American Industrial Hygiene Association Journal
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/aiha20

Assessing Airborne Aflatoxin B_1 During On-Farm Grain Handling Activities

Mustafa I. Selim [a], Alex M. Juchems [b] & William Popendorf [c]
[a] Institute for Rural and Environmental Health, Department of Preventive Medicine and Environmental Health, The University of Iowa, Iowa City, IA 52242
[b] Delta Environmental Consultants, Minneapolis, MN 55112
[c] Department of Biology, Utah State University, Logan, UT 84322

Version of record first published: 18 Jun 2010.

To cite this article: Mustafa I. Selim, Alex M. Juchems & William Popendorf (1998): Assessing Airborne Aflatoxin B_1 During On-Farm Grain Handling Activities, American Industrial Hygiene Association Journal, 59:4, 252-256

To link to this article: http://dx.doi.org/10.1080/15428119891010514

AMERICAN INDUSTRIAL HYGIENE ASSOCIATION JOURNAL 59:252–256 (1998) Ms. #449

APPLIED STUDIES

Assessing Airborne Aflatoxin B_1 During On-Farm Grain Handling Activities

AUTHORS
Mustafa I. Selim[a]
Alex M. Juchems[b]
William Popendorf[c]

[a]Institute for Rural and Environmental Health, Department of Preventive Medicine and Environmental Health, The University of Iowa, Iowa City, IA 52242
[b]Delta Environmental Consultants, Minneapolis, MN 55112
[c]Department of Biology, Utah State University, Logan, UT 84322

The presence of aflatoxin in corn and corn dust during relatively normal years and the increased risk of *Aspergillus flavus* infestation during drought conditions suggest that airborne agricultural exposures should be of considerable concern. Liquid extraction, thin layer chromatography, and high pressure liquid chromatography were used for the analysis of aflatoxin B_1 in grain dust and bulk corn samples. A total of 24 samples of airborne dust were collected from 8 farms during harvest, 22 samples from 9 farms during animal feeding, and 14 sets of Andersen samples from 11 farms during bin cleaning. A total of 14 samples of settled dust and 18 samples of bulk corn were also collected and analyzed. The airborne concentration of aflatoxin B_1 found in dust collected during harvest and grain unloading ranged from 0.04 to 92 ng/m^3. Higher levels of aflatoxin B_1 were found in the airborne dust samples collected from enclosed animal feeding buildings (5–421 ng/m^3) and during bin cleaning (124–4849 ng/m^3). Aflatoxin B_1 up to 5100 ng/g were detected in settled dust collected from an enclosed animal feeding building; however, no apparent correlation was found between the airborne concentration of aflatoxin B_1 and its concentration in settled dust or bulk corn. The data demonstrate that farmers and farm workers may be exposed to potentially hazardous concentrations of aflatoxin B_1, particularly during bin cleaning and animal feeding in enclosed buildings.

Keywords: aflatoxin, *Aspergillus flavus*, farm workers, grain handling operations

Although the most publicized route for human and animal exposure to aflatoxins is dietary, evidence has been accumulating to support the possibility that aflatoxins in airborne dust particles may also constitute both potential acute and chronic hazards from exposure via the respiratory route.(1–9)

Mycotoxins are commonly seen as the etiologic agent behind the clinical picture of farmers with sudden acute illness following silo uncapping or other work with moldy silage.(3,4) A negative immune reaction sharply distinguishes these patients from those with farmer's lung disease. Both classes of patients subsequently return to health after their exposures. Emanuel et al. originally referred to the observed lung disease as pulmonary mycotoxicosis, which they attributed to the inhalation of fungal toxins from the work environment.(3) This toxic response is now known as organic dust toxic syndrome.(4,5)

Dvorackova(6) reported a case of pulmonary adenomatosis in a chemical engineer who became acutely ill after he was exposed to high levels of the mold *Aspergillus flavus* for 3 months while working on a process to sterilize Brazilian peanut-meal. The condition of the patient became worse, and he died 3 months after the onset of his illness. Analysis of the lung tissue using the technique of thin layer chromatography (TLC) revealed the presence of aflatoxin B_1. Three patients, two agricultural workers and one textile worker, died of acute lung disease, clinically diagnosed as bronchopneumonia or influenza.(7) Postmortem histological investigation revealed interstitial fibrosis, and radio immunoassay analysis showed the presence of aflatoxin B_1 in samples of lung tissue in all three cases.(7) These cases provide plausible evidence for an acute occupational risk associated with aflatoxin inhalation.

This research was supported by the National Institute for Occupational Safety and Health (NIOSH) through two RO1 grants (1 RO1 OH02857-01 and 2 RO1 OH2857-02).

Documented cases of chronic effects associated with airborne exposure are limited in part by the lack of a method to detect low-level airborne aflatoxin exposure. Epidemiological studies of about 71 Dutch workers exposed to airborne aflatoxin at a peanut and linseed oil processing plant represents the earliest and most direct evidence for an occupational risk associated with airborne exposure to aflatoxin-contaminated grains.(8,9) These workers were exposed from 1961 to 1969 to dust from oil-press residues that were dried and bagged for use as animal feed. Aflatoxin exposures were estimated to be on the order of 5 pg/m^3 with the content of aflatoxin in the airborne dust in the range of 250 to 410 ng/g. The time-weighted average dose of baggers was estimated to be from 0.04 to 2.5 μg per week. Initially, researchers found rates of cancer generally and liver cancer specifically among exposed workers to be more than three times that in a matched control group, but the number of workers exposed was too small to provide statistical significance.(8) In 1984 Hayes et al.(9) analyzed the mortality data for the same exposed population and comparison group using the Standardized Mortality Ratios (SMR). The mortality occurring between 1963 and 1980 was found to be significantly elevated for cancer of all types (SMR=2.5) in comparison with matched controls at $p<0.01$, while the p for an SMR=2.5 for respiratory cancers was still less than 0.05 due to the small sample size.(9) The other (nonrespiratory) tumors identified were at a variety of sites but were primarily digestive.

Cancer studies among grain handlers or farmers are few and variable. Among 2649 recently studied Swedish grain millers, working during the period from 1961 to 1979, the incidence of liver cancer (but not overall cancer) was significantly elevated.(10) A recent review of U.S. statewide epidemiological studies of farmers and rural residents scattered over the past 15 years found low overall cancer rates,(11,12) low rates for the common cancers related to lung, esophagus, and mouth,(13) but high rates for various specific types such as leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, and stomach.(12) The low rates of respiratory cancer are attributed to low rates of smoking; approximately 17% of farmers smoke compared with 34% of the general population.(14) The high rate but diverse pattern of other cancers is commonly (and perhaps wishfully) attributed to pesticides but is not inconsistent with some of the above findings related to aflatoxin.(1,9)

Exposure levels among farmers are poorly documented and no doubt highly variable. In surveys by Donham et al.(15–17) dust levels in swine barns ranged from 2.4 to 16 mg/m^3, were largely of organic origin (ca. 25% protein content typically associated with feed corn), and included 16 *A. flavus* colony forming units/mg dry dust (no analyses were made for aflatoxin per se). Shotwell and Burg(18) found 12 to 200 ng/g and up to 25 ng/m^3 aflatoxin B_1 contamination in corn dust during their pilot surveys. Sorenson et al.(19) reported that while the dust may be coarse, aflatoxin B_1 was more concentrated on the smaller particles (in the 3–5 μm diameter characteristic of *A. flavus* spores).

Farmers are potentially exposed to grain dust year-round, beginning at harvest and grain storage, continuing during animal feeding in livestock buildings, and peaking in bin cleaning operations. There are no literature data currently available on aflatoxin B_1 exposure levels inside swine production buildings. This type of exposure is highly important in determining farmers' total annual exposure because animal feeding is a year-round activity. A dose capable of producing cancer could result from repeated exposure to low levels of aflatoxin over a long period of time. Therefore, the purpose of this article is to document preliminary findings on levels of aflatoxin B_1 in grain dust during several on-farm grain handling activities.

EXPERIMENTAL MATERIALS AND METHOD

Samples

Corn dust samples were collected from local farms within the state of Iowa between 1989 and 1993. Settled dust samples were gathered from floors or equipment in grain loading and unloading areas, storage bins, or feed grinding areas. Airborne dust samples were collected on fiber glass filters during harvest, animal feeding, and bin cleaning operations, using Andersen impactors and 37-mm cassette personal air samplers. In most cases representative samples of whole kernel corn were also collected from the respective corn-handling operation. The Andersen impactors were disassembled in the lab and the dust loaded filters transferred to labeled storage containers until ready for analysis. Personal air sampling cassettes were allowed to equilibrate overnight at room temperature, and the dust loaded filters were then weighed and stored in a 50-mL screw-cap tube until analyzed. All samples were stored in sealed plastic bags at −4°C. Before analysis, settled dust samples were sieved through a 40-mesh screen to remove large debris. Bulk corn samples were ground and sieved through a 1-mm mesh screen for consistent size.

Chemicals

(1) Organic solvents were HPLC grade, purchased from Baxter Health Corp., Scientific Products Division (McGaw Park, Ill.).

(2) Solid aflatoxin B_1 was obtained from Sigma Chemical Co. (St. Louis, Mo.) at 99.0% purity and was used for preparation of standards without further purification.

(3) Solid phase extraction cartridges containing 200 mg cyano packing were obtained from Alltech Associates (Deerfield, Ill.). Similar cartridges (Supelclean LC-CN, Supelco, Bellefonte, Penn.) were also used.

(4) Acid washed celite was obtained from Sigma Chemical Co.

(5) Silica gel, 100–200 mesh, was obtained from Fisher Scientific (Newlawn, N.J.).

(6) Anhydrous granular sodium sulfate was obtained from Matheson Coleman and Bell (Raleigh, N.C.).

Liquid Extraction

The liquid extraction method for bulk corn was adapted from the *Methods of the Association of Official Analytical Chemists,* section 968.22, 1990.(27) The liquid extraction of dust samples was adapted from the method developed by Shotwell et al.(28) Fifty grams of ground corn (<1 mm mesh size) were mixed with 15 g celite, 15 mL water, and 250 mL chloroform in a 500-mL conical flask. The flask was mounted on a Bruell wrist shaker and shaken for 30 minutes. After shaking, the contents of the flask were filtered through Whatman #1 filter paper. The content of the flask was washed with chloroform, and the washings were combined with the sample extract. The dust samples (< 1 g ea.) were extracted similarly, by shaking for 30 minutes with 15 g of celite, 15 mL of water, and shaken with 150 mL chloroform. Filtered extract was transferred to a round-bottom flask, evaporated on a rotary-evaporator to near dryness, and the residue was transferred to a graduated conical tube using 4 × 2 mL transfers in methylene chloride. The methylene chloride was evaporated under nitrogen, and the residue was redissolved in 1 mL methanol.

TABLE I. TLC Analysis of Aflatoxin B_1 in Airborne Dust on Samples Collected During Harvest and Handling of the 1988 Corn Crop in Iowa

	Aflatoxin B_1 Level in Airborne Dust (ng/m^3)		
Farm ID	Harvest	Unloading	Feeding
1	ND	ND	NA
2	67[A]	ND	NA
3	ND	ND	NA
4	ND	92	8

Note: NA = not available, samples were not collected; ND = not detected.
[A] Inside tractor cab

Extract Cleanup with Solid-Phase Extraction

The extract solution in methanol was cleaned from matrix interferences using glass columns packed with silica gel, following the procedure described in the literature.(28) Alternatively, Supelclean LC-CN columns were used for extract cleanup.(29) The clean extract was then analyzed by thin layer chromatography (TLC),(28,30) or high pressure liquid chromatography (HPLC).(29,31)

HPLC Analysis

The HPLC system consisted of two Waters model 600A pumps (Waters Associates, Division of Millipore Corp., Bedford, Mass.) and a Hewlett-Packard diode array detector, model 1050 (Hewlett-Packard Corp., Palo Alto, Calif.). The analytical column was a Supelcosil LC-18 column, 4.6 mm id × 25 cm long (Supelco, Inc.). Data acquisition and analysis was carried out using a Spectra Physics SP4270 computing integrator (Spectra-Physics, San Jose, Calif.). A mixture of water-methanol-acetonitrile (60+20+20) was used as the mobile phase, at a flow rate of 1.1 mL/min. The ultraviolet detector was set at a wavelength of 365 nm, band width of 8 nm, reference wavelength 450 nm, and band width 50 nm. The clean sample extract (after solid-phase extraction cleanup) was evaporated to near dryness using gentle nitrogen purge, the extract residue was redissolved in 0.2 mL of the HPLC mobile phase, and 30-mL aliquots were injected into the HPLC. At ambient temperature and the above instrumental conditions, the retention time for aflatoxin B_1 was around 9.0 minutes with a retention window of $< \pm 5\%$.

The identity of the aflatoxin B_1 was based on its HPLC retention time compared with the standard and control samples. The control samples consisted of matrix blank (corn with no detectable aflatoxin B_1) and matrix spike. Under the HPLC conditions used, a mixture of aflatoxins (B_1, B_2, G_1, G_2) is totally resolved and aflatoxin B_1 eluted as the last peak. With the extract cleanup procedure used, the identity of aflatoxin B_1 was not affected by matrix interferences except in cases of high background interferences or improper extract cleanup. In such cases the extraction and cleanup processes were repeated, or in some cases, TLC or fluorescence detection was used to confirm the presence of aflatoxin B_1.

TABLE II. TLC Analysis of Aflatoxin B_1 in Airborne Dust, Animal Feed, and Settled Dust in Animal Confinement Buildings, Summer 1989

	Aflatoxin B_1 Levels		
Farm No.	Feed ng/g	Settled Dust ng/g	Airborne Dust ng/m^3
5	ND	125 (n = 2)	6 (n = 2)
6	28 (n = 3)	227 (n = 3)	6 (n = 2)

Note: ND = not detected; TLC detection limits were ≈ 2 ng.

TABLE III. Aflatoxin B_1 Levels in Settled Corn Dust Collected from Swine Buildings in 1990

Sample ID	Location of Collected Dust	Aflatoxin B_1 Level (ng/g)
JM-S	sifted stored corn (1- and 2-mm sieve)	23
CW-C	ceiling vent in swine nursery building	56
DF-M	walls and equipment in swine farrowing building	91
JB-W	surface of tractor operated as feed mill	181
JM-Q	walls and equipment in swine gestation building	287
EH-N	walls and equipment in swine farrowing building	501
DF-K	tractor-operated feed mill	628
DF-L	walls and equipment in nursery building	870
JB-X	walls and equipment in swine nursery buildling	5101

The concentration of aflatoxin B_1 was calculated from the peak height of the unknown sample and the response factor, determined from the multilevel calibration with known standards. The HPLC detection limit was 1 ng based on the amount injected.

RESULTS AND DISCUSSION

Following the drought of 1988, four local farms were selected for collection of airborne samples during the harvest operation. The farms were selected based on the farmers' concern and willingness to collaborate during sampling. Personal air sampling pumps were used to collect dust samples on fiber glass filters at two locations, inside and outside the cab of the harvesting tractor. In one of the four farms extra air samples were collected during the unloading of the grain and inside a hog production building, where some of the harvested grains were used for animal feeding.

The results of the TLC analysis of these samples are summarized in Table I. Aflatoxin B_1 was detected in the airborne dust samples in two out of the four farms selected. In Farm 2, aflatoxin B_1 was detected in the dust sample collected from inside the tractor cab but was not detected in the outside sample, perhaps because of strong crosswinds. In Farm 4, aflatoxin B_1 was not detected in the field dust samples but was found in the dust samples collected during corn unloading and inside the hog production building, where the same corn was used for animal feeding.

TABLE IV. Aflatoxin B_1 Levels in Airborne Dust Collected from Enclosed Swine Facilities in the Fall of 1991

Sample ID	Dust Weight (mg)	Air Volume (m^3)	Aflatoxin B_1 in Air (ng/m^3)
CW-1 SF	5.09	1.92	ND
CW-2 SF	6.22	1.92	55
CW-3 SF	NA	1.92	ND
CW-4 SF	5.67	1.92	367
JB-1 SF	4.42	3.64	15
JB-2 SF	3.72	1.77	421
JB-3 SF	14.41	5.87	5
JB-4 SF	14.48	5.86	ND

Note: NA: Not available, weight was not accurately recorded; ND: not detected; HPLC detection limit: 1 ng injected.

TABLE V. Aflatoxin B_1 Levels in Bulk Corn and Airborne Dust Samples Collected During 1991 Harvest

Sample ID	Aflatoxin B_1 in Bulk Corn (ng/g)	Aflatoxin B_1 in Dust (ng/g)	Aflatoxin B_1 in Air (ng/m^3)
BI-1 H		ND	ND
BI-2 H		ND	ND
BI-3 H		161	2
BI-4 H		ND	ND
Bulk corn	15		
JB-1 H		ND	ND
JB-2 H		559	27
JB-3 H		ND	ND
JB-4 H		2	0.04
Bulk corn	11		
CW-1 H		6183	91
CW-2 H		ND	ND
CW-3 H		ND	ND
CW-4 H		ND	ND
Bulk corn	23		
BH-1 H		3	0.3
BH-2 H		ND	ND
BH-3 H		ND	ND
BH-4 H		ND	ND
Bulk corn	23		

Note: ND: Not detected; HPLC detection limit: 1 ng injected.

TABLE VI. HPLC Analysis of Aflatoxin B_1 Concentration in Bulk Corn and Airborne Dust During Bin Cleaning, Summer 1992 Cleaning

Farm No.	Aflatoxin B_1 Level in Bulk Corn (ng/g)	Aflatoxin B_1 Level in Air (ng/m^3)
BC1	16	348
BC2	309	3390
BC3	15	124
BC4	35	769
BC5	82	1210
BC6	10	512
BC7	4	276
BC8	207	447
BC9	120	1550
BC10	2	4849
BC11	ND	2488
BC12	ND	245
BC13	ND	ND
BC14	4.13	1421

Note: ND: Not detected; HPLC detection limit: 1 ng injected.

Aflatoxin B_1 concentrations of 67 ng/m^3 and 92 ng/m^3 are comparable with farm level measurements of Burg and Shotwell(25) and are significantly higher than the Netherlands epidemiological study (roughly 5 pg/m^3), which showed 2.5 times the risk of cancer among 71 peanut and flax seed processors.(9)

Improper storage of initially contaminated grain may also result in fungal spread and increased production of aflatoxin B_1. In addition, grains that may be unmarketable due to their contamination with aflatoxin B_1 producing fungi can be used for on-farm animal feeding. To investigate this possibility, airborne dust samples (approximately 4–5 m^3 of air) were collected and analyzed along with bulk feed samples and settled dust from two animal confinement facilities during the summer of 1989. The results of the analysis are provided in Table II. Although aflatoxin B_1 was not detected in the feed sample from Farm 5, it was detected in the duplicate samples of settled dust and airborne dust from the same farm. Aflatoxin B_1 was detected in all replicated samples of feed, settled dust, and airborne dust from Farm 6.

Continued sampling and analysis of more farms in 1990 revealed aflatoxin B_1 levels ranging from 23 to 5100 ng/g in settled dust from swine buildings (Table III). Analysis of airborne dust samples collected from the swine buildings during the fall of 1991 (Table IV) showed aflatoxin B_1 concentrations in airborne dust ranging from 5 to 421 ng/m^3. These preliminary data demonstrate that even in less drought-stressed corn, aflatoxin B_1 is present at detectable levels in many on-farm grain handling operations. Settled dust from animal confinement buildings contained the highest level of aflatoxin B_1 detected among all samples collected (983 ng/g).(24) This is probably due to uncontrolled changes in temperature and humidity, which are conducive to increased fungal growth of *Aspergillus* spores. Exposure to such dust may be highly significant when it becomes airborne as a result of air or mechanical disturbances.

Analysis of airborne dust collected during the 1991 harvest (Table V) showed concentrations of aflatoxin B_1 in the air ranging from 0.3 to 91 ng/m^3. The concentration of aflatoxin B_1 in bulk corn in the same farms ranged from 11 to 23 ng/g; however, there is no apparent correlation between the concentration of aflatoxin B_1 in the air and the bulk corn collected during the harvest.

A comparison between aflatoxin B_1 concentration in bulk corn and airborne dust samples collected during bin cleaning on 11 Iowa farms is shown in Table VI. Aflatoxin B_1 was detected in the airborne dust from 13 out of the 14 samples collected. The level of aflatoxin B_1 in the sampled air ranged from 124 to 4849 ng/m^3.

Although bin cleaning was the dustiest among all sampled farm activities, enclosed hog production building aerosols (principally feed and other organic dusts(17)) constitute the highest potential hazard to farmers by virtue of their combination of dust concentration and total annual duration of exposure in comparison with other activities, as shown in Table VII. (Harvest data are based on best judgment as are respiratory rates, which are higher for the

TABLE VII. Comparison of Annual Dose of Dust While Farming

	Exposure					
Activity	Concentration (mg/m^3)	Duration (hours)	Frequency/ Year	Respiratory Rate (m^3/hr)		Annual Dose of Dust (mg/year)
Livestock feeding	~4	~2/day	365 days	1.25	=	3650
Storage bin cleaning	~40	4/bin	2 bins	2.5	=	800
Harvest	~1	12/day	7 days	1.25	=	105

manual labor involved in bin cleaning.) The cumulative annual dose of aerosolized dust is an indicator of potential dose of aflatoxin B_1 if the toxin concentration is the same at each stage. At this time, it can only be hypothesized that aflatoxin B_1 concentrations in dust from bin cleaning may be higher than in dust from other on-farm grain handling activities.

CONCLUSION

The detection of aflatoxin B_1 in airborne dust during harvest, grain loading and unloading, bin cleaning, and animal feeding operations provides evidence that farmers may be exposed to potentially hazardous levels of aflatoxin B_1. Although the highest level of aflatoxin B_1 in airborne dust was measured during bin cleaning, animal feeding in confined buildings could contribute the major part of the exposure since it is a year-round grain handling activity. Accurate assessment of the annual exposure requires repeated measurements under different conditions of temperature, humidity, and wind velocity and direction. The content of aflatoxins in the dust should be compared with the level of aflatoxins in bulk corn from the same sampling location.(2) Such correlation may then be used in a regional survey to estimate the annual exposure dose to aflatoxin B_1 from various farm activities.

REFERENCES

1. **Baxter, C.S., H.E. Wey, and W.R. Burg:** A prospective analysis of the potential risk associated with inhalation of aflatoxin-contaminated grain dust. *Food Cosmet. Toxicol. 19*:765–769 (1981).
2. **Popendorf, W., K.J. Donham, D.N. Easton, and J. Silk:** A synopsis of agricultural respiratory hazards. *Am. Ind. Hyg. Assoc. J. 46*:154–161 (1985).
3. **Emanuel, W.A., B.S. Wenzel, and B.R. Lawton:** Pulmonary mycotoxicosis. *Chest 67*:293–297 (1975).
4. **Donham, K.J.:** Hazardous agents in agricultural dusts and methods of evaluation. *Am. J. Ind. Med. 10*:205–220 (1986).
5. **Rylander, R.:** Lung diseases caused by organic dust in the farm environment. *Am. J. Ind. Med. 10*:221–227 (1986).
6. **Dvorackova, I.:** Aflatoxin inhalation and alveolar cell carcinoma. *Br. Med. J. 1*:691–693 (1976).
7. **Dvorackova, I., and V. Pichova:** Pulmonary Interstitial fibrosis with evidence of aflatoxin B_1 in lung tissue. *J. Toxicol. Environ. Health 18*: 153–157 (1986).
8. **Van Nieuwenhuize, J.P., R.F.M. Herber, A. Debruim, I.P.B. Meyer, and W.C. Duba:** Aflatoxins—epidemiological study on the carcinogenicity of prolonged exposure to low levels among workers of a plant. *Tijdschr. Soc. Geneesk. 51*:754–759 (1973).
9. **Hayes, R.B., J.P. Van Nieuwenhuize, J.W. Raatgever, and F.J.W. Ten Kate:** Aflatoxin exposures in the industrial setting: An epidemiological study of mortality. *Food Chem. Toxicol. 22(1)*:39–43 (1984).
10. **Alavanja, M.C., H. Malker, and R.B. Hayes:** Occupational cancer risk associated with the storage and bulk handling of agricultural foodstuff. *J. Tox. Environ. Health 22*:247–254 (1987).
11. **Blair, A., and D.W. White:** A death certificate study of leukemia among farmers from Wisconsin. *J. Nat. Cancer Inst. 66*:1027–1030 (1981).
12. **Blair, A., H. Malker, K.P. Cantor, L. Burmeister, and K. Wiklund:** Cancer among farmers: A review. *Scand. J. Work, Environ. Health 11*:397–407 (1985).
13. **Burmeister, L.F., G.D. Everett, S. Van Lier, and P. Isacson:** Selected cancer mortality in farm practices in Iowa. *Am. J. Epidemiol. 118*:72–77 (1983).
14. **Pomrehn, P.R., R.B. Wallace, and L.F. Burmeister:** Ischemic heart disease mortality in Iowa farmers. *J. Am. Med. Assoc. 248*:1073–1076 (1982).
15. **Donham, K.J., M. Rubino, T.D. Thedell, and J. Kammermeyer:** Potential health hazards to agricultural workers in swine confinement buildings. *J. Occup. Med. 19*:383–387 (1977).
16. **Donham, K.J., and W. Popendorf:** Ambient levels of selected gases inside swine confinement buildings. *Am. Ind. Hyg. Assoc. J. 46*:658–661 (1985).
17. **Donham, K.J., L.J. Scallon, W. Popendorf, M.W. Treuhaft, and R.C. Roberts:** Characterization of dusts collected from swine confinement buildings. *Am. Ind. Hyg. Assoc. J. 47*:404–410 (1986).
18. **Shotwell, O., and W. Burg: Aflatoxin in corn:** Potential hazard to agricultural workers. *Ann. Am. Conf. Gov. Ind. Hyg. 2*:69–86 (1982).
19. **Sorensen, W.G., J.P. Simpson, M.J. Peach, T.D. Thedell, and S.A. Olenchock:** Aflatoxin in respirable corn dust particles. *J. Toxicol. Environ. Health 7*:669–672 (1981).
20. **Schmitt, S.G., and C.R. Hurburgh:** Measurement and distribution of aflatoxin in 1983 Iowa corn (ASAE paper no. 85-3533). St. Joseph, MI: American Society Agricultural Engineers, 1985.
21. **Shank, R.C., N. Bhamarapravati, J.E. Gordon, and G.N. Wogan:** Dietary aflatoxins and human liver cancer IV. Incidence of primary liver cancer in two municipal populations in Thailand. *Food Cosmet. Toxicol. 10*:170– (1972).
22. **Stoloff, L.:** Aflatoxin—An overview. In *Mycotoxins in Human and Animal Health.* J.V. Rodricks, C.W. Hesseltine, and M.A. Mehlman (eds.). Park Forest South, IL: Pathotox Publishers, Inc., 1977. pp. 7–28.
23. **Van Rensburg, S.J., J.J. Van der Watt, I.F.H. Purchase, L. Pereira Coutinho, and R. Markham:** Primary liver cancer and aflatoxin a high cancer area. *S. Afr. Med. J. 48*:2508a (1974).
24. **Selim, M.I., and M-H Tsuei:** Development and optimization of a supercritical fluid extraction method for the analysis of aflatoxin B_1 in grain dust. *Am. Ind. Hyg. Assoc. J. 54*:135–141 (1993).
25. **Burg, R.W., and O.L. Shotwell:** Aflatoxin levels in airborne dust generated from contaminated corn during harvest and at an elevator in 1980. *J. Assoc. Off. Anal. Chem. 67*:309–312 (1984).
26. **Emanuel, D.A., J. Marx, Jr., B. Alt, M. Reuhaft, R. Roberts, and M. Kryda:** Pulmonary mycotoxicosis revisited. *Am. J. Ind. Med. 10*: 305–306 (1986).
27. **Association of Official Analytical Chemists (AOAC):** *Methods of Analysis (1990),* 14th ed. Arlington, VA: AOAC, 1990. Sec. 968.22 (d).
28. **Shotwell, O.L.,W.R. Burg, and T. Diller:** Thin layer chromatographic determination of aflatoxin in dust corn. *J. Assoc. Off. Anal. Chem. 64*:1060–1063 (19981).
29. **Supelco Inc.:** *Separate and Quantify Aflatoxins by HPLC* (Bulletin No. 800A). Bellefonte, PA: Supelco Inc., 1988.
30. **Trucksess, M.W., W.C. Brumley, and S. Nesheim:** Rapid quantification and confirmation of aflatoxins in corn and peanut butter, using a disposable silica gel column, thin layer chromatography, and gas chromatography/mass spectrometry. *J. Assoc. Off. Anal. Chem. 67*: 973–975 (1984).
31. **Scott, P.M.:** Liquid chromatography in the analysis of mycotoxins. In *Trace Analysis,* vol. 1. Academic Press, 1981.