preventive measures should be taken at all stages of culture, harvest, transportation, storage and processing. If prevention has failed contaminated material may still be salvaged, but at a cost by mechanical removal of contaminated seed, by extraction with polar solvents or by destruction of aflatoxins with appropriate chemicals. Aflatoxins that may be present in crude oil are effectively removed in conventional refining operations.

REFERENCES

1. Keyl, A.C., and A.N. Booth, JAOCS 48:599(1971)
2. U.S. Agricultural Research Service, U.S. Department of Agriculture, 1968, ARS 20-16, 10 p.
3. U.S. Agricultural Research Service, U.S. Department of Agriculture, 1968, Farmer's Bulletin No. 2238, 16 p.
4. Mayne, R.Y., G.A. Harper, A.O. Franz, Jr., L.S. Lee and L.A. Goldblatt, Crop Sci. 9:147 (1969).
5. Rao, K.S., and P.G. Tulpule, Nature 214:738 (1967).
6. Kulkarni, L.G., Y. Sharief and V.S. Sarma, Indian Farming 17 (2):11 (1967).
7. Doupnik, B., Jr., Phytopath. 59:1554 (1969).
8. Troeger, J.M., E.J. Williams and C.E. Holaday, Oleagineux 25:213 (1969).
9. Golumbic, C., and M.M. Kulik, in "Aflatoxin—Scientific Background, Control and Implications," Edited by L.A. Goldblatt, Academic Press, New York, 1969, p. 315-316.
10. Deyoe, C.W., and S.F. Quadri, Feedstuffs 42 (31):58 (1970).
11. Dollear, F.G., in "Aflatoxin—Scientific Background, Control and Implications," Edited by L.A. Goldblatt, Academic Press, New York, 1969, p. 359-391.
12. Wogan, G.N., Fed. Proc. 27:932 (1968).
13. Pattinson, I., P. Crowther and H. El Noubey, Trop. Sci. 10 (4):212 (1968).
14. Kensler, C.J., and D.J. Natoli, in "Aflatoxin—Scientific Background, Control and Implications," Edited by L.A. Goldblatt, Academic Press, New York, 1969, p. 334-358.
15. National Peanut Council, "Voluntary Code of Good Practices for Purchasing, Handling, Storage, Processing and Testing of Peanuts," National Peanut Council, Washington, D.C., 1970, 4 p.
16. Duggan, R.E., FDA Papers 4 (3):13 (1970).
17. Ashworth, L.J., Jr., J.L. McMeans, J.L. Pyle, C.M. Brown, J.W. Osgood and R.E. Ponton, Phytopath. 58 (1):102 (1968).
18. Katz, R., E.P. Farrell and M. Milner, Cereal Chem. 31:316 (1954).
19. Holzenthal, L.L., E.L. D'Aquin, H.J. Molaison, W.T. Gentry, Jr., and H.L.E. Vix, Oil Mill Gaz. 6 (6):19 (1956).
20. Dollear, F.G., and H.K. Gardner, Jr., "Proceedings of the Fourth National Peanut Research Conference," Tifton, Georgia, 1966, p. 72.
21. Cucullu, A.F., L.S. Lee, R.Y. Mayne and L.A. Goldblatt, JAOCS 43:89 (1966).
22. Parker, W.A., and D. Melnick, Ibid., 43:635 (1966).
23. Sreenivasamurthy, V., A. Jayaraman and H.A.B. Parpia, in "Mycotoxins in Foodstuffs," Edited by G.N. Wogan, Mass. Inst. of Technol. Press, 1965, p. 251-260.
24. Robertson, J.A., Jr., L.S. Lee, A.F. Cucullu and L.A. Goldblatt, JAOCS 42:467 (1965).
25. Goldblatt, L.A., and J.A. Robertson, Jr., U.S. Patent 3,515,736 (1970).
26. Gardner, H.K., Jr., S.P. Koltun and H.L.E. Vix, J. Agr. Food. Chem. 16 (6):990 (1968).
27. Vorster, L.J., Rev. Fr. des Corps Gras. 13 (1):7 (1966).
28. Pons, W.A., Jr., and P.H. Eaves, JAOCS 44:460 (1967).
29. Rayner, E.T., and F.G. Dollear, Ibid. 45:622 (1968).
30. Rayner, E.T., F.G. Dollear and L.P. Codifer, Jr., Ibid. 47:26 (1970).
31. Feuell, A.J., Trop. Sci. 8:61 (1966).
32. Pons, W.A., Jr., J.A. Robertson and L.A. Goldblatt, JAOCS 43:665 (1966).
33. Andrellos, P.J., A.C. Beckwith and R.M. Eppley, J. Ass. Offic. Anal. Chem. 50:346 (1967).
34. Arthur, J.C., Jr., and J.A. Robertson, Jr., U.S. Patent 3,506,452 (1970).
35. Mann, G.E., L.P. Codifer, Jr. and F.G. Dollear, J. Agr. Food Chem. 15:1090 (1967).
36. Lee, L.S., A.F. Cucullu and L.A. Goldblatt, Food Technol. 22:1131 (1968).

37. Lee, L.S., A.F. Cucullu, A.O. Franz and W.A. Pons, Jr., J. Agr. Food Chem. 17:415 (1969).
38. Ciegler, A., E.B. Lillehoj, R.E. Peterson and H.H. Hall, Appl. Microbiol. 14:934 (1966).
39. Ciegler, A., and E.B. Lillehoj, U.S. Patent 3,428,458 (1969).
40. Fischbach, H., and A.D. Campbell, J. Ass. Offic. Agr. Chem. 48:28 (1965).
41. Trager, W., and L. Stoloff, J. Agr. Food Chem. 15:679 (1967).
42. Sreenivasamurthy, V., H.A.B. Parpia, S. Srikanta and A.S. Murti, J. Ass. Offic. Anal. Chem. 50:350 (1967).
43. Masri, M.S., H.L.E. Vix and L.A. Goldblatt, U.S. Patent 3,429,709 (1969).
44. Dollear, F.G., "Proceedings of the 1967 Cottonseed Processing Clinic," Agricultural Research Service, U.S. Department of Agriculture, ARS 72-61, 1967, p. 17-21.
45. Dollear, F.G., G.E. Mann, L.P. Codifer, Jr., H.K. Gardner, Jr., S.P. Koltun and H.L.E. Vix, JAOCS 45:862 (1968).
46. Gardner, H.K., Jr., S.P. Koltun, F.G. Dollear and E.T. Rayner, Ibid., 48:70 (1971).

[Received November 10, 1970]