Regulatory Aspects of Mycotoxins in Soybean and Soybean Products

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ABSTRACT: More than 50 countries have enacted or proposed regulations for the control of aflatoxins in foods and/or feeds, and at least 15 of these countries also have regulations for permitted levels of contamination by other mycotoxins. Since 1965, the U.S. Food and Drug Administration has used action levels to control aflatoxins in its compliance programs. Cooperative programs with the U.S. Department of Agriculture, state agencies, and industry also have been used to keep exposure to aflatoxins as low as practical. Soybeans support the growth of many mold species, which can produce toxins such as aflatoxins, trichothecenes (such as T-2), and cytochalasins. The natural occurrence of these toxins in soybeans has not been a problem. Limited surveys of soybeans and soy-based infant formulas have not revealed significant contamination. The sequence of events that leads to consideration of a mycotoxin for control programs and other regulatory activity includes determination of a toxic response, isolation and identification of the toxin, development of a sampling plan and method of analysis, and determination of incidence and levels of contamination of the susceptible commodity. The quality of soybeans can vary widely, depending on environmental, agronomic, and storage conditions. Products susceptible to contamination from improper storage are subject to regulatory action on a case-by-case basis. The government–industry cooperative programs have been successful in limiting human exposure to aflatoxins. *JAOCS 72*, 1421–1423 (1995).

KEY WORDS: Aflatoxin, analysis, mycotoxins, regulation, sampling, soybeans.

The occurrence of mycotoxins in foods and feeds, produced in various countries of the world, has been well documented. Many genera of fungi are capable of producing mycotoxins on suitable substrates under favorable conditions of humidity and temperature. The mycotoxins are of great concern because of their reported toxicological effects in humans and in animals. The toxins may be considered unavoidable contaminants in susceptible food and feed crops. It is not possible to predict their presence nor to completely prevent their occurrence during preharvest, storage, and processing when using normal agronomic practices.

Aflatoxins have received more attention than other mycotoxins because of their acute toxicity and potent carcinogenic effects in susceptible animal species. Many countries have enacted or proposed regulations (1) to limit the amounts of these toxins in foods and feeds. The legal basis for regulating poisonous or toxic substances in food in the United States is the Federal Food, Drug, and Cosmetic Act. In Section 402(a)(1) of this Act, a food is considered to be adulterated "if it bears or contains any poisonous or deleterious substance which may render it injurious to health." By enforcing this statute, the U.S. Food and Drug Administration (FDA) can prohibit the entry of, and remove from interstate commerce, any food or feed so adulterated.

RESULTS AND DISCUSSION

A definitive program to control aflatoxins in foods was initiated in 1965. The rationale for taking action at that time was based on the observation that aflatoxins were potent hepatocarcinogens in some animal species, notably duck, rat, and trout. It was not certain that the aflatoxins caused primary liver cancer in humans, but it was prudent to limit exposure to these potential human carcinogens to the lowest level possible (2). An informal action level of 30 μg/kg total aflatoxins (B_1, B_2, G_1, and G_2) was established for peanut products. The selection of this limit was based on considerations relating to the sampling procedures and analytical methodology to identify, measure, and confirm the presence of aflatoxins in contaminated food. The action level was reduced to 20 μg/kg in 1969 and applied to all foods and feeds susceptible to aflatoxin contamination.

Different action levels have since been established for aflatoxin M_1 in fluid milk products (3), for aflatoxins B_1, B_2, G_1, and G_2 in cottonseed meal as a feed ingredient (4), and for corn and peanut products intended for specific food-producing animals (5,6) (Table 1). The rationale for these action levels is described in the cited references (see Refs. 3–6).

The major commodities that are susceptible to aflatoxin contamination include corn, peanuts, cottonseed, and tree nuts. Soybeans and small grains, such as wheat, rye, oats, bar-

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TABLE 1
FDA Regulatory Levels for Total Aflatoxins[a]

Commodity	Concentration (ng/g)
All products, except milk, designated for humans	20
Corn for immature animals and dairy cattle	20
Corn and peanut products for breeding beef cattle, swine, and mature poultry	100
Corn and peanut products for finishing swine	200
Corn and peanut products for finishing beef cattle	300
Cottonseed meal (as a feed ingredient)	300
All other feedstuffs	20
Milk	0.5[b]

[a]Food and Drug Administration (FDA) Compliance Policy Guides 7120.26, 7106.10, 7126.33 (revised 1994).
[b]Aflatoxin M_1.

ley, sorghum and rice, historically have not been significant sources of aflatoxin exposure, unless abused in storage or after processing. Because soybeans are a good source of edible oil and soybean meal is widely used as a protein source for human consumption as well as an animal feed, the susceptibility of soybeans to aflatoxin contamination has been investigated. In early surveys conducted by the U.S. Department of Agriculture (USDA), 1,046 soybean samples collected from different regions of the United States, including all grades and different crop years, were examined for aflatoxins (7,8). Aflatoxin was confirmed at low levels (7–14 μg/kg) in only two of the test samples analyzed. These findings, along with other observations, suggested that soybeans were not a good substrate for aflatoxin production. However, many of the test samples showed evidence of contamination with *Aspergillus flavus*, a main contributor to aflatoxin production under certain conditions. Therefore, the potential for aflatoxin formation during adverse storage conditions does exist.

In a study designed to determine if some varieties of soybeans were more susceptible to aflatoxin contamination than others, 16 commercial varieties of soybeans were inoculated with five isolates of fungi from the *A. flavus* series and incubated for 10 d (9). All varieties of soybeans supported the production of aflatoxins under optimal conditions, but the extent of toxin production was dependent on the variety of the soybeans and the toxigenic potential of the fungal isolate used. The average yields of aflatoxin B_1 ranged from 0.7 to 34 μg/g. Although soybeans grown in the United States over the years have been relatively free of aflatoxins, they are still routinely included each year for testing by the FDA in its compliance programs. In the last two years, 64 samples of soybeans or soybean meals were tested; none contained detectable aflatoxins.

In an attempt to determine the potential of soybeans to support the growth of fungi that produced mycotoxins other than aflatoxins, 385 unprocessed soybean samples were examined to determine their mold flora (10). The predominant mold flora were species of *Aspergillus, Penicillium, Alternaria,* and *Cladosporium.* Although there are reports in the literature of mycotoxins being produced by species of these genera, information on the susceptibility of soybeans to contamination by mycotoxins other than aflatoxins is meager. Some other mycotoxins have been found to occur naturally on soybeans. In 1986, after a wet autumn delayed the harvest of soybeans in northwestern Illinois, deoxynivalenol was identified in seven of seven samples of damaged beans in amounts from 160 to 490 ng/g. Zearalenone contamination ranged from 80 to 750 ng/g in six of the test samples; diacetoxyscirpenol, from 0 to 40 ng/g in five of seven samples; T-2 toxin, from 0 to 130 ng/g in six of seven samples; and HT-2 toxin, up to 1000 ng/g (11).

In 1985 and early 1986, when harvest was delayed in North Carolina because of late-season rains, there were widespread reports of moldy beans and sprouting in the pods. There also were reports of reproductive and other health problems in swine and poultry in both North and South Carolina. Mold counts indicated invasion of the soybeans by *Fusarium* spp. Twenty-four samples were analyzed for zearalenone and deoxynivalenol. Seventeen of 24 test samples contained zearalenone at levels up to 1,800 ng/g, and 10 of 24 contained deoxynivalenol at levels up to 400 ng/g (12).

Exposure data, along with toxicological evaluations, are essential to establish the need for regulatory control programs. Past concerns by the FDA about certain mycotoxins, such as ochratoxin A, zearalenone, patulin, sterigmatocystin, deoxynivalenol, citrinin, and fumonisins, have resulted in surveys of susceptible commodities for these mycotoxins. From the results of these surveys and the available toxicological data, no formal regulatory programs have been warranted. The FDA currently has an advisory level for deoxynivalenol and is considering some type of regulatory guidance for the fumonisins and patulin. Because of the random, unpredictable contamination of food by known mycotoxins, as well as the potential occurrence of new mycotoxins, the control of mycotoxins in foods is a difficult task. Continuous efforts are being made by the FDA (through monitoring or surveillance) to minimize the extent to which consumers may be exposed to mycotoxins. The monitoring efforts are directed at regions and commodities that historically have had high levels of contamination, or in response to new information on contamination problems that may develop in regions or commodities not normally affected.

An effective monitoring program includes the use of an effective sampling plan and reliable analytical methods. The three basic steps involved in any monitoring program are: (i) sampling a given lot of food to obtain a sample representative of that lot, (ii) grinding and blending the primary sample to produce a homogeneous test sample from which to take a test portion, and (iii) performing the quantitative analysis. The first step is difficult to achieve. Because the distribution of mycotoxin contamination in agricultural commodities is very heterogeneous, large errors may occur due to concentration levels of aflatoxin in individual seeds that are as high as 25,000, 80,000, 1,000,000, and 5,750,000 μg/kg in Brazil nuts, corn, peanuts, and cottonseed, respectively (13). Individual seeds so heavily contaminated can contribute to variability in analytical results for sequential test samples taken

from a primary lot sample not thoroughly ground and blended. There are errors associated with each of these steps, but the largest error, that of sampling, can be reduced considerably if adequate random sampling plans are used.

FDA and USDA inspectors use established sampling plans for examining grains of various types so that each primary sample represents the lot from which it is taken. Instructions on how to sample a moving stream, or probe a carlot, a truck load, a bulk pile, or a stack of bags have been given in detail (13) in the Manual of the Federal Grain Inspection Service, USDA (14), and by the World Health Organization/Food and Agriculture Organization (WHO/FAO) of the United Nations (15). The primary lot sample obtained by these procedures is 50–100 kg. It is reduced by blending and splitting sequentially to 5 kg. The 5 kg is coarsely ground (to pass a No. 14 sieve), blended and riffled to 1–2 kg, reground to pass a No. 20 sieve, and thoroughly blended to constitute the test sample. At least a 50-g test portion is taken for extraction and analysis (16).

Most analytical methods employed for soybeans are specific for individual and groups of chemically related mycotoxins. The measurable levels of toxins may vary greatly, depending on how the toxins are detected, whether by visible, ultraviolet, fluorescence, mass spectrometry, or gas chromatography. The official method of analysis of the Association of Official Analytical Chemists (AOAC) for aflatoxin in soybeans, AOAC sec. 972.27 (16), is also used for peanuts and corn. More recent official methods for these commodities also would be applicable to soybeans, as would methods developed for other mycotoxins, such as deoxynivalenol, ochratoxin A, T-2, HT-2, and zearalenone.

In 1991, 54 million metric tons of soybeans were produced in the United States. Forty-two percent of this amount was exported for use as food, feed, and oil (17). Therefore, the soybean industry can be greatly affected by the regulatory activities of other countries. At least 60 countries have proposed or established regulations of some type to limit exposure to aflatoxins and other selected mycotoxins in susceptible commodities in domestic and import channels. In some instances, no rationale based on hazards to human or animal health has been cited as the basis for the levels imposed. In view of the regulatory limits in other countries, there is a need to continuously monitor soybeans for aflatoxins, as well as for other mycotoxins that may be produced under adverse weather and storage conditions.

In summary, mycotoxin contamination of soybeans has not been a significant problem as compared to commodities such as corn, cottonseed, peanuts, barley, and other grains. Problems arising from poor harvesting and storage procedures are handled on a case-by-case basis. Increased numbers of sensitive, specific, and reliable methods have become available for the aflatoxins and other mycotoxins that may be found on soybeans. For the more newly recognized mycotoxins, such as the fumonisins and cytochalasins, relatively few methods are available. Methods development and toxicological evaluations are necessary for these mycotoxins and for other unidentified mycotoxins, particularly those associated with human and livestock toxicoses.

REFERENCES

1. van Egmond, H.P., *Food Addit. Contam. 6*:139 (1989).
2. *Fed. Regist. 39*:42748 (1974).
3. *Ibid. 42*:61630 (1977).
4. *Ibid. 47*:33007 (1982).
5. *Ibid. 54*:22622 (1989).
6. *Ibid. 59*:17383 (1994).
7. Shotwell, O.L., C.W. Hesseltine, H.R. Burmeister, W.F. Kwolek, G.M. Shannon, and H.H. Hall, *Cereal. Chem. 46*:454 (1969).
8. Shotwell, O.L., M.L. Goulden, G.A. Bennet, R.D. Plattner, and C.W. Hesseltine, *J. Assoc. Offic. Anal. Chem. 60*:778 (1977).
9. Shotwell, O.L., E.E. Vandegraft, and C.W. Hesseltine, *Ibid. 61*:574 (1978).
10. Mislivec, P.B., and V.R. Bruce, *J. Food Prot. 40*:309 (1977).
11. Scott, P.M., in *Trichothecene Mycotoxicosis: Pathophysiologic Effects*, Vol. I, CRC Press, Boca Raton, 1989, p. 14.
12. Hagler, W.M. Jr., F.T. Jones, and D.T. Bowman, in *Biodeterioration Research 2*, Plenum Press, New York, 1989, pp. 337–349.
13. Park, D.L., and A.E. Pohland, *J. Assoc. Offic. Anal. Chem. 72*:399 (1989).
14. Federal Grain Inspection Service, in *Grain Sampling*, U.S. Department of Agriculture, Washington, D.C., 1979.
15. Food and Nutrition, in *Report of the FAO Technical Consultation on Sampling Plans for Aflatoxin Analysis in Peanuts and Corn*, Food and Agriculture Organization of the United Nations, Rome, 1994, pp. 1–75.
16. Scott, P.M., in *Official Methods of Analysis*, 15th edn., Vol. II, Association of Official Analytical Chemists, Arlington, 1990, pp. 1184–1212.
17. U.S. Department of Agriculture, in *Agricultural Statistics*, U.S. Government Printing Office, Washington, D.C., 1992, pp. 121–127.

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