Final Progress Report for

ASSESSMENT OF FARMERS' EXPOSURE TO AFLATOXIN B_1 AND OTHER NATURAL TOXINS IN GRAIN AND GRAIN DUST

Grant #5R01OH02857-3

Principal Investigator: Mustafa I. Selim

The University of Iowa
Department of Preventive Medicine and Environmental Health
100 Oakdale Campus, 137 IREH
Iowa City, Iowa 52242-5000
Telephone: 319-335-4424

EXECUTIVE SUMMARY

PROGRESS REPORT

Epidemiological studies from around the world provide evidence for an association between the incidence of lung and other cancers and exposure to airborne aflatoxins in contaminated grain and/or grain dust. Exposure data to assess the potential risk of cancer in farmers and grain workers due to the inhalation of aflatoxin contaminated grain dust are limited by the high cost and poor detection limits of current analytical methods. Exposure data to other mycotoxins (e.g. fumonisins, ochratoxins, vomatoxin, and zearalenones) which are commonly found in grain and grain dust, is virtually nonexistent. Past analysis of grain dust samples from the Midwest and Southeast corn growing belt has demonstrated the presence of aflatoxins in high volume air samples of airborne dust. Our preliminary studies indicated that if aflatoxin B_1 is present in airborne dust samples during harvest, it continues to be present and may in fact increase throughout subsequent grain handling and animal feeding operations; over 75% of our test farms had detectable airborne aflatoxin by the time the grain bins are cleaned out. Airborne levels in these bins were found in excess of those associated with a historic cohort exposed to airborne aflatoxin currently experiencing excess cancer. The completed project (5R01OH02857-3) was an extension of an earlier grant limited largely to laboratory method development (1R01OHO2857-1) which demonstrated the high sensitivity, efficiency, and reliability of the supercritical fluid extraction technique for the extraction of aflatoxin B_1 and fumonisin B_1 from grains and grain dust. In this project we proposed to investigate potential correlation between the level of aflatoxin B_1 (and other mycotoxins) in grain and its level in airborne dust. Such correlation provides a means by which the health risk of farmers' exposure to airborne aflatoxin B_1 (and other mycotoxins) can be predicted from the analysis of an appropriately processed sample of bulk corn. Such correlation will then be used to determine the exposure levels needed to conduct future epidemiologic assessments over a wide geographical area throughout the corn growing belt in the U.S. (including South Carolina, North Carolina, Illinois, Iowa, Kansas, Missouri, and Oklahoma).

The first phase of this project tested and optimized two methods for processing grain dust from bulk corn samples. These methods were then used to characterize the variability of aflatoxin B_1 and other mycotoxins in samples collected during on-farm grain handling activities within the state of Iowa. The data obtained provide information on the prevalence, activity mean exposure level, and the variability within the study area. During the second phase we have achieved significant improvements in the speed and sensitivity of our multitoxins analysis methods, using the upgraded ES-API/HPLC/MS system. In addition, the project examined the effect of intra-farm, intra-year variability (change) in the aflatoxin content of the grain over time as affected by the grain moisture content at storage and grain handling history. Obtained correlation for aflatoxin B_1 in aerosolized dust and bulk corn provides means to estimate farmers' exposure to aflatoxin in airborne

dust from its measurement in a processed bulk sample. The prevalence of other mycotoxins such as fumonisin, ochratoxin, vomatoxin, and zearalenone in bulk and prepared grain dust in these same on-farm activities was investigated. Correlations for other mycotoxins in bulk corn and aerosolized dust were also investigated. The correlations obtained, extend the utility of the new bulk processing methods as means to estimate farmers' exposure to other mycotoxins in aerosolized grain dust based on its measurement in bulk samples.

In addition to achieving the above project objectives, we have studied the fate and distribution of aflatoxin B_1 and its transformation products in soil and aerosolized soil dust. The data obtained provide information on the stability and prevalence of aflatoxin B_1 and its breakdown products in soil and soil dust. Such information is important for estimating the overall level of farmer's exposure to aflatoxin B_1 from various farming activities.

The possibility of climatic patterns of repeated hot and humid growing seasons creates an increasing need to define the possible role of aflatoxins in the etiology of lung or other cancers. We intend to use the results and methodology developed in this project to establish the exposure database and exposure model needed to initiate and/or participate in an epidemiologic assessment of the occupational risk to farmers resulting from their exposure to aflatoxin B_1 and other mycotoxins throughout the agricultural belt in the U.S.

PROJECT ACCOMPLISHMENTS

The broad objective of this project was to develop a better means to assess the exposures of farmers to aflatoxin B_1 in airborne grain dust. We believe repeated low level exposures during various on-farm grain handling activities represent a significant health risk to farmers and potentially to full-time grain handlers. We have previously developed an efficient, sensitive, and highly specific analytical method to determine low levels of aflatoxins in aerosolized grain dust samples. In this project we have established quantitative link between airborne aflatoxin and aflatoxin extracted from a specially prepared bulk corn sample. The accomplishments of this project have met and exceeded the previously proposed objectives as described below:

Accomplishments of Project Objectives:

1. Developed and optimized laboratory methods to assess the levels of aflatoxin B_1 in grain dust from analysis of bulk corn samples. These methods are based on the protocols described in the proposal. (Objective 1)

2. Established means to estimate farmers' exposure to aflatoxin in aerosolized grain dust based on its measurement in a bulk sample. A correlation was found between aflatoxin B_1 in specially prepared samples of bulk corn and in airborne dust collected during on-farm grain handling activities in a wide geographic area within the state of Iowa. This correlation will greatly extend the utility of future bulk grain in surveys of aflatoxin B_1 exposure. (Objective 1)

3. The results of this project provide information on the prevalence, activity mean exposure level, and the variability in aflatoxin B_1 concentrations pertinent to future epidemiologic assessments. Of particular importance is the intra-farm, intra-year variability (change) in the aflatoxin content of the grain over time as affected by the grain moisture content at storage and grain handling history. The effect these factors upon the correlation of aflatoxin between prepared bulk and airborne samples was examined. (Objective 2)

4. Identified and quantified the prevalence of other natural toxins such as fumonisin, ochratoxin, vomatoxin, and zearalenone which may be detectable during the course of aflatoxin analyses in bulk and prepared grain dust in these same on-farm activities and geographic areas. (Objective 3)

5. Tested and investigated the similarity of the correlation between these other toxins (fumonisin, ochratoxin, vomatoxin, and zearalenone) in bulk grain and in the processed dust with the correlation obtained for aflatoxin B_1. Useful correlations were obtained, for other toxins, which extends the utility of the new methods as means to estimate farmers' exposure to mycotoxins in aerosolized grain dust. (Objective 3)

Additional Accomplishments (Not in Proposed Objectives):

1. Developed and used new method for simultaneous analysis of aflatoxin B_1 and other mycotoxins. This method is based on the use of ES-API/HPLC/MS system, upgraded through this project funding.

2. Investigated and analyzed the distribution of aflatoxin B_1 in aerosolized soil dust. The results of this investigation are critically important for estimating farmers' exposure to aflatoxin from various on-farm activities.

3. Studied the fate of aflatoxin B_1 in soil and identified its transformation products and the conditions leading to their formation.

Papers Published or In Press Resulting from the Project:

1. El-Sharkawy SH, Selim MI, Afifi MS, Halawish FT. Microbial transformation of zearalenone IV - formation of zearalenone sulfate. Appl Environ Microbiol 1991; 57:549-52.

2. Selim MI, Tsuei MH. Development and optimization of a supercritical fluid extraction method for the analysis of aflatoxin B_1 in grain dust. Am Ind Hyg Assoc J 1993; 54(4):135-41.

3. Selim MI, Ibrahim MS. Effect of aflatoxin B_1 on steroid hormones in young male rats. J Environ Sci 1994; 7:125-40.

4. Ibrahim MS, Selim MI. Effect of aflatoxin B_1 on thyroid hormones metabolism in young male albino rats. J Environ Sci 1994; 7:141-58.

5. Ibrahim MS, Selim MI. Effect of aflatoxin B_1 on the carbohydrate metabolism and growth hormone in young male rats. J Egypt Ger Soc Zool 1994; 14:317-37.

6. Salem MI, Selim MI. Determination of aflatoxin B_1 in some Egyptian foodstuffs and medicinal plants. Mans Sci Bull 1994; 21:121-33.

7. Selim MI, Popendorf W, Ibrahim MS, EL-Sharkawy S, EL-Kashory S. Aflatoxin B_1 in common Egyptian foods. J AOAC Int 1996; 79(5):1124-9.

8. Selim MI, EL-Sharkawy S, Popendorf WJ. Supercritical fluid extraction of fumonisin B_1 from grain dust. J Agric Food Chem 1996; 44:3224-9.

9. Selim MI, Juchems AM, Popendorf WJ. Potential predictors of airborne concentrations of aflatoxin B_1. J Agromedicine 1997; 4:91-8.

10. Selim MI, Popendorf WJ, Juchems AM. Assessing airborne aflatoxin during on-farm grain handling activities. Am Ind Hyg Assoc J 1998; 59:252-6.

Books/Chapters/Manuals Resulting from the Project:

1. Selim MI. Application of supercritical technology to the analysis and treatment of hazardous waste. Hazardous Waste Research, University of Kansas, Manhattan, KS, 1990; 1:123-7.

2. Selim MI, Juchems AJ, Popendorf WJ. Potential predictors of airborne concentrations of aflatoxin B1. In: Donham KJ, Rautiainen R, Schuman SH. Agricultural Health and Safety, Recent Advances. Binghamton, NY: The Haworth Press, 1997: 91-8.

Papers Submitted for Publication Resulting from the Project:

Selim MI, Juchems AM, Popendorf W, Dawson J. Aflatoxin B_1 levels and size distribution in aerosolized grain dust. Am Ind Hyg Assoc J.

Papers in Preparation Resulting from the Project:

1. Selim MI, Starr JM. Levels and Prevalence of Mycotoxins (Aflatoxins, Fumonisins, Ochratoxins, Vomatoxin, and Zearalenones) in During on Farm Grain Handling Activities.

2. Selim MI, Popendorf, W, Starr JM. Correlation Between Aflatoxin B_1 Levels in Grain and Aerosolized Grain Dust.

3. Selim MI, Popendorf, W, Starr JM. Correlation Between Mycotoxin (Fumonisins, Ochratoxins, Vomatoxin, and Zearalenones) levels in Grain and Aerosolized Grain Dust.

4. Selim, MI, Brown, JD, Starr JM. Simultaneous Analysis of Aflatoxins, Fumonisins, Ochratoxins, Vomatoxin, and Zearalenones using SFE and ES-API/HPLC/MS.

5. Selim MI, Starr JM. Fate of aflatoxin B_1 in agricultural soil.

6. Selim MI, Starr JM. Identification of aflatoxin B_1 metabolites in soil using ES-API/HPLC/MS. J Am Soc Mass Spectrom.

7. Starr JM, Selim MI, O'Shaughnessy PT. Supercritical fluid extraction of aflatoxins from agricultural soil. JSFC.

8. Starr JM, Selim MI. Levels and distribution of aflatoxin B_1 and metabolites in soil and soil dust. Am Ind Hyg Assoc J.

Published Abstracts Resulting from the Project:

1. Selim MI, Weinrich AJ, Popendorf WJ. Occupational exposures to aflatoxins in agricultural workers. American Industrial Hygiene Conference; 1990 May 13-18; Orlando, FL. Abstract #60, p. 40.

2. Selim MI, Dhawan SK. Application of SFE to the extraction of mycotoxins from contaminated grains. [Invited] 105th Association of Official Analytical Chemist (AOAC) Meeting and Exposition; 1991 Aug 12-14; Phoenix, AZ. Abstract #288, p. 161.

3. Selim MI. Assessment of farmers' exposure to mycotoxins grain dust. American Industrial Hygiene Conference and Exposition; 1992 Jun 1-5; Boston, MA.

4. Selim MI. Matrix effects in the SFE of mycotoxins. [Invited] 4th International Symposium on Supercritical Fluid Chromatography and Extraction; 1992 May 20-22; Cincinnati, OH. pp. 191-2.

5. Selim MI, El-Sharkawy SH, Padanilam BM. Supercritical fluid extraction of fumonisins from grain and contaminated dust. [Invited] Midwest AOAC; 1992 Jun 8-11; Champaign, IL. No. 12, p. 7.

6. Selim MI, Popendorf W, Juchems AM. Levels and distribution of aflatoxin B_1 in grain dust. Agricultural Safety and Health: A National Conference on Detection, Prevention and Intervention; 1994 Aug 24-26; Columbus, OH. p. 12.

7. Selim MI, Juchems AM, Popendorf W, Dawson J. Levels and distribution of aflatoxin B_1 in aerosolized grain dust. Third Annual NIOSH Agricultural Health and Safety Conference; 1996 Mar 26. p. 60.

8. Selim MI, Popendorf W, Juchems AM. Assessing occupational exposure to aflatoxin B_1 during on-farm grain handling activities. Third Annual NIOSH Agricultural Health and Safety Conference; 1996 Mar 26. p. 61.

Presentations Resulting from the Project:

1. Selim MI, Weinrich AJ, Popendorf WJ. Occupational exposures to aflatoxins in agricultural workers. American Industrial Hygiene Conference; 1990 May 13-18; Orlando, FL.

2. Selim MI. Application of SFE to the extraction of mycotoxins from contaminated grains. [Invited] 105th Association of Official Analytical Chemist (AOAC) Meeting and Exposition; 1991 Aug 12-15; Phoenix, AZ.

3. Selim MI. Assessment of occupational exposure to mycotoxins in grain dust. [Invited] Silver Anniversary Chemistry Department Symposium, Murray State University; 1992 Apr 17-18; Murray, KY.

4. Selim MI. Assessment of farmers' exposure to mycotoxins grain dust. American Industrial Hygiene Conference and Exposition; 1992 Jun 1-5; Boston, MA.

5. Selim MI. Matrix effects in the SFE of mycotoxins. [Invited] 4th International Symposium on Supercritical Fluid Chromatography and Extraction; 1992 May 20-22; Cincinnati, OH.

6. Selim MI, El-Sharkawy SH, Padanilam BM. Supercritical fluid extraction of fumonisins from grain and contaminated dust. [Invited] Midwest AOAC; 1992 Jun 8-11; Champaign, IL.

7. Selim MI, Popendorf W, Juchems AM. Levels and distribution of aflatoxin B_1 in grain dust. Agricultural Safety and Health: A National Conference on Detection, Prevention, and Intervention; 1994 Aug 24-26; Columbus, OH.

8. Selim MI, Juchems AM, Popendorf W, Dawson J. Levels and distribution of aflatoxin B_1 in aerosolized grain dust. Third Annual NIOSH Agricultural Health and Safety Conference; 1996 Mar 24-26; Iowa City, IA.

9. Selim MI, Popendorf W, Juchems AM. Assessing occupational exposure to aflatoxin B_1 during on-farm grain handling activities. Third Annual NIOSH Agricultural Health and Safety Conference; 1996 Mar 24-26; Iowa City, IA.

Master's Thesis and Graduate Students Training Supported by the Project:

1. Mei Tsuei, M.S., 1991. Detection of Aflatoxins in Grain Dust Using Supercritical Fluid Extraction.

2. Alex Juchems, M.S., 1992. Distribution of Aflatoxin B_1 in Aerosolized Grain Dust.

3. Sunita Dhawan, M.S., 1993. No thesis.

4. Jenelle Brown, M.S. Analysis of Multitoxins Using SFE and ES-API/HPLC/MS (in preparation)

5. Chandran Achutan (M.S.), Binny Padanilam (M.S.) and Peter Svebakken (M.S.), Eric Svendsen (Ph.D.) have were partially supported by the project and participated in the filed sampling and laboratory work.

Doctoral Dissertation Resulting from the Project:

Jim Starr, Ph.D. Potential Human Health Associated with Aflatoxins in Soil and Soil Dust - Using SFE and ES-API/HPLC/MS. Completed September 1998.

Post-Doctoral Fellows

Saleh El-Sharkawy, post-doctoral, Summer 1991. Method Development and Analysis of Mycotoxins in Cereals and Grain Dust.

Mahmoud Salem Ibrahim, post-doctoral, July 1992 - February 1993. Effect of Aflatoxin B_1 on the Endocrine System in Young Albino Rats.

Other Student Training Resulting from the Project:

The project supported the training of the following high school students through the University of Iowa Secondary School Training Program (SSTP).

1992 Nicole Rascon, North High School, Davenport, IA
1994 Sorya Asadi, Central High School, Davenport, IA
Bertina Hooks, Columbus High School, Waterloo, IA
1996 Kevin Anthony, Wiyatta Freeman, Summer Research Experience Program

Each of the above students was received 6-8 weeks of training and research participation on the field and laboratory research activities of the project. Each students concluded his/her work documenting and presenting the results of his research experience at a local symposium at the University of Iowa. Nicole Rascon and Sorya Asadi have received national (NIH) awards and recognition for their summer research work on the project.

STUDIES AND RESULTS

Detection of Aflatoxin in Grain Dust:

Recent droughts of 1988 and 1989 have created favorable conditions for substantial growth of Aspergillus flavus on the corn crop in the Midwest, where approximately one-third of the world's corn is produced. Analysis of bulk corn samples revealed unacceptable levels of aflatoxins in one third of the official samples in Iowa, which caused many grain elevators to close their doors for receiving any corn. Many farmers were forced to keep their corn crop for animal consumption, and others were discouraged from harvesting their poor and unmarketable crop. The situation has generated tremendous concern regarding the possible health risk to farmers associated with handling aflatoxin contaminated grain. Several local farmers have called the Institute for Rural and Environmental Health at the University of Iowa, expressing their fear of harvesting or handling the contaminated corn. Field visits to some local farms showed substantial crop damage with visible widespread fungal growth.

Four local farms were selected for collection of airborne samples during the harvest operation. 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 confinement building, where some of the harvested grains were used for animal feeding.

Collected dust samples were analyzed for aflatoxin B_1 using a modification of the thin layer chromatography (TLC) procedure described in the literature. Aflatoxin B_1 was detected in the airborne dust samples in two out of the four farms selected. In farm number 2, aflatoxin was detected in the dust sample collected from inside the tractor cab but was not detected in the outside sample, primarily because of strong cross winds.

In farm number 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 confinement building where the same corn was used for animal feeding. Aflatoxin B_1 concentrations of 66.6 ng/m^3 and 92.6 ng/m^3 are comparable to recent farm level measurements of Burg and Shotwell, and are significantly higher than the Netherlands epidemiologic study (roughly 5 pg/m^3) which showed 2.5 times the risk of cancer among 60 to 70 peanut and flax seed processors.

There is no literature data currently available on the aflatoxins exposure levels inside animal confinement facilities (farms 4-6). This type of exposure is highly important in determining the total yearly exposure because the animal feeding operation is a year-round activity. In addition, grains which are unmarketable, due to their contamination with aflatoxin producing fungi, are usually used for on-farm animal feeding. Improper storage of such contaminated grain results in fungal spread and increased aflatoxin production. [A recent highly publicized shipment transported from Oklahoma to Iowa contained 32,000

ppb.] To investigate this possibility, we collected and analyzed airborne dust samples (approx. 4-5 m^3 of air) along with bulk feed samples and settled dust from two animal confinement buildings during the summer of 1989. These preliminary data demonstrate that even in less drought-stressed corn, aflatoxin B_1 is present at detectable levels throughout various on-farm grain handling operations. Therefore, year-round grain handling in animal confinement buildings may contribute the largest portion of the overall farmer's exposure to aflatoxin contaminated dust. More data is needed in order to determine the contribution of all possible exposure sources to the total dose of farmers' exposure to aflatoxins from their yearly farming activities.

Our experience with this limited number of samples has brought to our attention several factors to consider in future sampling. The effects of temperature, humidity, and wind velocity and direction are crucial in determining the amount of dust sampled. Multiple sampling of the same farm under different weather conditions should be used. The content of aflatoxins in the dust should be compared with the level of aflatoxins in bulk corn from the same sampling location. Moreover, the use of personal air sampling pumps is important for determining the actual dose delivered to the exposed worker. Thus, the broad objective of this project was to develop a better means to assess the exposures of farmers to aflatoxin B_1 in airborne grain dust. We believe repeated low level exposures during various on-farm grain handling activities represents a significant health risk to farmers and potentially to full-time grain handlers. However, analysis of the small dust samples collected by the personal air sampler (which collect ≤ 1 m^3/day) necessitated the use of a more sensitive analytical method.

The project was initially designed with both laboratory and field testing phases. The laboratory phase was primarily directed toward analytical method development and optimization for aflatoxin B_1 and other toxins (including ochratoxin, vomatoxin, fumonisin B_1, and zearalenone) in small airborne grain dust samples (2 RO1 OH2857-02). Despite start-up delays for the acquisition of and the installation of the GC,HPLC/MS system, and the needed lengthy facility renovation, a great deal of progress in the proposed method development activities has been achieved.

Bulk corn, settled grain dust, and airborne dust samples were collected from selected Iowa farms for the purpose of the method development and validation. These samples were analyzed using both classical liquid-liquid extraction and supercritical fluid extraction (SFE) followed by off-line HPLC analysis, using diode array UV detection. Fluorescent detection was later used on-line with the UV detection to enhance the detection sensitivity for aflatoxin B_1 and fumonisins. Using this technique we have developed and published two articles on the analysis of aflatoxin B_1 and fumonisins B_1 and B_2 in grain dust samples. A third article and book chapter were also published on aflatoxin environmental exposure during on-farm grain handling operations.

The laboratory methods also supported an M.S. thesis project which investigated the prevalence and distribution of aflatoxin B_1 in airborne grain dust during the manual clean-out of grain storage facilities. Aflatoxin B_1 was detected in 13 of the 14 operations

sampled, at an average concentration of 794 ng/m^3. Significant correlation (p=0.0024) was found between the measured concentration of aflatoxin B_1 and the diameter of the airborne dust particles. The average concentration of aflatoxin B_1 was 160 ppm in dust particles ≥ 1 μm, and 6.5 in dust particles ≤ 7 μm in diameter. Based on the data obtained in this study, a 1-hour exposure to grain dust during the manual bin clean-out process may result in a pulmonary dose of approximately 0.22 μg of aflatoxin B_1. This data is provided in Appendix IA, and is submitted for publication.

On-line SFE/HPLC analysis for aflatoxin B_1 was carried out using the ISCO SFX-10 on-line with a Hewlett-Packard 1090 Series II HPLC (Hewlett-Packard Co., Palo Alto, CA). The on-line SFE extraction procedure involves placing the sample into the extraction vessel (2.5 mL) which is inserted into the extraction chamber of the SFX-10 module. The sample is then extracted using 2 mL of supercritical carbon dioxide with 5% acetonitrile-methanol (2:1) at 1200 psi and 45°C. The SFE extract is passed through a concentration cartridge containing C-18 packing material (37-50μm). Following the SFE extraction, valve switching allows the C-18 cartridge to be connected on-line with the analytical HPLC column, where chromatographic separation takes place. The analytical column was an ODS-Hypersil reversed phase column, 100 x 2.1 mm., 5 μm particle size. The mobile phase was methanol-acetonitrile-water (35:35:30). Dual detection systems were used for these on-line experiments: UV (diode array) at a wavelength of 265 nm and fluorescent detection at an excitation wavelength of 366 nm and emission of 425 nm. The latter detection system was more useful for the lower concentration range.

Particle beam HPLC/MS detection was found to be less sensitive than both the diode array-UV and fluorescent detection and therefore was not used during the optimization of the on-line SFE/HPLC procedure for low concentration airborne dust samples. For this reason we intend to use fluorescent detection and Thermospray (TS)/MS for future mycotoxin analysis in grain and grain dust.

The first phase of the most recently funded work of the project involved the following:

1. Developing and optimizing two laboratory methods by which bulk corn can be processed to extract friable grain dust to be analyzed for aflatoxin B_1.

2. Developing laboratory analytic methods to screen these samples simultaneously for other mycotoxins (e.g. fumonisin, ochratoxin, and vomatoxin).

3. Collecting and analyzing field samples using laboratory developed methods.

The farms to be surveyed was intended to be chosen from among the 750 farms identified for a NIOSH-funded Farm Family Health Hazard Surveillance Project (U04 CCU70676063-05) which was then directed by the co-investigator in this project, Dr. William Popendorf. However, following the departure of Dr. Popendorf from the University of Iowa, it was not possible to coordinate the proposed sampling plan with the

new director of the Farm Family Health Hazard Surveillance Project. This unpredicted change has created serious constraints on the budget and proposed timetable of this project. Significant time, effort, and cost (for personnel, supplies, and field sampling equipment) were spent to recruit farmers and collect field samples.

The second phase of the project was intended to assess differences in the prevalence, concentrations, and intra-farm changes in aflatoxin concentrations across a wide as possible geographical area in Iowa. This also contained a longitudinal sample collection component in which several loads of corn will be tracked from harvest through storage and usage in order to assess intra-farm, intra-year variability (changes in aflatoxin content over time).

Phase I:

In the previous submission, we have budgeted and were funded $30,000 for the purchase of a Thermospray (TS) interface for the Hewlett-Packard 5989A HPLC/MS system. By the time the proposal was reviewed, revised, and approved for funding, the proposed TS/MS interface became obsolete, following the development of the much sensitive and versatile electrospray (ES)/MS.

On January 30, 1995, we requested supplemental funding of ($97,613) to upgrade the HPLC/MS interface with the Atmospheric Pressure Ionization - Electrospray (API-ES) interface. Alternatively, $ 13,940 were needed for the price difference of the TS upgrade. On March 22, 1995, we were notified by NIOSH that funds were not available to support our request for the API-ES upgrade ($97,613). However, NIOSH agreed to consider our request for the $13,940 toward the end of the fiscal year. On March 22, 1995, we requested a six month extension to delay the project start-up date. Our request for the no-cost extension was approved on April 10, 1995. The request for the supplemental funds for the TS price difference of $13,940 was approved by NIOSH on September 27, 1995. Therefore, the project schedule was revised to reflect the start-up date of October 1, 1995. Despite the delayed start-up, the revised timetable and sampling schedule reflects major improvement over the original timetable.

Task 1 - Acquisition of HPLC Fluorescent Detector and Thermospray HPLC/MS Interface: After placing the order for the TS interface, we were advised by Hewlett-Packard that the display instrument at the 1996 Pittsburgh Conference and Exhibition, held in Chicago, March 3-7, 1996, will be sold at a discounted price. We were able to negotiate and purchase API-ES interface with approximately 60 % discount. The delivery and installation of the new API-ES system is scheduled for May 10, 1996. However, delays in this task did not create significant impact on the progress of other tasks of the project.

Task 2 - Dislodgable and Roller Air Method Development: Two laboratory methods were developed and optimized for the separation of friable dust from bulk corn. The first method is based on solvent washing and gravimetric determination of the recovered dust,

and the second is based on the theory of the commercial fluidized bed particle extraction. The two methods were used to study potential correlation between aflatoxin levels in bulk corn and dislodgable dust.

Task 3 - Mycotoxin Method Development: We have completed method development for aflatoxins and fumonisins, as well as multitoxin screening (ochratoxins, vomatoxin, and zearalenones). We are currently progressing with the off-line method development for multitoxins using HPLC and fluorescence detection. The on-line SFE/HPLC/MS work has been postponed until the installation of the new API-ES/HPLC/MS interface. This will result in extending this method development task, but is not expected to cause any delay in subsequent tasks.

Tasks 4 and 5 - Feed Use and Bin Clean-out: Field sampling of feed use and bin clean-out for crop '94 (tasks 4 & 5) were revised to feed use and bin clean-out for crop '95 (tasks 7 & 8), which starts in the summer of '96.

Task 6 - Harvest, Crop '95: Field sampling of the harvest of Crop '95 was the first task in the revised timetable. This task was completed as scheduled except for some difficulties caused by unusual climatic conditions which resulted in the shortening of the harvest time and conflict in scheduling field sampling. Some of the farmers who agreed to call us before harvest did not do so. In addition, we have planned selecting sampling farms from among the 750 farms for the Farm Family Survey study (PI: William Popendorf), which is funded by NIOSH. In the summer of 1995, Dr. Popendorf left the University of Iowa and joined Utah State University. The unexpected departure of Dr. Popendorf resulted in some difficulties in coordinating planned farmers' recruitment and field sampling through the Farm Family Health Hazard Surveillance Project (U04 CCU70676063-05). As a result, recruitment of farms took place only immediately prior to, and during, the harvest season, which placed some time constraint on the initial sampling phase. These problems will be taken into consideration by recruiting more farmers and maintaining frequent contact with them before the next harvest season.

Task 7 - Feed Use, Crop '95: was completed as scheduled.

Task 8 - Bin Clean-out, Crop '95: was completed as scheduled.

During the earliest part of Phase I, two methods to prepare samples of bulk corn to obtain physically determinant friable portions was developed. One of these methods is based on the "dislodgable" pesticide extraction method of Iwata et al as modified by Popendorf and Leffingwell. The dislodgable method is an aqueous process designed to strip surface pesticide residues from foliage. The other method was based on a commercial fluidized bed particle extraction device known as the Roller Air Analyzer (Federal Pneumatic Systems, Chicago IL). This device uses an aerodynamic process similar to a fluidized bed to agitate a macro sample and to elute aerosolizable particles onto a filter connected to its top. Its operating flow rate was varied to control the aggressiveness of the agitation

process and optimized to match aerosolized dust results obtained during our preliminary field studies.

Similar to the preliminary studies reported earlier, a bulk corn sample, a ground corn sample if applicable, and a set of simultaneous area total airborne aerosol samples was collected in various agricultural operations on Iowa farms. Hog-confinement building aerosols (principally feed and other organic dusts) was the focus for this phase because such activities constitute the highest combination of dust concentration (circa 4 mg/m^3) and total annual duration of exposure (circa 2 hours/day virtually 365 days per year = 3650 mg/yr at a nominal respiratory rate of 1.25 m^3/hr). Other comparable activities include harvest (circa 1 mg/m^3 times 12 hours x 7 days per year = 105 mg/yr) and storage bin clean-out (circa 40 mg/m^3 times 4 hours/bin x 2 bins/year x 2.5 m^3/hr = 800 mg/yr).

The above sampling approximates an approach suggested by a previous reviewer to screen a bulk sample for detectable aflatoxin/mycotoxin before committing to a full field exposure assessment, presumably on a regional or more local basis. However, Wood points out that while "corn is the grain most susceptible to aflatoxin contamination in the United States...the unpredictable nature of the incidence and levels of contamination for a particular area from year to year is evident." While it is desirable to begin the exposure assessment at harvest, the length of harvest within a given field will not permit it to be "screened" (i.e. for a bulk sample to be collected, analyzed, and resampled with exposure monitoring. It is also difficult to collect representative screening samples prior to harvest due to the potential for aflatoxin producing spores to cluster on susceptible or damaged ears of corn which would be the pre-harvest sampled unit. Moreover, our previous study of grain bin clean-out using the low aflatoxin detection limits of the analytical methods proposed herein has found aflatoxin to be detectable in 11/14 sites (77%), about double that reported from FDA surveillance data.

In contrast to harvest, corn from these same farms used for livestock feeding is intrinsically a composite sample. A second visit to these farms will be conducted in mid-winter to obtain a similar set of bulk corn and airborne dust during livestock feeding operations. Some of the farms where aflatoxin was not detected during harvest could, in principle, be omitted from this visit; however, based on the high prevalence of aflatoxin in the grain bin clean-out activities during the pilot studies previously discussed, it is of interest during this phase to explore the progression of aflatoxin at this intermediate time (cf. Phase II where about 25% of the initial fields are scheduled to be dropped from further monitoring by its sampling plan).

The last visit during Phase I collected a third set of bulk corn and airborne dust during grain bin clean-out. Again, although it may be possible to access that corn left over near the bottom of a bin before clean-out actually takes place, our preliminary studies of Iowa grain bins being cleaned out in the summer of 1992, showed aflatoxin was detectable in 11 out of 14 bulk corn samples and 12 out of 14 air samples! Thus, the added complexity and cost of a preliminary sample and analysis was unlikely to be cost-beneficial in comparison to the low rate of non-detections expected using these methods.

Phase II:

The expected second phase of the project examined how the production of friable aflatoxin and other detected mycotoxins is affected by a broader range of environmental conditions than was possible in Phase I. In addition, an assessment of intra-farm, inter-year variability was intended to be conducted in Phase II by returning to collect a sample of bulk corn from those farms previously assessed in Phase I. This intra-farm, inter-year variability was compared to the inter-farm variability observed in both Phases I and II. If Phase II assessments of bulk corn were conducted on all 32 farms visited in Phase I, the intra-farm, inter-year variability could be estimated to within a precision of just over ±25%. However, the distribution of resources between sample collection and analysis was based upon the outcome of the aflatoxin detection in collected samples and the correlations between prepared bulk corn-dust and airborne dust in Phase I.

Due the lack of field contaminated samples during Phase I, it was not possible to establish correlations between aflatoxin concentrations in airborne dust and bulk grain samples. Our efforts were then directed toward more field sampling to investigate possible correlation between the concentrations of aflatoxins and airborne grain dust. Unfortunately (fortunately for the farmers' sake), good climatic conditions continued to prevail over the following harvest seasons, where the environmental conditions were not conducive to field contamination of harvested crops. Bulk corn and airborne dust samples were found to be completely free of aflatoxin contamination. Therefore, the remaining project resources were directed toward further development of the laboratory methods and investigating potential correlations in bulk corn and processed dust. The outcome of these laboratory development and field testing studies was found to be predictive of the relationship between the aflatoxin concentration in an appropriately prepared bulk corn dust sample and the aflatoxin concentration in the airborne dust to which a farmer is exposed. The data obtained provide information on the identity, quantity, and prevalence of aflatoxins and other mycotoxins (fumonisin B_{1},, ochratoxin, vomatoxin, and zearalenone) in bulk corn and dust samples. The optimized vacuum dislodgable method resulted in better correlations with bulk corn concentrations, for aflatoxins and other toxins. The solvent dislodgable method was more time consuming, less reproducible, and resulted in more variable results for different toxins. The reliability and statistical significance of the correlation coefficients between bulk corn-derived dust and airborne dust, between the aflatoxin content in bulk corn, prepared bulk corn dust and airborne dust, or between any other mycotoxins found on bulk corn and prepared bulk corn dust will be discussed in the publication of this project findings, as well future grant applications, which is currently in progress.

FIELD AND LABORATORY METHODS

Air Sample Collection:

Personal air samples were collected using the personal air sampling techniques used during our preliminary studies with the addition of parallel respirable and total sampling. Four samples of airborne dust were collected during harvest, one total and one respirable inside-the-cab and one total and one respirable outside-the-cab to control for the effects of no, partial, or full tractor cab enclosures on airborne dust levels (the status of the cab windows will also be recorded). For redundancy two air samples of each type were collected during mid-season feeding activities. Because particle size has already been investigated during bin clean-out and the sampling duration is sufficiently short that higher air flow rates may be needed, only total aerosol samples was collected. This approach was intended to expand our estimates of exposures within each of these activities and to allow annual time-weighted average [TWA] exposures to be calculated, as well as document changes in aflatoxin concentration within each farm over time, but most importantly will provide a basis to allocate sources of variability in exposure between inter-farm, inter-region, and inter-year for purposes of future epidemiologic studies.

Preliminary studies of the distribution of aflatoxin by particle size showed a significant tendency toward higher concentrations of aflatoxin to reside on smaller diameter dust particles (≤ 1 μm); however, the preponderance of larger particles in these agricultural environments causes the majority of airborne aflatoxin to be on particles ≥ 6 μm. Because large aflatoxin-contaminated particles when inhaled are deposited in the upper respiratory tract and later ingested, the principal sampling method was total aerosols collected on 37mm filters held in closed-face air monitoring cassettes. Samples were collected for between 1 and 4 hours. Area sampling in the work zone was used because it was also used in preliminary studies to maximize farmer participation, it is easier to collect the number of parallel samples proposed, and because the concentrations are generally locally uniform (e.g. not point sources). For consistency, the flow rate during collection with portable battery operated pumps was calibrated at 1.7 liters per minute for all samples; for short activities (e.g. grain bin clean-out), flow rates of up to 28 Lpm was maintained via larger vacuum pumps onto open faced filters.

Bulk Corn Sample Collection:

Sampling of bulk corn followed the procedures described by Woodget and Cooper and Park and Pohland for sampling solid materials. During harvest or feeding, bulk corn was sampled in motion (i.e. while loading or unloading) by running a hand scoop across the free falling grain. Sampling probes was used for sampling bins, piles, and wagons. In both cases, 6-8 samples were collected into large opaque plastic bags and mixed thoroughly before sub-sampling for laboratory analysis.

Bulk Corn Dust Preparation:

While the expectation of the project (based on both prior reports by Burg and Shotwell, Sorenson, and our own preliminary studies) is that an accurate predictor of airborne aflatoxin will require a specially designed pre-extraction separation step to be developed and tested in Phase I, it is also prudent to extract and analyze the bulk corn in a more traditional manner to provide a basis for comparability to on-going surveillance activities and to assess the predictive limitations of bulk corn.

In selecting a method to extract the friable dust from bulk corn, it is desirable that the method be inexpensive, reasonably quick, and that it minimize risk to the technicians from aerosolized aflatoxin. The "dislodgable" pesticide extraction method of Iwata et al as modified by Popendorf and Leffingwell was initially developed and has been used for a number of years as a predictor of pesticide residues for reentry hazards to harvesters. In addition to pesticides, correlations have also been established both between the mineral composition (viz. % quartz) of dislodgable foliar versus airborne dusts and between the mass density of dust on foliage ($\mu g/cm^2$) and its airborne concentration (mg/m^3) surrounding manual harvesters.

For dislodgable foliar dusts, the method involved shaking a representative sample of leaf disks (usually forty-eight 3 cm diameter disks) in an aqueous solution, followed by the collection of suspended dust onto a filter, and its gravimetric or other analyses. The pesticide residue is back extracted from the aqueous solution. A proposed method for grain might entail washing a known weight of bulk corn (100-200 g) in distilled water, and filtering the washings through organic-free filter paper (47 mm). The filter would be dried to a constant weight to determine the mass of the dislodged dust. The reproducibility of this method will be evaluated on replicate corn samples and settled dust samples spiked with known concentrations of aflatoxin. Because of the water solubility of aflatoxin, the mechanics and the chemistry (i.e. the washing solvent) of the dislodgable method may have to be modified for aflatoxin. Correlations will be sought primarily between the aflatoxin concentration in dislodgable dust versus airborne dust and secondarily between the mass of dislodgable versus airborne dust concentration.

The second dislodgable method was undertaken using the Roller Air Analyzer technique. This device was developed for powder technology research and was obtained on a loan from Dr. Beddow's laboratory at the University of Iowa. Although there are several advantages to using a non-aqueous process to separate loose dust from a macro-material such as corn, this procedure required somewhat more clean-up between batches. For this reason, a glass apparatus was specially designed and used in this study. The entire roller air apparatus was operated in a fume hood to avoid potential hazard of laboratory exposure. The mechanical theory of fluidized bed separators is reasonably developed, but to our knowledge the process has not been applied to assess an aerosolizable hazard.

Scope of the Analytical Methods:

Samples of total and airborne dust, bulk corn, ground corn and dislodgable dust were analyzed using a combination of liquid and supercritical fluid extraction (SFE) followed by HPLC and electrospray (ES)/HPLC/MS for qualitative and quantitative analysis of the mycotoxins of interest. Fluorescent detection was used in-line with the HPLC to enhance the detection sensitivity for aflatoxin B_1, followed by electrospray (ES)/MS interface for qualitative and quantitative determination of other toxins.

Although the analytical procedure for aflatoxin B_1 has been optimized to allow simultaneous identification and quantification of the other aflatoxins (B_2, G_1, & G_2), we will focus our screening of field samples to aflatoxin B_1 due to its natural prevalence and known carcinogenic potential. Other toxins (ochratoxin, vomatoxin, zearalenone, and fumonisins B_1 and B_2) were screened in the sample extracts during the course of the ES/HPLC/MS analysis. The use of ES/MS technique (ES) facilitated simultaneous identification of all toxins of interest in one chromatographic separation. This resulted in significant saving of time in sample preparation, derivatization (particularly for fumonisins), and analysis.

Other analytical protocols and reference analytical methods used were essentially the same as described in the proposal.