Thin Layer Chromatographic Analysis of Possible Aflatoxins Within Grain Dusts[1]

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ABSTRACT

The National Institute for Occupational Safety and Health is interested in assessing the hazards to grain workers associated with respirable grain dusts of all types. One of these hazards could involve the occurrence of mycotoxin producing fungi either upon or within grains. Aflatoxins, types of mycotoxins produced by both *Aspergillus flavus* and *A. parasiticus*, are hepatocarcinogens, mutagens, teratogens and toxins. Here, we report an attempt to determine whether settled and/or airborne dusts from barley, corn, flax, oats and Durum as well as spring wheats contain aflatoxins. These dusts were collected at port grain terminals in the Superior-Duluth regions of the United States. The dusts were extracted with and chromatographed upon thin layer plates in a variety of solvents which have been approved for the separation of aflatoxins. Two acceptable aflatoxin B_1 confirmatory tests were employed to verify suspected aflatoxin B_1 within the extracts. Each dust contained a chloroform-soluble, blue fluorescent compound(s) which possessed an R_f similar to that of aflatoxin B_1 upon chromatography of chloroform extracts in chloroform/95% methanol. Methylene chloride/H_2O) extracted a blue fluorescent compound(s) from each dust, and the compound(s) possessed R_f intermediate between those of aflatoxin B_1 and B_2 upon chromatography in acetone/methylene chloride. The methylene chloride/H_2O extracted compounds failed to turn yellow upon spraying with 25% sulphuric acid in methanol and subsequent viewing with an ultraviolet source. Our results confirm those of Sorenson et al., who reported that aflatoxins were absent from airborne grain dusts collected from the Superior-Duluth areas of the United States in the fall of 1977. In conclusion, we stress the need for extracting, detecting, and identifying aflatoxins by a variety of analytical procedures including thin layer and high performance liquid chromatography and "approved" confirmatory tests.

INTRODUCTION

Aflatoxins (AFT), types of mycotoxins, are secretions of *Aspergillus parasiticus* and *A. flavus* which can grow on a wide variety of agricultural commodities (1-4). These mycotoxins are hepatocarcinogens, mutagens, teratogens and toxins (5-7).

Epidemiological data suggest a correlation between the amount of AFT ingested and the lifetime risk (8) of liver cancer as well as the incidence of hepatocellular carcinoma (9, 10) in both human males and females.

From time to time, AFT have been reported to be present within various grains, e.g., corn (11-15), grain sorghum, oats, rye, rice and wheat (2).

Because AFT can be found either upon or within grains, the possible occurrence of AFT within certain grain dusts has been examined (16,17). In this connection, the milling of corn containing AFT (18-21) has been reported.

Other indications that inhalation of AFT can impair human health are those of Dvorackova (22) who reported a correlation between the appearance of AFT within the lungs and alveolar cell carcinoma in two individuals. In addition, Deger (23) observed two cases of colon cancer in those individuals who scraped AFT from thin layer chromatoplates without the aid of a safety hood.

This paper reports a confirmation of the results published within a technical note by Sorenson et al. (16) concerning the possible occurrence of AFT within grain dusts which were collected from the northern regions of the USA. In addition, we point out the need for the utilization of "approved" confirmatory tests to prevent the misidentification of AFT of blue fluorescent, organic-soluble, grain dust compounds which can exhibit R_f similar to certain AFT following thin layer chromatography (TLC). Also, we point out differences in AFT distributions within grain dusts which originate from the milling of northern and southern-derived USA grains.

EXPERIMENTAL PROCEDURES

Dust Collection

Airborne dusts of barley, corn, flax, oat, Durum and spring wheats were collected from the Superior-Duluth areas of the United States in the fall of 1977. The dusts were collected with an industrial vacuum cleaner (24, 25) during the transportation of whole grains upon conveyor belts within active, terminal grain elevators. Following their collection, the dusts were stored within sealed, plastic bottles at 4 C.

Settled, grain dusts were obtained from beams, rafters and ledges within the same elevators. Dusts, which ranged from 8 to 30 cm in depth (ca. 20 year accumulation based upon eyewitness testimony), were scooped, packaged and returned to the laboratory whereupon they were stored under the same conditions as the airborne dusts.

Extractions

Chloroform. One hundred mg fresh wt lots of dusts were extracted with 6 mL aliquots of chloroform for 1 hr and agitation on a New Brunswick gyrotory shaker (150 rpm). Following their extraction, the dusts were filtered through Whatman No. 1 filter paper and the filtrates evaporated to dryness prior to their reconstitution in 100 μL chloroform.

Chloroform/H_2O. One hundred mg fresh wt lots of dusts were added to 150 mL chloroform and 15 mL H_2O and then shaken for 30 min with a Magni Whirl, wrist-action shaker (Blue M Electric Company, Blue Island, IL). Follow-

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TABLE I

R_f of Fluorescent Compounds Extracted from Grain Dusts with Chloroform and Chromatographed on Adsorbosil+1 in Chloroform/Methanol[a]

Dust or reference standards	R_f			
	Band 1 (red)[b]	Band 2 (blue)	Band 3 (blue-green)	Band 4 (red)
Dusts				
Barley	0.12 ± 0.08	0.46 ± 0.06	0.64 ± 0.02	0.73 ± 0.03
Corn	0.00 ± 0.00	0.50 ± 0.05	0.69 ± 0.02	0.00 ± 0.00
Flax	0.17 ± 0.07	0.54[c]	0.67 ± 0.04	0.78 ± 0.03
Oat	0.15 ± 0.07	0.51 ± 0.05	0.68 ± 0.01	0.81 ± 0.01
Settled	0.14 ± 0.08	0.49 ± 0.06		0.81 ± 0.02
Wheat				
Durum	0.15 ± 0.08	0.48 ± 0.08	0.65 ± 0.03	0.78 ± 0.03
Spring	0.14 ± 0.08	0.47 ± 0.07	0.66 ± 0.02	0.77 ± 0.02
Reference standards				
AFT-B_1		0.46 ± 0.06		
AFT-B_2		0.40 ± 0.04		
AFT-G_1		0.36 ± 0.05		
AFT-G_2		0.32 ± 0.04		

[a]Data are means and standard deviations of three replicate experiments.
[b]Fluorescent color.
[c]One sample lost.

ing shaking, the dust residues were removed by filtration through Whatman No. 1 filter paper. The filtrates were evaporated to dryness and reconstituted in 100 μL chloroform.

Methylene chloride/H_2O. Five g samples were extracted by the "CB Method" (26), except that chemicals and solvents were reduced proportionately and methylene chloride was substituted for chloroform, since the former has been recommended by certain investigators (L. Stoloff, personal communication) as being both less toxic and more selective than chloroform for extraction of AFT. The second step of the "CB Method" employs "clean-up" by column chromatography.

Chromatography

Substances extracted with chloroform. Filtrates were evaporated to dryness and reconstituted in 100 μL chloroform and spotted onto heat-activated (100 C, 30 min), 250 μm thick Adsorbosil+1 plates. Ten μg each AFT-B_1, AFT-B_2, AFT-G_1 and AFT-G_2 (Applied Sciences Lab. Inc., State College, PA) were spotted onto the plates as reference standards. Table I shows that the standard deviations about the mean R_f for AFT-B_1, AFT-B_2, AFT-G_1 and AFT-G_2 were much less than half the mean. In view of this reproducibility, we believe that 10 μg of each toxin could be successfully separated without distortion of the R_f. The plates were developed in unlined chambers containing either chloroform/95% methanol (100:3) or toluene/ethyl acetate/formic acid (60:30:10) and then viewed with an ultraviolet (UV) source (UVS-12 mineral light, San Gabriel, CA). The limits of detection were 2 ppb (27).

Substances extracted with chloroform/H_2O. The reconstituted filtrates were spotted onto thin layer plates together with reference standards as above. Whereas the reference standards were spotted as a mixture, the unknowns were spotted side by side. The plates were developed in unlined chambers which contained 101 mL $CHCl_3$/acetone/water (91+9+1).

Substances extracted with the "CB Method". The column eluate was evaporated to dryness and reconstituted to a known volume. Repeated microliter aliquots were spotted onto Adsorbosil+1 hard thin layer plates until the entire reconstituted sample was spotted. The plates were developed in unlined chambers containing acetone/methylene chloride (1+9) or benzene/methanol/acetic acid (90+5+5). The plates were developed for 40 min at 23–25 C and the solvent removed from the plates by evaporation following removal of the latter from the chambers. The plates were viewed with the UV source.

Confirmation Tests

Thin layer plates containing presumed and authentic AFT-B_1 were either treated with trifluoroacetic acid (TFA) (28) or sprayed with 25% sulphuric acid (29) as confirmatory tests.

RESULTS AND DISCUSSION

Chloroform-soluble substances. Table I presents both the R_f and fluorescent colors of compounds extracted from grain dusts with chloroform and subsequent chromatography upon Adsorbosil+1 plates in chloroform/methanol. Each of the dusts contained four compounds whose fluorescent colors were red, blue, blue-green and red for the lowest, next to the lowest, adjacent to the highest and the highest R_f. The compound(s) composing band 2 possessed either the same or nearly the same R_f as that for AFT-B_1. This suggested that the dusts might contain AFT-B_1.

Both the R_f and fluorescent colors of chloroform-soluble substances following their chromatography in toluene/ethyl acetate/formic acid are displayed within Table II. A green fluorescent compound(s) with an R_f in excess of those for the reference standards was detected within each of the dust extracts.

To verify that the dusts might contain AFT-B_1, we extracted dusts with either chloroform/H_2O, chloroform/methanol or methylene chloride/H_2O and then chromatographed the extracts in a variety of solvents. Approved

TABLE II

R_f of Fluorescent Compounds Extracted from Grain Dusts with Either Chloroform/H_2O or Chloroform and Chromatographed on Adsorbosil+1

Dusts or reference standards	R_f		Fluorescent colors	
	Chloroform/H_2O[a]	Chloroform[b]	Chloroform/H_2O	Chloroform
Dusts				
Barley	0.15	0.80	red	green
Corn	ND	0.80		green
Flax	0.19	0.74	red	very faint green
Oat	0.15	0.76	red	very faint green
Settled	0.12	0.80	red	green
Wheat				
Durum	0.15	0.80	red	green
Spring	0.21,0.52	0.80	red, blue	green
Reference standards				
AFT-B_1	0.58	0.40	blue	blue
AFT-B_2	0.51	0.34	blue	blue
AFT-G_1	0.46	0.31	green	green
AFT-G_2	0.40	0.23	blue-green	blue-green

[a]Developing solvent—chloroform/acetone/H_2O (91+9+1).
[b]Developing solvent—toluene/ethyl acetate/formic acid (60+30+10).

TABLE III

R_f of Fluorescent Compounds Present within Methylene Chloride/H_2O-Soluble Extracts of Grain Dusts Following Thin Layer Chromatography on Adsorbosil+1 in Acetone/Methylene Chloride[a]

Dusts or reference standards	R_f
Dusts	
Spiked with standards	0.37, 0.43, 0.49, 0.51, 0.56, 0.62
Unspiked	
Barley	0.52[b] 0.63
Spring wheat	0.51 0.61
Reference standards	
AFT-B_1	0.56 ± 0.01
AFT-B_2	0.48 ± 0.01
AFT-G_1	0.42 ± 0.01
AFT-G_2	0.35 ± 0.01

[a]Developing solvent—acetone/methylene chloride (1+9).
[b]Fluorescent blue spot with an R_f between AFT-B_1 and AFT-B_2.

confirmatory tests were also employed.

Chloroform/H_2O-soluble substances. The data within Table II demonstrate that each dust except for corn contained a red fluorescent compound(s) whose R_f did not correspond to any of those for the reference standards. However, a blue fluorescent compound(s) exhibiting an R_f similar to that for AFT-B_2 was observed within chromatographed extracts of spring wheat.

Methylene chloride/H_2O-soluble substances. Table III compares the R_f of methylene chloride/H_2O-soluble substances from barley and spring wheat dusts with those for AFT-B_1, AFT-B_2, AFT-G_1 and AFT-G_2 subsequent to thin layer chromatography of the substances in acetone/methylene chloride (1+9). A blue fluorescent spot with an R_f intermediate between those for AFT-B_1 and AFT-B_2 was apparent within both the spiked and unspiked dust extracts except for corn, flax and Durum wheat. However, the spot failed to turn yellow upon spraying with 30% sulphuric acid in methanol. In addition to the blue fluorescent spot, a green fluorescent one with an R_f below that for any of the reference standards was apparent within the dust extracts. When the plates containing the blue fluorescent compounds were sprayed with 25% sulphuric acid in methanol (29), the compounds failed to turn yellow upon viewing with the UV source.

Blue fluorescent compounds with R_f either resembling those of AFT-B_1, AFT-B_2, AFT-G_1 or AFT-G_2 or in close proximity to the origin were not observed following TFA treatment of methylene chloride/H_2O extracted substances and their chromatography in acetone/methylene chloride.

The results derived from chromatography of chloroform/H_2O, chloroform/methanol and methylene chloride/H_2O extraction suggest that the examined grain dusts lacked AFT-B_1, AFT-B_2, AFT-G_1 and AFT-G_2. Therefore, the blue fluorescent compounds which were extracted by either chloroform or methylene chloride/H_2O and exhibited an AFT-B_1 R_f appear to be other than AFT.

These results confirm those of Sorenson et al. (16) who reported that AFT were absent from airborne dusts which were collected from the Superior-Duluth areas of the United States in the fall of 1977. This absence is to be sharply contrasted with the occurrence of AFT within

dusts collected from southern USA. For example, Sorenson et al. (16) observed that corn dust obtained from a dumping station of a feed mill plant near Tifton, Georgia, in August, 1978 contained 130 ppb AFT-B_1. They separated the dusts into particulates with "aerodynamic/diameters" of 7-11 and < 7 μm. The AFT contents of the latter were in excess of that of the former. Shotwell and her coworkers (30-32) recently reported the development of procedures for the detection of AFT-B_1 in airborne corn dusts. Their thin layer chromatographic procedures enabled the use of 0.1 g samples.

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