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Samples of airborne dust generated during the handling of aflatoxin-contaminated corn were collected and analyzed to assess potential exposures of farmers and other agricultural workers to these mycotoxins. Using high volume total dust samplers and a high volume Andersen sampler, downwind dust samples were collected on glass fiber filters when contaminated corn was transferred by augers from a storage bin into a wagon and back into the storage bin. The aflatoxin B_1 content of the 15 dust samples ranged from 12.5 to 204.3 ppb, with an average of 138 ppb; the aflatoxin B_2 content ranged from 1.1 to 41.6 ppb, with an average of 24.6 ppb. The B_1 and B_2 levels of contamination in the bulk corn were 223.9 and 17.5 ppb, respectively. The gravimetric dust concentration in the air ranged from 7 mg/m^3 to 417 mg/m^3. The samples taken with an Andersen sampler indicate the dust is relatively coarse with only approximately 17% less than 7 μm. An analysis of the dust from each stage showed higher levels of aflatoxins in the larger first-stage particles than in the finer particles on the succeeding stages. The results of this study indicate that the dust generated when handling contaminated commodities also may be contaminated and represent a potential inhalation hazard. This fact, coupled with the extreme toxicity and carcinogenicity previously demonstrated in animal studies, suggests that appropriate measures be taken to prevent worker exposure during handling of contaminated materials.

Measurements of airborne aflatoxins during the handling of contaminated corn

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introduction

The purpose of this study was to investigate the potential hazard from inhalation of aflatoxins in airborne dust to agricultural workers handling contaminated corn. Aflatoxins are a group of chemically similar compounds, bis-difurancourmarins fused to either a pentenone (B-series) or lactone ring (G-series), which are metabolites of two common fungi: *Aspergillus flavus* and *Aspergillus parasiticus*. Several of these compounds have been shown to be highly toxic, mutagenic, carcinogenic, teratogenic, and immunosuppressive in animal studies.

This article is not intended to be a review of aflatoxins, since the massive accumulation of information dealing with the chemistry, biological effects and occurrence of aflatoxins has been summarized in a number of excellent books and review articles.[1-4] However, because only a single reference was found dealing with the possibility that aflatoxins might be an occupational hazard, some background into the nature of these toxins as it relates to occupational exposure is presented.

Aflatoxins were discovered in England in 1960, as a result of investigations into the causes of the deaths of thousands of turkey poults and other livestock.[5,6] The severe economic factors, the interesting nature of the fungi producing the toxic factor, and the implications for human health prompted many investigations, which subsequently established the existence of 13 or more aflatoxins or closely related compounds formed by animal biotransformations. Several of the most abundant and commonly occurring aflatoxins are shown in Figure 1. The trivial name of aflatoxin (from *A*spergillus *fla*vus *toxin*) was originally proposed by a British group in 1962 and has become almost universal. The Chemical Abstracts Service code for aflatoxin B_1 is 1162-65-8. Other aflatoxins include: aflatoxins M_1, M_2, GM_1, GM_2, B_2a, P_1, R_0 and Q_1. The capital letters B and G refer to the blue and green fluorescence of the compounds, the numerical subscripts refer to their respective positions on a thin-layer chromatographic plate and the subscript "a" refers to the adduct. Aflatoxins M_1 and M_2 are compounds first isolated from milk and later also found in the urine of animals which had consumed aflatoxin contaminated feed.

Microorganisms have an impact on human health and welfare in many beneficial and harmful ways. For centuries, alcohol has been produced by fermentation. More recently fermentation processes have been used to produce medicinals and other commercial products. Other fermentation products from microorganisms also have been responsible for widespread illnesses and deaths in the general populace as well as for several occupational diseases. Fungal-related diseases may be divided into two main types: mycosis and mycotoxicosis. Mycosis is the resulting effect on an organism from the invasion of the tissues by the living fungi as seen in such diseases as histoplasmosis, candidiasis and farmer's lung.[7] Mycotoxicosis, on the other hand, is the effects on an organism by the metabolites produced by the fungi and is the cause of such diseases as alimentary toxic aleukia, yellow rice disease and aflatoxicosis. Aspergillosis is recognized as an occupational hazard[7] and aflatoxicosis is well

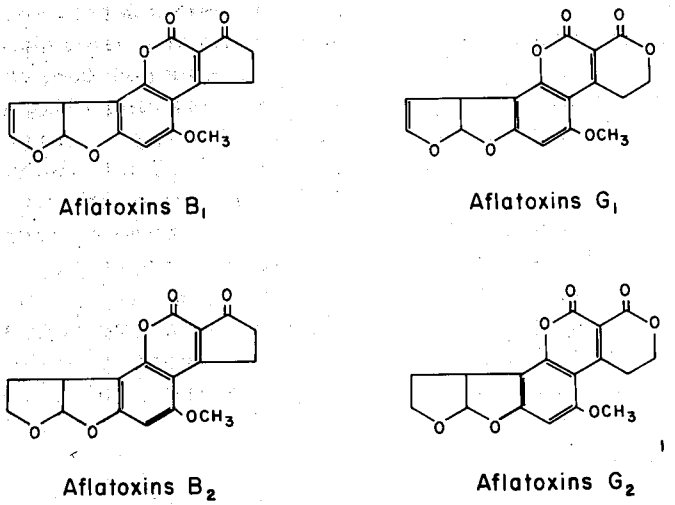

Figure 1 – The four major aflatoxins.

recognized as a public health threat,[8] but aflatoxicosis has not been extensively investigated as an occupational hazard.

The prolific *Aspergillus flavus* is ubiquitous in the soil and air throughout most of the world. Consequently, many foods and feed already contain spores; when the environmental conditions (time, temperature, moisture, nutrients, pH, etc.) are favorable, some strains of the fungi begin growing and producing aflatoxins. During the exponential growth rate phase, the metabolism is directed toward satisfying the energy requirements for cell growth, cell division and cell maintenance, and primary metabolites (CO_2, alcohols, H_2O, etc.) are produced. These fermentation products can be explained by one or more of the known biochemical pathways and generally result from degradations. Following this period, the metabolism of the fungi changes and it begins producing secondary metabolites. Secondary metabolites are generally complex molecules believed to be synthesized from acetate and malonate groups, but which serve no known useful function to the fungi. It has been proposed that the production of secondary metabolites serves as a mechanism for eliminating intermediates.[9] Aflatoxins are but a few of the secondary metabolites produced by those genetically capable strains; over 65 others have been identified. These other metabolites of *Aspergillus flavus* belong to the coumarins, flavenoids, macrolides, pyrroles and terpenoid families, and a number of these compounds show significant biological activity.

The prolific *Aspergillus flavus* is capable of developing a variety of substrates over a range of temperatures and humidities; therefore, contamination of commodities is always of concern. *A flavus* grows best in temperatures from 25 ° to 30 °C and a relative humidity of 80-85%. However, *A flavus* can produce aflatoxins from 12 ° to 41 °C at a relative humidity of 99%.[10] A partial list of foods and feed known to become contaminated includes: peanuts, pecans, peas, bread, cheese, rice, corn ears, barley, grain, sorghum, wheat, cotton seed and copra. In addition, residues of aflatoxin have been found in milk, eggs and the livers of animals that had consumed contaminated feed.[11-13] However, the most important sources of aflatoxins in human diets in the United States are from peanuts and corn products.[14]

A number of studies including those concerned with decontamination, have shown that the aflatoxins can remain in the substrates long after the fungi have died. Pure crystalline aflatoxins are heat stable up to the melting point (268 °C for aflatoxin B_1).[15] Over a period of time in the presence of moisture and with elevated temperatures (as in peanut roasting), some destruction of aflatoxin occurs but a fraction remains.[16] The aflatoxins are reactive at either end of the pH scale (pH < 3 or pH > 10) and with oxidizing agents such as chlorine, hydrogen peroxide and ozone.[17-19] Aflatoxins survive most home cooking preparations[20] and also have been found in home-brew beer (17 μg/L) in Kenya.[21] Recent studies suggest that gaseous ammonia may decontaminate livestock feed effectively while preserving acceptable odor, flavor and nutritional properties.[22,23] It may also be of interest that marihuana can support the production of aflatoxins by *A flavus*, although a chemical analysis of moldy "street" samples did not show the presence of aflatoxins.[24] An analysis of the condensates from the smoke of contaminated tobacco did not show the presence of aflatoxins.[25] Sunlight only very slowly decomposes pure aflatoxins, and the rate of photodecomposition appears to further decrease when the aflatoxins are in a feed matrix.[26]

The biological effects of aflatoxins on microorganisms, insects, plants, tissue cultures and several animals have been investigated. An outstanding feature is the great variation in sensitivity of the test systems to acute and chronic exposures, the nutritional state and route of entry. The extreme acute toxicity of several aflatoxins was demonstrated in oral 7-day LD_{50} values for day-old duckling (60 g body weight): B_1, 18.2 μg; B_2, 84.8 μg; G_1, 39.2 μg and G_2, 172.5 μg.[27] Other selected acute oral LD_{50} values for aflatoxin B_1 in mg/kg body weight are: 0.8, trout; 6.5, rat; 1.4, guinea pigs; 10.2, hamsters; 9.0, mice;[28] and 2.2, monkey.[29] The acute effects noted in the aflatoxin-caused Turkey X Disease – anorexia, lethargy and muscular weakness – are typical. Within a few days, many of the affected birds died; autopsies showed hemorrhages in many parts of the body (particularly the liver), pale and necrotic livers, engorged kidneys, lesions of the livers and kidneys and cirrhosis of the liver.

Probably of most concern to agricultural workers are the effects of long-term, low-level exposures to aflatoxins. Trout and other aquatic vertebrates are particularly sensitive. Daily levels of 0.4 ppb aflotoxin B_1 in the diet produced a significant number of liver cancers in trout in less than a year.[30] The extreme sensitivity of trout led to the development of a bioassay method in which studies have shown that trout embryos immersed in a solution of 0.5 μg/mL of aflatoxin B_1 for 60 minutes will develop a high incidence (40%) of liver cancer 10 months later.[31]

Rats are relatively resistant to acute effects of aflatoxin B_1 but very sensitive to the carcinogenic effects. Rats on a diet containing 15 ppb aflatoxin B_1 (0.2 μg/day) developed liver tumors in 476 days, with a 100% incidence rate.[32] Some of the rats had multiple tumors with neoplasms in the colon,

kidney and lungs. Doses of 300 μg of a mixture of aflatoxin B_1 and G_1 in peanut oil, administered intratracheally twice weekly for 30 weeks to six rats, produced squamous cell carcinomas of the trachea in three animals.[33] Primary pulmonary tumors were produced in strain A mice by intraperitoneal injections of aflatoxin B_1.[33] Monkeys and mice, however, appear to be more resistant to the carcinogenic effects.[34,35]

Several other important biological effects also have been reported. Aflatoxin B_1 given intraperitoneally to pregnant hamsters was a potent teratogen, causing a number of malformed and resorbed fetuses.[36] Secondary diseases were also reported due to altered immunity in poultry exposed to aflatoxins in the feed.[37] One study noted lesions after aflatoxins were applied singly to the skin of rabbits,[38] and a second study noted hepatic lesions after percutaneous applications of aflatoxins.[39] Most of the above studies have used a single purified aflatoxin; however, a possible synergistic action was suggested when B_1 and B_2 were fed together[40] and with other compounds.[30]

Considerable details about the mechanism of action of aflatoxins have been established using cell cultures and comparative animal metabolism studies. Evidence suggests that aflatoxin biodegradation occurs by several mechanisms. Different mechanisms appear to lead to detoxification, production of a proximate carcinogen, or creation of a metabolic reservoir of aflatoxin, and at least one mode appears to lead directly to the ultimate carcinogen. The variation in the susceptibility of animals may rest in the relative rates of the respective metabolic pathways. Radio-labeled derivatives showed that the aflatoxin binds to nucleic acid and is followed by altered protein production. Fifteen and twenty times as much aflatoxin were found in the DNA and RNA, respectively, than in proteins. The patterns of binding have also been correlated with susceptibility in additional experiments. *In vitro* experiments demonstrated that bacteria were killed by aflatoxin B_1 incubated with rat liver hemogenate but not by either alone.[41] Similar experiments using the liver microsomes of guinea pig, mouse, hamster and man also killed bacteria. Considerable evidence suggests the ultimate carcinogen is the formation of 2,3-epoxide of aflatoxin B_1.[42] Evidence also suggests that nutrition plays a major role in the metabolism;[43] accordingly, the carcinogenic aflatoxin B_1 appears to be metabolized by the NADPH-dependent microsomal enzyme system that includes cytochrome P-450.

The evidence of the effects of aflatoxin on human health is necessarily indirect. Five studies in Southeast Asia and Africa have shown a positive correlation between the incidence of cancer and aflatoxin contamination of foodstuffs. A recent article explored the fit of the epidemiologic data with different mathematical models.[44] In proposed rules it was noted that the cancer rates in the southeastern states, where the climatic conditions are conducive to aflatoxin contamination, do not differ significantly from other areas.[45]

Studies have linked Reye's syndrome[46] and liver cirrhosis with aflatoxin ingestion. Such a syndrome was reported in three children aged 22, 12 and 8 months.[47] Diffuse fatty changes of the liver parenchyma, kidney tubules, myocardium and hypoplasia of the thymus, lymph nodes and adrenals that were observed in these children are common in Reye's syndrome. Samples of liver tissue from the first and third cases showed the presence of aflatoxin by thin-layer chromatography. In the study it was considered significant that the mother of the last two children worked on a poultry farm during the pregnancies, where she was in daily contact with infected fodder; the two children may have been exposed to aflatoxins during gestation and/or breast feeding.

Evidence of hazards associated with the inhalation of aflatoxins is scarce and generally inconclusive. Only three cases were found in the literature linking aflatoxin inhalation with health effects. A 68-year-old chemical engineer working on a method of sterilizing Brazilian peanut meal became ill and died of pulmonary adenomatosis. A chemical analysis of lung tissue by thin-layer chromatography showed a blue fluorescent spot in 365 nm UV light similar to that of commercial B_1, with the same thin-layer chromatographic RF value as aflatoxin B_1 and the same color change when the spot was treated with 50% H_2SO_4. A colleague of this patient who previously had been doing the same work died three years before of pulmonary adenomatosis, but no chemical analysis was performed in his case.[48] A second report linked the death of a biochemist and the illness of his graduate student to colon cancer possibly caused by inhalation of aflatoxin while purifying aflatoxins by thin-layer chromatography. The graduate student recalled that some dust might have been generated in scraping the spots from the plates in an unventilated area.[49]

The most persuasive evidence of the effects of inhalation of aflatoxin contaminated dust is provided by an epidemiological study of workers at a plant where peanuts and linseed were processed for their oils.[50] The nuts and seeds were cleaned, conditioned with steam, rolled, and the oil pressed out by wringers. The dry residue came in the form of a cake called shavings and was transported by augers into a bin. The shavings were then bagged or removed in bulk for feed. Attempts were made to correlate the aflatoxin content in airborne dust samples with the aflatoxin in the bulk peanut shavings. Airborne dust particles varied in size with location, but on the average 8.4% of the particles were less than 5 μm in diameter. The aflatoxin content of the airborne dust ranged from 250 to 410 ppb and of the peanut bulk shavings ranged from 330 to 495 ppb. Linseed shavings were found to be virtually free of aflatoxins. Baggers were estimated to be exposed to from 0.039 to 2.5 μg of aflatoxins per 45-hour week based on a respiration rate of 1 m^3 per hour. It is notable that this dose is lower than the oral dose common in Thailand by a factor of 20. Urine samples were collected and analyzed, but aflatoxins could not be detected.

The rate of multiple kinds of cancer and liver cancer disease taken together was more than three times that in the matched control group. There was only one liver cancer in eleven malignancies, with an unusual variety of other tumors such as colloid-carcinoma of the sinus maxillaris,

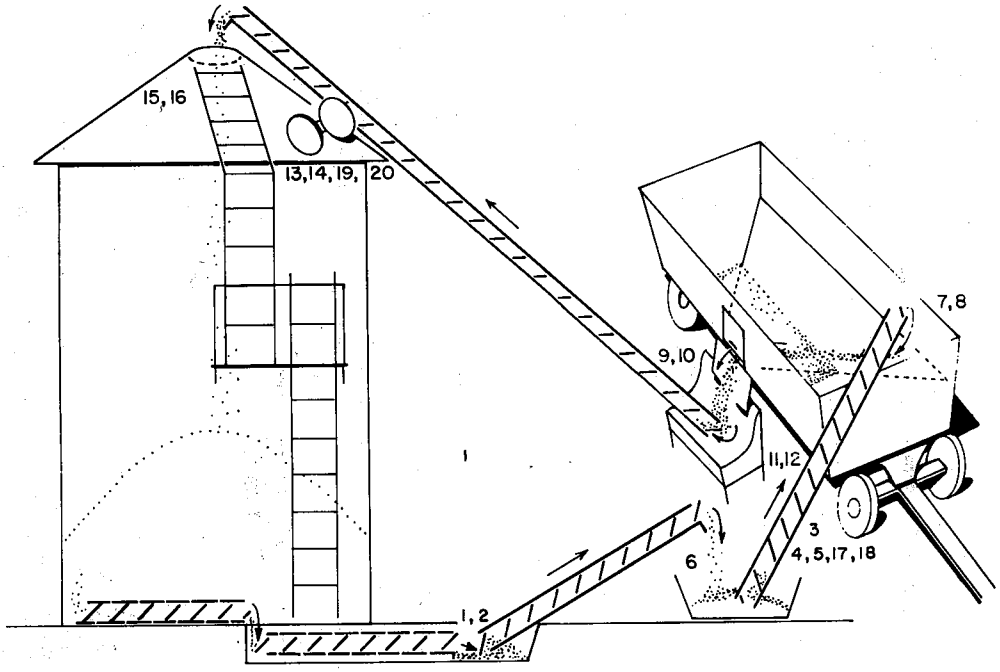

Figure 2 – Schematic drawing of the corn transport system and the sampling sites at a storage bin.

reticulocarcinoma, cholangio carcinoma and mesotheleoma pleural. The authors concluded that there was strong indications of the presence of carcinogenic factors, but the number of workers exposed was too small to provide positive proof. It was also pointed out that the workers consumed raw peanuts as they worked.

occurrence of aflatoxin in corn and peanuts

Studies show that the major causes of fungal growth in stored grain were: delayed drying of the commodity after harvest, inadequate ventilation allowing moisture of respiration to accumulate, inadequate blending of moist and dry grain so that "hot" spots developed, leaky storage bins, migration of moisture and the storage of physically damaged kernels. Recently it was shown that the ears of corn still standing in the field also can become highly contaminated.[51] As a result, farmers and grain handlers may be exposed to airborne dust containing aflatoxins generated in harvesting and handling contaminated corn. Several factors contribute to the growth of *Aspergillus flavus* prior to harvest, including: hot and humid weather, plant stress, insect damage to the kernels (the insects are carriers of *Aspergillus flavus* and may inoculate the ears by transporting spores to the kernels), and possibly by failure of the ears to drop thereby allowing moisture to enter. Higher temperatures, essential for fungi growth, probably are responsible for the prevalence of aflatoxins on the corn in the southeastern parts of the United States. The production of aflatoxins in fields is quite variable, fluctuating not only from year to year and field to field but also from one location to another within a field. In fact, the contamination within an ear of corn is not uniform, but generally only a few highly contaminated kernels are found in an ear. One kernel of corn was found to have 207 000 ppb B_1. Even the distribution of aflatoxin within a kernel may not be uniform, and evidence suggests that the germ of a kernel may be more contaminated than the remainder of the kernel. Contamination in peanuts is similar in that the variation in a lot of naturally mold-damaged peanut kernels was found to range from 250 to 740 000 ppb w/w.

aflatoxin standards and dietetic levels

Standards have been established only for ingestion of aflatoxins. These presently represent the only available means for evaluating other types of exposures.

In the United States, aflatoxin-contaminated materials are regulated by the Food and Drug Administration. The FDA takes the view that aflatoxins are not natural components of any food (even though they are produced naturally) and therefore fall within their jurisdiction. From 1965 to 1969, a guideline of 30 ppb was enforced, which was mainly based on the limit of analytical detection. Improvements in analytical methods have now forced a more rational approach. The present FDA guideline is 20 ppb, but a proposal to reduce it to 15 ppb was introduced in 1974.[45] In milk and milk products, in which the principal contaminant is aflatoxin M_1, the FDA guideline is 0.5 ppb.[52] The FDA can seize and destroy any food in interstate commerce found to be contaminated with aflatoxin above the guideline.

Most countries have established standards for aflatoxin contamination of food. Many of these standards are complex but the following levels are the essential ones: 10 ppb total, West Germany; 15 ppb total, Canada; 5 ppb B_1, France; 20 ppb B_1, Israel. A joint committee of the Food and Agriculture organization (FAO) of the United Nations and World Health Organization (WHO) now called the Protein

TABLE I
Dust Generated by Pouring Ground Corn Through a Bourner Divider

Sample Number	Location	Sampler Type	Sampling Time, min	Sample Volume, m^3	Weight of Dust, mg	Dust Conc., mg/m^3	Aflatoxin Concentration, ppb w/w			Airborne Aflatoxin Conc., (Total) ng/m^3
							B_1	B_2	Total	
(1A)	0.9 m above floor, 0.3 m from divider inlet	Hi Vol	38	69.7	121.0	1.74	2440.0	114.9	2555.0	4.45
(2A)	0.5 m above floor, 0.6 m from divider	Hi Vol	38	65.5	558.0	8.51	4380.0	180.2	4560.0	38.8
(3A)	Worker's collar	Personal	38	0.075	0.022	0.30	[A]	[A]	[A]	[A]
(4A)	0.6 m above floor, 0.6 m from divider	Andersen	43	24.4	152.8	6.3				5.71
		1st Stage			23.6	0.9	3991.0	548.4	4540.0	
		2nd Stage			109.3	4.4	160.3	14.7	175.0	
		3rd Stage			6.7	0.2	298.0	-	298.0	
		4th Stage			2.4	0.1	417.0	-	417.0	
		Final Filter			10.8	0.4	926.0	-	926.0	
(5A)	Bulk corn (29.6 Kg)								2250.0	

[A]Sample too small for chemical analysis of aflatoxins

Advisory Group for the United Nations Systems, has recommended a level of no more than 30 ppb in supplemental food. This recommendation is a compromise between combating malnutrition and avoiding the chronic effects of aflatoxins.

It is of interest that some groups in the United States recommend enforcement of the Delaney Amendment for aflatoxin contaminated foods. Industry has resisted the zero tolerance for aflatoxins on the basis that they are naturally occurring and are not an artificial food additive. In practice, industry and government have kept the levels of aflatoxins much lower than 20 ppb. It was estimated in 1975 that consumption of peanuts in the United States was 2.7 kg per person per year which was contaminated with approximately 1.5 ppb aflatoxin. When this quantity of aflatoxin is divided by the average amount a person eats (2 kg of food per day) the average concentration of ingested aflatoxin is 0.005 ppb per day.

detection of aflatoxins

Because some strains of *Aspergillus flavus* do not produce aflatoxins, and because other strains which produce aflatoxins do so only after the exponential growth period, moldiness does not necessarily mean the presence of aflatoxins. In other cases, the fungi develops on the interior of the kernels, either by entering through small cracks in the pericarp or by an unknown mechanism, so that a kernel may be highly contaminated without externally visible mold. Cracked and damaged kernels are much more prone to fungal contamination than undamaged kernels, and greater care must be taken to prevent fungal growth during storage. Because of the need for quick and convenient tests for aflatoxins, three categories of methods are employed:[51]

1. *Presumptive Test* – In this test, corn is examined under ultraviolet light (365 nm) for the presence of a bright greenish-yellow fluorescence. The fluorescence is not due to the presence of aflatoxin but to a substance produced concurrently by the fungi. The corn should be cracked before inspection to be sure the contamination is exposed to the surface so that fluorescence can be observed. Because the fluorescence is not due to aflatoxin, false positives occur. A negative test indicates the aflatoxin is not likely to be present in levels equal to or greater than 20 ppb.[53]
2. *Screening Procedure* – A small glass column containing an adsorbent such as Florisil silica gel or alumina may be used to determine the presence or absence of aflatoxin at a predetermined level (usually 20 ppb). False positives generally do not occur; however, the test takes from 0.25 to 1 hour.[54]
3. *Quantitative Method* – This procedure is based on thin-layer chromatography or high pressure liquid chromatography and measures levels down to 1-3 ppb. This procedure is recommended by the Association of Official Analytical Chemists (AOAC) and the American Association of Cereal Chemists (AACC). Equipment and an experienced technician are required for the proper performance of the screening and quantitative procedures.

sampling and analysis of dusts from contaminated corn

This study was conducted to determine occupational hazards from fungus-contaminated corn and focused on sampling and analysis of dusts generated during its

TABLE II
Dust Generated During the Transport of Corn into and out of a Storage Bin[A]

Sample Number	Location	Sampling Time, min	Sample Volume, m^3	Weight of Dust, mg	Dust Conc., mg/m^3	Aflatoxin Concentration, ppb w/w			Airborne Aflatoxin Conc., (Total) ng/m^3
						B_1	B_2	Total	
(1)	0.9 m from bin, 0.5 m above slab	9.0	15.6	432.0	27.6	147.9	24.8	172.7	4.78
(2)	0.9 m from bin, 0.5 m above slab	7.5	13.1	1034.0	79.0	104.5	20.3	124.8	9.85
(3)	1.0 m from auger drop bin, 0.8 m above slab	11.0	17.3	688.0	39.8	164.3	25.9	190.2	7.57
(4)	1.0 m from auger drop bin, 0.8 m above slab	13.0	20.2	8453.0	417.8	123.9	24.1	148.0	61.84
(5)	1.0 m from auger drop bin, 0.8 m above slab	9.5	15.9	4449.0	279.8	125.8	31.6	157.4	44.04
(6)	1.0 m from auger drop bin, 0.8 m above slab	6.5	10.2	2487.0	244.0	111.4	35.5	146.9	35.85
(7)	Top of wagon	13.5	23.9	1689.0	70.7	127.0	41.6	168.6	11.91
(8)	Top of wagon	8.5	14.6	672.0	46.0	139.6	19.5	159.1	7.32
(9)	Side of wagon, 0.9 m from outlet	5.5	9.7	181.0	18.8	11.4	1.1	12.5	0.23
(10)	Side of wagon	9.0	15.8	105.0	6.6	trace	trace	trace	trace
(11)	Side of wagon	5.5	9.5	320.0	33.7	119.9	32.2	152.1	5.13
(12)	Side of wagon	9.0	15.3	397.0	25.9	173.4	30.9	204.3	5.29
(13)	Inside at top of bin	5.0	7.5	5392.0	719.0	108.2	20.3	128.5	92.39
(14)	Inside at top of bin	9.0	12.5	10 793.0	866.2	96.6	18.8	115.4	100.0
(15)	Outside on top of bin	5.5	10.1	389.0	38.6	152.6	23.4	176.0	6.8
(16)	Outside on top of bin	9.0	17.9	849.0	47.4	112.2	20.3	132.5	6.3
(17)	Andersen - 1.0 m from auger drop bin	13.25	7.5	1248.2	166.43				20.1
	1st Stage			1017.3	135.6	126.5	15.9	142.4	19.3
	2nd Stage			69.2	9.2				
	3rd Stage			37.3	5.0	33.4	2.4	35.8	
	4th Stage			23.6	3.1				
	Final Filter			100.8	13.4	9.5	0.9	10.4	
(18)	Andersen - 1.0 m from auger drop bin	9.0	5.10	690.2	135.3				23.7
	1st Stage			574.7	112.7	181.5	25.0	206.5	
	2nd Stage			50.3	9.9				
	3rd Stage			25.3	5.0	17.8	2.9	20.7	
	4th Stage			14.3	2.8				
	Final Filter			25.6	5.0				
(19)	Andersen - inside at top of bin	5.0	2.83	1539.4	543.9				96

TABLE II Cont.
Dust Generated During the Transport of Corn into and out of a Storage Bin[A]

	1st Stage			1432.7	506.2	111.4	20.5	131.9	
	2nd Stage			63.7	22.5				
	3rd Stage			21.7	7.6	83.0	1.6	84.6	
	4th Stage			13.6	4.8				
	Final Filter			7.7	2.7				
(20)	Andersen - inside at top of bin	9.0	5.10	3786.6	742.4				107
	1st Stage			3576.0	701.1	113.0	26.1	139.1	
	2nd Stage			142.8	28.0	94.1	11.1	105.2	
	3rd Stage			29.7	5.8				
	4th Stage			15.9	3.1	neg.[B]	neg.[B]	neg.[B]	
	Final Filter			22.2	4.3				
(21)	Bulk corn					231.9	17.5	241.4	

[A]Samples 1-16 collected with High-Volume sampler, 17-20 with Andersen Sampler
[B]Below the limit of detection

handling. Tests were made at three different types of operations with corn known to be contaminated with aflatoxin, in order to evaluate the upper ranges of possible exposures.

hazards from handling grain in a laboratory

To assess the potential exposure to laboratory workers handling contaminated products, ground corn was poured manually through a Bourner divider. A Bourner divider is a device for mixing, blending or randomly dividing pulverized samples. Airborne dust was collected while 3.4 kg of contaminated corn was poured four times through the divider. Although some dust is generated by the corn impacting on the metal surfaces as it falls through the divider, the majority was dust generated previously during grinding that was simply dislodged during pouring.

hazards from grain conveying and storage

Contaminated corn was moved out of a metal storage bin by a series of augers into a holding wagon and then transferred back into the bin. A schematic drawing of the system and the sampling sites is shown in Figure 2. The metal storage bin contained a traveling auger, which rotated around the bin and carried the corn to the center. There the corn fell into a second stationary horizontal auger, which moved the corn to a third inclined auger. This auger elevated the corn approximately 1.5 m where it then fell into the drop bin and a fourth auger delivered it to the wagon. Air samples were collected at the sites indicated in Figure 2. The corn was held in the wagon until the sampling equipment was moved to the new sites. During unloading of the wagon, the corn fell approximately 0.5 m from the gate into a drop bin and was then augured back into the original bin. Approximately 250 bu of corn was moved by this system during the sampling period. The processes are typical of those performed by farmers and grain elevators using commercial equipment. Each step in the process subjected the kernels to strong abrasive and shear forces which began a comminution process. A fraction of this dust can become airborne at the end of each auger.

Because of the large variability of aflatoxin levels found between corn kernels, a series of small random samples were collected from the bulk corn flowing from the wagon. A total of approximately 4 kg was collected. One kg of corn contains approximately 3500 kernels, and it is possible that a single kernel containing all of the aflatoxins would be missed in a smaller sample (*e.g.* 0.5 kg).

hazards from a drying bin

In order to harvest grain at the most convenient time, drying is widely employed to reduce moisture content to levels at which mold does not grow during storage. Corn with ~ 22% moisture content is placed in the bin and dried to ~ 13% moisture content. A drying bin forces heated air through a perforated floor while mechanically stirring the grain to avoid overheating and to achieve a uniform moisture content. However, the stirrer can only approach within ~ 5 cm of the wall; in the fall, moisture can migrate and condense on the inside wall and molding can occur. In one particular bin, an area of ~ 14 m^2 was in the advanced stages of decay. In the manual scraping and disposing of the moldy corn, the farmer generated and worked in a visible cloud of dust. A sample of this moldy corn was picked up outside the bin during the cleaning operation for the analysis of aflatoxins B_1, B_2, G_1 and G_2 and of the mycotoxin, zearalenone.

collection of samples

Total dust samples were collected on 8 in. × 10 in. type "A" glass fiber filters, using high-volume samplers to collect sufficient dust for a chemical analysis. The sampling rates were approximately 0.029 m^3/s and were determined individually for each pump with a Magnehelic gauge ®.

A 4-stage, high-volume Andersen sampler with an 8 in. × 10 in. backup filter was used at a flow rate of 0.0096 m^3/s (20 cfm) to collect size fractions on perforated glass-fiber type

"AE" filters. Because of interest in the aflatoxin content of different size particles, relatively large quantities of dust were collected with the possibility that collection characteristics of the sampler were somewhat altered. When the filters were changed, the impacted dust cones and the perforated collection filters were visually inspected to detect the possible occurrence of re-entrainment. When there were no visible "tracks" on the filters and the cones retained their shape, the sample was analyzed for aflatoxins. A brush or a vacuum cleaner was used to clean the stage plates between samples.

Power in remote locations was supplied for both the total and Andersen high-volume samplers by a portable 4 HP gasoline-powered generator capable of supplying 125 volts and 14.0 amperes. The air flow through the Andersen sampler was adjusted to the flow rate recommended by the manufacturer, using a variable voltage transformer and a Magnehelic gauge.

sample treatment and storage

The filters and a control filter were allowed to stand for at least 24 hours in an air conditioned room for moisture equilibration, and then accurately weighed before use and stored in individual envelopes. After sampling in the field, the filters were folded and placed in 7.5 × 2.5 cm round seamless tin cans for storage and transportation. At the end of each day, the sample cans were opened and placed overnight in an oven maintained at 65 °C to halt the growth of the fungi and to dry the sample, after which the covers were replaced. In the laboratory, the covers were removed and the samples were then placed in the air-conditioned room for 24 hours for a second equilibration. The filters were then transferred, along with any loose dust, into a second preweighed specimen can for reweighing. Changes in the weight of the control filter were used to correct for moisture variations. It was recognized that in transporting glass fiber filters to and from the field, small weight losses occurred. However, these losses were small in comparison to the sample weights and were neglected in this study. The bulk corn samples were placed in flat open trays for overnight drying at 85 °C. The bulk corn samples were then placed in paper bags and stored in an air conditioned room.

determinations

The filter samples were analyzed for aflatoxin B_1, B_2, G_1 and G_2 contents by a modification of a method approved by the AOAC and AACC for analyzing bulk corn. The approved method was modified and scaled down for application to small dust samples (0.1 to 10 g). The analysis consisted of a chloroform extraction and partial purification on a miniature silica gel column. Quantification of the aflatoxins was accomplished by integrating the fluorescence of the "spot" on thin-layer chromatographic plates of Adsorbosil-1 developed with acetone:chloroform:water, 90:92:2 V/V/V. The limit of detection for a 0.1 g sample was 9 ng/g. Recoveries of 1.5 μg and 3.0 μg standard B_1 from glass filters (8 × 10) were 98% and 67%, respectively. Recoveries of B_1 from naturally contaminated corn on glass filters were 107% for the 0.1 g portion; 62%, 0.25 g; 75%, 0.5 g; and 87%, 1.0 g. It is emphasized that the levels reported in Tables I and II are "as found" and have not been corrected for recoveries. Additional details of the analytical procedure and methods for determining recoveries are available.[56] The bulk corn samples were analyzed by the AOAC approved method.[54]

results and conclusions

The results of the analysis of airborne dust generated by the Bourner Divider are shown in Table I. The total aflatoxin content of the dust ranged from 2560 to 4560 ppb with an average of 3886 ppb. The 3886 ppb average aflatoxin concentration in the dust is higher than the 2250 ppb aflatoxin content of the bulk ground corn. During grinding, highly contaminated kernels tend to shatter and may account for the increased aflatoxin content in the airborne dust. Supporting evidence of an enrichment process is provided by a study of the dry milling process to determine whether aflatoxins could be mechanically separated.[55] It was shown that aflatoxin distribution varied in the product fraction, with the lowest in the grits and the highest in the germ, hull and fines. The size distribution of airborne particles collected during this operation is shown in Figure 3 (Sample 4A). The reason for the shape of this curve is unknown but a possible explanation is that the distribution resulted from the grinding. Concentration of the aflatoxins in the dust collected on the first stage was significantly higher than the levels found in the succeeding stages or the final filter. The calculated distributions should be used with caution because of the possibility that overloading may have occurred and altered the collection pattern.

The results of the sampling conducted at the storage bin are shown in Table II. In the total dust samples, 1-16, the aflatoxin concentrations ranged from a trace to 204.3 ppb. An average level of aflatoxins was calculated to be 155.5 ppb for the 15 samples containing significant levels of aflatoxins. This average of 138 ppb is less than the aflatoxin concentration in the bulk corn of 241.4 ppb. This indicates that the aflatoxin concentration in the airborne dust is approximately 1/2 of the level of aflatoxin in the bulk corn. The particle size distributions shown in Figure 3 represent samples 17, 18, 19 and 20. The first samples collected were at sites 17 and 18 and the smaller particles may have become airborne so that the size distributions were shifted towards larger particles at the sampling sites 19 and 20. Because of the large extrapolations of available data that would be necessary, standard geometric deviations and the median diameters were not calculated. The distribution curves show that only 5 to 17 percent of the particles were less than 7 μm. Therefore, the amount of aflatoxin capable of penetrating to the lower lung was a small fraction of the total. The large size of the particles also suggested that fallout would be rapid and that aflatoxins would not be a general air pollution problem.

The sample of bulk material removed from the drying bin wall contained 127 ppb aflatoxin B_1, 21 ppb aflatoxin B_2 and 28.1 ppm zearalenone. Although no airborne samples were collected, considerable dust was generated by sweeping, etc.

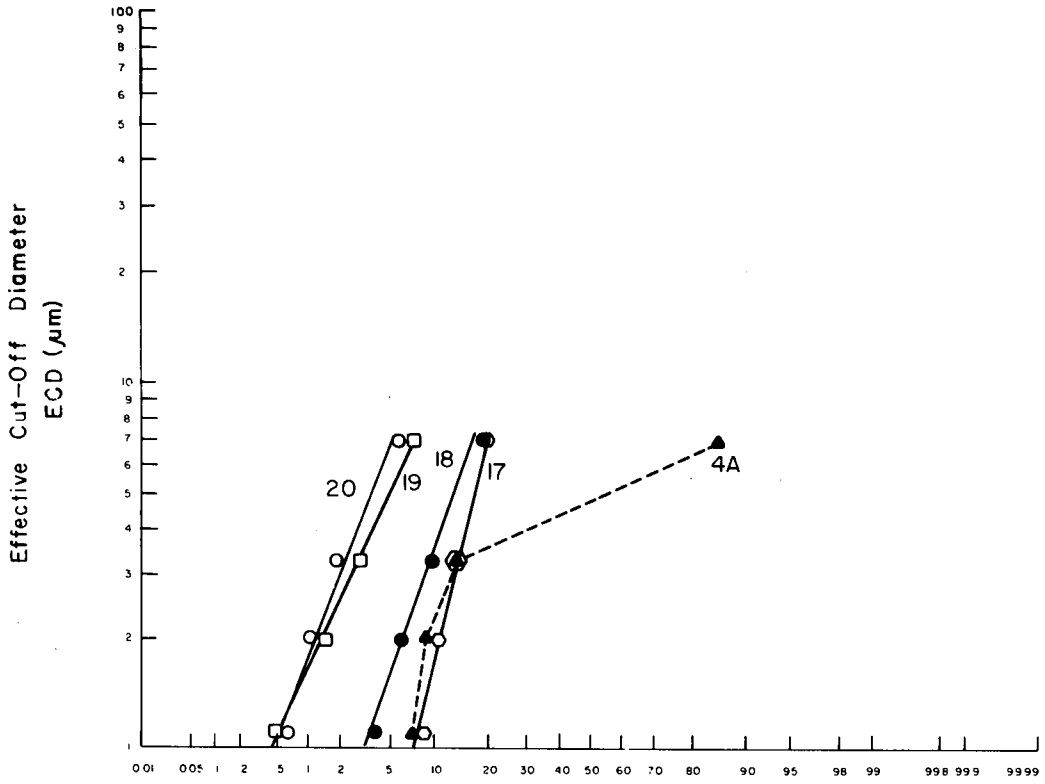

Figure 3 – Size distribution of corn dusts collected with an Andersen Sampler.

in the bin with virtually no ventilation. The farmer spent approximately 2 hours working in the bin.

It is not possible with existing toxicological data to accurately assess the hazards associated with the inhalation of aflatoxin-contaminated dust. There are likely to be considerable differences in the risks from inhalation compared to the risks from ingestion. The absorption rates and biological activations of aflatoxins in the lungs are unknown. It may also be of importance to consider the relative concentrations of aflatoxins in the lungs compared to the intestinal system. By calculation, the average concentration of aflatoxin in the stomach from eating peanuts subsequently diluted with other food is considerably lower than the concentration of aflatoxin at a site in the respiratory system produced by inhaling contaminated corn dust. The level of aflatoxin in the airborne dust represents an average concentration. However, distribution of aflatoxin is not uniform and probably is similar to that in the bulk corn. Thus, a single kernel with an aflatoxin concentration of 207 000 ppb in 3500 kernels (1 kg) would result in an average concentration of only 78 ppb in the bulk corn. This suggests that extremely high concentrations of aflatoxins could exist at the deposition sites of some highly contaminated particles. Defensive mechanisms might be unable to deal with such levels.

The rationale for the proposed guideline established by the FDA for aflatoxins in food appears to be logically sound. In arriving at this level, the FDA attempted to bring four factors into balance:

1. the need to minimize human exposure;
2. the capabilities of sampling procedures and analytical methods;
3. the capabilities to prevent and remove contaminated peanuts; and
4. the need for a continued source of low cost protein.[45]

This carefully constructed proposal concluded that a zero level was impossible and recommended a 15 ppb level.

The central factor in the FDA rationale for its aflatoxin guideline, the maintenance of a protein supply that may unavoidably become contaminated, is not a consideration for establishing an occupational exposure level. By the use of control technology and/or personal protective devices, occupational exposures may be kept below almost any desired level and, therefore, should be set on the basis of expected health effects. In attempting to establish a level of exposure to aflatoxin-containing dust several factors should be noted:

1. The significance of available evidence: there is a question of the potency of aflatoxins to produce cancers in man. In spite of the fact that aflatoxins are carcinogenic to mice, rats, fish, ducks, tree shrews and monkeys (producing mainly cancers of the liver, colon and kidney) and that epidemiologic

studies have shown positive correlations between average human dietary concentrations of aflatoxins and the incidences of liver cancer, this evidence is circumstantial. No studies have linked an increased risk to actual intake in a specific individual. The International Agency for Research on Cancer Working Group, therefore, gave aflatoxins a Group 2 (probably carcinogenic to humans), subgroup A classification (highest degree of evidence);

2. The sites of deposition and the concentrations: This is important because the aflatoxin is unevenly distributed on dust particles;
3. The concentrations of the aflatoxin in the dust and of the dust in air;
4. Other toxic effects;
5. Mode of entry: Entry via inhalation may produce different results than via the oral mode;
6. Other sources of exposure: The occupational exposure may be in addition to dietary exposures.[57]

Even though the results of the epidemiologic study in the oil pressing plant were statistically inconclusive, the strong implications and severe effects warrant prudent measures. Until additional data becomes available, it is recommended that the Dutch experience serve as an alert and that precautions be taken if the commodity to be handled contains more than 20 ppb total aflatoxin. This level is slightly below the levels found in the Dutch plant and most commodities can be handled in the usual manner. Commodities with higher concentrations, which might result from environmental conditions or sorting processes, should be handled with caution.

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Nickel producers research organization formed . . .

An international group of nickel producers has formed an organization to develop and publish scientific information concerning occupational health and safety and environmental matters related to the production of nickel. It is called the Nickel Producers Environmental Research Association, Inc. (NiPERA).

The following thriteen companies are members: AMAX Inc., Pt Aneka Tambang, Falconbridge Nickel Mines Limited, The Hanna Mining Company, Impala Platinum Limited, Inco Limited, LARCO, Nippon Mining Company Limited, Outokumpu Oy, Sherritt Gordon Mines Limited, Societe Metallurgique Le Nickel - SLN, Sumitomo Metal Mining Company, Limited, Western Mining Corporation Limited.

Some projects will be carried out by the members of NiPERA but most of the research will be conducted by experts from outside the industry, mainly from the academic community. NiPERA is now searching for a multi-disciplinary organization capable of providing the administrative and technical support needed to develop and suppervise a comprehensive program of scientific research. In the meantime, NiPERA will maintain an office at Box 44, 1 First Canadian Place, Toronto, Ontario M5X 1C4, Canada.