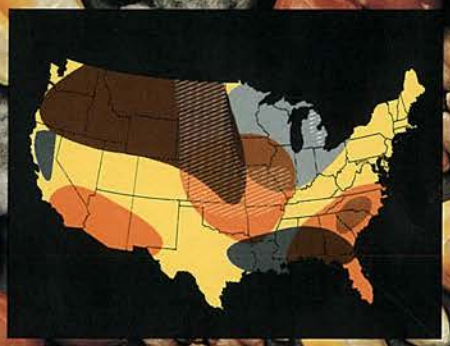
MYCOTOXINS

ECONOMIC AND HEALTH RISKS

REPORT NO. 116 NOV. 1989



COUNCIL FOR AGRICULTURAL
SCIENCE AND TECHNOLOGY

CAST®

The Science Source for Food, Agricultural, and Environmental Issues

NOTE: *The information contained in this publication is based on data and methodologies available at the time of publication and may be outdated. Newer research or updated publications may supercede some information in backlisted publications.*

4420 West Lincoln Way, Ames, Iowa 50014-3447 • Phone: (515) 292-2125 • Fax: (515) 292-4512
www.cast-science.org E-mail: cast@cast-science.org

Council for Agricultural Science and Technology

137 Lynn Avenue, Ames, Iowa 50010-7197 • (515) 292-2125

Mission and Policies

The Council for Agricultural Science and Technology (CAST) is a nonprofit organization comprised of 29 member scientific societies and many individual, company, nonprofit, and associate society members. CAST's Board of Directors is composed of 48 representatives of the scientific societies and individual members, and an executive committee. CAST provides scientific information on key national issues in food and agriculture to policymakers, the news media, and the public. As an educational organization CAST takes no advocacy positions on issues.

The primary mission of CAST is the publication of task force reports written by scientists from many disciplines. The National Concerns Committee screens proposals from all sources and recommends to the Board certain topics for approval as task force projects.

Through representatives on the CAST Board of Directors, the member societies are responsible for the policies and procedures followed in developing, processing, and disseminating the documents produced. Depending on the nature of the publication, the society representatives nominate qualified persons from their respective disciplines for participation in the preparation. Aside from these involvements, the member societies have no responsibility for the content of any CAST publication.

Careful selection of topics, diverse writing groups, and active participation by all task force members assures readers that a balanced statement on agriculturally related social, economic, environmental, energy, and health issues will result.

The authors named in each publication are responsible for the contents. Task force members serve as independent scientists and not as representatives of their employers. They receive no honoraria, but are reimbursed for expenses. CAST publishes and distributes the documents.

Membership

Member Societies

American Academy of Veterinary and Comparative Toxicology
American Association of Cereal Chemists
American Dairy Science Association
American Forage and Grassland Council
American Meat Science Association
American Meteorological Society
American Peanut Research and Education Society
American Phytopathological Society
American Society for Horticultural Science
American Society of Agricultural Engineers
American Society of Agronomy
American Society of Animal Science
American Veterinary Medical Association
Aquatic Plant Management Society
Association of Official Seed Analysts
Council on Soil Testing and Plant Analysis
Crop Science Society of America
Institute of Food Technologists
International Society of Regulatory Toxicology and Pharmacology
North Central Weed Science Society
Northeastern Weed Science Society
Plant Growth Regulator Society of America
Poultry Science Association
Rural Sociological Society
Society of Nematologists
Soil Science Society of America
Southern Weed Science Society
Weed Science Society of America
Western Society of Weed Science

Associate Societies

Individual Members

Sustaining Members

Companies and Cooperatives
Nonprofit Associations

The complete task force report, *Mycotoxins: Economic and Health Risks* is available for $15.00 from CAST, 137 Lynn Avenue, Ames, Iowa 50010-7197, (515) 292-2125. The 8-page Summary is $1.00. Discounts are available for quantity purchases: 6-99 copies, 25% discount; 100 or more copies, 35% discount.

Mycotoxins
Economic and Health Risks

Council for Agricultural Science and Technology
Printed in the United States of America
95 94 93 92 4 3 2

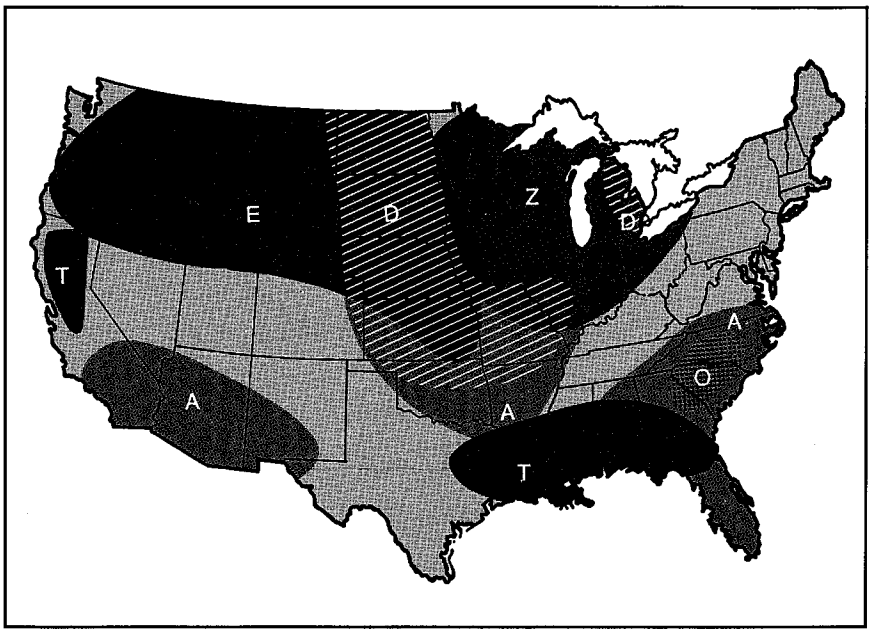
Library of Congress Cataloging-in-Publication Data

Mycotoxins: economic and health risks.

p. cm. — (Task force report, ISSN 0194-4088; no. 116)
"November 1989."
Includes bibliographical references.
1. Mycotoxicoses. 2. Food poisoning. 3. Food contamination. 4. Feeds—Contamination. 5. Mycotoxins—Decontamination. I. Council for Agricultural Science and Technology. II. Series: Task force report (Council for Agricultural Science and Technology); no. 116.
RA1242.M94M95 1989
615.9'5292—dc20 89-25448
ISSN 0194-4088 CIP

Task Force Report
No. 116 November 1989

Council for Agricultural Science and Technology



Major mycotoxins associated with field crops by general geographic areas in the United States.

A = aflatoxins	O = ochratoxin A
D = deoxynivalenol	T = tremorgens
E = ergot	Z = zearalenone

Courtesy of A. C. Pier and J. L. Richard.
Cover design and art work by L. Ekblad, Chichaqua Bend Studios, Ames, Iowa

Task Force Members

John L. Richard, Cochair of Task Force, USDA, ARS, National Animal Disease Center, Ames, Iowa

Richard J. Cole, Cochair of Task Force, USDA, ARS, National Peanut Research Laboratory, Dawson, Georgia

Sandra O. Archibald, Food Research Institute, Stanford University, Stanford, California

Lloyd B. Bullerman, Department of Food Science and Technology, University of Nebraska, Lincoln

John D. Groopman, School of Hygiene and Public Health, Department of Environmental Health Sciences, Johns Hopkins University, Baltimore, Maryland

Winston M Hagler, Jr., Department of Poultry Science, North Carolina State University, Raleigh

Clifford W. Hesseltine (retired), USDA, ARS, Northern Regional Research Center, Peoria, Illinois

Dennis P. H. Hsieh, Department of Environmental Toxicology, University of California, Davis

Fredrick W. Oehme, Comparative Toxicology Laboratories, VCS-Kansas State University, Manhattan

James J. Pestka, Department of Food Science and Nutrition, Michigan State University, East Lansing

Timothy D. Phillips, Department of Veterinary Public Health, Texas A&M University, College Station

Albert E. Pohland, Division of Contaminants Chemistry, Food and Drug Administration, Washington, D.C.

Steven P. Swanson, Eli Lilly and Company, Indianapolis, Indiana

Allan K. Watson, MacDonald College, McGill University, Quebec, Canada

Thomas B. Whitaker, USDA, ARS, Department of Biological and Agricultural Engineering, North Carolina State University, Raleigh

David M. Wilson, Department of Plant Pathology, Georgia Coastal Plain Experiment Station, Tifton

Fu-Li Yu, Department of Biomedical Sciences, University of Illinois College of Medicine, Rockford

Technical Advisors

Scott Appleton, Fries and Fries, Cincinnati, Ohio

David H. Bechtel, Best Foods Research Center, Union, New Jersey

Stephen A. Ziller, The Procter and Gamble Company, Cincinnati, Ohio

Additional Contributors

W. W. Carlton, Department of Veterinary Pathobiology, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana

T. Kuiper-Goodman, Toxicological Evaluation Division, Health and Welfare Canada, Ottawa, Ontario

S. D. Lenz, Department of Veterinary Pathobiology, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana

W. M. Peden, USDA, ARS, National Animal Disease Center, Ames, Iowa

A. C. Pier, Department of Veterinary Science, University of Wyoming, Laramie

N. R. Schneider, Veterinary Diagnostic Center, University of Nebraska-Lincoln

F. N. Thompson, College of Veterinary Medicine, University of Georgia, Athens

K. A. Voss, USDA, ARS, Toxicology and Mycotoxin Research, Richard B. Russell Agricultural Research Center, Athens, Georgia

Foreword

A request for an update of the 1979 CAST task force report on aflatoxins was received in February 1988. Since 1979, many additional mycotoxins have been discovered and new problems have been associated with certain mycotoxins. There is considerable recent information regarding occurrence, toxicity, epidemiology, detection, analysis, economic impacts, and control and management of mycotoxins.

The CAST National Concerns Committee recommended to the Board of Directors that CAST prepare a more comprehensive report addressing the entire area of mycotoxins. This topic was approved by the CAST Board of Directors at the February 1988 board meeting.

Nominations for the task force members were received from the representatives of the CAST Board of Directors. Two cochairs with expertise in complimentary areas were selected; John L. Richard, whose research is on effects of mycotoxins on animal health, and Richard J. Cole, who is associated with mycotoxin research on an important commodity (peanuts). A highly qualified and broad-based task force of scientists was chosen that includes persons with expertise in such diverse fields as plant pathology, toxicology, chemistry, economics, human health and medicine, veterinary medicine, microbiology, engineering, and contaminants regulations. Additionally, three scientists from industry agreed to serve as technical advisors and are included herein as part of the task force. Another eight scientists also contributed to various sections of the report.

Drs. Richard and Cole proposed an outline of the subject matter and submitted it to the task force prior to the task force meeting held in Kansas City in July 1988. At the meeting, the members reached a consensus on the scope of the report, developed a detailed outline, established a calendar of completion dates for the first and second drafts of the report, and selected subgroups (each with a chair) to be responsible for writing the chapters of the report. The entire task force revised each draft of the report and reviewed the proofs. The CAST Executive and Editorial Review Committees reviewed the final draft. The CAST staff provided only editorial and structural suggestions. The cochairs and task force are responsible for all scientific content in the report.

During the latter part of 1988 and throughout 1989, task force members were spending considerable time investigating problems associated with mycotoxin contamination of grain crops in the United States caused by the 1988 drought. The public, government agencies, and others were aware that CAST was producing a report on mycotoxins and CAST received many inquiries regarding the problems. CAST had recently organized this task force and was able to refer the callers to the cochairs and members of the task force, who readily provided assistance.

On behalf of CAST, we thank the task force members, who gave of their time and talents to prepare this report as a contribution of the scientific community to public understanding. We thank also the employers of the task force members, who made the time of the members available at no cost to CAST. The members of CAST deserve special recognition because the unrestricted contributions they have made in support of the work of CAST have financed the preparation and publication of this report.

This report is being distributed to members of Congress, the Food and Drug Administration, the Environmental Protection Agency, the U.S. Department of Agriculture, the Office of Technology Assessment, the Office of Management and Budget, and the Agency for International Development; to media personnel; and to institutional members of CAST. All individual members of CAST receive a summary of the report and may order a copy of the full report. The report may be republished or reproduced in its entirety without permission. If copied in any manner, credit to the authors and CAST would be appreciated.

Kayleen A. Niyo
Interim Executive Vice President
Scientific Editor

Virgil W. Hays
President

Contents

General Summary

Toxic fungal metabolites, known as mycotoxins, are chemically diverse and occur in a wide variety of substrates including feeds and foods. They impair human health and cause economic losses in livestock through disease and reduced efficiency of production.

The fungi that produce mycotoxins can invade the food and feed supply during production, processing, transport, or storage. These fungi may be pathogenic to plants or saprophytic. The three major genera of mycotoxin-producing fungi are *Aspergillus, Fusarium,* and *Penicillium.* Several factors influence mycotoxin production by fungi, including substrate, moisture, temperature, pH, and stresses such as drought and associated growth of other fungi or microbes. The major crops affected in the United States are corn, peanuts, and cotton.

The aflatoxins are produced primarily by *Aspergillus flavus* and *A. parasiticus.* Sterigmatocystin is a precursor in the biosynthesis of aflatoxins, but it can be produced as an end product by several species of *Aspergillus.* The large family of mycotoxins known as trichothecenes are produced notably by *Fusarium* spp. The estrogenic metabolite zearalenone is produced by *F. graminearum.* The ochratoxins are produced by *A. ochraceous,* from which they derived their name, but several *Penicillium* spp. also may produce ochratoxins. Some species of *Penicillium* and *Aspergillus* are capable of producing either citrinin, citreoviridin, or cyclopiazonic acid. The newly described fumonisins presently are known to be produced only by *F. moniliforme.* The group of mycotoxins, collectively known as tremorgens, are produced by species of *Penicillium, Aspergillus, Claviceps,* and *Acremonium.*

The toxicity of mycotoxins to animals range from acute death to chronic disease and interference with reproductive efficiency. The aflatoxins can cause liver damage or cancer, decreased milk and egg production, and immune suppression. The young of a species are most susceptible to the effects of aflatoxins, which may be expressed as gastrointestinal disturbances, anemia, jaundice, and/or reduced feed consumption and efficiency. Nursing animals may be affected by exposure to aflatoxin metabolites secreted in milk.

The trichothecenes primarily cause necrosis and hemorrhage throughout the digestive tract, depression of blood regenerative processes in the bone marrow and spleen, and changes in reproductive organs. Signs of disease include weight loss, reduced feed consumption and utilization, vomiting, diarrhea, abortion, and death. Immune suppression may be important in trichothecene-affected animals.

Ochratoxin A at concentrations usually found in feeds causes kidney damage. However, higher concentrations may cause liver damage as well as intestinal necrosis and hemorrhage. Like aflatoxins and sterigmatocystin, ochratoxin A is immunosuppressive and carcinogenic.

Zearalenone is an estrogenic metabolite which induces feminization at dietary concentrations of less than 1 ppm. Higher concentrations can interfere with conception, ovulation, implantation, fetal development, and the viability of newborn.

A variety of other effects in animals have been attributed to mycotoxins, including embryonic death, inhibition of fetal development, and abortions attributed to aflatoxins and zearalenone as well as the ergot toxins and rubratoxin. Teratogenicity has been described for aflatoxins, ochratoxin, T-2 mycotoxin, zearalenone, sterigmatocystin, and rubratoxin. Nervous system functioning is adversely altered by at least nine mycotoxins, including the tremorgenic mycotoxins. Clinical signs include tremors, uncoordinated movements, weakness in the limbs, staggering, and sudden muscular collapse. The neurological signs may be complicated with seizures, hemorrhage, diarrhea, perfuse salivation, feed refusal, and gangrene of the extremities.

Human mycotoxicoses are documented for relatively few of the many mycotoxins. Of considerable importance are those mycotoxins, such as the aflatoxins, that are potentially carcinogenic to humans. Major epidemiological studies regarding the aflatoxins have been conducted, primarily in Asia and Africa, and some have shown a positive association, while others have indicated a lack of positive association between exposure of aflatoxins and disease outcome. Major criticism of many of these studies surrounds the role of hepatitis B virus as an agent for liver cell cancer (LCC) in the populations studied. Therefore, the exact relationship of aflatoxins with liver cell cancer has not been fully established in humans. Nevertheless, in 1988, the International Agency for Research on Cancer (IARC) placed aflatoxin B_1 on their list of human carcinogens.

Unlike Africa and Asia, the incidence of LCC in the United States is relatively low, as is the exposure to aflatoxins and the prevalence of most alternate risk factors. Although there have been no complete investigations conducted in the United States, a limited retrospective population study demonstrated no apparent association between consumption of aflatoxins

and LCC. New epidemiology studies in the United States, utilizing recently developed techniques, could provide a better determination of the extent of exposure to populations. They also would have the potential to aid in clarifying the role of aflatoxins in LCC and other disease states in the relative absence of alternate risk factors. However, conduct of these studies may not be practical. The LCC incidence in the United States is so low that such studies would require untenably large numbers of participants to yield meaningful statistics. In contrast, cases of acute aflatoxicosis leading to death or disease involving gastrointestinal disturbances, hemorrhage, vomition, and hepatic changes have been well documented in humans.

Mycotoxins can contaminate crops before harvest, in transport, and in storage. Thus, raw or processed foods and feeds can become contaminated. With the exception of the aflatoxins, the frequency of contamination by mycotoxins is unknown. The aflatoxins are frequently detected in a variety of feeds and foods produced in the United States, as well as in imported commodities and products. Contamination of milk, eggs, and meat can result from animal consumption of mycotoxin-contaminated feed. Aflatoxins, ochratoxin, and some trichothecenes have been given considerable attention, because they are either carcinogenic or of economic concern in animal health.

Additional mycotoxins produced by various *Aspergillus, Penicillium, Fusarium,* and other fungal genera can contaminate foods; however, their importance to animal and human health has not been established.

The economic losses due to mycotoxins are multifaceted, involving direct crop and livestock losses through reduced health and production efficiency, regulatory programs, processing, and diagnostic expenses. The incidence of mycotoxins varies among commodities, climatic conditions, and regions. For these reasons, the economic importance of mycotoxins is difficult to quantify. Product losses likely occur, but except for certain corn and peanut products, milk, and eggs, there is no documentation. Increased costs resulting from mycotoxin-contaminated commodities are likely passed on to the buyer or consumer. Such costs have significant direct or indirect effects on the control of mycotoxins and international trade of commodities and processed feeds and foods.

Because mycotoxins are unavoidable, naturally occurring compounds, regulations provide an important means to control the quality of the food and feed in which they may occur. Presently, in the United States, only the aflatoxins are regulated. To accomplish such controls, the concentration of mycotoxins in foods and feeds must be accurately assessed. Such accuracy involves adequate sampling of the food or feed, chemical extraction, cleanup, and quantitative analysis of the mycotoxins. Although most of the procedures are developed for the laboratory and often utilize sophisticated equipment, field-practical screening tests are available for rapid detection of selected mycotoxins in certain commodities.

Regardless of our best efforts to control the occurrence of mycotoxins in commodities, they do occur, and therefore strategies have been developed to decontaminate or detoxify the commodity. Presently, this is true primarily for the aflatoxins. These methods for decontamination/detoxification include physical separation, thermal inactivation, irradiation, microbial degradation, and chemical treatment. Ammoniation has been safely and effectively used for aflatoxins in some commodities used for animal feed, but has not yet been sanctioned by the U.S. Food and Drug Administration. A new approach using selective adsorption of aflatoxins by dietary compounds has resulted in reducing or preventing some adverse effects of aflatoxins. Continued efforts are needed to establish these kinds of methods to practically, safely, and effectively control mycotoxins in foods and feeds.

Future needs in mycotoxin research include:

1. Surveillance of commodities, foods, and feeds for presence and quantity of mycotoxins.
2. Surveillance of human populations to assess the exposure level to selected mycotoxins.
3. Assessment of control methods of mycotoxins involving methods of decontamination/detoxification.
4. Development of standard regulatory controls of mycotoxins for international trade.
5. Development of resistant plants to fungal and mycotoxin occurrence.
6. Development of biocompetitive agents for toxigenic fungi.
7. Improvement of sampling and analysis for mycotoxins.
8. Research on the effect of mycotoxins on animal disease through immunosuppression and interaction with other disease agents.
9. Toxicological evaluation of newly discovered mycotoxins.
10. Examination of the possible therapeutic and biocontrol nature of mycotoxins.
11. An integrated worldwide assessment of the economic aspects of mycotoxins.

1. Introduction

Definitions

Mycotoxins comprise a structurally diverse family of naturally occurring, fungal-elaborated toxins, many of which have been strongly implicated as chemical precursors of toxicity in humans and animals. Consequently, there is a growing awareness of the potential hazards of these substances as contaminants of food and feed. Interest in mycotoxins and mycotoxicoses has resulted in publication of a vast literature since the early 1960s. The interest continues as new mycotoxins are discovered and as the information is developed on their possible involvement in animal and human disease. Mycotoxin contamination of food and feed supplies could increase the economic and health risks to humans and animals. To assess these risks requires knowledge or understanding of: (1) pre- and postharvest contamination, (2) natural occurrence of mycotoxins in foods and feeds, (3) human and animal exposure to the toxins, and (4) the toxicology of the compounds and the recorded effects on naturally exposed human and animal populations (epidemiology).

The word mycotoxin apparently was derived from mycotoxicosis, as first used by Forgacs and Carll (1955). They described mycotoxicoses as diseases of animals caused by fungal toxins. Subsequently, mycotoxin was defined as a toxin produced by a fungus. The term is used in this report to mean any fungal metabolite that causes adverse effects in humans or other animals and referred to as mycotoxicoses. (This excludes poisonings from ingestion of toxic mushrooms, because these are purposely ingested as food.) Ingestion of foods and feeds containing mycotoxins is the usual route of exposure. However, dermal or inhalation exposure may occur as well.

The direct effects of mycotoxins on humans and other

Table 1.1 Types of economic losses and costs associated with contamination of foods or feeds by mycotoxins as expressed in general terms (adapted from Jemmali, 1987)

Bearer of economic losses and costs	Economic losses and costs
National level	
Primary producer	Outright food and feed loss. Contaminated crops provide less income and may lead to potential loss of outlet. Reduced productivity of livestock from (1) lower quantity and quality of animal products, (2) smaller litters, (3) reduced work output, (4) loss of pregnancy, (5) reduced feed efficiency, (6) impaired resistance to disease, and (7) loss of vaccination efficacy.
Intermediary	Less income from products refused, condemned, or sold at discount. Increased storage, transport, and packing costs on such products. Potential loss of market, trading reputation, and raw material source. Increased costs due to litigation (may exceed cost of product). Increased costs due to surveillance and control.
National government	Lower foreign exchange earnings from reduced exports. Increased costs involved in shipment, sampling, and analysis of exported goods that are subsequently refused import entry; potential loss of overseas outlets. Increased costs of detoxification or reconditioning abroad. Increased costs for food or feed imports; staple food subsidies. Increased costs of surveillance and control. Increased need for expenditures on human and animal health facilities and activities. Increased costs involved in training and extension programs.
Consumer (human or animal)	Consumption may lead to impaired health and productive capacity. Lack of food may lead to undernutrition or higher food prices resulting from outside purchase of foods or feeds. Possible medical and veterinary costs associated with the above conditions in previous two statements. Possible consumer-initiated litigation costs.
International level	Inconsistent supply resulting in price fluctuation and loss of market. Increased difficulty involved in arriving at sound international agricultural production adjustments and commodity and food security agreements.

animals may include: (1) growth retardation, impaired immunity, and reduced resistance to infection, (2) acute toxicity and death following exposure to high levels of a mycotoxin, (3) reduced milk and egg production, or (4) chronic disease including tumor formation after prolonged exposure to small quantities of toxin. Although more difficult to define, the latter is of greater concern in developed countries where the food and feed supplies are of high quality and, therefore, probably contain lesser amounts of toxin(s). Wide diversity in susceptibility to a particular mycotoxin occurs among animal species, and this is influenced by age, sex, strain, and nutritional status.

Indirect exposure of humans to mycotoxins is known to occur when toxic residues of mycotoxins or their metabolites persist in milk, eggs, and animal tissues, and these products are consumed. Effects of such exposure to mycotoxins may include: (1) impaired immunity and resistance to disease, (2) lowered growth rates, and (3) reduced reproductive efficiency. All are of definite economic significance (Table 1.1).

Occurrence of Mycotoxin-producing Fungal Genera

Many of the fungi capable of producing mycotoxins are also frequent contaminants of food and agricultural commodities. These include members of the genera *Aspergillus, Penicillium, Fusarium, Alternaria, Claviceps, Stachybotrys, Pithomyces, Phoma, Diplodia, Trichothecium, Phomopsis, Cladosporium, Byssochlamys, Chaetomium, Rhizopus,* and *Sclerotinia.* These organisms may grow on a variety of substrates and under diverse conditions of moisture, pH, and temperature. Thus, most foods and feeds are susceptible to invasion by fungi during some stage of production, processing, transport, or storage. Mycotoxins may be produced when fungal growth occurs. However, the presence of propagules of these fungi in or on a food product does not automatically mean the presence of mycotoxins but, rather, that a potential for mycotoxin contamination exists. On the other hand, the absence of propagules of toxigenic fungi does not guarantee that the commodity is free of mycotoxins, because the toxins may persist long after the fungi have lost viability. Mycotoxins often occur in crops in the field prior to harvest. Postharvest contamination can occur if crop drying is delayed and during storage of the crop, if water activity is allowed to exceed critical values for mold growth. Mycotoxin-producing fungi have been isolated from diverse foods and commodities (Table 1.2).

Factors Involved in Fungal Growth and Mycotoxin Development

Fungal growth and mycotoxin contamination are the consequence of an interaction among the fungus, the host, and the environment. The appropriate combination of these factors determine infestation and colonization of the substrate, and the type and amount of mycotoxin produced.

Many of the toxigenic fungi overwinter as mycelium or resting spore stages on plant debris or in the soil (Figure 1.1). Sporulation in or on this overwintered material results in an inoculum that is often dispersed by air currents, splashing rain, or insects (Figure 1.2). Different strains of a toxigenic fungus can vary in virulence, growth rate, competitiveness, ability to produce toxins, and the type and quantity of toxin produced. Strain variability is common in most toxigenic fungi

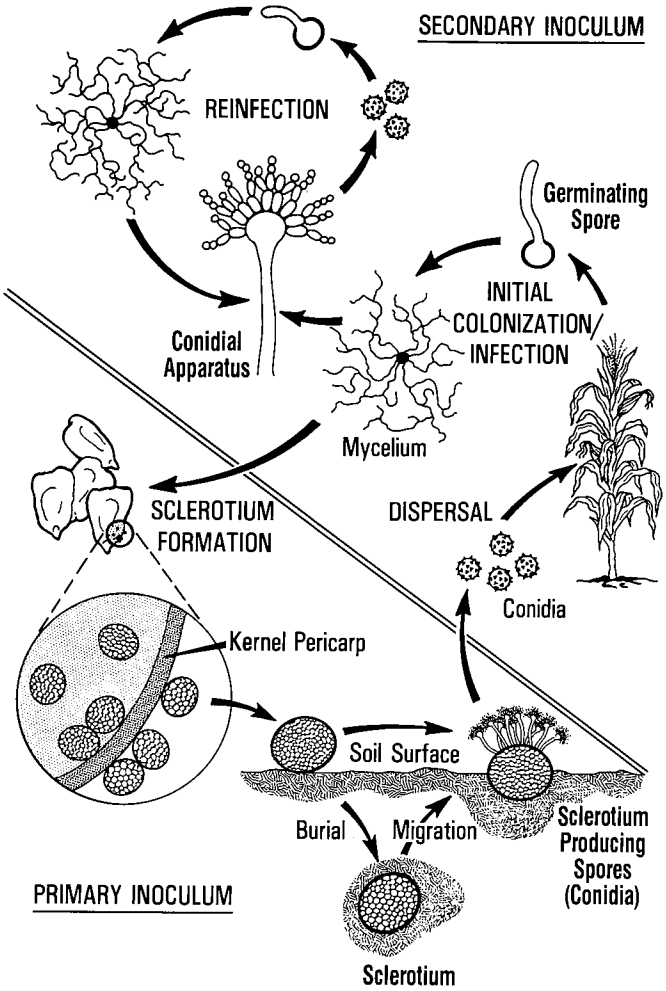


Figure 1.1. Ecological relationships of asexual and vegetative elements of *Aspergillus flavus* with infection of corn (Wicklow and Donahue, 1984).

Table 1.2 Summary of selected reports of potentially toxic molds from various food or agricultural commodities (Bullerman, 1979, 1986)

Commodity	Potentially toxic genera/species found		Potential mycotoxins
Wheat, flour, bread cornmeal, popcorn	*Aspergillus* *flavus* *ochraceus* *versicolor* *Fusarium* spp.	*Penicillium* *citrinum* *citreo-viride* *cyclopium* *martensii* *patulum* *puberulum*	Aflatoxins, ochratoxin A, sterigmatocystin, patulin, penicillic acid, deoxynivalenol, zearalenone
Peanut, in-shell pecans	*Aspergillus* *flavus* *parasiticus* *ochraceus* *versicolor* *Fusarium* spp.	*Penicillium* *cyclopium* *expansum* *citrinum*	Aflatoxins, ochratoxin A, patulin, sterigmatocystin, trichothecenes, cytochalasins, oosporein
Meat pies, cooked meats, cocoa powder, hops, cheese	*Aspergillus* *flavus*	*Penicillium* *viridicatum* *roqueforti* *patulum* *commune*	Aflatoxins, ochratoxin A, patulin, penicillic acid
Aged salami and sausage, country cured ham, moldy meats, cheese	*Aspergillus* *flavus* *ochraceus* *versicolor*	*Penicillium* *viridicatum* *cyclopium*	Aflatoxins, ochratoxin A, patulin, penicillic acid, sterigmatocystin, penitrem
Black and red pepper, macaroni	*Aspergillus* *flavus* *ochraceus*	*Penicillium* spp.	Aflatoxins, ochratoxin A
Dry beans, soybeans, corn, sorghum, barley	*Aspergillus* *flavus* *ochraceus* *versicolor* *Alternaria*	*Penicillium* *cyclopium* *viridicatum* *citrinum* *expansum* *islandicum* *urticae*	Aflatoxins, ochratoxin A, sterigmatocystin, penicillic acid, patulin, citrinin, griseofulvin, alternariol, altenuene
Refrigerated and frozen pastries	*Aspergillus* *flavus* *versicolor*	*Penicillium* *cyclopium* *citrinum* *martensii* *olivino-viride* *palitans* *puberulum* *roqueforti* *urticae* *viridicatum*	Aflatoxins, sterigmatocystin, ochratoxin A, patulin, penicillic acid, citrinin, penitrem
Moldy supermarket foods	*Penicillium* *cyclopium* *Fusarium* *oxysporum* *solani*	*Aspergillus* spp.	Penicillic acid, trichothecenes, aflatoxins, possibly other *Aspergillus* and *Penicillium* toxins
Foods stored in homes, both refrigerated and nonrefrigerated	*Penicillium* spp.	*Aspergillus* spp.	Alfatoxins, kojic acid, ochratoxin A, penitrem, patulin, penicillic acid
Apples and apple products	*Penicillium* *expansum*		Patulin

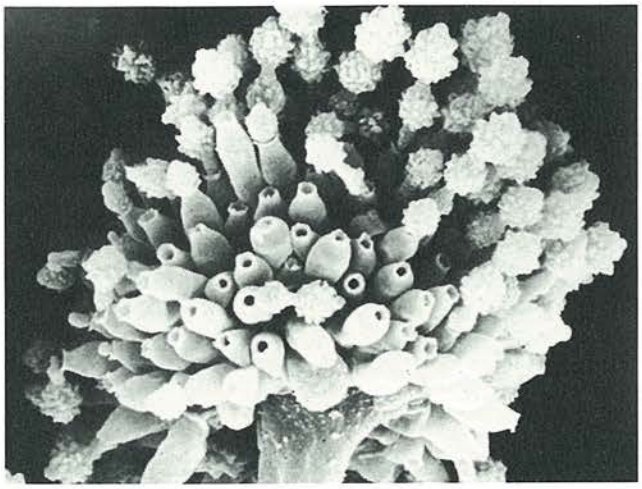


Figure 1.2. Asexual sporulation of *Aspergillus* as viewed by scanning electron microscopy. Rough conidia serve as inoculum for infecting crops. Photograph courtesy of D. P. H. Hsieh, University of California, Davis.

studied. This makes identification at the species level difficult and confounds the interpretation of much of the reported research (Diener et al., 1987; Lacey, 1986).

A suitable substrate is required for fungal growth and subsequent toxin production, although the precise factor(s) that initiates toxin formation is not well understood (Diener et al., 1987). Stress and subsequent reduced vigor often predispose crop plants to infestation, colonization, and contamination by toxigenic fungi. Water stress, high-temperature stress, and insect damage (Figures 1.3, 1.4, and 1.5) of the host plant are major determining factors in mold infestation and toxin production. Similarly, specific crop growth stages, poor fertility, high crop densities, and weed competition have been associated with increased mold growth and toxin production (Diener et al., 1987; Lacey, 1986; Tuite, 1979). Toxin formation is also affected by associated growth of other molds (Trucksess et al., 1988; Mislivec et al., 1988).

Preharvest mold growth and production of aflatoxins in peanuts and corn are favored by warm ambient temperatures and prolonged drought conditions typical of many parts of the world, including the southern United States (Diener et al., 1979). Postharvest production of aflatoxins on corn and peanuts is favored by warm temperatures and high humidity, which also is typical in the southern United States. The greatest problems with contamination by aflatoxins have occurred in corn and peanuts in the southeastern area and in cottonseed in the southwestern area of the United States. Grains produced in the midwestern area of the United States generally have been found to be relatively free of aflatoxins, with the exception of growing seasons characterized by drought stress, such as 1983 and 1988.

Major Classes of Mycotoxins

A wide diversity of toxic metabolites have been obtained from fungal laboratory cultures. Most of these compounds are not known as causes of human or animal disease. The mycotoxins currently considered to pose the greatest potential risk to human and animal health, as food and feed contaminants, are listed in Table 1.3 (see Appendix 2 for chemical structures), together with their effects in animals and the

Figure 1.3. Ear of corn with insect hold in husk. Insects function as vehicles for transmission of fungi from the environment into the ear of corn. Photograph courtesy of J. L. Richard, USDA, ARS, National Animal Disease Center, Ames, Iowa.

Figure 1.4 Same ear of corn as in Figure 1.3, except husk has been peeled back to show the brown insect larva and fungal contamination of the corn. Photograph courtesy of J. L. Richard, USDA, ARS, National Animal Disease Center, Ames, Iowa.

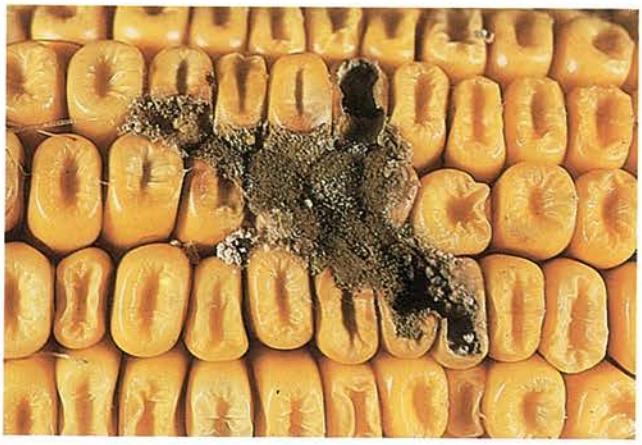


Figure 1.5. Growth of *Aspergillus flavus* in an area of insect damage in an ear of corn. Photograph courtesy of J. L. Richard, USDA, ARS, National Animal Disease Center, Ames, Iowa.

commodities in which the mycotoxins have been found.

Aflatoxins are produced primarily by some strains of *A. flavus* and most, if not all, strains of *A. parasiticus,* plus a related species, *A. nomius,* (Kurtzman, et al, 1987). There are four major aflatoxins, B_1, B_2, G_1, and G_2, plus two additional metabolic products, M_1 and M_2, that are of significance as direct contaminants of foods and feeds. The aflatoxin M toxins were first isolated from the milk of lactating animals fed aflatoxin preparations; hence, the M designation.

The mycotoxin **sterigmatocystin** is produced by several species of *Aspergillus, Penicillium luteum,* and a *Bipolaris* species. Chemically, sterigmatocystin resembles the aflatoxins and is a precursor in the biosynthesis of aflatoxin (Hsieh et al., 1973). Sterigmatocystin has been detected at low concentrations in green coffee, moldy wheat, and in the rind (normally discarded) of hard Dutch cheese (Bullerman, 1981; Scott, 1985; Vesonder and Horn, 1985).

The **trichothecenes** are a family of over 148 structurally related compounds (Grove, 1988) produced by several fungal genera (*Fusarium, Cephalosporium, Myrothecium, Stachybotrys,* and *Trichoderma*). There are several naturally-occurring trichothecene mycotoxins produced in foods and feeds by *Fusarium* species, including deoxynivalenol, T-2 mycotoxin, nivalenol, and diacetoxyscirpenol. Deoxynivalenol contamination of corn and wheat has been significant in some crop years. Natural contamination of foods and feeds by T-2 mycotoxin in the United States has been reported in only one incident involving heavily molded corn. The T-2 mycotoxin is the trichothecene studied most extensively, probably because it is the trichothecene most readily available.

In 1980 and 1981 in Canada and 1982 in the United States, deoxynivalenol was found in wheat as the result of severe infestations with the wheat scab fungus, *F. graminearum*. In both Canada and the United States, the soft winter wheats were the most severely affected. In Canada, dried corn was found to contain levels of deoxynivalenol that were slightly higher than those found in the wheat (Trenholm et al., 1985). In some parts of the United States, zearalenone was found to occur with deoxynivalenol in scabby wheat; in most cases of *Gibberella* ear rot of corn, zearalenone and deoxynivalenol are found together.

Zearalenone, an estrogenic mycotoxin, causes vulvovaginitis and estrogenic responses in swine. Zearalenone is produced primarily by *F. graminearum,* occurring naturally in high moisture corn, and zearalenone has been found also in moldy hay and pelleted feeds. Physiological responses in swine occur when the zearalenone level in corn used for feeds exceeds about 1 ppm (Kurtz and Mirocha, 1978). Zearalenone can be transmitted to piglets in sows' milk and cause estrogenism in the young pigs. The natural occurrence of zearalenone is favored by high humidity and low temperatures. These conditions often occur in the Midwest during autumn harvest (Christensen et al., 1977; Diener et al., 1979).

Ochratoxins are a group of structurally related metabolites that are produced by *A. ochraceus* and related species, as well as *P. viridicatum* and certain other *Penicillium* species. The major mycotoxin in this group is ochratoxin A. Ochratoxin has been suggested to be a factor in the etiology of a human disease known as Balkan endemic nephropathy (Krogh, 1977; Smith and Moss, 1985).

Citrinin is a yellow-colored mycotoxin that is produced by several *Penicillium* and *Aspergillus* species, including *P. viridicatum* strains that produce ochratoxin. Like ochratoxin A, citrinin causes kidney damage in laboratory animals similar to swine nephropathy, and may interact synergistically with ochratoxin A in cases of swine nephropathy as found in Denmark (Krogh, 1977).

Other less studied mycotoxins found at low frequency or under unusual circumstances are included in the following paragraphs.

Citreoviridin was originally isolated from cultures of molds obtained from rice associated with a disease called cardiac beriberi that had occurred for three centuries in Japan (Ueno and Ueno, 1972). The

Table 1.3 Commodities in which mycotoxin contamination has been found and the resulting effects on animals and humans (adapted from Bullerman, 1979, 1981, 1986)

Mycotoxin	Commodities found contaminated	Effects of mycotoxins	
		Affected species	Pathological effects
Aflatoxins (B_1, B_2, G_1, G_2, M_1, M_2)	Peanuts, corn, wheat, rice, cottonseed, copra, nuts, various foods, milk, eggs, cheese	Birds Duckling, turkey, poult, pheasant chick, mature chicken, quail Mammals Young pigs, pregnant sows, dog, calf, mature cattle, sheep, cat, monkey, human Fish Laboratory animals	Hepatotoxicity (liver damage) Bile duct hyperplasia Hemorrhage Intestinal tract Kidneys Carcinogenesis (liver tumors)
Citrinin	Cereal grains, (wheat, barley, corn, rice)	Swine, dog, laboratory animals	Nephrotoxicity (tubular necrosis of kidney) Porcine nephropathy
Cyclopiazonic acid	Corn, peanuts, cheese, kodo millet	Chicken, turkey, swine, rat, guinea pig, human (?)	Muscle necrosis Intestinal hemorrhage and edema Oral lesions
Ochratoxin A	Cereal grains (wheat, barley, oats, corn), dry beans, moldy peanuts, cheese, tissues of swine	Swine, dog, duckling, chicken, rat, human	Nephrotoxicity (tubular necrosis of kidney) Porcine nephropathy Mild liver damage Enteritis Teratogenesis Carcinogenesis (kidney tumors)
Patulin	Moldy feed, rotted apples, apple juice, wheat straw residue	Birds Chicken, chicken embryo, quail Mammals Cat, cattle, mouse, rabbit, rat Others Brine shrimp, guppie, zebra fish larvae	Edema Brain Lungs Hemorrhage Lungs Capillary damage Liver Spleen Kidney Paralysis of motor nerves Convulsions Carcinogenesis Antibiotic
Penicillic acid	Stored corn, cereal grains, dried beans, moldy tobacco	Mouse, rat, chicken embryo, quail, brine shrimp	Liver damage (fatty liver, cell necrosis) Kidney damage Digitalis-like action on heart Dilates blood vessels Antidiuretic Edema in rabbit skin Carcinogenesis Antibiotic

Table 1.3 (continued)

Mycotoxin	Commodities found contaminated	Effects of mycotoxins: Affected species	Effects of mycotoxins: Pathological effects
Penitrem	Moldy cream cheese, English walnuts, hamburger bun, beer	Dog, mouse, human	Tremors, death, incoordination, bloody diarrhea
Sterigmatocystin	Green coffee, moldy wheat, Dutch cheeses	Mouse, rat	Carcinogenesis Hepatotoxin
Trichothecenes (T-2 toxin, diacetoxyscirpenol, neosolaniol, nivalenol, diacetylnivalenol, deoxynivalenol, HT-2 toxin, fusarenon X)	Corn, wheat, commercial cattle feed, mixed feed	Swine, cattle, chicken, turkey, horse, rat, dog, mouse, cat, human	Digestive disorders (emesis, diarrhea, refusal to eat) Hemorrhage (stomach, heart, intestines, lungs, bladder, kidney) Edema Oral lesions Dermatitis Blood disorders (leucopenia)
Zearalenone	Corn, moldy hay, pelleted commercial feed	Swine, dairy cattle, chicken, turkey, lamb, rat, mouse, guinea pig	Estrogenic effects (edema of vulva, prolapse of vagina, enlargement of uterus) Atrophy of testicles Atrophy of ovaries, enlargement of mammary glands Abortion

natural occurrence of this mycotoxin in corn and other foods and feedstuffs has been observed recently (Wicklow et al., 1988). Several species of *Penicillium* and a single species of *Aspergillus* have been reported to produce this mycotoxin. Interestingly, citreoviridin and aflatoxin were found to occur simultaneously in corn, and this allows for possible interaction of these two mycotoxins in producing animal disease (Wicklow et al., 1988). Citreoviridin causes paralysis, dyspnea, cardiovascular disturbances, and loss of eyesight in experimental animals (Ueno, 1974).

Cyclopiazonic acid (CPA) was originally isolated from a culture identified as *P. cyclopium* during routine screening for toxigenic molds (Holzapfel, 1968). The potential significance of CPA as a natural contaminant of foods and feeds became apparent with reports that it was produced by several molds found commonly on agricultural commodities or by molds used in fermented food production. These included *A. flavus, A. versicolor,* and *A. tamarii,* and several *Penicillium* species used in the production of fermented sausages in Europe. This includes *P. camemberti,* used in the production of Camembert cheese, and *A. oryzae,* used in the production of soy sauce in the Far East. This mycotoxin has been shown to occur naturally in corn (Gallagher et al., 1978), cheese (LeBars, 1979), and peanuts (Lansden and Davis, 1983), and occurred in Kodo millet (Rao and Husain, 1985) that was implicated in a natural human intoxication in India. Cole (1986) retrospectively presented evidence that CPA may have been involved, along with the aflatoxins, in the "Turkey X" syndrome in England in 1960.

Fumonisins are newly described mycotoxins isolated from *F. moniliforme* (Gelderblom et al., 1988). This organism is involved in producing equine leukoencephalomalacia, and the fumonisins (B_1) were described recently as being capable of reproducing the disease in horses (Marasas et al., 1988). Also, one of the fumonisins (B_1), isolated from culture material of *F. moniliforme,* was shown recently to have cancer-promoting activity in rats (Gelderblom et al., 1988). Leukoencephalomalacia commonly occurs in horses in the United States, and the organism, *F. moniliforme,* is a frequent (almost universal) inhabitant of corn (Haliburton and Buck, 1986).

Fungi capable of producing **tremorgenic mycotoxins** belong to the genera *Penicillium, Aspergillus, Claviceps,* and *Acremonium.* The disease caused by these mycotoxins in cattle is called staggers. Clinical signs include muscle tremor, uncoordinated movements, and general weakness in the hind legs, with stiff, stilted movements of the forelegs. Severely affected animals may not be able to stand. Other intoxications involving fungal tremorgens have been reported from moldy cream cheese, a hamburger bun, and walnuts consumed by dogs. Another case involved a human who consumed mold-contaminated beer (Cole, 1986).

Penicillium roqueforti and *P. caseicolum (P. camemberti),* used to produce mold-ripened cheeses, have been shown to produce several toxic compounds, including **penicillic acid, roquefortine, isofumigaclavines A** and **B, PR toxin, mycophenolic acid,** and **cyclopiazonic acid** (Scott, 1981). The significance of the various toxins produced by *P. roqueforti* and *P. caseicolum* to public health is not clear because of the lack of scientific research on compound stability, production in agricultural commodities, and toxicity.

Objectives

The ultimate benefit in studying the mycotoxins and mycotoxicoses is to provide a safe food supply. While we realize that a 100% safe food supply (that is, a food supply without risk) is not achievable, we must base a portion of our acceptance of our food supply on the knowledge of mycotoxin effects in humans and animals and on the costs associated with marketing an acceptable food supply. Nearly a decade has passed since the last task force compiled information and published a CAST report on mycotoxins (Diener et al., 1979). Since then, much new information on the occurrence, toxicology, analysis, and control of mycotoxins has been published. Therefore, our intent was to carefully gather and assemble herein important information on the economic and health risks associated with selected mycotoxins. While this compilation is intended primarily for education of those making decisions that affect regulation and control of foods and feeds, we anticipate that the scientific accuracy of the report is such that it can be utilized by the scientific community.

Undoubtedly, mycotoxicoses of animals occur, affecting livestock health and production efficiency, and subtle effects of mycotoxins occur that are difficult to assess regarding economic losses. However, the effects of mycotoxins on the human population are unknown, and the best estimates in this area are extrapolations from animal mycotoxicoses or experimentation with animals and data from epidemiological studies. Efforts continue in determining the occurrence of mycotoxins in foods and feeds through diagnostic procedures, surveys, and epidemiologic studies. Utilizing the information in studies involving the areas outlined above, an estimate of the economic and health risks can be made.

Imperative to the task of minimizing mycotoxin contamination of the food supply is the development of methods to control the formation of mycotoxins and to detoxify or decontaminate the mycotoxin-contaminated commodity. Underlying our abilities to control and manage the contamination of mycotoxins in our feeds and foods is the development of rugged, precise, and sensitive procedures for analyzing mycotoxins in a variety of food and feed substrates.

Finally, following a compilation of this knowledge base, an assessment of future needs for research on mycotoxins and mycotoxicoses can be made to further increase the basis for making a better evaluation of economic and health risks associated with contamination of foods and feeds by mycotoxins.

2. Mycotoxicoses and Animal Health

Summary

Fungal toxins produce a wide range of injurious effects in animals, in addition to serving as food-borne hazards to humans. The economic impact of reduced productivity, increased disease incidence because of immune suppression, subtle but chronic damage to vital organs and tissues, and interferences with reproductive capacity is many times greater than that of acute livestock death.

The aflatoxins cause liver damage, decreased milk and egg production, and suppression of immunity in animals consuming low dietary concentrations. While the young of a species are most susceptible, all ages are affected, and clinical signs include gastrointestinal dysfunction, reduced reproductivity, decreased feed utilization and efficiency, anemia, and jaundice. Nursing animals may be affected by exposure to aflatoxin metabolites secreted in the milk.

The trichothecene mycotoxins are a large group of mycotoxins that cause necrosis and hemorrhage throughout the digestive tract, depress blood regenerative processes in the bone marrow and spleen, and cause changes in reproductive organs. Affected animals show signs of weight loss, poor feed utilization, apparent inappetence, vomiting, bloody diarrhea, abortion, and death. Suppression of immunity is a significant feature of certain trichothecene intoxications.

Ochratoxin A damages the kidneys of a wide variety of domestic and wild animals that consume contaminated feed. High concentrations of dietary ochratoxin A also can cause liver damage as well as intestinal necrosis and hemorrhage. Ochratoxin A has been shown to suppress immunity and to be carcinogenic.

Zearalenone mimics the effects of the female hormone estrogen and induces feminization at dietary concentrations of less than 1 ppm, while higher concentrations will interfere with conception, ovulation, implantation, fetal development, and the viability of newborn animals.

A variety of other effects has been attributed to mycotoxins, and these are briefly described in the following paragraphs.

Embryonic death, inhibition of fetal development, and abortions have been associated with ergot, aflatoxin, rubratoxin, and zearalenone in the rations of pregnant animals.

Teratogenicity has been documented in at least one mammalian species for aflatoxin, ochratoxin, rubratoxin, T-2 mycotoxin, zearalenone, and sterigmatocystin.

Nervous system functions are adversely altered by at least nine mycotoxins, inducing such clinical signs as tremors, uncoordinated movements, weakness of one or more limbs, staggering, and sudden muscular collapse from the consumption of contaminated forage, silage, cereal grains, or dietary supplements. In some instances, the neurological effects are complicated by seizures, diarrhea, and hemorrhage of the digestive tract, profuse salivation, feed refusal, and gangrene of the limbs, ears, or tail.

At least three mycotoxins (aflatoxins, ochratoxins, and sterigmatocystin) are known to induce tumors in one or more species of animal. The cancers have developed in liver, kidney, urinary system, digestive tract, and lung. The effects of some mycotoxins in producing skin irritations on contact and causing direct death of nervous tissue in the brain highlight the sensitivity of selected organs in certain species to these unique poisons.

The range and potency of mycotoxins in causing damage in animals make this group of naturally-occurring toxins an ongoing animal health hazard and a constant risk for contamination of the food supply.

Introduction

The impact of fungal toxins upon animals extends beyond their obvious effect in producing death in the wide variety of animals that are likely to consume mycotoxin-contaminated grain or feeds. The economic impact of lowered productivity, reduced weight gain (Figure 2.1), reduced feed efficiency, less meat and egg production, greater disease incidence because of immune system suppression, subtle damage to vital body organs, and interferences with reproduction is many times greater than that of immediate morbidity and lethality. Potential threats of cancer induced by mycotoxins in feeds and human foods, along with the unknown subtle effects of these mycotoxins, are coupled to the universal concerns about health risks. This section will present the range of mycotoxin-induced toxicities expected in animals, beginning with the potential for acute effects through organ-specific damage to the often unrecognized chronic effects.

Figure 2.1. Effects of dietary T-2 mycotoxin on growth of turkey poults. The turkey poult on the left was given normal ration, and the poult on the right was given a ration containing 10 ppm of T-2 mycotoxin for 3 weeks. Photograph courtesy of J. L. Richard, USDA, ARS, National Animal Disease Center, Ames, Iowa.

Acute Lethal Toxicity

Table 2.1 presents the lethal doses for 50% of various animal test populations (LD_{50}) given single doses of selected mycotoxins, and are included here as an example of the relative toxicity of some of the mycotoxins.

The Effects of Mycotoxins on Productivity and Animal Health

Aflatoxins

Aflatoxins are potent liver toxins, and their effects in animals vary with dose, length of exposure, species, breed, and diet or nutritional status (Figure 2.2). These toxins may be lethal when consumed in large doses. Sublethal doses produce a chronic toxicity, and low levels of chronic exposure can result in cancer (Wogan and Newberne, 1967; Sinnhuber et al., 1977), primarily liver cancer, in some animal species (Wogan, 1973; Busby and Wogan, 1984). Generally, young animals of any species are more susceptible to the toxic effects of the aflatoxins than are older animals. Of all the mycotoxins presently known, the aflatoxins have elicited the greatest public health concern because of their widespread occurrence in several dietary staples (peanuts, milk, and corn) and their potential as human carcinogens.

Acute aflatoxicosis in cattle has been thoroughly described. Clinical signs consisted of reduced feed consumption, dramatic drops in milk production, weight loss, and liver damage (Bodine and Mertens, 1983). However, chronic exposure of dairy and beef cattle to naturally occurring levels of aflatoxins may have an even greater economic impact as a result of reduced feed efficiency, immunosuppression, and reduced reproductivity (Bodine and Mertens, 1983).

Another characteristic of aflatoxin exposure in dairy cattle is the conversion of aflatoxin B_1 to the hydroxylated metabolite, aflatoxin M_1, and the excretion of aflatoxin M_1 in milk. Milk from Holstein cows given aflatoxin B_1 daily for seven days contained aflatoxin M_1, but no aflatoxin M_1 was detected in milk four days after termination of aflatoxin B_1 administration (Applebaum et al., 1982; Price et al., 1985). Mertens (1979) determined that the average excretion of aflatoxin M_1 in milk was approximately 0.9% of the total aflatoxin intake by the animal.

Chronic exposure of a dairy herd to aflatoxin-contaminated corn (120 ppb) resulted in severe herd health problems (Guthrie, 1979). Breeding efficiency decreased 2% for a five-month period after exposure, and milk production increased 28% after removal of the aflatoxin-contaminated corn from the diet. Other problems included the birth of smaller and unhealthy calves, diarrhea, acute mastitis, respiratory disorders, prolapsed rectum, hair loss, and reduced feed consumption (Guthrie, 1979).

Aflatoxins have been shown to affect rumen function *in vitro* and *in vivo* by decreasing cellulose digestion, volatile fatty acid formation, and proteolysis (Fehr and Delage, 1970; Dvorak et al., 1977). Cook and his associates (1986) demonstrated reduced rumen motility in steers given single doses of aflatoxins. They also characterized the clinical response and determined various tissue and fluid concentrations of aflatoxin B_1 and aflatoxin M_1 after dosing.

The toxicity of aflatoxins has been reported in suckling piglets, growing and finishing swine, and breeder stock. Clinical and pathological signs included decreased rate of weight gain, decreased feed conversion efficiency, toxic hepatitis, nephrosis, and systemic hemorrhages (Hoerr and D'Andrea, 1983; Miller et al., 1981, 1982). The effects of aflatoxins in pigs are varied, and may be more or less pronounced, depending upon the age of the animal, diet, concentration of aflatoxins, and length of exposure. Swine appeared to be resistant to dietary levels of aflatoxins up to 300 ppb fed from time of weaning to marketing (Monegue et al., 1977).

Although aflatoxins can be transferred *in utero* from sows to the piglets and can affect the biological and

Table 2.1 LD_{50}'s[a] of selected mycotoxins

Toxin	Animal	Sex	Age/Size	Route	LD_{50}	Reference
Aflatoxin B_1	Duckling	M	Day-old	PO[b]	0.37 mg/kg	Butler, 1964;
	Rat	M-F	Day-old	PO	1.0 mg/kg	Wogan, 1965
	Rat	M	21 days	PO	5.5 mg/kg	
	Rat	F	21 days	PO	7.4 mg/kg	
	Rat	M	100 g	IP[c]	6.0 mg/kg	
	Rat	F	150 g	PO	17.9 mg/kg	
	Dog	M-F	Adult	IP	ca. 1.0 mg/kg[d]	
	Dog	M-F	Adult	PO	ca. 0.5 mg/kg	
	Hamster	M	30 days	PO	10.2 mg/kg	
Aflatoxin B_2	?					
Aflatoxin G_1	Duckling	M-F	Day-old	PO	0.79 mg/kg	Lijinsky and Butler, 1966
Aflatoxin G_2	Duckling	M-F	Day-old	PO	172.5 ug/ duckling	Lijinsky and Butler, 1966
Aflatoxin M_1	Duckling	M-F	Day-old	PO	16.6 ug/ duckling	Purchase, 1967
Aflatoxicol	?					
Aflatrem	?					
Citreoviridin	ddys[e] mice	M	—	SC[f]	11.0 mg/kg	Ueno and Ueno,
	ddys mice	M	—	IP	7.2 mg/kg	1972
	ddys mice	M	—	PO	29.0 mg/kg	
Citrinin	Rat	—	—	SC/IP	67.0 mg/kg	Ambrose and
	Mice	—	—	SC/IP	35.0 mg/kg	DeEds, 1946
	Guinea pig	—	—	SC	37.0 mg/kg	
	Rabbit	—	—	IV[g]	19.0 mg/kg	
Cyclopiazonic acid	Rat	M	—	IP	2.3 mg/kg	Purchase, 1971;
	Rat	M	—	PO	36.0 mg/kg	Wilson et al.,
	Rat	F	—	PO	63.0 mg/kg	1989
	Chick	M	Day-old	PO	12.0 mg/kg	
	Chick	F	Day-old	PO	12.1 mg/kg	
	Poult	M	Day-old	PO	19.0 mg/kg	
	Poult	F	Day-old	PO	17.9 mg/kg	
	Duckling	M-F	Day-old	PO	38.6 mg/kg	
	Quail	M	6 wk-old	PO	69.6 mg/kg	
Cytochalasin H	Chick	M	Day-old	PO	12.5 mg/kg	Wells et al., 1976
Moniliformin	Chick	M	Day-old	PO	4.0 mg/kg	Burmeister et al.,
	Mice	M	25 g	IP	29.1 mg/kg	1979; Cole, 1973
	Mice	F	25 g	IP	20.9 mg/kg	
Ochratoxin A	Rat	—	Weanling	PO	22.0 mg/kg	Doster et al.,
	Trout	—	6 mos	IP	5.53 mg/kg	1974
Penitrem A	Mice	—	—	IP	1.05 mg/kg	Cole and Cox, 1981
PR toxin	Rat	—	Weanling	IP	11.0 mg/kg	Wei et al., 1973
	Rat	—	Weanling	PO	115.0 mg/kg	
	Mice	—	—	IP	5.8 mg/kg	
Roquefortine A	Mice	—	—	IP	340.0 mg/kg	Cole and Cox, 1981

Table 2.1. (continued)

Toxin	Animal	Sex	Age/Size	Route	LD_{50}	Reference
Slaframine	Chick	—	Day-old	PO	81.6 mg/kg	Johnson et al., 1986
Sterigmatocystin	Rat	M	—	PO	166.0 mg/kg	Cole and Cox, 1981
	Rat	F	—	PO	120.0 mg/kg	
	Rat	M	—	IP	60.0 mg/kg	
	Monkey	M	—	IP	32.0 mg/kg	
Tenuazonic acid	?					
Trichothecene toxins:						
Deoxynivalenol	ddys mice	M	—	IP	70.0 mg/kg	Cole and Cox, 1981
	ddys mice	F	—	IP	76.7 mg/kg	
Diacetoxyscirpenol	Rat	M-F	—	IP	0.75 mg/kg	Bamburg, 1972;
	Rat	M-F	—	PO	7.3 mg/kg	Ueno et al., 1973
	Mice	M-F	—	IP	23.0 mg/kg	
	Mice	M-F	—	IV	10.0 mg/kg	
Fusarenon-X	ddys mice	M	—	IP	3.3 mg/kg	Ueno et al., 1972; Ueno et al., 1973
HT-2	ddys mice	M-F	—	IP	9.0 mg/kg	Ueno et al., 1973
Monoacetoxy-scirpenol	Rat	F	20 days	SC	0.75 mg/kg	Cole and Cox, 1981
Neosolaniol	ddys mice	M-F	—	IP	14.5 mg/kg	Ueno et al., 1973
Scirpenetriol	Rat	—	—	IP	0.81 mg/kg	Cole and Cox, 1981
T-2	Rat	M-F	21 days	IP	3.0 mg/kg	Ueno et al., 1972;
	ddys mice	M-F	—	IP	5.2 mg/kg	Ueno et al., 1973
	Swine	M-F	—	PO	4.0 mg/kg	
	Chick	M	Day-old	PO	1.84 mg/kg	
T-2 tetraol	?					
Zearalenone	?					
Verruculogen	Mice	F	20 g	IP	2.4 mg/kg	Cole et al., 1972
	Mice	F	20 g	PO	126.7 mg/kg	
	Chick	M	Day-old	IP	15.2 mg/kg	
	Chick	M	Day-old	PO	265.5 mg/kg	

[a] LD = lethal dose
[b] PO = per os (by mouth)
[c] IP = intraperitoneal
[d] ca. = approximately
[e] ddys = recombinant congenic strain of mice used in the study of the major histocompatibility complex region of chromosomes.
[f] SC = subcutaneous
[g] IV = intravenous

immunological responsiveness of the neonatal pigs (Pier et al., 1985), reproductive effects of aflatoxins in swine would appear to be lacking or minimal (Armbrecht et al., 1972; Hintz et al., 1967). Spermatogenesis was not affected in aflatoxin-treated boars (Hintz et al., 1967). No carcinogenicity has been reported in naturally-occurring aflatoxicoses in swine.

Aflatoxicoses have produced severe economic losses in the poultry industry, affecting ducklings, broilers, layers, turkeys, and quail. Clinical signs of intoxication included anorexia, decreased weight gains, decreased egg production, hemorrhage, embryotoxicity, and increased susceptibility to environmental and microbial stressors (Edds and Bortel, 1983).

Histopathologic changes, including fatty liver, necrosis, and bile duct hyperplasia, were found in chickens given a high level of (1.5 ppm) dietary aflatoxins (Carnaghan et al., 1966). In chickens given half of this amount of aflatoxins, clinical responses included hypoproteinemia, reduced hemoglobin, and decreased serum triglycerides, phospholipids, and cholesterol (Brown and Abrams, 1965; Tung et al., 1972). Aflatoxins can cause a decrease in activities of several enzymes important in digestion of starches, proteins, lipids, and nucleic acids in broiler chickens (Osborne and Hamilton, 1981). The decreased activities of pancreatic amylase, trypsin, lipase, RNAse, and DNAse

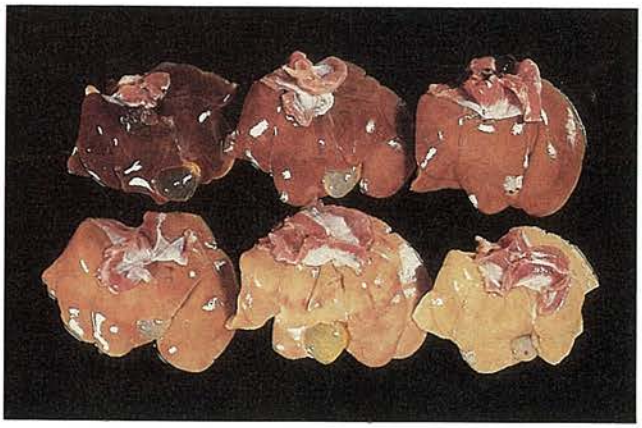


Figure 2.2. Livers from guinea pigs given increasing doses of aflatoxins over the same period of time. From left to right beginning in the upper left corner with the liver from a guinea pig given no aflatoxins, to the lower right corner with a liver from a guinea pig given the greatest dose of aflatoxins. Note the increasingly pale livers with increasing doses of aflatoxins. Photograph courtesy of J. L. Richard, USDA, ARS, National Animal Disease Center, Ames, Iowa.

could contribute to the malabsorption of nutrients associated with aflatoxicoses.

Hamilton (1971) reported a reduction to 5% of normal in egg production in laying hens that were given near LD_{50} levels of aflatoxins in naturally-contaminated corn. Aflatoxin-containing feed (up to 10 ppm) consumed by layers for 4 weeks caused a decrease in egg production and egg size (Huff et al., 1975). There was a decrease in total yolk weight and yolk as a percentage of total egg weight, accompanied by an increase in yolk and plasma carotenoid concentrations (Huff et al., 1975).

Clinical signs of aflatoxicosis in adult male Shetland ponies given daily doses of aflatoxins (0.3, 0.15, and 0.075 mg/kg body weight) consisted of lowered feed consumption, depression, and terminal prostration. Horses given the highest dosage died in approximately 25 to 30 days, preceded by an increase in prothrombin time, sulfabromophthalein clearance time, total plasma bilirubin, icteric index, and plasma activity of asparatate amino transferase. Target organs appeared to be the liver and kidneys (Cysewski et al., 1982).

Trichothecenes

General signs of trichothecene toxicity in animals include weight loss, decreased feed conversion, feed refusal, vomiting, bloody diarrhea, severe dermatitis (Figure 2.3), hemorrhage, decreased egg production, abortion, and death (Morehouse, 1985). Trichothecenes may also inhibit protein and DNA synthesis. Histologic lesions consist of necrosis and hemorrhage in proliferating tissues of the intestinal mucosa, bone marrow, spleen, testis, and ovary.

Mild enteritis and loose feces developed in calves given 0.08 to 0.64 mg of T-2 mycotoxin/kg body weight/day orally for nine days, but the highest dose levels of 0.32 and 0.64 mg T-2 mycotoxin/kg body weight produced bloody diarrhea in treated calves. Clinical signs of toxicity were loss of appetite, weight loss, and an increase in prothrombin time and serum glutamic oxalacetic transaminase (aspartate amino transferase) activity. Ulcerative lesions were present in the abomasum (Figure 2.4) and rumen, but there was no depression of leukocyte count, and no hemorrhaging, or histologic bone marrow lesions (Pier et al., 1976).

Commercial broiler starter containing 4.8 to 16 ppm T-2 mycotoxin fed to day-old broiler chicks for 3 weeks caused oral lesions described as circumscribed, proliferative, caseous-like plaques developed in treated birds (Wyatt et al., 1973b). The T-2 mycotoxin caused a decrease in growth rate, but feed conversion was not affected. There was an increase in organ weight of the spleen and pancreas, but the bursa decreased in weight in the high-dosed birds. The T-2 mycotoxin did not affect the levels of hemoglobin, serum proteins, cholesterol, total lipids, plasma glucose, or uric acid (Wyatt et al., 1973b). Neural toxicity and altered feathering occurred in chickens fed dietary T-2 mycotoxin for 3 weeks (Wyatt et al., 1973c, 1975a).

Figure 2.3 Severe oral lesion in a turkey poult that consumed a ration containing 10 ppm T-2 mycotoxin for 3 weeks. Photograph courtesy of J. L. Richard, USDA, ARS, National Animal Disease Center, Ames, Iowa.

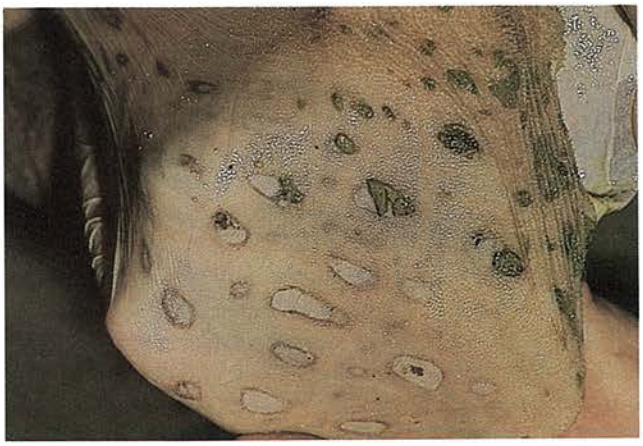


Figure 2.4. Abomasal ulcers in a calf given dietary T-2 mycotoxin (Pier et al., 1976).

Clinical symptoms were abnormal positioning of the wings, hysteroid seizures, and an impaired righting reflex.

The T-2 mycotoxin added to the feed (20 ppm) of laying hens caused a decrease in egg production and thinner egg shells (Wyatt et al., 1975b). Other signs of toxicity consisted of oral lesions, decreased feed consumption, low leukocyte counts, and decreased plasma protein and total lipid. Ten days after dosing began, T-2 mycotoxin (3 ppm) also caused a cessation of egg laying in geese (Palyusik and Koplik-Kovacs, 1975). Egg fertility from geese treated with T-2 mycotoxin was 54%, compared with 77% for control geese.

The mechanism of action of T-2 mycotoxin is not fully understood. This toxin is thought to be an amphipathic molecule, and to interact initially with the outer phospholipid bilayer of the cell (Gyongyossy-Issa et al., 1986a, 1986b, 1986c). The binding of T-2 mycotoxin to cell membrane receptors may interfere with nucleic acid and protein synthesis (Gyongyossy-Issa and Khachatourians, 1984, 1985; Rosenstein and LaFarge-Frayssinet, 1983). The mycotoxin inhibited protein chain initiation in intact ribosomes (Bamburg, 1983; Cundliffe and Davis, 1977). Murine lymphocytes were affected by T-2 mycotoxin; however, resting cells required a longer period to demonstrate the effect than actively dividing cells (Gyongyossy-Issa and Khachatourians, 1985). Cell membrane lysis may be caused by T-2 mycotoxin given at greater dosages (Gyongyossy-Issa et al., 1985); lysis may result from the formation of free radicals (Segal et al., 1983). Proliferative cells (Gyongyossy-Issa and Khachatourians, 1985) and cells containing many free polysomes (Terao, 1983) (hematopoietic, lymphoid, intestinal crypt, and bursa of Fabricius) are more susceptible to T-2 mycotoxin (Rosenstein and LaFarge-Frayssinet, 1983; Terao, 1983; Thurston et al., 1986) than are nonproliferative undifferentiated cells or cells with few free polysomes, such as parenchymal tissue cells.

Radiolabeled T-2 mycotoxin was rapidly metabolized by the liver and eliminated into the intestinal tract through the biliary excretion system, primarily as glucuronide conjugates (Beasley et al., 1986; Corley et al., 1985, 1986; Yoshizawa et al., 1981). Niyo and coworkers (1988a, 1988b) reported pathologic changes in the liver of rabbits given T-2 mycotoxin.

Results from studies on the mutagenic and carcinogenic activity of T-2 mycotoxin are contradictory (Bamburg, 1983). Teratogenic effects have been known to occur, and T-2 mycotoxin readily crosses the placenta (Hayes, 1981).

Natural contamination by T-2 mycotoxin of feeds given to chickens resulted in severe edema of the body cavity and hemorrhage of the large intestine, along with neurotoxic effects, oral lesions, and finally death. The T-2 mycotoxin has been implicated as one of the trichothecene mycotoxins involved in the human disease (Woronin, 1891), alimentary toxic aleukia (ATA) (Joffe, 1983; Mayer, 1953a, 1953b), and the so-called "yellow rain" samples collected from southeast Asia (Rosen, 1984; Wade, 1981). Several trichothecenes produce dermal necrosis in all animals tested, including humans, when in contact with the skin.

The seven-day oral LD_{50} in day-old broiler chicks given deoxynivalenol was approximately 140 mg/kg body weight. Acute signs included widespread hemorrhaging and deposition of urates, neural toxicity, and irritation of the upper gastrointestinal tract (Huff et al., 1981). Deoxynivalenol has been implicated in a disease known as moldy corn toxicosis of swine. Clinical signs include feed refusal, poor weight gain, and digestive disorders, including diarrhea and vomition (Morehouse, 1985).

Diacetoxyscirpenol (0, 2, 3, 8, and 9 ppm) fed to young pigs for 9 weeks caused multifocal, proliferative, gingival, buccal, and lingual lesions at all levels. There was also hyperplasia of the small intestine. Reduction in weight gain and feed consumption occurred at all dose levels and 10 ppm diacetoxyscirpenol caused complete feed refusal. Hematocrit, red and white blood cell counts, and serum enzyme levels were not affected. Also, diacetoxyscirpenol did not cause widespread hemorrhaging in the pigs (Weaver et al., 1981).

Ochratoxin A

Ochratoxin A, a nephrotoxic mycotoxin produced by

several *Aspergillus* and *Penicillium* species, only affected the kidneys in animals exposed to naturally occurring levels of this mycotoxin (Krogh, 1977). Changes in the renal function of pigs exposed to ochratoxin A included impairment of proximal tubular function, impairment of urine concentration, and increased excretion of urine glucose (Krogh, 1976). Extrarenal effects may have occurred in animals exposed to levels of ochratoxin A in feed greater than 5 to 10 ppm. These effects included enteritis, necrosis of lymphoid tissue, and fatty change in liver (Szczech et al., 1973).

Turkeys and chickens both exhibited lowered productivity during field outbreaks of ochratoxicosis. Symptoms included retarded growth, decreased feed conversion, nephropathy, and mortality, plus there was an apparent increased susceptibility to air sacculitis caused by *Escherichia coli* among affected birds (Hamilton et al., 1982). Feed refusal was demonstrated in turkeys exposed to ochratoxin A, but chickens did not exhibit any feed refusal (Burditt et al., 1984).

Ochratoxin A also had an effect on egg production in laying hens. There was a dose-related decrease in egg production in layers experimentally fed ochratoxin A (0.25 to 2.0 ppm) for 12 weeks. Higher levels of ochratoxin A caused a decrease in egg shell quality and increased the percentage of eggs with blood and meat spots (Shirley and Tohala, 1983).

Immune Suppression by Mycotoxins

One of the targets of several important mycotoxins is the immune system. Affected animals contract infections as a result of impaired acquired immunity or impaired native resistance to infectious agents. Although the entire episode may be primarily a mycotoxin-facilitated disease, recognition of such is often overshadowed by the infectious disease itself. Therefore, a descriptive or analytical epidemiological evaluation of the role of mycotoxins in the health of farm animals and humans is difficult or impossible to accomplish. Only when mycotoxicoses are acute manifestations of disease is there a chance at such an evaluation. However, the concentration of mycotoxins in foods and feeds is usually such that chronic, low-dose effects are more likely.

Experimental evidence conclusively reveals that selected mycotoxins, especially aflatoxins, some trichothecenes, and ochratoxin A, play a role in immunosuppression in domesticated and laboratory test animals. Thus, immunosuppression is potentially involved in economic losses due to mycotoxins. This same experimental evidence shows that immunosuppressive effects of aflatoxins, T-2 mycotoxin, and deoxynivalenol are experienced at levels that produce other adverse effects, and that normal immunity apparently returns after cessation of toxin exposure.

Aflatoxins

Most investigations of the effects of aflatoxins on immunity have revealed that these compounds primarily affect the cellular aspect of the immune process, although some studies have shown an effect on certain humoral factors involved in immunity.

The general features of immunosuppression by aflatoxins are summarized in Table 2.2. The interpretation of results from the studies of aflatoxins and immunity requires particular care, because mixtures of the aflatoxins were used in some studies, while in other studies, a single purified aflatoxin (aflatoxin B_1) was used. Differences have been demonstrated in this regard with aflatoxin B_1 and its metabolites (Bodine et al., 1984).

Immune phenomena mediated by cells, rather than by antibody or other humoral factors is referred to as cell-mediated immunity. Aflatoxins are known to suppress the function of T cells, which are specific cells of the lymphocyte system. T cells are apparently more sensitive to aflatoxins than B cells, which are responsible for humoral immunity. The effects on T cells are apparently of a functional nature, rather than that of morphological or populational changes.

Aflatoxins impair the function of more than one cell type of the mononuclear phagocyte system. The reduction of this activity would appear to be related to effects on the phagocytic cells (Figure 2.5), but, perhaps more importantly, to a heat-stable substance in the serum that is necessary for the activity of phagocytes (Richard et al., 1978a).

Complement, a serum constituent produced by the liver, plays an important role in several immune reactions and impairment of this activity means

Table 2.2 Effects of aflatoxins on immunity

Effects on cellular responses
Phagocytosis by macrophages reduced
Delayed cutaneous hypersensitivity reduced
Lymphoblastogenesis reduced (response to mitogens)
Graft versus host response reduced
Effects on humoral factors
Immunoglobulins (IgG and IgA) concentrations in serum may be reduced
Complement activity reduced
Bactericidal activity of serum reduced

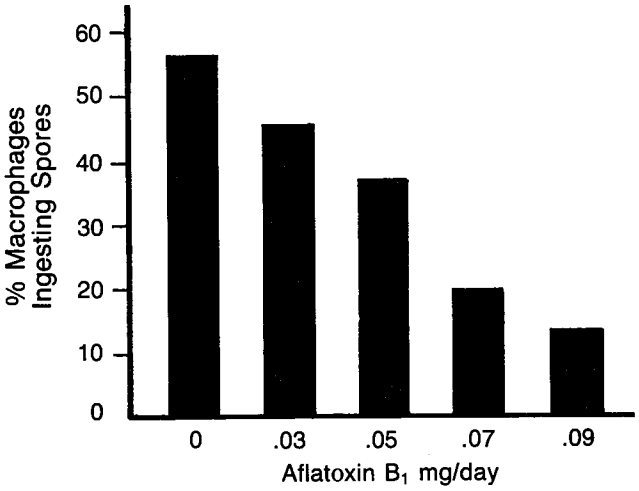


Figure 2.5 Reduction in phagocytosis of macrophages with increased doses of aflatoxins in rabbits (Pier et al., 1980b).

the reduction of an integral part of the immunologic potential of the host animal. Aflatoxins reduce the hemolytic complement activity in guinea pigs (Richard et al., 1978a; Thurston et al., 1972) and other species (Pier, 1986).

From limited epidemiological data, aflatoxins apparently are involved in outbreaks of infectious disease in domesticated animals. Salmonellosis, a bacterial infection, and candidiasis, a yeast infection, were associated with outbreaks of Turkey X disease caused by aflatoxins in 1960 (Siller and Ostler, 1961). Outbreaks of salmonellosis occurred in swine in the southeastern United States following the appearance of high concentrations of aflatoxins in the 1977 corn corp in that region (Miller et al., 1978). There is extensive experimental evidence that aflatoxins are very capable of lowering the resistance of several animal species to bacterial, fungal, and parasitic infections. A summation of these effects is part of several reviews of this subject (Pier, 1981, 1986; Pier et al., 1980a; Richard et al., 1978b).

Trichothecenes

The major effects of trichothecenes on immunity appear to be associated with the cellular immune response (Table 2.3). Also, the interaction of trichothecenes with infectious disease agents occurs when the resistance mechanisms would appear to be dependent on cellular immunity (Table 2.3). An example of this was shown by Niyo and coworkers (1988a, 1988b), who reported a reduction in phagocytosis by alveolar macrophages (Table 2.4) and an increased severity of experimental aspergillosis in rabbits treated with T-2 mycotoxin (Figure 2.6). Most experimental models for the study of these effects have utilized T-2 mycotoxin; however, a limited amount of work has been done with deoxynivalenol, diacetoxyscirpenol, and fusarenon X. The T-2 mycotoxin would appear to be as important as the aflatoxins in causing immunosuppression, and both the trichothecenes and aflatoxins appear to have the greatest effect on cellular immune phenomena.

An interesting feature of trichothecene toxicoses, related to immunity, is that deoxynivalenol can cause increased serum concentrations of IgA in mice (Forsell et al., 1985), and this can result in accumulation of IgA in the kidney glomerulus (Pestka and Moorman, 1988) in a manner analogous to human IgA nephropathy, the most common form of glomerulonephritis in the world.

Ochratoxin A

Immunosuppression by ochratoxin A has been studied mostly in poultry, with evidence suggesting that this mycotoxin has an effect on immunoglobulins and phagocytic cells (Burns and Dwivedi, 1986). Unlike aflatoxin and T-2 mycotoxin, the reduction in phagocytosis was not associated with a serum factor. Also, ochratoxin A does not appear to have an effect on complement activity (Richard et al., 1975).

Cellular immune responses in poultry were affected by ochratoxin A more in broilers than in quail or turkeys (Burns and Dwivedi, 1986); species differences are apparent.

Air sacculitis caused by *Escherichia coli* was notably increased in turkeys and broilers involved in an outbreak of ochratoxicosis (Hamilton et al., 1982). Interestingly, antibiotic therapy for the air sacculitis was ineffective in birds consuming the ochratoxin A-containing feed, but became effective when the diet was changed. This phenomenon could be economically important, because of added cost with little or no benefit in treating disease.

Hematopoietic Effects of Mycotoxins

Aflatoxins

Hemorrhagic anemia syndrome, caused by the consumption of moldy feed by poultry, was linked to the presence of aflatoxin-producing fungi in the feed (Forgacs and Carll, 1962). The syndrome is characterized by massive hemorrhagic lesions in the major organs and musculature. A possible hemolytic anemia

Table 2.3 Trichothecene effects on immunity

Effects	References
Effects on cellular immunity and inflammatory responses	
Chemotactic migration of neutrophils decreased	Buening et al., 1982; Yarom et al., 1984
Phagocytosis by alveolar macrophages decreased	Niyo et al., 1986, 1988a, 1988b; Gerberick and Sorenson, 1983; Gerberick et al., 1984
Skin graft rejection time increased	Rosenstein et al., 1979
Mitogen-induced blastogenesis of lymphocytes inhibited	Buening et al., 1982; Forsell et al., 1985; Cooray, 1984; Pang et al., 1987
Platelet function inhibited	Chan and Gentry, 1984; Gentry et al., 1987
Cytotoxic to lymphocytes	Rosenstein and LaFarge-Frayssinet, 1983; LaFarge-Frayssinet et al., 1979; Corrier and Ziprin, 1986; DiNicola et al., 1978; Yarom et al., 1984; Jagadeesan et al., 1982; Lutsky et al., 1978
Increased sensitivity to bacterial endotoxin	Tai and Pestka, 1988
Depressed delayed-type hypersensitivity	Pestka et al., 1987
Effects on infectious diseases	
Resistance to mycobacterial infection in mice decreased	Kanai and Kondo, 1984
Mortalities of chickens and mice to *Salmonella* spp increased	Boonchuvit et al., 1975; Tai and Pestka, 1989
Susceptibility to herpes simplex virus in mice increased	Friend et al., 1983a, 1983b
Mortality of mice with listeriosis increased (dependent upon time of exposure to toxin)	Corrier and Ziprin, 1986, 1987; Ziprin et al., 1987; Ziprin and Corrier, 1987
Infectious response in rabbits with aspergillosis increased	Niyo et al., 1988b

Table 2.4. Mean number of *Aspergillus fumigatus* conidia ingested in 1 hour *in vitro* by rabbit lung alveolar macrophages (Niyo et al., 1988a)

Rabbit dosage groups (mg. of T-2/kg/day) from which alveolar macrophages were obtained	Rabbit dosage groups (mg of T-2/kg/day) from which sera were obtained		
	0.0	0.25	0.5
0.0	2.4	1.5	1.1
0.25	2.4	1.6	ND[a]
0.5	2.1	ND	1.1
	2.3[b] (0.12)	1.5 (0.9)	1.1[c] (0.11)

[a] Not determined.
[b] Mean (SEM).
[c] $P < 0.01$.

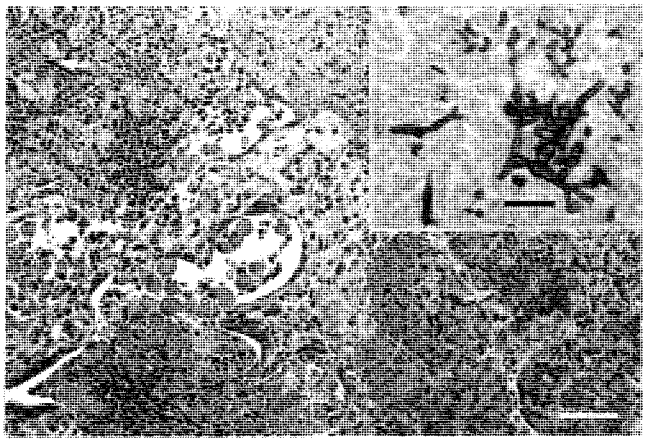


Figure 2.6. Photomicrograph of a section of the lung from a rabbit given T-2 toxin orally for 17 days, exposed to aerosols of *A fumigatus* conidia on days 7 through 16, and necropsied on day 17. There are multifocal to confluent necrotic granulomas within alveoli and bronchioles. Notice fungal elements surrounded by radiating clubs (white arrows) within some granulomas. H&E stain; bar = 50 μm.
Inset reveals tangled masses of *A. fumigatus* hyphae in this lung specimen. Gridley stain; bar = 25 μm. (Niyo et al., 1988b).

with bone marrow hyperplasia and an increase in bone marrow nucleic acid occurred in broiler chicks consuming aflatoxin. There were significant decreases in hemoglobin, packed cell volume, and circulating erythrocytes (Tung et al., 1975a). Aflatoxins also caused significant increases in whole blood clotting time, recalcification time, and prothrombin time in broiler chicks fed aflatoxins for 3 weeks (Doerr et al., 1974). However, aflatoxins in the feed (20 ppm) of mature broilers for 4 weeks caused only a slight anemia without increasing erythrocyte fragility (Wyatt et al., 1973a). Lanza and associates (1980) presented data suggesting that the development of anemia in aflatoxin-treated animals was a secondary effect of severe hypoproteinemia. These effects may be secondary to primary liver damage, as discussed in the following section.

Exposure to aflatoxins may also affect hemostasis in developing embryos. Hatched chicks, following embryonic exposure to aflatoxin B_1, had significantly reduced cell counts, hematocrits, and hemoglobin concentrations (Dietert et al., 1983). However, there were no differences in erythrocytes of treated versus control groups.

Trichothecenes

Trichothecenes, as exemplified by T-2 mycotoxin, caused histologic lesions in experimental animals, including cellular necrosis and karyorrhexis in actively dividing tissues of the intestinal mucosa, bone marrow, spleen, testis, and ovary (Ciegler, 1978; Niyo et al., 1988a, 1988b). Therefore, a reduction in production and numbers of macrophages, lymphocytes, and erythrocytes may occur with prolonged exposure to trichothecenes. Erythrocyte numbers can also be reduced by trichothecene-induced hemolysis (Segal et al., 1983). The T-2 mycotoxin caused complete hemolysis of rat erythrocytes following a lag period dependent on the concentration of T-2 mycotoxin. Hemolysis was thought to occur by the formation of free radicals, because darkness and free radical scavengers, including vitamin E, mannitol, and histidine, inhibited erythrocyte hemolysis by T-2 mycotoxin (Segal et al., 1983).

Trichothecenes may also alter hemostasis. The T-2 mycotoxin increased prothrombin time significantly, but not recalcification time or whole blood clotting time, in broiler chickens (Doerr et al., 1974). However, there was a trend to prolong recalcification (formation of a fibrin clot) by T-2 mycotoxin. A single intravenous administration of T-2 mycotoxin in rabbits caused a reduction in circulating activity of certain plasma coagulation factors (Gentry, 1982). Similar results were shown in calves given a single IV dose of T-2 mycotoxin (Gentry and Cooper, 1983).

Hepatotoxicity of Mycotoxins

Aflatoxins

Aflatoxicosis is primarily an hepatic disease. The susceptibility of individual animals to aflatoxins varies considerably, depending on species, age, sex, and nutrition (Butler, 1974; Edds, 1979; Hsieh et al., 1977b; Newberne and Butler, 1969; Newberne and Gross, 1977). Hepatic injury in aflatoxicosis can be demonstrated by changes in clinical chemistry values associated with liver function (Norred, 1986; Richard et al., 1975; Thurston et al., 1980) and by histopathology, where lesions of bile duct proliferation, hepatocellular degeneration, necrosis, and fibrosis are seen in virtually all instances of clinical and experimental disease (Butler, 1974; Edds, 1979; Hoerr et al., 1986).

The carcinogenicity of aflatoxin B_1 is believed to be due to metabolic activation of the parent compound by microsomal enzymes in the liver that generate an epoxide (Garner, 1973a; Swenson et al., 1974). This electrophilic intermediate metabolite binds to macromolecules (DNA, RNA, proteins) within hepatocytes (Garner, 1973b; Lin et al., 1977; Neal, 1973; Swenson et al., 1974). Further metabolism of the epoxide yields another metabolite (Swenson et al., 1974) that may be, in part, responsible for the acute toxicity (Neal et al., 1981).

Binding of aflatoxin B_1 to these hepatocyte macromolecules could interfere with transcription (Clifford and Rees, 1966; Neal, 1973; Yu, et al, 1988) and with protein synthesis (Clifford and Rees, 1966; John and Miller, 1969; Sarasin and Moule, 1975). Binding of aflatoxin to hepatocellular proteins may result in the formation of Schiff bases, reduce enzyme activity, and lead to acute toxicity (Patterson, 1973; Patterson and Roberts, 1970).

Jaundice, decreased performance (milk production, egg production, and weight gain), and hemorrhage are but a few of the clinical signs often seen in experimental and field cases of aflatoxicosis (Edds, 1979; Norred, 1986). Hepatic disease and dysfunction are reflected in decreased hepatic synthesis and increased consumption of coagulation factors, leading to hemorrhage (Baker and Green, 1987), decreased synthesis of liver-specific proteins such as complement and albumin (Richard et al., 1975, 1978a; Thurston et al., 1972; Tung et al., 1975b), and fatty infiltration of

hepatocytes, due to disturbed lipid and protein metabolism (Donaldson et al., 1972; McLean and McLean, 1967; Tung et al., 1972).

Ochratoxin A

Ochratoxins are primarily nephrotoxic. However, they can cause damage to the liver of animals, particularly at higher doses. In studies with broiler chicks, liver damage was present in concert with nephrotoxicity (Smith and Moss, 1985). Lymphocyte infiltration occurred in the liver along with lymphocytolysis in lymphoid organs. Necrotic changes in periportal cells were observed in rats given the LD_{50} (20 mg/kg body weight) dose of ochratoxin A (Terao and Ueno, 1978). The effects of ochratoxins on liver are apparently much less pronounced and specific than those of aflatoxins. Interestingly, ochratoxin A apparently prevented fatty degeneration of the liver caused by aflatoxins when the two toxins were given simultaneously to broiler chickens (Huff et al., 1984).

Nephrotoxicity of Mycotoxins

Ochratoxin A

Ochratoxin A causes kidney damage in rats, dogs, and swine. The ochratoxins are thought to be involved in a disease of swine known as porcine nephropathy, which has been associated with the feeding of moldy corn or barley (Cook et al., 1986; Krogh, 1977). Also, ochratoxin A has been reported to be teratogenic to mice, rats, and chicken embryos.

Reproductive Effects of Mycotoxins

Estrogenism

The major effects of zearalenone are estrogenic (Figure 2.8) and primarily involve the urogenital system. Swine are the most commonly affected animals. Cattle, poultry, and laboratory rodents are also affected, but to a lesser degree. Hyperestrogenism in female swine may be manifested as swelling of the vulva and enlargement of the mammary glands, especially in prepubescent gilts. Dietary concentrations of 1.0 ppm zearalenone or more may produce hyperestrogenism in pigs (Kurtz and Mirocha, 1978). Zearalenone has been associated with feminization in young male swine, including testicular atrophy, swollen prepuce, and mammary gland enlargement; decreased libido may be a variable sequela, but mature boars apparently have enough testicular reserve to avoid decreased spermatogenesis. This syndrome may progress to rectal and vaginal prolapse in severe cases. Other effects related to higher concentrations include anestrus, nymphomania, and pseudopregnancy. High concentrations of zearalenone in cattle diets have been associated with infertility, teat enlargement, and udder secretions, which were most apparent in immature dairy heifers; vent enlargement and enhanced secondary sex characteristics are noted in turkeys and chickens. Zearalenol, the reduced form of zearalenone, has about four times greater estrogenic activity than zearalenone. The commercially synthesized zearalanol, (Ralgro^R), is approved for use as an anabolic agent in cattle and sheep.

Reproductive Efficiency

High concentrations of zearalenone (50 to 100 ppm) in swine diets have been reported to adversely affect cycling, conception, ovulation, and implantation. Placental membrane and fetal development may also be disrupted, resulting in reduced litter size and diminished viability of neonates (Sundlof and Strickland, 1986; Miller et al., 1973; Chang et al., 1979). Zearalenone also reduced conception rates in dairy heifers. Clinical signs of T-2 mycotoxin and diacetoxyscirpenol exposure in domestic animals are usually manifested as reduced egg production or rate of weight gain. The T-2 mycotoxin consumption by breeding sows has caused drastically reduced conception rates and weak piglets with reduced litter sizes in those

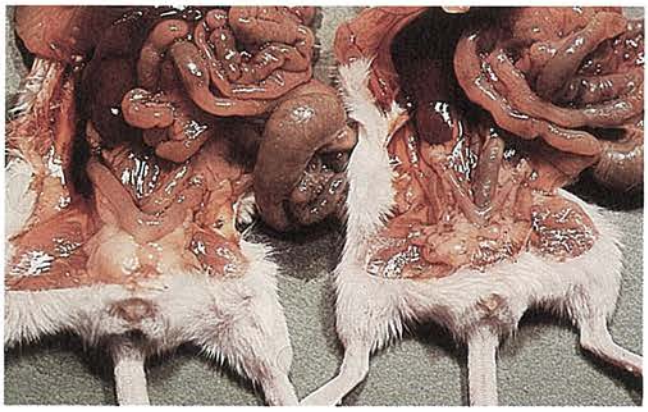


Figure 2.7. Enlarged uterus in mouse (left) given an intraperitoneal injection of zearalenone. Compare with uterus of control mouse on the right. Photograph courtesy of J. L. Richard, USDA, ARS, National Animal Disease Center, Ames, Iowa.

sows conceiving.

Ergot-contaminated diets of pregnant swine are associated with decreased piglet birth weights and increased stillborn rates; gestation length may be shorter or longer than normal. Ergot also causes inhibition of prolactin secretion in pregnant swine, resulting in diminished udder development at farrowing with minimal or absent milk production; severity of effects is directly related to dietary concentrations, and feeds containing $\geq$0.3% ergot sclerotia have definite detrimental effects on overall reproductive performance (Loveless, 1967; Burfening, 1973). Altered reproduction patterns and reproductive failures in swine are associated with more than 450 ppb dietary aflatoxins, and lesser concentrations may adversely impact neonatal resistance to disease (Bailey et al., 1973).

Fetal Resorption/Death

Zearalenone caused embryonic death, inhibition of fetal development, and decreased numbers of fetuses present in exposed swine. Abortion may be a variable and controversial sequela to ergot ingestion in animals, depending on species affected and alkaloid content of sclerotia. Aflatoxin B_1 has been associated with bovine abortions, and may adversely affect nursing neonates through exposure to metabolites in milk. When administered parenterally to pregnant swine, T-2 mycotoxin elicited embryo-toxic effects, whereas that fed in the diet did not. Ochratoxin A, rubratoxin B, secalonic acid D, and sterigmatocystin are all reported to affect embryonic survival in various animal species.

Teratogenicity

Experimentally, aflatoxin B_1, ochratoxin A, rubratoxin B, T-2 mycotoxin, sterigmatocystin, and zearalenone are teratogenic in at least one mammalian species (Hayes, 1981). When administered in combination during organogenesis, ochratoxin A enhanced T-2 mycotoxin-induced teratogenicity. Intraperitoneal administration of ochratoxin A to pregnant mice had a teratogenic effect on surviving pups, increased fetal death, and decreased fetal weight (Hayes et al., 1974).

Neurotoxicity of Mycotoxins

Tremorgens

Fungi represent a rich source of secondary metabolites that are capable of eliciting a tremorgenic response in animals. Fungal tremorgens have been implicated in the etiology of several tremorgenic syndromes (Table 2.4). Some tremorgenic syndromes that occur in pastures involving cattle are referred to as "staggers syndromes". Typically, these are characterized by muscle tremor, uncoordinated movements, and a general weakness in the hind legs with stiff, stilted movements of the forelegs. More severely affected animals are not able to stand. All the effects of these toxins are aggravated when the animals are forced to move or are excited in any way. Deaths are indirect, usually from dehydration, pneumonia, or drowning while attempting to drink from a pond or large watering container. The animals generally recover, with no apparent damage, 24 to 48 hours after removal from the toxic feed source. Known staggers syndromes include paspalum staggers, ryegrass staggers, corn staggers, and Bermuda grass staggers (Table 2.4). With the exception of Bermuda grass staggers, the implication of fungal tremorgens in the etiology of these diseases has been fairly well established.

Another tremorgenic syndrome in cattle occurs when corn silage from a trench-type silo is improperly unloaded using a front-end loader. This aerates the silage,

Table 2.5 Listing of intoxication, species involved, suspected or incriminated etiological agent, and their fungal source (Cole and Dorner, 1986; Hocking et al., 1988)

Intoxication	Animal	Suspected or incriminated mycotoxin	Fungal source
Paspalum staggers	Cattle	Paspalitrems	*Claviceps paspali*
Ryegrass staggers	Cattle	Lolitrems	*Lolium* endophyte
Bermuda grass tremors	Cattle	Unknown	Unknown
Corn staggers	Cattle	Aflatrem; paspalinine	*Aspergillus flavus*
Moldy silage	Cattle	Verruculogen/fumitremorgens	*Aspergillus fumigatus*
Moldy cream cheese	Dog	Penitrem A	*Penicillium crustosum*
Moldy walnuts	Dog	Penitrem A	*Penicillium crustosum*
Moldy hamburger bun	Dog	Penitrem A	*Penicillium crustosum*
Moldy beer	Human	Penitrems	*Penicillium crustosum*

causing a fermentation involving toxigenic strains of *A. fumigatus*. These fungi are capable of producing alkaloids and tremorgens belonging to the verruculogen/fumitremorgen group. The disease is characterized by a general deterioration typical of protein deficiency and malnutrition, even though ample pasture and supplemental feed may be available. Other prominent clinical signs are diarrhea, irritability, and abnormal behavior.

The fungal tremorgen, penitrem A, has recently been strongly implicated in three different tremorgenic intoxications involving dogs (Arp and Richard, 1979, 1981; Hocking, et al., 1988; Richard et al., 1981; Richard and Arp, 1979).

The first episode involved two dogs from the same neighborhood that were both exhibiting clinical signs characterized by muscle tremors and clonic seizures (Arp and Richard, 1979; Richard and Arp, 1979). The animals were both treated with sodium pentobarbital (anesthesia) and dextrose-electrolyte solution (to maintain vital functions). Both dogs were clinically normal 12 hours after treatment, except for slight incoordination and moist pulmonary rales.

The apparent source of the intoxication was a discarded package of moldy cream cheese. The fungus isolated from the cream cheese was identified as *P. crustosum*. Penitrem A was isolated from the cream cheese and from cultures of the *P. crustosum* isolate.

The second, rather unusual, episode involved ingestion of moldy English walnuts by a dog (Arp and Richard, 1981; Richard et al., 1981). The "moldy walnut" toxicosis in dogs has been recognized for several years in walnut-producing areas of California, and involvement of a mycotoxin was suspected, but not proved, prior to this episode. Approximately 2 to 3 hours after ingesting moldy overwintered walnuts, the dog exhibited generalized convulsions and other signs, including ataxia, urination, defecation, polypnea, hyperthermia, and mydriasis. Full recovery occurred in 36 hours, presumably due to removal from the source of the tremorgen, rather than from any applied therapy.

The fungus isolated from the moldy walnuts was *P. crustosum*, and penitrem A was identified from both the moldy walnuts and the fungus when grown on a synthetic medium.

The third episode occurred in Australia, and involved a dog that consumed a moldy hamburger bun (Hocking et al., 1988). The clinical signs in this case were very similar to the previous two cases. The fungus was *P. crustosum*, and the toxin was penitrem A.

Penitrem A and *P. crustosum* have also been implicated in a human intoxication involving moldy beer (Table 2.4). The individual involved was a physician, who became acutely ill approximately four hours after consuming about 30 cc of the contaminated beer. He described the symptoms as a tremor, throbbing frontal headache, feverish feeling, nausea, vomiting, double vision, weakness, and bloody diarrhea. All symptoms disappeared, and no apparent residual effects were noted after 30 hours.

Slobber Syndrome - Slaframine

Slobbers or salivary syndrome, characterized by excessive salivation in horses and cattle, has been caused by the consumption of red clover hay infected with *Rhizoctonia leguminicola*. Other clinical signs of toxicity include diarrhea, piloerection, increased lacrimation, feed refusal, and respiratory failure in very severe cases (Aust, 1974, 1986; Crump et al., 1967; O'Dell et al., 1959; Rainey et al., 1965; Smalley, 1977a, 1977b). Outbreaks of salivary syndrome have caused a decrease in the cultivation of red clover in the Midwest (Aust, 1974; Gough and Elliot, 1956).

Slaframine, the compound responsible for salivary syndrome, was isolated and identified from two strains of *Rhizoctonia* (Aust and Broquist, 1965; Gardiner et al., 1968; Rainey et al., 1965).

Slaframine is a parasympathomimetic metabolite, and the active ketoimine liver metabolite of slaframine has an affinity for muscarinic receptors of the nervous system (Aust, 1969; Guengerich and Aust, 1977). Slaframine has a particularly high affinity for receptors in the gastrointestinal tract. Chicks and cattle exposed to this compound showed an increase in salivation, pancreatic flow, and growth hormone release (Aust, 1974; Fernandez et al., 1985; Froetschel et al., 1985; 1986). This research indicates that slaframine may prove to be an important therapeutic agent in treating some digestive animal disorders.

Ergot

Ergotism is a classic mycotoxicosis caused by products of the plant parasitic fungi, *Claviceps purpurea*, *C. paspalli*, and *C. fusiformis*. These fungi invade the female portion of the host plant, particularly small grains such as rye and barley, and replace the ovary with a mass of fungal tissue called a sclerotium. These sclerotia (ergots) (Figure 2.9) contain alkaloids produced by the fungus, and the amounts of alkaloids vary, dependent upon the host plant and environmental factors (Robbins et al., 1986). When the sclerotia are ingested by animals or humans, two major signs of disease (gangrene or convulsions and

Figure 2.8. Black sclerotia (ergots) caused by *Claviceps purpurea* infection in barley. Photograph courtesy of J. L. Richard, USDA, ARS, National Animal Disease Center, Ames, Iowa.

gastrointestinal disorders) may occur. Additionally, production losses have been noted in animal feeding studies. Gangrenous symptoms were described in medieval episodes of ergotism in humans, where early symptoms were swollen limbs with burning sensations (St. Anthony's fire), with subsequent necrosis leading to loss of appendages. Signs of lameness and necrosis of ears, tails, and feet have occurred in animals pastured on infected grasses or fed grains containing the ergot sclerotia.

Nervous signs, predominant in the convulsive form of ergotism, have occurred primarily in animals and consist of ataxia, convulsions, and paralysis (Marasas and Nelson, 1987).

The effects of ergot on production in animals have included reduced weight gains, milk production, reproductive efficiency, and agalactia (Robbins et al., 1986).

Ergotism occurs in the United States, particularly in the areas where rye, barley, and other susceptible small grains or grasses are grown (i.e., midwestern states to the northwestern states).

Because the sclerotia may be inadvertently included in grains processed for human consumption, there is a potential threat for disease in humans and the need for continued surveillance of grains used for such purposes. Two outbreaks have occurred in recent times involving human patients. In Africa, 93 cases of gangrenous ergotism were reported involving grain infected with *C. purpurea* (Derneke et al., 1979), and 78 human cases of gastrointestinal ergotism occurred in India involving millet infected with *C. fusiformis* (Krishnamachari and Bhat, 1976). Although the sclerotia may contain variable quantities and proportions of the ergot alkaloids, the tolerances established in the United States are based on the percentage by weight (<0.3%) of sclerotia in graded grains (Marasas and Nelson, 1987).

Carcinogenesis

Aflatoxins

The induction of neoplasms (abnormal growth of tissue) by aflatoxins has been extensively studied. Aflatoxin B_1, as well as the secondary aflatoxin B_1 metabolites, aflatoxin M_1 (present in milk of cows ingesting *A. flavus*-contaminated feed), aflatoxicol, and aflatoxin G_1, have caused hepatic, renal, and colonic neoplasms in rats (0.2 to 0.12 μg/day) (Wogan and Newberne, 1967), hepatic neoplasms in trout (0.4 ppb for 9 months) (Jackson, 1968), ducks (30 ppb for 2 to 4 weeks) (Carnaghan, 1965), ferrets (0.3 ppm) (Lancaster, 1968), mice (20 μg/week for 76 weeks) (Dickens and Jones, 1965), guppies, and monkeys (Berry, 1988). Nasal neoplasms were induced in sheep at 1.75 ppm for 3½ years (Lewis et al., 1967). Pulmonary neoplasms were produced in mice given 0.2 μg of aflatoxin B_1/day for 4 weeks (Wieder et al., 1968). Results of several epidemiological studies have implicated aflatoxins as potential factors in the increased incidence of human gastrointestinal and hepatic neoplasms in Africa, the Philippines, and China. Aflatoxin B_1 has also been implicated as a cause of human liver cell carcinoma (LCC) (Berry, 1988; Stark, 1980) (see Chapter 3).

In *in vitro* mutagenicity tests, aflatoxin B_1 activated by rat liver microsomal preparations has caused point mutations and frame shift mutations in host cell DNA (Berry, 1988). Aflatoxin B_1 also chemically (covalently) binds to DNA *in vitro*. This tight (covalent) binding of aflatoxin B_1 to DNA caused structural alterations in the DNA. Similar covalent interactions between chemicals and DNA and the resultant structural DNA alterations have caused genomic mutations in *in vitro* and *in vivo* systems.

Sterigmatocystin is a precursor of aflatoxin, and its toxicity is lower than aflatoxins. However, its carcinogenic potential, which experimentally is about one-tenth that of aflatoxin B_1, is still of concern.

Ochratoxin A

Although mutagenicity of ochratoxin A has not been established in several *in vitro* assays (Ames test and Rec assay), tumorigenesis/carcinogenesis was reported

in laboratory animals (Ueno, 1984b). Renal and hepatic neoplasms have been induced in laboratory mice fed 40 ppm ochratoxin A for 20 months (Bendele et al., 1985; Ueno, 1984b). These results were confirmed in a study performed under the direction of the National Institute of Environmental Health Sciences as a function of the National Toxicology Program (Boorman, 1988). Stark (1980) has reported a 40% increase in the incidence of urinary tract cancer of eastern Europeans with Balkan nephropathy, a condition now believed to be the result of ingestion of locally grown grains contaminated with ochratoxin.

Sterigmatocystin

Sterigmatocystin, biosynthetic precursor of the aflatoxin family of mycotoxins, has caused liver cell carcinoma in laboratory rats (Purchase and Van der Watt, 1970). It was also mutagenic by several *in vitro* tests, such as the Ames test, the Rec assay, and the *Bacillus subtilis* assay (Berry, 1988). Tumorigenicity of sterigmatocystin was considerably less than aflatoxin B_1 to rats (0.1 to 0.01) (Berry, 1988). Sterigmatocystin covalently bonded to DNA at approximately 20 to 30% of the level observed with aflatoxin B_1.

Dermal Toxicity

Trichothecenes

Several trichothecene mycotoxins have been shown to be skin irritants. Skin tests on animals have been used to qualitatively determine the biologic activity of this class of compounds (Gilgan et al., 1966; Bamburg et al., 1968).

Ueno (1984a) determined the minimum effective dose for dermal toxicity of 18 trichothecenes and compared their structure-activity relationships. The T-2 mycotoxin, the most toxic compound tested, caused erythema on the shaved backs of guinea pigs at a minimum dose of 5 ng. The T-2 mycotoxin and macrocyclic trichothecenes caused an edema approximately 24 hours after application. Trichothecenes, such as nivalenol and deoxynivalenol, exhibited very low dermal toxicity (Ueno, 1984a).

The cutaneous penetration rate and the metabolism of diacetoxyscirpenol, verrucarin A, and T-2 mycotoxin varied in human and guinea pig skin, and was dependent on structure, dosage, and carrier (Kemppainen et al., 1987a).

The *in vivo* cutaneous absorption of T-2 mycotoxin in guinea pigs was compared with the *in vitro* cutaneous penetration of T-2 in static and flow-through diffusion cells (Kemppainen et al., 1987b). They found that penetration data from static studies may underestimate the rate and amount of absorption that actually occurs through intact animal skin.

No T-2 mycotoxin was present in plasma or urine of guinea pigs in the *in vivo* study; however, metabolites of this parent compound were found in urine (Kemppainen et al., 1987b).

Other Mycotoxicoses

Equine Leukoencephalomalacia

A relationship between moldy corn and sporadic outbreaks of equine leukoencephalomalacia (ELEM), a unique neurotoxic syndrome of Equidae, has been known for many years (Badiali et al., 1968; Biester and Schwarte, 1939; Biester et al., 1940; Butler, 1902; Schwarte et al., 1937). However, leukoencephalomalacia in deer was recently described and was associated with *F. moniliforme*-infested corn (Howerth, et al., 1989). More recently, ELEM was shown to be caused by the fungus *F. moniliforme* that is commonly found in corn (Boccas et al., 1985; Brownie and Cullen, 1986; Buck et al., 1979; Kellerman et al., 1972; Kriek et al., 1981; Marasas et al., 1976; Pienaar et al., 1981). Hepatosis can also result from *F. moniliforme* ingestion by equines, and may occur alone or together with neurotoxicity (Brownie and Cullen, 1986; Haschek and Haliburton, 1986; Kellerman et al., 1972; Kriek et al., 1981; Marasas et al., 1976).

Neurotoxic symptoms, including loss of feed consumption, lameness, ataxia, oral and facial paralysis, head pressing, and recumbency, may begin within days after the initial exposure to moldy feed. Seizures may be present, and morbidity can occur within hours after the onset of clinical signs. Focal malacia and liquefication of cerebral white matter with peripheral hemorrhage is the pathognomonic necropsy finding. Microscopically, there is liquifactive necrosis and gliosis. Edema, hemorrhage, and perivascular cuffing of leukocytes are often present in the surrounding neuropil.

The hepatotoxic syndrome often occurs concurrently with facial edema, jaundice, pruritis, somnalence, loss of feed consumption, petechia of the mucous membranes, and/or cyanosis. Grossly, the liver is small, firm, and may have a yellowish discoloration. Visceral petechiae, ecchymoses, and jaundice are also typical necropsy findings. Centrilobular necrosis, fibrosis, and bile duct proliferation with increased mitoses,

acute inflammation, and fatty degeneration of the liver are characteristic histopathologic findings.

Recently, Gelderblom and associates (1988) discovered a new class of compounds, the fumonisins, that are hepatotoxic and have liver cancer promoting activity in rats. Malacic lesions have been experimentally induced in an equine exposed to fumonisin B_1 (Marasas et al., 1988). Independent confirmation of this discovery has not as yet been reported, although fumonisins have been found in corn naturally contaminated with *F. moniliforme* that was associated with field cases of ELEM (Norred et al., 1989; Voss et al., 1989).

Fescue Foot Toxicoses

Tall fescue is a perennial grass grown on approximately 40 million acres in the United States. In much of its growing range, it is the only cool season perennial forage. Although this forage is able to withstand heavy grazing and environmental stresses, body weight gains and fertility in cattle grazing fescue have been disappointing. The reason for the decreased animal performance is due to consumption of a fungus-infected grass. Surveys indicate that much of the fescue in the United States is infected. Newer varieties of fescue are available without the fungus; however, these varieties are susceptible to drought conditions. Therefore, the fungal-infected varieties are utilized predominantly.

The fungus secretes several alkaloids that may be important in fescue toxicosis; the ergopeptine alkaloids are the most frequently incriminated. Approximately 22% of the nation's cattle are on fescue pastures. With a reduction of 25% in body weight of weaned calves due to the effects of the fungus, this translates into a loss exceeding $500 million annually. A further loss of $300 million annually is due to decreased calving rate.

Additional losses are caused by fescue foot, a gangrenous loss of the extremities. Generally, this occurs during the colder periods, and affects a minority of the herd. Nevertheless, affected animals are lost from the herd (Robbins et al., 1986).

A final condition attributable to fescue is fat necrosis. This condition may be a herd problem affecting mature animals. Masses of hard, necrotic fat in the abdomen obstruct the flow of ingesta and lead to difficulty in calving. Profitable research should identify agents that will combat the toxicosis and means of altering the potency of the fungus.

Lupinosis

Lupinosis is a hepatotoxic condition characterized by severe liver damage and icterus in animals ingesting *Lupinus* species infected with the fungus *Phomopsis leptostromiformis* (Edgar and Culvenor, 1985). The mycotoxin phomopsin A is the major metabolite of the fungus responsible for lupinosis, and phomopsin B is probably involved as well. The disease is most common in sheep in Australia, New Zealand, and South Africa, but apparently occurs in the United States (Marsh et al., 1916). While the disease occurs primarily in sheep grazing *Lupinus* species, the disease does occur in cattle, horses, and pigs, and has been produced experimentally in goats, rabbits, dogs, and mice (Marasas, 1974).

Rubratoxicosis

Rubratoxin B is a mycotoxin that produces hepatic degeneration, centrilobular necrosis, and hemorrhage of the liver and intestine. These conditions occur in experimental animals given rubratoxin, but the natural occurrence of both rubratoxin and the disease rubratoxicosis remains to be documented. Rubratoxin B is known to be produced by *P. rubrum* and *P. purpurogenum* on corn, and both organisms have been found in moldy grains, causing intoxication in domestic animals. Analytical methods appear to be insensitive for the detection of rubratoxin in grains, especially at low concentrations. Rubratoxin was suspected as the cause of an hepatotoxic, hemorrhagic disease of cattle and pigs fed moldy corn (Sippel et al., 1953). Rubratoxin apparently exerts a synergistic effect with aflatoxin in guinea pigs, and rubratoxin alone does affect complement activity in this species (Richard et al., 1974).

Cyclopiazonic Acid Toxicosis

The biological effects of cyclopiazonic acid (CPA) in rats, dogs, pigs, and chickens have been thoroughly described. Clinical signs of intoxication include anorexia, diarrhea, pyrexia, dehydration, weight loss, ataxia, immobility, and extensor spasm at the time of death (Dorner et al., 1983; Lomax et al., 1984; Nuehring et al., 1985; Purchase, 1971). Histologic examination of tissues from CPA exposed animals revealed alimentary tract hyperemia, hemorrhage, and focal ulceration, and there was widespread focal necrosis in most tissues, including liver, spleen, kidneys, pancreas, and myocardium (Cullen et al., 1988; Dorner et al., 1983; Lomax et al., 1984; Nuehring et al., 1985;

Purchase, 1971). Cullen and coworkers (1988) found skeletal muscle degeneration characterized by myofiber swelling or fragmentation in broiler chicks given CPA.

Cyclopiazonic acid has the ability to chelate metal cations due to the structure of the tetramic acid moiety (Gallagher et al., 1978; Holzapfel, 1968). Approximately 50% of a dose of CPA administered orally or intraperitoneally is distributed to skeletal muscle of rats and chickens within 3 hours (Norred et al., 1985, 1988). The chelation of such cations as calcium, magnesium, and iron may be an important mechanism of toxicity of CPA. Cyclopiazonic acid occurs naturally in corn, peanuts, cheese, and kodo millet; the latter was involved in a human intoxication (Richard et al., 1989).

Because CPA is produced by *A. flavus* and can be found with aflatoxins produced by the same isolate (Gallagher et al., 1978; Trucksess et al., 1987), and the toxicity of some feeds contaminated by *A. flavus* can be greater than expected from the levels of aflatoxins in those feeds, the possible role of CPA in diagnosed aflatoxicoses should be explored.

3. Mycotoxins and Human Health

Summary

Humans are exposed to mycotoxins by consuming foods contaminated with products of fungal growth. Such exposure is difficult to avoid because fungal growth in foods is not easy to prevent. Some of the mycotoxins have been shown to have potent acute and chronic biological activities as determined in animal studies and *in vitro* bioassays.

Consumption of foods heavily contaminated with mycotoxins has resulted in acute intoxication episodes in human populations. Conditions enhancing the likelihood of such occurrences include limited kinds of food and food supplies, environmental conditions that favor fungal development in crops and commodities, and the absence of effective mycotoxin monitoring and control systems.

Such occurrences are not likely to occur often in the United States because heavily contaminated and uncontrolled food is not permitted in the market. Concern still remains, however, for possible adverse effects resulting from long-term exposure to low levels of mycotoxins in the food supply.

The major concern has focused on the possible contribution of aflatoxins to the development of liver cell cancer (LCC). The assessment of possible adverse effects resulting from long-term low level exposure to aflatoxins is a difficult task due to methodological problems and uncertainties inherent in the currently available data and evaluation procedures. Some human population studies conducted in Africa and Asia have reported positive associations between estimated exposure to aflatoxins and incidence of LCC, although such correlations have not been demonstrated in the United States. Studies conducted in still other countries have yielded mixed results.

Additional information suggests that certain other alternate risk factors for human LCC (e.g., hepatitis B virus) may play an important role in determining the relative susceptibility of humans to the suspected effects of aflatoxins. This information has complicated the efforts to define the role of aflatoxins *per se* in human LCC risk. When considered in a "weight of evidence" approach for safety evaluation, however, it suggests that in the United States, aflatoxins may not be important as a risk factor for human LCC as might be inferred from the epidemiological studies conducted in Africa and Asia.

Biological monitoring techniques are under development to strengthen future human population studies by increasing the confidence in estimates of exposure to aflatoxins.

Other documented health problems associated with mycotoxins include ergotism caused by fungal alkaloids, alimentary toxic aleukia caused by *Fusarium* toxins, and human nephropathy caused by ochratoxin A. These problems, some historical and others geographically restricted, do not seem of practical significance in the United States. The possibility cannot be ruled out that exposure to these toxins may contribute to the incidence of human cancers. Therefore, our current regulatory system of mycotoxin monitoring and control must be maintained to assure that exposure to mycotoxins in the United States remains low.

Introduction

The historical record provides evidence that under some circumstances, toxic residues from mold growth in food can be deleterious to human health (Table 3.1). However, the recorded episodes are relatively rare, generally occurred under conditions of economic or environmental stress, and, in total, involved a limited number of mold species and implicated toxins. The primitive conditions that allowed these episodes to occur are not as likely to be found in the United States as elsewhere in the world. The recent spate of interest in mold toxins, generated by the observed distribution and hepatocarcinogenicity of the aflatoxins, has resulted in the isolation and characterization of over 100 compounds (Busby and Wogan, 1981). Relatively few compounds, half of which had been characterized prior to this period, have been identified as natural contaminants of food (Table 3.2). Incidence and toxicological data, from which to derive some type of hazard analysis, is not available for most of those identified. Those mycotoxins that have been found in foodstuffs consumed in the United States have little more in common than their mold origin. The challenge to human health from each one must be considered separately. This approach is also required by the differences in the incidence and toxicity data bases available for each toxin.

Table 3.1. Diseases of humans commonly recognized as having been caused by moldy food, the molds involved, and the mycotoxins that have been implicated

Disease	Molds involved	Toxins implicated	Reference
Aflatoxicosis	*Aspergillus flavus, A. parasiticus*	Aflatoxins	Stoloff, 1986
Ergotism	*Claviceps purpurea, C. paspali, C. fusiformis*	Ergot alkaloids	Barger, 1931
Alimentary toxic aleukia	*Fusarium sporotrichioides F. poae*	(T-2 toxin)[a]	Joffe, 1986
Urov or Kashin-Beck	*Fusarium* ssp.	—	Joffe, 1986
"Drunken bread" toxicosis (Scabby grain toxicosis)	*Fusarium* spp.	—	Marasas et al., 1984
Stachybotryotoxicosis	*Stachybotrys atra*	(Various macrocyclic trichothecenes)[a]	Rodricks & Eppley, 1974
Yellow rice (cardiac beri beri)	*Penicillium citreoviride, P. citrinum, P. islandicum*	(Luteoskyrin, islanditoxin, cyclochloritine, citrinin, citreoviridin)[a]	Uraguchi, 1978

[a] Produced by molds isolated from implicated commodities; no reported detection in implicated food.

Aflatoxins

Since the discovery in the 1960s of the potent carcinogenicity of aflatoxins in animals, especially aflatoxin B_1 (Wogan and Newberne, 1967), and the subsequent detection of the toxins in a wide variety of food commodities (Stoloff, 1976), there has been strong scientific and regulatory interest in mycotoxins.

The suspected health consequences range from acute hepatic toxicity to chronic disease such as liver cancer. Much effort has been made to investigate the association between aflatoxin exposure and long-term health effects in humans.

While suspected acute and chronic mycotoxin-related effects have been reported from several developing countries, similar effects have not been documented in humans in countries such as the United States where the food supply is well-regulated.

Acute Aflatoxicosis

Evidence of acute aflatoxicosis in humans has been reported from Taiwan and Uganda (Shank, 1977, 1981). The syndrome was characterized by vomiting, abdominal pain, pulmonary edema, and fatty infiltration and necrosis of the liver. More extensive documentation of an outbreak of putative aflatoxin poisoning was provided in 1974 from western India (Krishnamachari et al., 1975a, 1975b). Unseasonal rains and scarcity of food prompted the consumption of heavily molded corn [five specimens analyzed contained 6 to

Table 3.2. Mycotoxins that have been identified as natural contaminants of foodstuffs and the availability of data from which to derive some type of hazard analysis

Mycotoxin	Contaminated food	Translatable toxicology	Incidence data
Aflatoxins	Corn, peanuts, cottonseed, tree nuts	+	+
Altenuene	Tomato	–	–
Citrinin	Barley, oats	+	–
Cyclopiazonic acid	Peanuts	–	–
Deoxynivalenol	Corn, wheat, barley	+	+
Ergot alkaloids	Rye, wheat	+	–
Ochratoxin	Barley, oats, corn, sorghum, wheat, rice, green coffee	+	+
Patulin	Apples	+	+
Penicillic acid	Corn, dried beans	+	–
T-2 toxin	Barley, corn, sorghum	+	+
Trichothecin	Wine	–	–
Zearalenone	Corn, wheat	+	+

16 mg aflatoxin/kg (ppm) corn] by people in over 200 villages. Of the 994 patients examined, there were at least 97 fatalities, with death in most instances due to gastrointestinal hemorrhage. The illness was not infectious and occurred only in households where the contaminated corn was consumed. Histopathology of liver specimens revealed extensive bile duct proliferation, a lesion often noted in experimental animals after acute aflatoxin exposure. However, the possibility of the concurrent presence of other mycotoxins, such as cyclopiazonic acid, was not investigated and multifactorial etiologies cannot be ruled out. On a 10-year follow-up of the survivors, there was no evidence of increased incidence of residual liver lesions in these populations.

An incident of acute aflatoxicosis in Kenya (Hgindu et al., 1982) was also associated with consumption of maize highly contaminated with aflatoxins. There were 20 hospital admissions with a 20% mortality.

A disease of children in Thailand with symptoms identical to that of Reye's syndrome was conjecturally associated with aflatoxicosis (Shank, 1977; van Rensburg, 1977). The disease was characterized by vomiting, convulsions, coma, and death with cerebral edema and fatty involvement of the liver, kidney, and heart. Aflatoxin poisoning was suggested as a possible cause, because the symptoms of Reye's Syndrome in humans closely approximated signs observed with acute aflatoxicosis in monkeys. In one case, consumption of aflatoxin-contaminated rice in a Thai household was apparently associated with a Reye's syndrome-like fatality. Later, this rice sample was shown to be contaminated with other toxigenic *Aspergillus* species, one of which (*A. clavatus*) produced cytochalasin E and two tremorgens. Subsequently, Shank and colleagues (1971) demonstrated aflatoxin B_1 in the liver, brain, kidney, bile, and gastrointestinal tract contents in 22 out of 23 Thai fatalities. In seven of these cases, the tissue content of aflatoxin B_1 was substantially elevated, compared with the levels of aflatoxin B_1 detected in 10 out of 15 patients dying from other causes.

Clearly, under certain circumstances such as those likely to occur in developing countries, periodic episodes of acute mycotoxicoses can occur. Conditions increasing the likelihood of such occurrences include limited availability of food, environmental conditions that favor fungal development in crops and commodities, and lack of regulatory systems for mycotoxin monitoring and control.

Such episodes are unlikely to occur in this country, because there is a variety of foods with independence of primary staples, and uncontrolled and heavily contaminated food normally is not found in the market in the United States. Concern still exists, however, for possible adverse effects in humans resulting from chronic low-level exposure to mycotoxins.

Chronic Aflatoxicosis

Because aflatoxins, especially aflatoxin B_1, are potent carcinogens in some animals (Figures 3.1, 3.2, and 3.3), there is interest in the effects of long-term exposure to low levels of this mycotoxin.

Presently, only aflatoxin B_1 and sterigmatocystin are considered by the International Agency for Research on Cancer (IARC) as having produced sufficient evidence of carcinogenicity in experimental animals (IARC, 1986); aflatoxin B_1 has also been classified as a probable human carcinogen (IARC, 1987). Both mycotoxins probably have induced liver tumors in more than two species of experimental animals. Also, the National Toxicology Program recently reported "clear evidence" that ochratoxin A produced kidney cancer in rats (National Toxicology Program, 1989). Aflatoxins are the only mycotoxin for which a sizeable body of human epidemiological data has been developed; therefore, assessment of the carcinogenic potential of dietary mycotoxins in humans focuses on aflatoxins.

While aflatoxins have been implicated as a possible cause of liver cell cancer (LCC) in humans, the assessment of health effects of mycotoxins in humans is not an easy task. These effects must be determined by extrapolation from animal data or interpretation of epidemiological data. In either case, the absence of adequate methodology contributes to the uncertainty of conclusions. Indeed, conflicting results have been reported for studies of the effects of aflatoxin ingestion in humans.

The interpretation of the observations reported by various investigators is complicated by their use of differing study methodologies, as well as difficulty in accurately measuring the intensity and duration of

Figure 3.1. Liver tumor from rat given dietary aflatoxins. Photograph courtesy of W. Norred, USDA, ARS, Russell Research Center, Athens, Georgia.

Figure 3.2. Normal liver of trout given control diet. Photograph courtesy of R. Sinnhuber, Oregon State University, Corvallis.

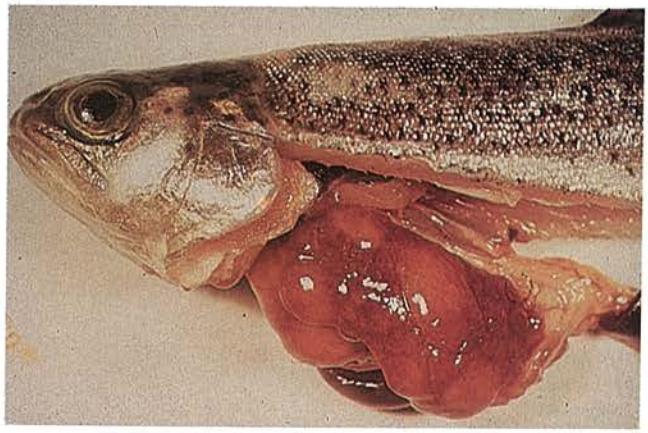


Figure 3.3 Liver of trout with tumors. Trout was given diet containing 20 ppb aflatoxins for 12 months. Photograph courtesy of R. Sinnhuber, Oregon State University, Corvallis.

aflatoxin exposure. Additionally, the expression of suspected aflatoxin-related disease in humans may be influenced by factors such as age, sex, nutritional status, and/or concurrent exposure to other causative agents such as viral hepatitis or parasitic infestation. Presently, it is not possible to precisely characterize the impact of these modulating factors on the development of diseases suspected of being related to aflatoxin exposure.

Relative susceptibility of humans to the chronic adverse effects of aflatoxins may be an additional important consideration. In the animal species thus far tested, susceptibility to the cancer-causing effects of aflatoxins ranges from extremely susceptible to extremely resistant (Wogan, 1973). Monkeys, for example, are considerably more resistant to the cancer-causing effects of aflatoxins than rats (Seiber et al., 1979). Although the relative susceptibility of humans is not known, metabolic and biochemical evidence indicates that humans, like monkeys, are relatively less sensitive to the chronic effects of aflatoxins (Buchi et al., 1974; Hsieh et al., 1977a, 1977b). However, caution must be exercised in extrapolating in vitro observations to an in vivo response.

Liver cell cancer is one of the leading causes of cancer mortality in Asia and Africa. For example, in the People's Republic of China, LCC accounts for approximately 100,000 deaths per year and is the third leading cause of cancer mortality (Armstrong, 1980). In parts of West Africa, annual LCC mortality can be up to 100 cases/100,000/year. Liver cell cancer rates vary worldwide by at least 500- to 1,000-fold (Okuda and Beasley, 1982). Those regions with the highest LCC rate correspond to areas where the climate favors contamination of foodstuffs by aflatoxins.

There have been a number of epidemiological studies designed to obtain information on the relationship of current estimated dietary intake of aflatoxins to the incidence of LCC. Unfortunately, for studies initiated before 1980, the importance of hepatitis B virus (HBV) infection as a confounding factor had not yet been established. Information is available from Uganda (Alpert et al., 1968, 1971), the Philippines (Campbell and Salamut, 1970), Swaziland (Peers and Linsell, 1973), Kenya (Peers and Linsell, 1973), Thailand (Shank et al., 1972a, 1972b), and Mozambique (van Rensburg, 1985). The latter country had the highest incidence of LCC in the world at the time of these studies, as well as an estimated daily per capita ingestion of aflatoxins several times greater than the next highest intake regions of Thailand and Swaziland (Szmuness, 1978) (Table 3.3). Ingestion of aflatoxins (mean of population) varied over a range of values from 3 to 222 ng/kg body weight/day. Estimated LCC incidence values extended from a minimum of 2 to a maximum of 35 cases/100,000 population/year. There was a positive association between high intakes of aflatoxins and high incidence rates. The association was most apparent in connection with incidence rates for adult men (Carlborg, 1979). The incidence of LCC in these studies was a linear function of the log of dietary intake of aflatoxins (Shank, 1977; Linsell and Peers, 1977).

This information provided a strong motivation to further investigate the circumstantial relationship between ingestion of aflatoxins and LCC incidence. Several of these recent epidemiologic studies on the

Table 3.3 Ingestion of aflatoxins and liver cancer incidence in humans (Szmuness, 1978 by permission from S. Karger A G, Basel)

Population	Dietary aflatoxins intake (mg/kg of body weight/d)	Cases of liver cancer in adults (>15 years old)			
		Men		Women	
		No./100,000 population/ year	Incidence	No./100,000 population/ year	Incidence
Kenya					
High altitude	3 - 5	1	3.1	0	0
Medium altitude	6 - 8	13	10.8	6	3.3
Low altitude	10 - 15	16	12.9	9	5.4
Swaziland					
Highveld	5 - 9	9	7.0	2	1.4
Middleveld	9 - 14	24	14.8	5	2.2
Lebombo	15 - 20	4	18.7	0	0
Lowveld	43 - 53	35	26.7	7	5.6
Thailand[a]					
Songkhala	5 - 8	—	—	—	—
Ratburi	45 - 77	—	—	—	—
Mozambique[b]	222	—	35.0	—	15.7

Note: Data were compiled for 1 year in Thailand, 3 years in Mozambique, and 4 years in Kenya and Swaziland.

[a] Statistics for the total population were cases/100,000 population/year with an incidence of 6.0. In Ratburi, Thailand, statistics for the total population were 2 cases/100,000 population/year with an incidence of 2.0.

[b] Incidence for the total population was 25.4 cases/100,000 population/year.

association of aflatoxin exposure and LCC take HBV infection into account. Bulatao-Jayme and coworkers (1982) compared the dietary intakes of confirmed primary liver cancer cases with age-sex matched controls. Using dietary recall, the frequency and amounts of food items consumed were calculated into units of aflatoxin exposure per day using a standardized Philippine table of aflatoxin values. Eighty percent of the subjects' total aflatoxin exposure was due to cassava, corn, peanuts, and sweet potato. The mean aflatoxin exposure per day for the LCC cases was 440% of the controls. Upon grouping dietary aflatoxin exposure (and alcohol intakes) into heavy and light, the relative risk of developing LCC was found to be statistically significant in the following high-to-low order of rank: cassava, peanuts, sweet potato, corn, and alcohol. Combining aflatoxin load and alcohol intake gave a synergistic and statistically significant effect. However, this study was not controlled for HBV infection. These researchers concluded that a direct effect of alcohol and aflatoxin consumption existed, especially among heavy drinkers. High exposure to aflatoxins in the diet increases risk, with a synergistic effect from increased alcohol consumption.

Concurrent with the studies of LCC incidence in Mozambique, 2,183 samples of prepared food were randomly collected from six districts of Inhambane, Mozambique, as well as from Manhica-Magude, Mozambique, a region of lower LCC incidence to the south. A further 623 samples were taken during 1976 and 1977 in Transkei, much further south, where an even lower incidence of liver cancer had been recorded. The mean aflatoxin dietary intake values calculated from these samples were significantly related to liver cancer rates.

Peers and colleagues (1987) reported on a study in Swaziland to assess the relationship between aflatoxin exposure, HBV, and the incidence of LCC. Levels of aflatoxin intake were evaluated in dietary samples from households across the country, and from crop samples taken from representative farms. Prevalence of HBV was estimated from the serum of blood donors. The LCC incidence was recorded for the years 1979 to 1983 through a national cancer registration system. Among 4 geographic regions, the estimated daily intake of aflatoxins ranged from 3.1 to 17.5 micrograms. The proportion of HBV-exposed individuals was 86% in men, with little geographic variation. The prevalence of carriers of the HBV surface antigen averaged 23% in men. The LCC incidence was strongly associated with estimated levels of aflatoxins. In 10 small subregions, aflatoxin exposure was reported to be a more important determinant of liver cancer incidence than the prevalence of hepatitis.

In a paper just published by Yeh et al. (1989), the role of aflatoxin exposure in the diet, hepatitis B virus

infection, and LCC in Guangxi Province, China, was examined. The conclusion from this cohort study was that no positive association between hepatitis B virus antigen (HBsAg) positivity and corresponding LCC mortality could be found. However, a positive and linear correspondence for aflatoxin exposure and LCC was determined. These data support the contention that in regions where aflatoxin contamination is extremely high, risk of developing liver cancer is also very high.

Although a positive correlation between aflatoxin exposure and LCC incidence has thus been reported in African and Asian populations, there is no evidence that aflatoxins contribute similarly to LCC incidence in the United States. Unlike Africa and Asia, the incidence of LCC in the United States is relatively low. In the United States, a geographical association between LCC and dietary aflatoxin intake has not been found. In a retrospective study, rural white males from the southeastern, northern, and western regions were selected for the comparison of lifetime risk of death from LCC and estimated past dietary exposure to aflatoxins (Stoloff, 1983). For an approximately 1,000-fold interregional difference in aflatoxin intake, there was only a 6 to 10% increase in LCC death risk in the expected direction of comparison.

Other studies in areas outside the United States have yielded mixed results. A dose-response association was not evident in a case-control study conducted in Hong Kong (Lam et al., 1982). In regions in Mozambique where heavy aflatoxin contamination of foodstuffs occurred, only a subset of the population had a high risk of LCC (van Rensburg, 1977; van Rensburg et al., 1985). In Taiwan, illness and mortality have been associated with acute levels of aflatoxin intake (Tung and Ling, 1968). Yet in a large prospective study of LCC conducted by Beasley and colleagues (1981), there was no evidence of aflatoxin involvement. Also, Eskimos from Alaskan villages had a high LCC incidence (Lanier et al., 1976). Presumably, the aflatoxin intake of this population is very low, due to the normal diet of animal products and the cold environment, which is unfavorable for the growth of aflatoxin-producing fungi. In both of these last two populations, there was a very strong correlation between LCC and chronic HBV infection. Orientals resident in the United States, Chinese males in particular, constitute another population with a very high incidence of LCC and low likelihood of exposure to aflatoxins (Stoloff, 1983).

As indicated previously, establishing a causal relationship between aflatoxin exposure and human disease is a difficult task because of some uncertainties associated with human epidemiological studies. The data base, methodology, and interpretation of the studies that provide positive associations between aflatoxin exposure and LCC have been criticized (Linsell, 1982; Wagstaff, 1985; Stoloff, 1986).

One of the major sources of uncertainty associated with these studies is accurate assessment of human aflatoxin exposure. Development of techniques for more accurate assessment of actual exposure to aflatoxins could significantly increase the level of confidence derived from studies assessing the correlation between aflatoxin exposure and disease incidence.

Monitoring Techniques for Assessing Human Exposure to Aflatoxins

In the last few years, new technologies have been developed to more accurately monitor individual exposures to aflatoxins. Particular attention has been paid to the analysis of aflatoxin DNA adducts (Figure 3.4) and albumin adducts as surrogates for genotoxicity in people. Autrup and colleagues (1983) have pioneered the use of synchronous fluorescence spectroscopy for the measurement of aflatoxin DNA adducts in urine. Preliminary data on urine samples collected in Murang's district, Kenya, showed that they contain 2,3-dihydro-2-(N^7-guanyl)-3-hydroxyaflatoxin B_1 (AFB-

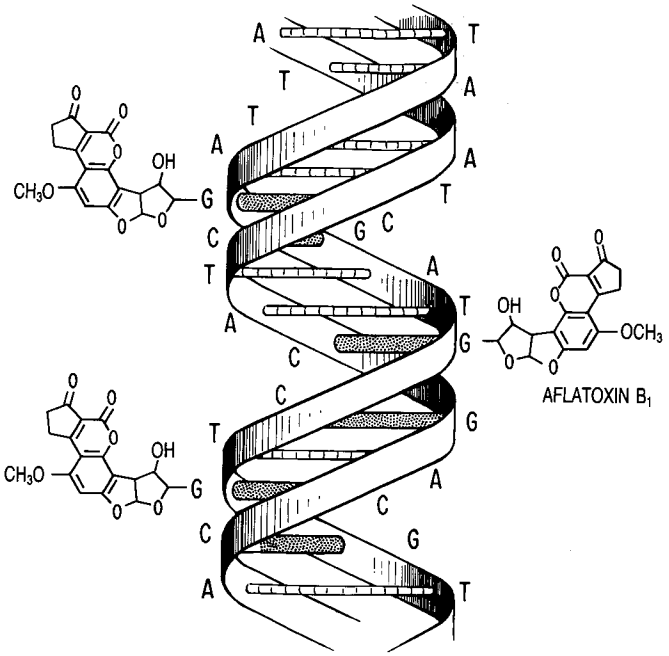


Figure 3.4. Aflatoxin B_1 binds to DNA at the guanine base in liver cells, corrupting the gentic code that regulates cell growth. Out-of-control cells grow into tumors that eventually become cancerous. Photograph courtesy of D.P.H. Hsieh, University of California, Davis. Drawing by G. Hedberg, USDA, ARS, National Animal Disease Center, Ames, Iowa.

Gual). Using high performance liquid chromatographic methods, six of 81 samples had a detectable level of a compound whose fluorescence spectrum was identical to chemically synthesized AFB-Gual. This work was followed by a more extensive study conducted from 1981 to 1984 and published by Autrup and coworkers (1987). A thousand urine samples from all over Kenya were analyzed. Of all tested individuals, 12.6% were positive for aflatoxins based on urinary excretion of AFB-Gual. The incidence of nationwide hepatitis infection was 10.6%, revealing a wide regional variation. A multiplicative and additive regression analysis to investigate the synergistic effect of hepatitis and aflatoxin exposure on the induction of LCC was negative. However, a moderate correlation between aflatoxin exposure and LCC was observed when the study was limited to certain ethnic groups.

Wild and coworkers (1986) used highly sensitive immunoassays to quantitate aflatoxins in human body fluids. An enzyme-linked immunosorbent assay (ELISA) was used to quantitate aflatoxin B_1 over the range 0.01 ng/ml to 10 ng/ml, and was validated in human urine samples. Urine samples from the Philippines were analyzed and found to contain a mean of 0.875 ng/ml aflatoxin B_1 equivalents (range = 0 to 4.25 ng/ml). This compared with a mean of 0.666 ng/ml aflatoxin B_1 equivalents in samples from France.

Zhu and associates (1987) analyzed a total of 252 urine samples from 32 households in Fushui county of the Guangxi autonomous region of the People's Republic of China. A good correlation between total dietary aflatoxin B_1 intake and total aflatoxin M_1 excretion in human urine was observed during a 3-day study. Between 1.23 and 2.18% of dietary aflatoxin B_1 was found to be present as aflatoxin M_1 in human urine. A good correlation was also observed between the aflatoxin B_1 concentration in dietary corn and the aflatoxin M_1 concentration in human urine. This is the same county where Yeh and other researchers (1989) reported the annual mortality rate of LCC being 15.4/100,000. They showed marked variation in different counties, ranging from 5/100,000 to 55/100,000. The incidence of LCC was found to be correlated with the severity and extent of aflatoxin B_1 contamination of foodstuffs. Hepatitis infection rates tended to be somewhat higher in the high-incidence area.

Finally, Groopman and colleagues (1985, 1987), using a monoclonal antibody affinity column and high pressure liquid chromatography procedures showed that by comparing aflatoxin intake with an aflatoxin metabolite in urine from both males and females in Guangxi Province, aflatoxin DNA adduct excretion into urine was positively correlated with dietary intake, and that the major aflatoxin B_1-DNA adduct excreted in urine is an appropriate dosimeter for monitoring aflatoxin dietary exposure.

The need for noninvasive screening methods to assess human exposure to dietary aflatoxins has prompted investigations into the mechanisms of aflatoxins binding to proteins, particularly albumin. A preliminary study (Gan et al., 1988), based on the work of Sabbioni and coworkers (1987), was undertaken to determine if serum albumin from persons environmentally exposed to aflatoxin B_1 contained measurable levels of aflatoxin adducts.

People living in Fusuai County, in Guangxi Province, were identified because of their high dietary exposure to aflatoxins through corn consumption. The albumin adduct levels in 42 subjects are illustrated in Figure 3.5 (Groopman, 1985, 1987).

These findings confirm that aflatoxin B_1 was metabolically activated *in vivo* in humans to a form that bound to DNA and to serum albumin. The amounts of adduct measured agreed very closely with the values predicted by an animal model and, thus, apparently the adducts formed with serum albumin, as well as DNA adducts in urine, are useful for determining recent exposure to aflatoxin B_1.

In conclusion, several human population studies have reported positive correlations between estimated aflatoxin consumption and LCC risk, while others have reported no such association. There still remains a great deal of uncertainty as to the role (extent and under what circumstances) aflatoxins play in the etiology of LCC. This uncertainty arises because of inherent methodological problems associated with human epidemiological studies, lack of understanding of the significance of alternate risk factors for human

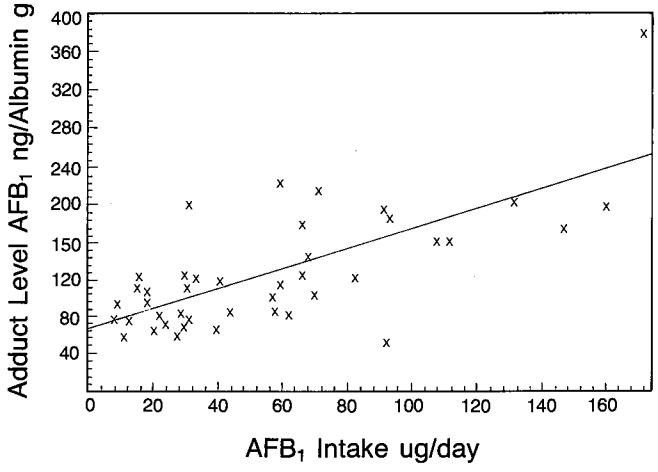


Figure 3.5. Adduct levels of aflatoxin B_1 detected in the albumin in serum of 42 humans in China as correlated ($p < 0.000001$) with dietary intake of the toxin (Gan et al., 1988).

LCC, and inability to quantitatively extrapolate effects from high level exposure to low level exposure scenarios both within and across species.

In the United States where past exposure to aflatoxins was relatively low and alternate LCC risk factors are not prevalent, LCC incidence is low and there is no apparent positive association between aflatoxin exposure and LCC risk.

Our current system of aflatoxin monitoring and control must be maintained to assure that exposure to aflatoxins remains low. A detailed discussion of the monitoring and control system in the United States is presented in Chapter 6.

From the uncertainties associated with the involvement of aflatoxins in the etiology of human liver cancer and other diseases, it is evident that the significance of other mycotoxins as the cause of human diseases is not easy to determine. Ideally, it requires the following four criteria to be satisfactorily met before a mycotoxin can be identified as an etiological factor of a human disease (Hsieh, 1989):

1. The mycotoxin is occurring in food commodities under field conditions and human exposure to it can be established.
2. The mycotoxin produces, in experimental animals, the symptoms and effects characteristic of the human disease in question.
3. Epidemiological studies indicate a dose-response association in exposed populations.
4. Mode of action studies demonstrate similarities between humans and animal models in the biochemical lesions produced by the mycotoxin.

It should be reemphasized that these criteria are ideal and that, in reality, they are difficult to achieve. Even when applying similar criteria to an animal mycotoxicoses, there is difficulty in accomplishing them. To date, the available scientific data are not sufficient for even the most extensively studied aflatoxin B_1 to meet all of the four criteria for a particular disease. However, based on accumulated information, some mycotoxicoses have been generally regarded as important human diseases in certain populations. These are Balkan endemic nephropathy caused by ochratoxin A, alimentary toxic aleukia caused by trichothecene mycotoxins, and ergotism caused by some fungal alkaloids. In addition, the contribution of mycotoxins to various forms of human cancer continues to be of concern because of practically unavoidable human exposure to low levels of mycotoxins and their diversified genetic and cellular toxicities.

Ochratoxin

Ochratoxin is the subject of two recent reviews (Marquardt et al., 1989; Pohland et al., 1990), in which information on its occurrence and toxicology were presented. Although "ochratoxin" refers to a family of related compounds, only ochratoxin A commonly occurs naturally and is of toxicological significance, and will be the compound meant whenever the term "ochratoxin" is used.

Ochratoxin is a nephrotoxin and teratogen in all species in which it was tested, and there is evidence that ochratoxin impairs some elements of the immune system (Kuiper-Goodman and Scott, 1989). In mice and rats, ochratoxin produced tumors of the urinary system but there is an open question whether the tumors were the result of a direct oncogenic effect or the sequelae of the observed nephritis. There has been speculation that a nephritis, and associated urinary system tumors, endemic in the Balkans may be related to the incidence and level of ochratoxin in the local foodstuffs. The evidence to date is suggestive, but a solid epidemiological study has not yet been conducted in the area. Although the surveys of U.S. foodstuffs susceptible to ochratoxin contamination indicate human exposure to this toxin in the U.S. is not likely to be of toxicological significance, the epidemiological evidence from the Balkans warrants a limited repeat of the survey effort, directed to regions and crops susceptible to ochratoxin contamination that are similar to those in northern Europe and the Balkans.

Trichothecenes

Combinations of some of the trichothecenes are thought to be involved in the human disease known as alimentary toxic aleukia, which affected people in parts of the Soviet Union during World War II. This disease was characterized by severe hemorrhage, leukopenia, necrotic angina, and exhaustion of bone marrow. These symptoms have been seen in victims of "yellow rain", an alleged chemical warfare episode in Southeast Asia, in which the trichothecene mycotoxins are implicated (Watson, 1984).

The most prevalent trichothecenes found in commodities in the northern United States are deoxynivalenol and nivalenol. They are implicated in a veterinary problem known as moldy corn toxicosis of swine, the symptoms of which include a refusal to eat, digestive disorders, and diarrhea; ultimately leading to death. Their significance in human health effects is yet to be demonstrated.

Ergot Alkaloids

An historical perspective of human mycotoxicoses would probably begin with descriptions of epidemics of ergotism in Europe that occurred from the 9th to the 18th century (Lewis, 1977). Typical gangrenous ergotism was described in an Ethiopian epidemic in 1978 after human consumption of grains contaminated with *Claviceps purpurea* infected wild oats (Demeke et al., 1979; King, 1979; Pokrovsky and Tutelyan, 1982). While approximately 50% of the affected individuals died, others revealed dry gangrene of the limbs, swollen limbs, skin desquamation, and loss of limbs. Common symptoms were weakness, burning sensations, nausea, vomition, and diarrhea.

Recurring episodes in India have been reported following human ingestion of pearl millet infected with *C. fusiformis* (Krishnamachari and Bhat, 1976). In these cases, there were prominent symptoms of nausea, vomition, and giddiness attributed primarily to the clavine group of alkaloids produced by the organism.

The cases of ergotism produced by *C. purpurea* included more severe effects attributable primarily to the ergotamine group of alkaloids, while the clavine alkaloids appeared to produce gastrointestinal symptoms without deaths or loss of limbs as a sequelae to gangrenous necrosis. Apparently, the cleaning and milling processes remove the ergot sclerotia from grain and food preparation processes, such as baking destroys most alkaloids of the ergotamine group. Also, wheat, which is less susceptible to ergot infestation than rye, has become more of a staple that the latter. Therefore, human exposure, although widespread, is to very low concentrations of these toxins and perhaps would be significant only where there is locally grown and consumed grain. Even then, ergot contamination is not a consistent event.

Zearalenone

A thorough review of the hazard from zearalenone and the risk from zearalenone levels found in Canadian foods has been developed by the Health and Welfare Canada group (Kuiper-Goodman et al., 1987). Because the exposure information they present is applicable to the U.S. population, their conclusions are also pertinent to that population. Zearalenone and its related compounds, such as zearalenol, are weak estrogenic and anabolic agents when compared to diethylstilbestrol. Zearalenone elicited no acute toxic response in the mouse, rat, guinea pig, or hen at the highest oral doses employed (5 to 20 mg/kg body weight). Carcinogenicity studies in rats and mice produced lesions in the highest dose groups (rats, 3 mg/kg body weight; mice 19 mg/kg body weight) that were typically estrogen related. Zearalenone is found in U.S. and Canadian foods, most commonly in corn-based products such as breakfast cereals and cornmeal. Zearalenone and zearalenol, theoretically, could be a residue in beef from cattle consuming zearalenone-contaminated corn. A related substance, zearalanol (Ralgro^{R}), used as an anabolic agent in cattle, may also be present. However, no actual surveys have been done to determine the presence of zearalenone, zearalanol, or their metabolites and conjugates in beef tissues and other animal food products. On the basis of very conservative safety factors and estimates of no effect levels, the Canadian group concluded no regulatory action currently is warranted for zearalenone.

4. Occurrence of Mycotoxins in Foods and Feeds

Summary

Many foods and feeds can be contaminated with mycotoxins before harvest, during the time between harvesting and drying, and in storage. A few mycotoxins, such as those associated with ergotism, are produced exclusively in the field. Many other mycotoxins can contaminate crops before harvest and, under certain circumstances, progress from that point. Aflatoxins can be found in the field before harvest, and contamination can increase during postharvest activities, such as crop drying, or in storage. However, the aflatoxins can contaminate stored products in the absence of field contamination. Many other fungi that produce mycotoxins contaminate crops in much the same way.

With the exception of the aflatoxins, the frequency of contamination of feeds and foods by other mycotoxins is not known. Aflatoxins are detected occasionally in milk, cheese, corn, peanuts, cottonseed, brazil nuts, copra, almonds, pecans, figs, spices, and a variety of other foods and feeds. Corn, peanuts, and cottonseed are the most frequently analyzed domestic crops, while copra, pistachio nuts, brazil nuts, figs, and spices are of concern in imported products. Milk, eggs, and meat products are sometimes contaminated because the animal has consumed mycotoxin-contaminated feed.

Besides the aflatoxins, several other mycotoxins may contaminate foods and feeds. The ochratoxins and the *Fusarium* mycotoxins have been given the greatest attention. These mycotoxins tend to occur in the more temperate regions of the United States. Zearalenone and deoxynivalenol are more of an economic concern to animal producers in the United States than ochratoxin. Many other mycotoxins produced by various *Aspergillus, Penicillium, Fusarium,* and *Alternaria* species can contaminate products, and the incidence and relative importance to animal and human health of these many different mycotoxins has not been established.

Because mycotoxin contamination of foods occurs in a random manner, it is impractical to think that a truly mycotoxin-free food supply can be guaranteed. The ability to identify and remove all naturally-occurring mycotoxin contamination or even all of a specific mycotoxin from foods and feeds is limited. Thus, it is important to establish realistic goals for mycotoxin management.

Introduction

The published mycotoxin data is replete with reports of the natural occurrence of various mycotoxins in foods and feeds. The intent of this section is not to summarize this information, but rather to put into perspective the reports of natural occurrence and their implications.

After considering the literature on mycotoxin occurrence, several conclusions can be made:

1. Numerous reports of the fungal flora on foods and feeds document the frequent presence of potentially toxigenic fungi (Table 1.2). These reports define the conditions conducive to or restrictive of fungal growth and mycotoxin formation.
2. The natural occurrence of most mycotoxins has not been adequately documented (Table 4.1). Therefore, with the exception of aflatoxins, the frequency and level of contamination of foods and feeds is not known.
3. Human exposure to mycotoxins is less in the developed countries where food resources are plentiful, food handling and preservation technology is well developed, and regulation of food quality restricts exposure to mycotoxins.
4. The major problems with mycotoxins in developed countries are associated with animal health, because animal feeds are largely unregulated and animal mycotoxicoses frequently occur. The veterinary literature has been a rich source of information on possible mycotoxin problems.

Mycotoxins can be categorized as naturally occurring in: (1) raw agricultural products, (2) processed foods, and (3) imported products.

Natural Occurrence in Raw Agricultural Products

Mycotoxins can contaminate raw agricultural products before harvest and/or after harvest. Some mycotoxins, such as the aflatoxins, can occur in the field as well as increase after harvest, if conditions are conducive to mold growth. Other mycotoxins, such as ergot toxins, are produced only prior to harvest. Many mycotoxins may be produced in stored products if conditions are favorable.

Table 4.1 Selected examples of natural occurrence of mycotoxins in processed foods

Mycotoxin	Food	Country	Average levels of contaminated samples (ug/kg)	Incidence	Reference
Aflatoxins	Peanut butter	U.S.	14	17/104	Wood, 1989
Aflatoxins	Peanut butter	U.K.	—	—	Jelinek, 1987
Aflatoxins	Peanut butter	Philippines	213	145/149	Diener, 1981
Aflatoxins	Peanut candies	Philippines	38	47/60	Diener, 1981
Aflatoxins	Peanut candies	U.S. (Imported)	10	10/18	Wood, 1989
Aflatoxins	Roasted, shelled peanuts	U.S.	68	6/55	Wood, 1989
Aflatoxins	Corn	U.S.	30	49/105	Jelinek, 1987
Aflatoxins	Corn	U.S.	20	12/28	Jelinek, 1987
Aflatoxins	Corn	Philippines	110	95/98	Diener, 1981
Aflatoxins	Corn products	Philippines	32	22/32	Diener, 1981
Aflatoxins	Spaghetti	Canada	13	1	van Walbeek et al., 1968
Aflatoxins	Wheat flour	France	0.25-150	20/100	Lafont and Lafont, 1970
Aflatoxins	Milk	Germany	—	79/419	Kiermeier et al., 1977
Aflatoxins	Non-fat dry milk	Germany	2.0	—	Polzhofer, 1977
Aflatoxins	Cheddar cheese	U.S., Germany	—	—	Bullerman, 1981
Deoxynivalenol	Corn meal	Canada	110	35	Scott, 1984
Deoxynivalenol	Corn flour	Canada	180	27	Scott, 1984
Deoxynivalenol	Popcorn	Japan (Imports U.S.)	84,000	10/14	Tanaka et al., 1985
Deoxynivalenol	Wheat flour	Japan	38,000	26/36	Tanaka et al., 1985
Deoxynivalenol	Wheat flour	Canada	400	43	Scott, 1984
Deoxynivalenol	Wheat bran	Canada	170	14	Scott, 1984
Deoxynivalenol	Cookies	Canada	120	35	Scott, 1984
Deoxynivalenol	Bread	Canada	80	21	Scott, 1984
Deoxynivalenol	Crackers	Canada	270	20	Scott, 1984
Deoxynivalenol	Wheat breakfast cereals	Canada	86	36	Scott, 1984
Deoxynivalenol	Baby cereal	Canada	43	30	Scott, 1984
Ochratoxin A	Pork kidney	Denmark	>25	9.8%	Leistner, 1984
Ochratoxin A	Pork kidney	West Germany	< 3	18.1%	Leistner, 1984
Ochratoxin A	Pork blood	West Germany	< 3	15.3%	Leistner, 1984
Patulin	Apple juice	Canada	1000	—	Scott et al., 1972
Patulin	Pears, stone fruits	U.S.	—	—	Buchanan et al., 1974
Penitrem A	Cream cheese	U.S.	—	1 sample	Richard and Arp, 1979
Sterigmatocystin	Gouda cheese	Holland	5-600	9/39	Northolt, et al., 1980

Ergotism is the oldest known mycotoxicosis of humans and animals. The ergot mycotoxins are present in the sclerotia (ergot) of the fungus, which replaces the grain seed. The mycotoxins associated with ergot are not often quantitated; therefore, little information is available on alkaloid concentration in ergot-contaminated products. Van Rensburg (1977) stated that as little as 0.2% (by weight) ergot in grain could cause mild symptoms or signs of ergotism in humans, and death from gangrene could follow consumption of about 100 g of ergot over a few days. Canadian scientists have suggested a maximum level of 0.05% of ergot particles by count in flour may be acceptable (Peace and Harwig, 1982).

Aflatoxins have been found to contaminate many crops, sometimes at very high concentrations. The commodities with a high risk of aflatoxin contamination include corn, peanuts (Figure 4.1), cottonseed, brazil nuts, pistachio nuts, and copra (Jelinek, 1987).

Figure 4.1 Growth of *Aspergillus flavus* (yellow-green fungus) from two of five surface sterilized peanuts placed on a nutrient culture medium. Photograph courtesy of R. J. Cole, USDA, ARS, National Peanut Research Laboratory, Dawson, Georgia.

Commodities with a lower risk of aflatoxin contamination include figs, almonds, pecans, walnuts, raisins, and spices. Soybeans, beans, pulses, cassava, grain sorghum, millet, wheat, oats, barley, and rice are resistant or only moderately susceptible to aflatoxin contamination in the field. However, aflatoxins can occur when any of these commodities are stored under high moisture and temperature conditions. Insect or rodent infestations can create microclimates that facilitate mold invasion of some stored commodities.

In the United States, raw, shelled peanuts are routinely tested for aflatoxin content. In >95% of peanut lots, the mean aflatoxin content is much less than the 20 ppb FDA guideline (National Peanut Council, 1988). The frequency distribution of aflatoxins in U.S. peanuts and North Carolina corn is presented in Figures 4.2 and 4.3 (Stoloff, 1986).

In the United States, the FDA (through its Compliance Program) randomly analyzes raw agricultural products for selected mycotoxins. These studies indicated the presence of mycotoxins other than the aflatoxins. For example, in 1983, 27 samples of corn and sorghum were analyzed, and 17 (63%) were positive for zearalenone at levels of 12 to 1,373 ppb. In 1984, 4 of 53 (7.5%) samples of corn were found to contain 10 to 25 ppb of ochratoxin A.

Besides aflatoxins, the most often reported group of mycotoxins to occur in raw agricultural commodities are those produced by various *Fusarium* species. In the United States and Canada, contamination of grains by deoxynivalenol and zearalenone is frequently encountered. In Japan, high concentrations of both deoxynivalenol and nivalenol have been found in corn, wheat, and barley; zearalenone sometimes occurs as a co-contaminant (Jelinek, 1987). Deoxynivalenol may be found in corn at levels >1,000 ppb. Deoxynivalenol is frequently found in U. S. and Canadian wheat, especially associated with cool, wet growing and harvest seasons that favor the formation of scab, the result of grain invasion by *F. graminearum* (Figure 4.4). Lower amounts of deoxynivalenol have been found in barley, rice, and rye. The T-2 mycotoxin and diacetoxyscirpenol have been reported to occur in raw products less frequently, which may be related to inadequate analytical methodology. Data on diacetoxyscirpenol is usually only qualitative.

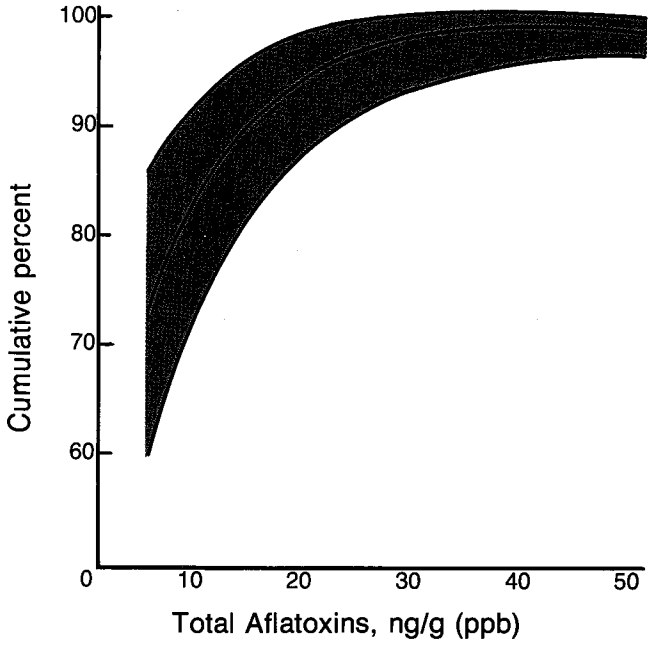


Figure 4.2. Frequency distribution (cumulative % less than indicated level) of total aflatoxins levels in raw shelled peanuts produced in the United States. Data are from the crop year reports of the Peanut Administrative Committee. Center plot is the average for the 12 crop years 1973-1984; boundary plots are for the year (1981) with the least aflatoxin contamination and the year (1980) with the most aflatoxin contamination (Stoloff, 1986).

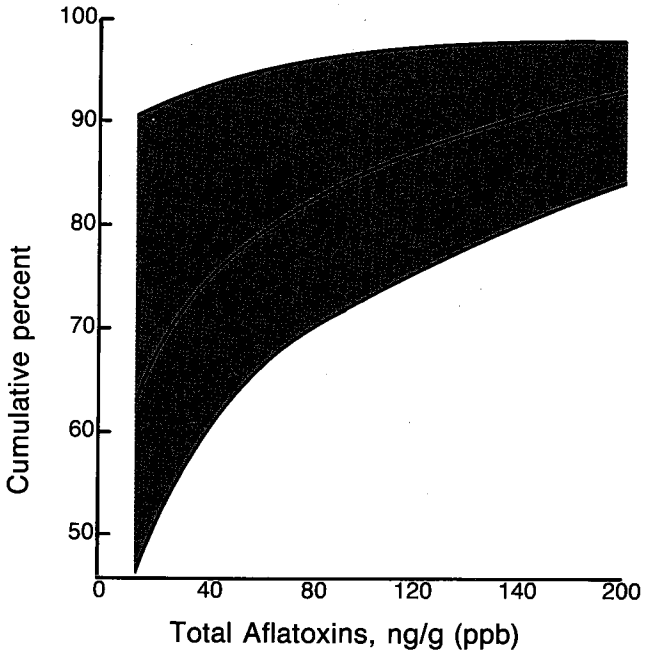


Figure 4.3. Frequency distribution (cumulative % less than indicated level) of total aflatoxins levels in shelled maize produced in the state of North Carolina. Data are from 8,653 farmer-submitted and elevator-survey samples assayed by the state analysts for the 6 crop years 1977-1978, 1980-1983. Center plot is the average of the 6 years; boundary plots are for the year (1982) with the least aflatoxin contamination and the year (1977) with the most aflatoxin contamination (Stoloff, 1986).

Figure 4.4 Pink scab of wheat resulting from invasion of grain by *Fusarium graminearum*. Deoxynivalenol may be present in such infected grain. Photograph courtesy of B. Doupnik, University of Nebraska, Lincoln.

Zearalenone is produced by several *Fusarium* species and is a frequent contaminant of corn, wheat, barley, and grain sorghum in the United States. Generally, zearalenone concentrations are well below 1 ppm (the level in feed that can cause oestrus in swine) in processed cereal foods, but higher amounts can be encountered in some feeds (Morehouse, 1985). Other toxic metabolites produced by *Fusarium* species that have been detected in corn or wheat include moniliformin and butenolide (Jelinek, 1987). The toxic metabolites of *F. moniliforme* are not well defined, nor have analytical methods for them been developed. Because of this, the incidence of mycotoxins derived from this fungus is poorly documented, although *F. moniliforme* is the most common *Fusarium* species found on corn. Newly discovered mycotoxins from *F. moniliforme* were characterized in 1988 by South African investigators and given the trivial name of fumonisins. The fumonisins have been implicated in equine leukoencephalomalacia and have been shown to be precancerous in rats (Gelderblom et al., 1988). Many *Fusarium* toxins have been found in commodities susceptible to contamination with aflatoxins, ochratoxins, or other mycotoxins, but significant co-occurrence of mycotoxins from different mold species has not been found.

Preharvest aflatoxin contamination of corn and peanuts is associated with high temperatures, high insect activity, and severe and prolonged drought stress, whereas contamination by the *Fusarium* mycotoxins and ochratoxins are associated with cool temperatures and high moisture conditions at harvest. Ochratoxin has been reported as naturally occurring in corn, wheat, sorghum, oats, rice, and green coffee. Ochratoxin contamination of coffee, corn, and wheat is generally <500 ppb, while barley and oats grown in Denmark and other Scandinavian countries are particularly susceptible to high levels of ochratoxin contamination (Krogh et al., 1973, 1974). Animal feeds in Canada, Europe, and Australia may be highly contaminated with ochratoxin (>5000 ppb) (Jelinek, 1987; Tsubouchi et al., 1988). The highest reported incidence and levels have been in barley, oats, wheat, and corn produced in northern European (United Kingdom, Denmark, and Sweden) or Balkan (Yugoslavia) countries and in India. The reports indicate that levels approaching the parts per million range, at an incidence of over 20%, can be expected to occur in random samples of these grains in the affected areas. Surveys of barley, corn, oats, sorghum, and wheat in the United States over a number of years found some ochratoxin in all but sorghum, but at a low incidence (corn 0.5%, wheat 1%, oats 2%, barley 14%) and low level (all samples <200 ppb) of contamination.

Citrinin can occur alone or with ochratoxin. However, there is no suitable quantitative method for citrinin analysis, so that current data is an estimation of the actual amount. Citrinin has been reported in peanuts, tomatoes, corn, barley, and other cereal grains (Jelinek, 1987). The importance of citrinin in human and animal health is difficult to determine without reliable estimates of the actual contamination frequency or levels.

Alternaria species can produce several mycotoxins including tenuazonic acid, alternariol, and alternariol methyl ether. Tenuazonic acid has been found in tomatoes in the United States and Canada, and alternariol methyl ether and alternariol have been found in apples in West Germany (Jelinek, 1987). *Alternaria* species frequently invade fruits and grains in the field and *Alternaria* metabolites could easily contaminate grains such as wheat, oats, barley, and grain sorghum. Again, we lack survey data from the United States to make an accurate appraisal of the occurrence of these mycotoxins in food and feeds.

Other mycotoxins such as cyclopiazonic acid, sterigmatocystin, sporidesmins, rubratoxin B, cytochalasins, penitrems, and slaframine have been reported in the literature as natural contaminants of agricultural commodities. Some of the above mycotoxins are of limited occurrence. More surveys designed to determine the incidence and levels of mycotoxins in foods and feeds are sorely needed, but surveys depend on the availability of suitable analytical methods. Patulin and penicillic acid are often considered in mycotoxin reports, but will not be considered here because of their inability to cause disease when given to animals

by a natural route.

Natural Occurrence in Processed Foods

Limited information is available on the natural occurrence of mycotoxins in processed foods (Jelinek et al., 1989; Scott, 1984). Most information available is for the aflatoxins. There is a dearth of information for ochratoxin A, trichothecenes, zearalenone, and citrinin. Apparently, ochratoxin A is not as great a problem in the United States as it is in some European countries.

Foods processed from peanuts, corn, and milk have received the greatest attention because corn and peanuts are the major raw products that have been previously surveyed for mycotoxin contamination (Campbell and Stoloff, 1974; Wood, 1989). Also, wheat, barley, oats, apples, and tomatoes occasionally have been found to be contaminated with mycotoxins.

Corn is probably the commodity of greatest worldwide concern, because it is grown in climates where it is likely to have perennial contamination with aflatoxins and it is the staple food in many countries. However, procedures used in the processing of corn help to reduce aflatoxin contamination of the resulting food product. Also, corn may be contaminated with *Fusarium* toxins, and foods made from corn (corn grits and popcorn) have been shown to be contaminated with deoxynivalenol (Davis and Diener, 1987; Trucksess et al., 1986). Deoxynivalenol contamination of corn and corn products has been correlated with cool, wet weather conditions occurring just before harvest.

Deoxynivalenol contamination of wheat can occur when preharvest weather conditions favor development of wheat scab. Wheat-based processed foods shown to be contaminated with deoxynivalenol include flour, bread, snack foods, baby foods, breakfast cereals, bran, and wheat germ (Jelinek et al., 1989; Trucksess et al., 1986). Citrinin and ochratoxin have been found in moldy bread (Davis and Diener, 1987; Visconti and Bottalico, 1983). Aflatoxins were found in one sample of spaghetti (van Walbeek et al., 1968).

The natural occurrence of mycotoxins in fruits and vegetables has been found most frequently in apples, tomatoes, and their processed products. *Alternaria* toxins including tenuazonic acid, alternariol, and alternariol methyl ether have been found in apple and tomato products (Jelinek et al., 1987). Patulin is a common contaminant of apple cider (Stoloff, 1975).

Aflatoxin-contaminated corn and cottonseed meal in dairy rations have resulted in aflatoxin M_1-contaminated milk and milk products, including nonfat dry milk, cheese, and yogurt. Natural occurrence of mycotoxins in cheeses, as a result of mold growth on the cheeses, also has been reported. Sterigmatocystin has been detected in the rind of Dutch cheese (Northolt et al., 1980), and metabolites of *P. roqueforti* and *P. caseicolum* (camemberti) in blue and Camembert-type cheeses have been reported (Bullerman, 1981; Leistner, 1984; Scott, 1981).

The natural occurrence of mycotoxins and/or their metabolites in meats and meat products can occur as residues from consumption of the toxins in moldy feed or as the result of fungal growth on certain meat products, such as aged hams and cured sausages. Ochratoxin A has been the mycotoxin most commonly found as a residue in pork and poultry meat. Aflatoxin residues have been demonstrated experimentally in animal organs, tissues, and eggs (Leistner, 1984). Ochratoxin A has been detected at an 84% incidence in the blood of pigs raised and slaughtered in western Canada, but at measurable levels in only $<5\%$ of the samples. Ochratoxin A has been detected in blood, kidneys, liver, and muscle tissue from slaughtered hogs in several European countries (Leistner, 1984). Ochratoxin A has been detected in apparently normal hog kidneys that had passed meat inspection and were obtained from butcher shops in West Germany (Leistner, 1984). European type sausages and country cured hams frequently have intentional or adventitious fungal growth on the outer surfaces. Most of these fungi have been shown to be *Penicillium* species, many of which are capable of mycotoxin production. Leistner (1984) in Germany has found patulin, penicillic acid, ochratoxin A, and cyclopiazonic acid to be the predominant mycotoxins produced by these fungi. Patulin and penicillic acid cannot be detected in the meat, due to their interaction with sulfhydryl compounds in the tissue.

Scott (1984) extensively reviewed the effects of food processing on selected mycotoxins. He showed that a number of factors, including the process, the food, moisture content, additives, and amount and manner of contamination can affect the stability of mycotoxins during food processing. Also, different mycotoxins have widely different degrees of stability in foods under processing conditions. For example, aflatoxins tend to be stable to moderately stable in most food processes, but are unstable in processes employing alkaline conditions or oxidizing steps. Deoxynivalenol is stable during bread baking, whereas ergot alkaloids are partially destroyed. Ochratoxin A appears to be moderately stable to most food processes. Thus, the effects of processing on mycotoxins in foods would be expected to be variable depending on many factors.

Natural Occurrence in Imported Foods

A number of different mycotoxins could be present in various imported foods and feeds; however, the FDA regularly tests only for aflatoxins. Because fungal growth and mycotoxin production are highly dependent on environmental conditions, continual vigilance is required to control this contamination in relation to FDA action guidelines. In the United States, the FDA compliance programs for aflatoxins in imported foods and feeds are designed to provide that control. Under these programs, the FDA annually inspects about 300 samples of imported foods and about 200 samples of imported feeds. The results of these analyses are shown in Table 4.2 for the years 1982 to 1986 (only samples positive for aflatoxin are shown) (Wood, 1989). Jelinek (1987) has summarized information on the worldwide occurrence of mycotoxins, using data from the FAO/WHO/UNEP food contamination monitoring program and other sources.

Table 4.2 Occurrence of aflatoxins in foods and feeds imported into the United States from 1982-1986 (Wood, 1989)

Commodity	No. contaminated samples	No. >20 ng/g	Max. (ng/g)
Human foods			
Almonds	26	2	372
Brazil nuts	123	10	133
Filberts	113	1	60
Peanuts	20	8	273
Peanut products	87	56	803
Pistachio	17	12	298
Melon seeds	28	17	299
Pumpkin seeds	11	6	123
Sesame seeds	2	1	22
Sunflower seeds	1	1	179
Chilies	9	1	30
Nutmeg	5	2	27
Paprika	1	0	3
Cashew bar	1	0	10
Coffee bean	1	0	14
Corn flour	1	0	6
Lotus seed	1	0	5
Marzipan	2	1	30
Cocoa products	2	1	37
Pipitora candy	3	1	26
Pipian paste	3	3	78
Calve barrel noodles	1	0	2
Chili powder	1	0	5
Corn meal	1	1	126
Animal feeds			
Copra Pellets	25	25	263
Corn	4	3	158
Cottonseed meal	6	2	753
Sorghum	2	2	1873
Mixed feeds	23	10	135

5. Economic Impacts of Mycotoxins

Summary

One quarter of the world's food crops are affected by mycotoxins annually. Mycotoxins and their control impose discernible economic costs on United States crop, livestock and poultry producers, grain handlers, and food and feed processors. The economic impacts of mycotoxins derive directly from crop and livestock losses as well as from regulatory programs designed to reduce risks to animal and human health. Yet, despite the economic importance of mycotoxins, we are unable to quantify losses adequately.

The incidence of mycotoxins in the United States varies among commodities, years, and regions. Years of extreme drought and other environmental conditions favorable to aflatoxin development are more likely to result in higher economic losses, although even then, losses may occur randomly, making prediction and aggregate cost estimates difficult. Although mycotoxins are naturally occurring chemicals, mycotoxin levels are potentially affected by economic as well as environmental factors, including choice of mechanical harvesting technology, relative prices of insecticides, fungicides, and labor and agricultural price supports.

The crops most affected economically in the United States are corn, peanuts (including peanut meal), and cottonseed and cottonseed meal. Cottonseed grown in the Southwest and corn in the Southeast are more frequently affected than in the Midwest. Annually, less than 2% of the total U.S. corn production is affected economically, yet losses for individual producers can be significant. Peanuts in all growing regions can potentially incur economic losses attributable to aflatoxins. Losses can also occur in corn and animal products such as cornmeal, grits, milk, and eggs. Fruits and vegetables can be affected, but no economic losses have been documented. Effects on products of fermentation, including beer, wine, and fruit juices, appear not to be an economic problem in the United States.

Mycotoxins produce a wide range of adverse economic effects, including lower yields for food and fiber crops. Mycotoxin losses to livestock and poultry producers from mycotoxin-contaminated feeds include death and the more subtle effects; among them immune suppression, reduced growth rates, and losses in feed efficiency. Prices are often lower for animal products and higher for feeds, and regulations can restrict the use of food and feed crops when acceptable levels are exceeded. The economic result is that output is reduced, production costs are increased, and returns to growers are reduced.

Costs to consumers can include higher product prices in years of severe outbreaks and increased health risks when regulations fail.

Social costs to control mycotoxins include testing, monitoring, and regulatory enforcement expenses, in addition to research and extension efforts. Economic losses are inextricably linked to regulatory standards. FDA guidelines are conservative to assure risks are minimal, but flexibility to balance health risks with an adequate food and feed supply exists. To this end, exemptions to guidelines have been made on a case-by-case basis when market disruption is threatened by widespread aflatoxin incidence. Although this policy of restricting known naturally occurring carcinogens to the lowest feasible level is consistent with regulations governing other food use chemicals, there is on-going scientific and policy debate regarding the appropriateness of these levels.

International regulatory standards have critical economic implications. More stringent standards result in higher costs for producers and exporters, and can discriminate against developing countries. In the long run, they may lead to increased demand for commodities by ensuring consistency and quality. On the other hand, overly restrictive and inconsistent standards can introduce significant barriers to trade. Because regulatory restrictions significantly impact losses, expected benefits of these restrictions must be accurately addressed. Standards must be based on sound health-based scientific data. To this end, improved economic information on incidence and associated losses are essential. Additionally, the increase in internationally traded agricultural commodities necessitates consistent cost-effective control of mycotoxins.

Economic Impacts of Mycotoxins

The Food and Agriculture Organization (FAO) estimates that 25% of the world's food crops are affected by mycotoxins (Mannon and Johnson, 1985). Despite the postulated economic importance of mycotoxins, a recent survey of researchers in 30 countries revealed that none was able to quantify losses (Hesseltine, 1986). Mycotoxins and their control impose discernable economic costs on U.S. crop, livestock and poultry producers, grain handlers, and food and feed

processors. The economic impacts of mycotoxins are derived not only from crop and livestock losses, but directly from regulatory programs to reduce the risks to animal and human health. Since the mid-1970s, regulatory programs set standards on the maximum tolerated level of mycotoxins in food, animal products, and feed. Thus, analysis of the economic impact of mycotoxins must include not only the private costs, but the social costs as well. These include the costs of regulatory programs to producers, consumers, and taxpayers and, theoretically, any residual health risks to consumers. The occurrence of mycotoxins in the United States varies from commodity to commodity, year to year, and region to region. Years of extreme drought or other environmental conditions favorable to mycotoxin development will result in higher economic losses.

The crops most affected economically in the United States are corn, peanuts, and cottonseed. Cottonseed grown in the Southwest, corn in the Southeast (more so than the Midwest), and peanuts in all three growing regions, potentially incur economic losses attributable to mycotoxins. Mycotoxins infrequently are reported to contaminate sorghum, wheat, and other oilseeds in the United States (Park and Pohland, 1986). Economic losses from ingestion of mycotoxin-contaminated feedstuffs in swine and poultry have been estimated. Losses also occur in corn products, such as cornmeal, grits, and in animal products, such as milk (Figure 5.1) and eggs. Apples and tomatoes are the fruits most commonly affected (Smith and Moss, 1985), but no economic losses have been documented. Mycotoxin contamination of fermentation products, including beer, wine, and fruit juices, does not appear to be an economic problem in the United States (Hesseltine, 1988). Incidents of zearalenone and ochratoxin contamination have been documented, but there have been no resulting economic impact analyses. Economic analyses have focused almost exclusively on aflatoxins.

Figure 5.1. Irrigation water containing milk that had to be dumped because of excess contamination with aflatoxins. Photograph courtesy of D. P. H. Hsieh, University of California, Davis.

Mycotoxin contamination levels can be indirectly affected by economic and environmental factors, including choice of production technology, relative prices, and agricultural policy. For example, rising labor costs relative to capital encouraged adoption of mechanical harvesting, that has changed the corn harvesting process significantly. Field-shelling and forced-air drying in bins, which can result in condensation and pockets of high-moisture in storage, facilitates fungal growth (Diener et al., 1979). Mechanical shelling of wet corn can cause a greater percentage of breakage, providing more points for entry of fungi. Large increases in yields and policies promoting on-farm storage complicate postharvest crop management (Hesseltine, 1986). Other economic factors that potentially contribute to mycotoxin formation include selection of hybrids for yield potential and disease control, rather than for mycotoxin resistance. The economics of pest control potentially affect mycotoxin levels as well.

Mycotoxins can produce a wide range of adverse economic effects, occurring at various stages of crop production, processing, transport, and storage. These effects are inventoried in Table 5.1. The fundamental economic impact of mycotoxins is that they increase risk at all levels. Mycotoxins can result in more variable per unit output, as well as in greater price uncertainty for commodities, because supplies are short and markets are restricted when contamination exceeds regulatory standards. Lower returns result from lower yields and increased production costs. Total costs of mycotoxin losses are difficult to estimate, due to the coincidence of pest and drought damage; however, other costs are quantifiable, given incidence data and regulatory standards (Nichols, 1987).

Economic losses for livestock and poultry producers from mycotoxin-contaminated feeds are less easily quantified. Cost analysis is complicated because mycotoxin effects on poultry, livestock, and other animals vary among individuals. Mortality losses due to death from aflatoxicosis can be estimated if laboratory analyses are available, but the more subtle chronic effects (reduced growth rate and losses in feed efficiency, infertility, immunological problems, and general loss of quality in animals and animal products) associated with contaminated feed are difficult to

Table 5.1 Adverse economic effects attributable to mycotoxins

Producer costs
- Crops
  - Yield losses
  - Restricted markets
  - Nonmarketable product
  - Price discounts
  - Increased production costs
    - Pest control
    - Irrigation
  - Increased postharvest costs
    - On-farm drying
    - On-farm testing and sampling
    - On-farm detoxification
  - Increased transportation costs
  - Inability to obtain loans on stored grain
  - Disposal of useless crops (buried, burning)
- Livestock (beef, swine, poultry) producers
  - Higher mortality rates
  - Reproductive failures
  - Reduced feed efficiency
    - Higher feed costs
    - Lower live weight
    - Infertility syndrome
    - Increased susceptibility to disease
  - Overall quality loss
  - Monitoring and testing
- Dairy
  - Higher mortality rates
  - Reproductive failures (abortions)
  - Reduced feed efficiency (as above)
  - Lower milk production
  - Nonmarketable milk
  - Monitoring and testing

Handler/Distributor costs
- Extra drying costs
- Excess storage capacity
- Losses in transit
- Loss of markets

Processor costs
- Milled corn products
  - Restricted markets
  - Product loss
- Peanut products
  - Insurance premiums
  - Restricted markets
  - Product loss
- Fermentation products

Consumer costs
- Less nutritious food
- Higher product prices
- Reduced income due to lost work days from acute aflatoxicosis
- Long-term chronic effects from low-level contamination

Social costs
- Regulatory costs
  - Establishing standards and tolerances
  - Surveillance and assay
  - Enforcement
- Research and extension
- Education
- Lower foreign exchange earnings
- Increased costs of imports

document (Nichols, 1987). Production costs are higher, and returns to producers are lower.

Costs to consumers may include higher product prices and increased health risks in years of severe mycotoxin contamination, resulting in a variety of associated economic and human costs. No estimates of these costs are available currently.

The costs of controlling mycotoxins include testing, monitoring, and regulatory enforcement expenses that are incurred at all levels of production, processing, and distribution by both the private and public sector. The costs of research and extension for mycotoxin control should also be included. Additionally, economic losses for producers, processors, and consumers are inextricably linked to regulatory standards as these standards directly impact product prices and define markets for agricultural commodities contaminated with mycotoxins. Regulatory agencies set standards for maximum tolerated levels of aflatoxins in foods and feeds, and this restricts uses of agricultural commodities when regulatory limits are exceeded. As a result of market restrictions, returns received by producers, distributors, and processors for products with the potential for mycotoxin contamination will be reduced.

The FDA administrative guidelines establish acceptable aflatoxin levels in agricultural commodities to protect human and animal health, as will be discussed in Chapter 6. The FDA action level for aflatoxins in agricultural products are based on the opinion made in the late 1960s that an unavoidable potential human carcinogen in important foodstuffs should be controlled at the lowest practical level. For products for human consumption, the current action level is 20 ppb total aflatoxins in a finished product, while that for milk and milk products is 0.5 ppb aflatoxin M_1. When these levels are exceeded, commodities are restricted to lower value uses, usually animal feeds, oil products, and alcohol production. More recently, the FDA has

used occurrence, metabolism, and animal toxicity information to set action guidelines for aflatoxins in animal feedstuffs (Park and Pohland, 1986). The policy of restricting known unavoidable animal carcinogens to the lowest practical level is not consistent with regulations requiring no detectable amounts of other food use chemicals shown to be animal carcinogens. There is an on-going scientific and policy debate over the appropriateness of all current regulations of carcinogens.

International regulatory standards also have critical economic implications for U.S. agriculture. The increase in internationally traded agricultural commodities necessitates consistent cost-effective control of mycotoxins. A recent survey of trading countries indicated that 56 out of 66 countries have or are proposing regulations to control aflatoxins (van Egmond, 1988). Presently, these standards vary from country to country. Although U.S. regulatory guidelines consider only aflatoxins, 13 countries have established tolerances for other mycotoxins (van Egmond, 1988) with potential economic impacts for the United States.

Mycotoxin contamination can lead to loss of important export markets. As one example, consumption of peanut feedcake in European Economic Community (EEC) countries dropped by over 60% in a four-year period (from 988 million metric tons in 1979 to 368 million metric tons in 1982) as a direct result of economic losses to livestock producers associated with high levels of aflatoxins in imported feedcake (Jemmali, 1987). Serious economic losses were sustained by exporting countries as a consequence of reduced demand. The United States annually rejects imported goods for noncompliance of standards, with mold and mycotoxin contamination cited as the primary violation. A 1983 Joint FAO/WHO Committee on Food Safety reported that between October 1979 and September 1980, the FDA rejected products valued at $206 million (World Health Organization, 1983). Similarly, in the period of October 1980 to September 1981, products valued at $253.5 million were rejected (Jemmali, 1987).

On the other hand, overly restrictive and inconsistent standards can introduce significant barriers to trade. In 1986, the Codex Alimentarius Commission reported maximum tolerance limits for aflatoxin B_1 in raw materials used for livestock feed by Denmark (0.05 ppm), Federal Republic of Germany (0.2 ppm), Italy (0.5 ppm), The Netherlands (1 ppm), and France (0.1 ppm) (Jemmali, 1987). Given scientific uncertainty and analytical constraints, these disparities might be explained more by economic conditions in the restricting countries than by probable risk (Jemmali, 1987). The EEC has proposed a community-wide maximum aflatoxin tolerance level of 0.2 ppm of aflatoxins in raw feed materials in an attempt to find common ground.

Stricter regulatory standards result in higher costs to producers and exporters, and can discriminate against developing countries. In the long run, stricter standards may lead to increased demand for commodities by ensuring consistent product quality (Ybarra and Webb, 1982). However, regulatory levels must balance costs and benefits. Research has found no benefit to controlling aflatoxin levels below 20 ppb, yet the costs of changing standards from 20 to 5 ppb for United States peanut producers alone is significant, and would be prohibitive to many developing nations (Jemmali, 1987; National Peanut Council, 1988).

Although an aggregate estimate of the total range of economic impacts outlined in Table 5.1 does not exist, isolated economic analyses do exist. These analyses provide one with a sense of the magnitude and distribution of costs associated with specific levels of contamination for a given product within a given region. It is also possible to develop a broader understanding of the critical relationship between economic losses and existing regulatory standards designed to protect human and animal health.

Economic Losses in Crops

Corn (Maize)

Economic losses since the mid-1970s to U.S. producers, handlers, and processors from aflatoxin in corn have been documented, primarily in the Southeast where conditions are conducive to aflatoxin development. Since the 1970s, corn production has increased yearly in all regions of the United States. Total area planted in corn in the United States increased by 25% in the first half of the 1970s, while yield per harvested acre rose from 72.4 bushels in 1970 to a high of 120.3 bushels in 1987 (U.S. Department of Agriculture, 1987). Corn production in the eight-state Southeast Region represents between 3 and 4% of total U.S. production, with Georgia, Virginia, and North Carolina producing 60% of all southeastern corn.

Additionally, more corn is being stored, which increases mycotoxin contamination risk. On-farm storage rose by over 40% from 1970 to 1987, while increases in off-farm storage rose 60% (U.S. Department of Agriculture, 1987). In the Southeast, one-half of the corn crop remained on the farm and most of the corn produced in the region was utilized for feed (Nichols, 1983). Overall, about 50% of U.S. corn production was utilized for domestic feed, down about 20% since 1970.

The U.S. per capita consumption of corn has increased from 21 pounds in 1972 to 77 pounds in 1986, largely due to the increased use of corn sweeteners in foods (U.S. Department of Agriculture, 1987). However, aflatoxins are not present in the starch fractions that are converted to sweeteners, but are present in the fractions utilized for animal feeds.

Exports of corn increased from 517 million bushels in 1970 to 1.7 billion in 1987, or over 325% (U.S. Department of Agriculture, 1987); thus, increasing the importance of international standards on aflatoxins and other mycotoxins.

Economic impacts of aflatoxins in the cornbelt have not been estimated but, in the past, likely have been minimal, with 1988 an exception because of the extreme drought conditions. Shotwell and colleagues (1973) reported that a low incidence of low levels of aflatoxin contamination (1.7 to 2.3 ppb) occurred in midwestern corn in 1964, 1965, and 1967. Most of the contamination was confined to the poorest grades. Only slightly less than 10% of corn sampled exceeded 20 ppb, indicating small economic losses. No losses have been reported for the 1970s. In 1988, the presence of aflatoxins in corn samples was reported in 9 states, including Texas, Oklahoma, Iowa, Indiana, Illinois, South Dakota, Maryland, Wisconsin, and Minnesota (*Wall Street Journal,* 1988). In a survey of the 1988 corn crop from seven midwestern states, more than 30% of the corn samples from two major corn-producing states, Iowa and Illinois, contained concentrations of aflatoxins above 20 ppb, with 7.2 and 11.6%, respectively, above 100 ppb (Hurburgh and Stahr, 1989).

No recent estimates of economic losses from aflatoxins in the Southeast are available. Given incidence data and present regulatory standards, clearly some economic losses occur annually in southeastern corn, although they are highly variable by states and year. Losses due to zearalenone and ochratoxin have not been evaluated for corn in this region (Shotwell and Hesseltine, 1983).

A five year study to determine aflatoxin levels in dent corn from Virginia (1976 to 1980) indicated that 78% of the corn sampled annually contained aflatoxin levels below the 20 ppb standard, with 14% exceeding 100 ppb (Shotwell and Hesseltine, 1983). A six-year study of pre-harvest corn in Georgia (1977 to 1982) reports that 9.4 to 75.2% of the samples were less than 20 ppb (McMillan et al., 1985). Ten years of data on North Carolina corn indicated that, dependent on the year, 65 to 90% of the corn samples contained less than 20 ppb; 2 to 9% exceeded 100 ppb. For the 3 years with high aflatoxin levels (1977, 1980, and 1983), 40 to 60% of the corn crop incurred some economic loss (20 ppb or more aflatoxin levels), with 12 to 20% containing levels of more than 100 ppb. In the other years, 90 to 99% of the crop averaged less than 100 ppb of aflatoxin (Nichols, 1987).

Nichols (1983) estimated costs for the eight-state southeastern region for 1977 and 1980; two years with serious aflatoxin outbreaks. Losses to corn producers were estimated at $80 million in 1977 and $97 million in 1980. This represented nearly 8% of the value of crops produced in the region (U. S. Department of Agriculture, 1985a). Loss estimates for 1980 are shown in Table 5.2, and include:

Table 5.2 Cost of aflatoxins to corn producers and handlers, southeastern states, 1980 (Nichols, 1983)

State	Corn production 1,000 bu.	Contamination range[a] 0-20	21-100	101-200	201+	Farmer losses: Nonmkt. grain and discounts[b]	Restricted markets[c]	Inability to store[d]	Extra drying[e]	Handler losses: Extra drying, handling and testing[f]	Total
Alabama	14,580	1.7	27.2	38.6	32.5	17,296	255	3,742	569	536	22,398
Florida	15,416	45.2	24.7	12.0	18.1	9,487	432	1,494	407	906	12,726
Georgia	54,600	71.3	15.6	11.3	1.8	3,440	1,310	4,582	1,704	393	11,429
Mississippi	2,464	76.8	12.5	5.4	5.3	464	17	228	127	5	841
North Carolina	103,800	34.3	48.1	10.7	6.9	6,432	3,581	11,352	1,931	7,520	30,816
South Carolina	24,720	33.5	35.9	16.4	14.2	12,286	853	2,405	460	1,791	17,795
Tennessee	28,800	81.2	18.8	—	—	—	619	1,852	985	1,300	4,756
Virginia	32,725	72.7	10.9	12.2	4.2	4,536	736	2,495	1,080	1,546	10,393
Total	277,105	52.1	24.2	15.9	11.9	53,941	7,803	28,150	7,263	13,997	111,154

[a]Correspondence or telephone conversation with State Chemists or State Veterinarians.
[b]Percent of crop greater than 201 ppb x average market price.
[c]Quantity sold x 5 cents per bushel.
[d]Percent of crop greater than 20 ppb but less than 201 ppb x 60 cents per bushel.
[e]Quantity stored on farm x 6 cents per bushel.
[f]Quantity sold x 10.5 cents per bushel.

1. Lost revenue due to nonmarketable grain (that in excess of 201 ppb).
2. Discounts on grain that exceeded the 20 ppb action level.
3. Extra drying costs for corn stored on the farm.
4. Unstorable corn (that portion of crop in excess of 20 ppb, but less than 201 ppb).

Nichols estimated that in 1980, these losses to producers amounted to approximately $0.40 per bushel of corn produced in the eight-state region.

Grain handler losses attributable to extra drying and testing were estimated to be $13.9 million, or approximately $0.10 per bushel (Nichols, 1983). Producer and handler costs exceeded $100 million in 1980.

Economic losses due to lower yields were not included in these estimates; neither were the costs of producing corn that was not marketable, nor the opportunity costs of land, labor, and capital. While estimates of economic losses due to lower yields from aflatoxins are not available, presumably this would be negligible because mold contamination occurs at the starch stage, when yields have already been established. Also, aflatoxin effects are difficult to isolate from drought factors. Annual yields were compared with the percent of sampled fields contaminated with aflatoxin levels in excess of 100 ppb for North Carolina from 1976 to 1985 (Figure 5.2). Yields were lower and aflatoxin levels were higher in 1977, 1980, and 1983, when drought conditions prevailed over much of the Southeast (Nichols, 1987). (Note: High levels of aflatoxins logically correlate with drought, and drought correlates with low yield, but high aflatoxin levels do not necessarily correlate with low yield.)

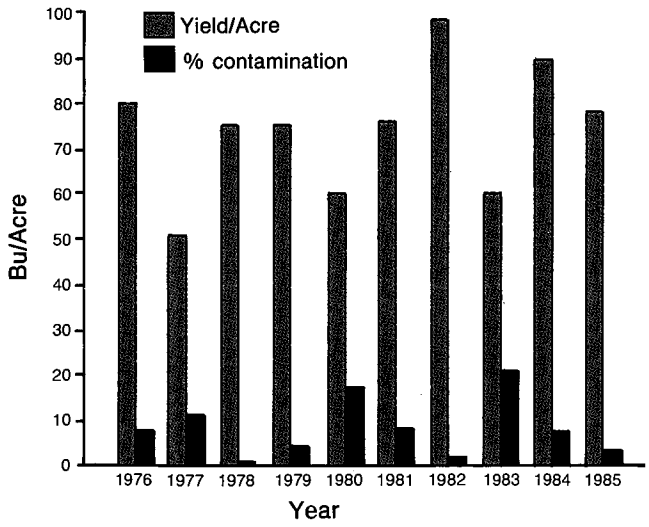


Figure 5.2. Relationship between average maize yield per acre and aflatoxin contamination, North Carolina, 1976-1985 (1 bu/acre = 62.78 kg/ha) (Nichols, 1987).

Regulatory costs for surveillance and assay programs were estimated at about $600,000 per year for the eight southeastern states (Nichols, 1983). In addition to these direct regulatory costs, regulatory standards for aflatoxins caused significant economic losses in corn. In 1977, 53% of the 249.7 million bushel southeastern states corn crop exceeded the 20 ppb FDA action level. Exemptions were made for feed grains, allowing intrastate feeding of corn contaminated with up to 100 ppb for nondairy livestock and poultry. Nearly one-third of the total crop (that quantity with aflatoxin levels of more than 100 ppb) could not be used as feed. In 1980, corn contaminated with 100 ppb or less was allowed to be fed intrastate (Nichols, 1987). If regulatory guidelines had not been changed, losses for corn producers would have increased drastically, indicating the importance of sound health-based regulatory standards.

The 1988 outbreak of aflatoxins in midwest corn has exposed additional regulatory issues. Because aflatoxin outbreaks are rarely important in the Midwest, testing capabilities were limited, slowing the process and causing marketing delays. The unknown extent of contamination can lead to uncertainty for domestic and international purchasers, and potentially impact financial markets. The FDA informally eased action levels in early October for interstate corn shipment to avoid market disruptions, raising limits to 100 ppb for grain fed to breeding livestock, 200 ppb to mature hogs, and 300 ppb or less for mature cattle. Action levels for corn fed to dairy cows and many young animals remained fixed at 20 ppb. These standards are more in line with recent scientific assessments of risk (Park and Pohland, 1986; Richard et al., 1983; Stubblefield et al., 1981).

With current regulatory standards, the southeastern corn crop is annually at economic risk from aflatoxins, while affecting less than 2% of the total U.S. corn crop. Because most of the southeastern corn is used for intrastate feeds, regulatory relief is available through state actions that follow the FDA recommendations, even if these recommendations are not incorporated into guidelines for interstate commerce. Losses can be significant for affected producers. In years of severe aflatoxin outbreaks, economic risks are compounded by yield losses associated with drought conditions. Market restrictions must be accurately assessed, because they can cause producer losses. Estimates of postharvest losses are not available.

Processed Corn Products

About 8% of corn production is consumed as food products. Only about 2 to 3% of corn is processed into

cornmeal and grits for human consumption with approximately 1.5% consumed as cereal, sugar, or starch. Over 12% of corn is now consumed as corn syrup (U.S. Department of Agriculture, 1987). Approximately one-third of the milled corn products from the Southeast analyzed from 1977 to 1981 contained detectable aflatoxin levels with an average level of 30 ppb (Nichols, 1983). The FDA action level for these products is 20 ppb, requiring that products identified to have levels greater than this must be diverted to animal feed or destroyed.

Annual data for the years 1977 to 1980 from North Carolina indicated that a significant quantity of cornmeal products can be affected. For example, in 1977, over 32% of product samples contained aflatoxins exceeding 20 ppb, resulting in losses to millers and processors, but reducing risks to consumers. In 1977, 2.7 million pounds of whole corn grain were diverted to animal feed; approximately 170,000 pounds of whole corn were destroyed and 117,000 pounds of product were diverted to feed. Slightly less than 35,000 pounds of product were recalled from commerce, involving 17 product recalls. In 1980, 25% of samples exceeded the 20 ppb level, resulting in recalls for 78 products, totaling approximately 25,000 pounds. Over 629,000 pounds of whole grain corn with a value of $145,000 was diverted to feed or destroyed (Nichols, 1983). Likely, other states suffered comparable losses during this period.

Peanuts

Aflatoxin contamination of peanuts has been of worldwide concern since the 1960s. A complex grading and marketing scheme that is part of the USDA Peanut Marketing Order each year diverts contaminated peanuts and minimizes aflatoxin losses to shellers[1]. Based on actual data for the period 1976 to 1985, the current U.S. peanut marketing system assured that approximately 94% of the delivered, shelled lots contained 20 ppb or less of aflatoxins (National Peanut Council, 1988).

Losses to peanut growers, shellers, processors, and the taxpayers from aflatoxin-contaminated peanuts for the years 1972 to 1978 were estimated to be $10 to 12 million (Diener et al., 1979), approximately 0.2% of the $5.2 billion total crop value over the period (U.S. Department of Agriculture, 1979). Producer costs include crop loss, along with lower prices received from sale of contaminated crops for other uses (oil and export feed). In 1975, the quantity of peanuts rejected at the buying point was estimated to be 0.15% of the total crop value, or about $0.5 million (Jemmali, 1987). Segregation 3 peanuts are those peanuts rejected for human consumption because of visible *A. flavus* contamination. The average quantity of Segregation 3 peanuts over the years 1970 to 1985 was 32,000 tons per year (U.S. Department of Agriculture, 1986). The benefits of reducing aflatoxins in farmers' stock peanuts have been reported by the Peanut Quality Task Force (National Peanut Council, 1988), which estimated that on-farm cleaning of peanuts could increase the value of the quota peanuts by $8 to $24 per ton, or up to 5% of the value per ton given 1985 prices (U.S. Department of Agriculture, 1986). The number of tons of U.S. Segregation 3 peanuts varies with the year. For example, in 1975, only 5,388 tons (0.02% of the crop) were Segregation 3, while in 1972 and 1980, over 130,000 tons (8 and 11.8% of the crop, respectively) were Segregation 3 peanuts.

When farmers' stock peanuts sold to shellers contain higher than tolerated *A. flavus* contamination, extra cleaning, handling, and screenings are required by both shellers and processors. In 1975, estimated costs for analysis and sampling were $3.20 per ton. Blanching (removal of the peanut skins), followed by color sorting, can remove most of the contaminated peanuts from the edible peanut supply at a cost of about $0.05 per pound (National Peanut Council, 1988). Shellers that have signed the marketing agreement are insured against losses under the marketing agreement administered by the Peanut Administrative Committee (PAC). The peanut marketing agreement involves edible quality farmers' stock peanuts that have been purchased by shellers but, after shelling, yield kernels that on analysis exceeded 25 ppb total aflatoxins.

The quantity and market value to peanuts indemnified (compensated for loss) under the marketing agreement administered by the PAC are documented in Table 5.3. For the years 1965 to 1985, approximately 430,000 tons of peanuts were indemnified (U.S. Department of Agriculture, personal communication). These peanuts were valued at $50.7 million. Some of these costs are recovered from the sale of peanut oil and exported meal, but these data are not available. Indemnification insurance premiums paid for the years 1972 to 1978 totaled $4.5 million (Diener et al., 1979); comparable data are not available for the years 1978 to 1985.

While the per capita consumption of peanuts in the United States has increased only slightly, the quantity of peanuts harvested for nuts has increased from 1.45 million tons in 1970 to 2.25 million tons in 1987. An increasing proportion of this production is exported, with shelled peanut exports doubling from 7% of total

[1]Until about 1980, compliance with the order was mandatory for all growers and shellers participating in the federal price support for peanuts.

Table 5.3 Costs of Peanut Administrative Committee (PAC) indemnification for aflatoxin contamination in shellers' peanut stocks, 1965-1985 (estimates from Peanut Administrative Committee, National Peanut Council, 1988)

Crop year	Tons of shelled peanuts > 50 ppb indemnified (000's tons)	Total value of loss from indemnifications (000's dollars)
1965	2.6	213.4
1966	3.2	343.3
1967	25.6	1,500.0
1968	38.0	2,000.0
1969	13.6	1,232.9
1970	27.4	2,763.0
1971	18.7	1,766.5
1972	44.6	3,242.4
1973	23.8	1,002.8
1974	10.3	882.9
1975	18.8	2,241.4
1976	5.4	643.3
1977	25.0	2,750.0
1978	34.2	4,451.6
1979	11.8	2,685.0
1980	25.8	2,545.3
1981	8.1	2,765.9
1982	11.4	4,080.3
1983	13.6	3,097.8
1984	39.4	9,356.2
1985	28.2	2,967.0

production in 1970 to 14% in 1987 (U.S. Department of Agriculture, 1987). Thus, lowering international standards for aflatoxins could significantly increase costs to the peanut industry, and indeed the domestic shellers are considering lowering tolerated levels to meet the European tolerances.

In international markets, acceptable aflatoxin levels for raw shelled peanuts vary by country from 0 to 10 ppb, with continuing pressure by some countries to move to a zero standard (the level is actually established by the minimum quantifiable level of the specified analytical method), according to a recent report (National Peanut Council, 1988). Currently, nearly 70% of the U.S. shelled peanuts have no detectable aflatoxin. From Figure 5.3, approximately 92.5% of the shelled peanut supply meets an upper limit of 20 ppb of aflatoxin; thus, 7.5% of supply exceeds that limit currently. If the upper limit was set at 10 ppb, an additional 10% of production would exceed it. This increases another 18% under an upper limit of 5 ppb (National Peanut Council, 1988). These regulatory changes would result in an estimated cost of $95 and $170 million, respectively, based on USDA data on the value of peanuts for nuts (U.S. Department of Agriculture, 1986); again demonstrating that regulatory standards are critical to economic losses. With

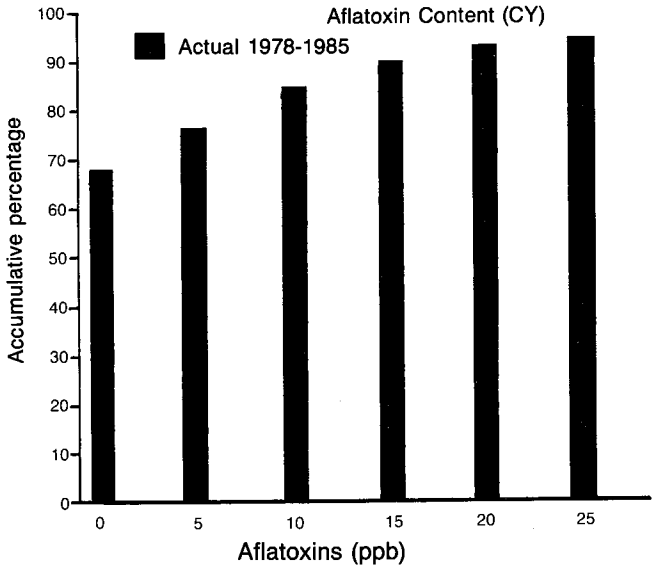


Figure 5.3. Percentage of aflatoxin found in U.S. shelled peanuts from 1978 to 1985 (National Peanut Council, 1988).

standards for the domestic market differing from those of the export market, it is difficult to design common sampling, analysis, and acceptance levels (National Peanut Council, 1988).

Feedstuffs

The FDA has examined maximum use levels in mixed feeds of components susceptible to aflatoxin contamination, including corn, peanut meal, cottonseed meal, and cottonseed (Table 5.4) in considering possible action levels for aflatoxins in these commodities.

Economic threshold levels to minimize the impact of aflatoxin on feed efficiency and productivity can be determined from the No Observable Effect Levels (NOEL) for livestock and poultry. (The NOEL is the highest dosage of a substance administered that does not produce toxic effects.) The NOELs were determined by Edds in 1979, on the basis of available published evidence, and reported by Park and Pohland (1986).

Table 5.4 Maximum use levels in U.S. mixed feeds of components susceptible to aflatoxin contamination (adapted from Park and Pohland, 1986)

	Maximum use levels (% by weight)			
Animal	Corn	Peanut meal	Cottonseed meal	Cottonseed
Beef cattle	75	10	11	20
Dairy cattle	56	14	14	20
Swine	75	11	5	—
Layers	68	12	9	—
Broilers	66	13	10	—

These NOELs are shown in Column 1 of Table 5.5, along with productivity and efficiency effects of higher contamination levels in Column 2. These are probably conservative, in that a number of studies have shown that cattle tolerated feeds contaminated with 300 ppb or less of aflatoxins with no apparent deleterious effects; decreased feed efficiency was observed at levels greater than 700 ppb (Richard et al., 1983; Stubblefield, et al., 1981). However, corn in interstate shipment with a given acceptable level of aflatoxin for feeds must be used for the designated species.

Frequency distribution plots of aflatoxin contamination in each commodity provides a measure of the control level that will allow use of the major portion of the crop (Stoloff, 1986). If this aflatoxin level in the commodity at its maximum use level is well below the NOEL, both animal safety and economic considerations are satisfied. If at the same time, this maximum exposure to aflatoxins results in no detectable residue in the animal tissue after slaughter, the human safety consideration is satisfied. Losses in feed efficiency are not expected to result, when these NOELs and maximum use levels are met.

Because the relative prices of feedstuffs change constantly, so does feed composition, requiring knowledge of contamination levels in feed (Hesseltine, 1986).

Economic Losses in Livestock

Swine Industry

Feeding grain contaminated with mycotoxins at toxic levels can lead to significant economic losses that are largely due to chronic effects (e.g., poor feed conversions, lower productivity, and decreased resistance to infections) (Nichols, 1983).

Deoxynivalenol-contaminated corn can have detrimental effects on swine production, but has relatively little effect on beef cattle or poultry. There is some evidence that economic losses have occurred due to occurrence of deoxynivalenol in cereal grains (Vesonder and Hesseltine, 1980). Documented outbreaks of contamination of corn in the corn belt with *Fusarium* spp. (Indiana in 1958 and 1965, and northwest Ohio in 1970, 1972, 1975 and 1977) caused feed refusal and vomiting in swine, and resulted in extensive losses. In northwest Ohio in 1975, 46% of the grain sampled from four counties was contaminated, resulting in additional feed costs of $0.50 per bushel. Vesonder and Hesseltine (1988) reported that high losses were sustained and many producers faced financial disaster.

Nichols (1983) estimated losses in 1980 to hog producers in the southeastern United States, from the use of aflatoxin-contaminated feeds, at $100 million. In that year, over 13.6 million hogs were produced in the region for a value of $500 million [or 8.3% of total U.S. hog value (U.S. Department of Agriculture, 1986)]. Average losses per hog estimated by Nichols were $7.37, or 10% of the value per hog. North Carolina and Georgia, the largest swine producers, suffered losses of $28 million and $22 million, respectively. In the Southeast, the overall mortality rate was estimated at 1.5% but, in North Carolina and Georgia, mortality rates were 23%, presumably due to aflatoxins. Losses due to reduced feed conversion and productivity (weight gain) for the eight-state area were estimated at $80.0 million. Losses in 1977 totaled $60 million, with an average loss of $6 per hog.

A 3-year study by Wilson and coworkers (1984) of 54 swine herds in Georgia provided limited evidence that smaller herd sizes suffer greater economic losses. This may be a consequence of feeding from one source for longer periods. Mortality was 10% in herds of 200 or more, but was 28% in herds with 20 to 50 pigs. Thirty to 45% of the pigs in these sampled herds were unthrifty or visibly ill from consuming grain with aflatoxin levels greater than 350 ppb.

Dairy Industry

Direct estimates of economic losses to dairy producers due to aflatoxin-contaminated feed grains are not available. Losses in efficiency are not expected when aflatoxin levels in feed are less than 20 ppb (Park and Pohland, 1986). Since 1977, FDA action levels require that milk have aflatoxin M_1 concentra-

Table 5.5. Levels of aflatoxins in feed rations and their toxicological result (adapted from Park and Pohland, 1986)

Ration	Estimated No Effect Level (NOEL), ng/g feed	Toxicological effects (level in feed, ng/g; exposure, wks)		Contamination level (B_1, ng/g) to result in 0.2 ppb			
				Corn	Peanut meal	Cottonseed meal	Cottonseed
Beef cattle	200	Stunting, decreased	(200+)	1,800	14,000	12,725	14,000
Dairy cattle	300	feed efficiency	(700+)	14	54	54	38
Swine	200	liver damage	(200-400;9)	105	730	1,600	
Layer	250		(200-800;10)	325	1,835	2,445	
Broiler	—	Reduced hatchability	(600:33)	180	925	1,200	

tions of less than 0.5 ppb. Research has shown that an aflatoxin level of 30 ppb in feed will result in residue levels of less than 1 ppb in milk (Park and Pohland, 1986; Price et al., 1984; Applebaum et al., 1982). Frobish and coworkers (1986) found less than 0.5 μg/l in milk of cows given total dietary aflatoxins of 33 ppb.

Poultry Industry

Losses to the U.S. poultry industry from aflatoxins for the early 1970s were in excess of $100 million per year (Nichols, 1983). Slow weight gain and reduced feed efficiency were the principal causes. This was consistent with Hesseltine's (1986) estimate of a $143 million loss to the U.S. broiler industry in 1984. Although potentially a serious problem due to some mycotoxins, economic losses due to adverse effects on the immune system are difficult to assess and, therefore, have not been estimated.

There should be few economic losses with turkeys when aflatoxin contaminant levels in feed are limited to 100 ppb. Richard and coworkers (1986) fed aflatoxin-contaminated corn to turkeys at levels of 50 and 150 ppb, and indicated no mortality nor significant differences in weight after 11 weeks, although those fed 150 ppb showed some loss in weight gain after six weeks. Results did indicate suppressed antibody response, but no changes in feed conversion were indicated.

Economic Losses in Fermentation Products

There is no evidence that mycotoxins (except patulin in apple juice) are present in detectable levels in wines, beers, and fruit juices in the United States (Hesseltine, 1988). No studies of any economic losses due to mycotoxin-contaminated fermentation substrates are available. However, there is some evidence that the presence of some mycotoxins (e.g., T-2 mycotoxin) in the substrate can reduce fermentation efficiency and inhibit yeast efficiency (Hesseltine, 1988). However, aflatoxin-contaminated grains do not appear to interfere with fermentation efficiency (Hesseltine, 1988).

Aflatoxin-contaminated grain is used to produce ethanol. Approximately 400 million gallons of ethanol are produced annually by fermentation in the United States. Efficiency is not reduced, but mycotoxin concentrations in spent grain can make them unsuitable for animal feed without detoxification (Hesseltine, 1988). The cost of these losses is unknown.

6. Control and Management of Mycotoxins

Summary

In the United States, the aflatoxins are the only mycotoxins that are formally and specifically regulated. Aflatoxins are considered unavoidable contaminants of food and feed where Good Manufacturing Practices (GMP's) have been followed and, as such, are regulated under the Food, Drug, and Cosmetic Act [Section 402 (a) (1)]. The FDA has established specific guidelines on acceptable levels of aflatoxins in human food and animal feed by establishing action levels that allow for the removal of violative lots from interstate commerce. The action levels for human food are 20 ppb total aflatoxins, with the exception of milk, that has an action level of 0.5 ppb for aflatoxin M_1 (a metabolite of aflatoxin B_1).

For feeds, the action level for aflatoxins is also 20 ppb, with the exception of a 300 ppb action level for aflatoxins in cottonseed meal used in feeds, 300 ppb action level for corn used for finishing (feedlot) beef cattle, 200 ppb for corn destined for finishing swine (i.e., >100 lbs), and 100 ppb aflatoxins in feeds used for breeding cattle, breeding swine, and mature poultry.

To effectively monitor food and feed for a particular mycotoxin, it is important to be able to accurately estimate its concentration. However, this is very difficult to accomplish in a large quantity of material, because of the variability associated with established testing procedures. The total variability of these procedures is equal to the sum of errors associated with each step (i.e., sampling, comminuting/subsampling, and analysis of aflatoxins). Because of these errors, the true aflatoxin concentration in a lot cannot be determined with 100% certainty. The only way to achieve a better estimate of the lot concentration is to reduce the total variance associated with test results. The sampling variance can be reduced by increasing the sample size. The subsampling variance can be reduced by increasing the size of the subsample or by increasing the degree of comminution (number of particles per unit mass in the subsample). The analytical variance can be reduced by increasing the number of analyses.

Presently, a high degree of technical expertise and laboratory sophistication [e.g., thin-layer chromatography (TLC), gas and high performance liquid chromatography (GLC and HPLC), and GLC/mass spectrometry, etc.] are widely utilized for the detection and confirmation of identity of various mycotoxins. The development of rapid and field-practical screening tests for mycotoxins (e.g., black light, minicolumns, and immunoassays) has greatly facilitated control through effective monitoring programs that allow for the detection and diversion of contaminated crops and animal feeds. A variety of structure-selective techniques have emerged to the forefront of research in chemistry and immunology. These include methods of selective adsorption of multi-mycotoxins (SAM) and immunoassays. The immunoassays utilize mycotoxin-specific antibodies that can discriminate between minor differences in chemical structure. A number of commercial kits (including minicolumns, SAM detectors, and immunoassays) are currently available for the field-practical analysis of mycotoxins.

Although rigorous guidelines have been long established for the preventive management of aflatoxins in crops (i.e., recommended practices for growing/production, harvesting, handling, storage, processing/manufacturing, and sampling and analysis), significant contamination can still occur. It is important to understand that good crop management techniques and practical methods of aflatoxin detection (although significant tools) do not provide a complete solution, because aflatoxin contamination is unavoidable, and sampling problems may easily bias aflatoxin analysis. Consequently, we must also develop and utilize safe and effective procedures for the decontamination/detoxification of aflatoxin-containing food and feed.

Numerous strategies for the detoxification of aflatoxins have been proposed. These generally include methods of physical separation, thermal inactivation, irradiation, microbial degradation, and treatment with a variety of chemicals. The detoxification strategy that has received the most attention is the treatment of aflatoxin-contaminated feed with ammonia (i.e., ammoniation). The ammoniation procedure is currently being utilized in Arizona and California to reduce the parent aflatoxin levels in cottonseed products and in France, Senegal, and Brazil for the treatment of aflatoxin-contaminated peanut meal. Although ammoniation was apparently safe and effective in earlier studies, it has not been sanctioned by the U.S. Food and Drug Administration, due to the potential toxicity and carcinogenicity of reaction products. A new approach to the detoxification of aflatoxins is the addition of inorganic adsorptive compounds such as hydrated sodium calcium alumino silicate (HSCAS or NovaSil,™ Engelhard Chemicals,

Cleveland, Ohio) in the diet that possess the ability to tightly bind and neutralize aflatoxins in the gastrointestinal tract of animals. Recent studies indicate that HSCAS can prevent the adverse effects of aflatoxins in chickens and swine and decrease the level of aflatoxin M_1 residues in the milk of lactating dairy cattle. The use of HSCAS for binding of dietary aflatoxins has not yet been approved.

A cooperative effort on the part of academia, government, and industry to identify and implement state-of-the-art control and preventive management strategies to solve mycotoxin problems is clearly warranted. The effective combination of safe and field-practical tools for the detection and detoxification of mycotoxins (along with optimal methods of food and feed production, harvesting, handling, storage, and processing/manufacturing) provides an integrated approach to the control and preventive management of mycotoxins.

Regulatory Control

A broad spectrum of mycotoxins have been identified in the environment associated with human food and animal feeds, and new ones will undoubtedly be discovered in the future, because improved scientific methods for analysis and safety evaluation are constantly being developed. Presently in the United States, only aflatoxins are formally and specifically regulated. For the other known mycotoxins, the observed level, incidence, estimated consumption, and toxicological profiles have not warranted regulation by the FDA beyond the general requirements for safe and wholesome food and feeds. As new information is generated, additional controls may be justified.

In the United States, aflatoxin is considered an unavoidable contaminant in food and feed where Good Manufacturing Practices (GMP's) have been followed. The FDA has regulated aflatoxins under the Food, Drug, and Cosmetic Act, Section 402 (a) (1), which states, "A food shall be deemed to be adulterated...if it bears or contains any poisonous or deleterious substance which may render it injurious to health..." The FDA established more specific guidance on acceptable levels of aflatoxins in human food and animal feed by establishing action levels allowing for removal of a violative lot from interstate commerce (Table 6.1).

Currently, the action levels for human food are 20 ppb total aflatoxins, except for milk, which has an action level of 0.5 ppb of aflatoxin M_1. For feeds, the action level for aflatoxins is also 20 ppb, with the exception of a 300 ppb action level for aflatoxins in cottonseed meal used in feeds, 300 ppb for corn for finishing (feedlot) beef cattle, 200 ppb for corn destined for finishing swine (i.e., >100 lbs), and 100 ppb aflatoxins in feeds used for breeding cattle, breeding swine, and mature poultry.

Sampling, Sample Preparation, and Analytical Variability

Accurate estimates of mycotoxin concentrations in food products are important for quality control purposes, for research on mycotoxin control procedures, and for evaluation of the effects of a mycotoxin on animals. However, it is difficult to estimate accurately the mycotoxin concentration in a large quantity of material (lot), because of the great variability associated with the testing procedure (Whitaker et al., 1974a, 1976a, 1979). The testing procedure is a complicated process, and generally consists of 3 steps:

1. A sample is taken from the lot.
2. In the case of a granular product, the sample is comminuted to reduce particle size, and a subsample is removed from the comminuted sample for analysis.

Table 6.1 U.S. Food and Drug Administration guidelines for acceptable levels of aflatoxins in food and feed

Action level (ppb)	Commodity	Species
0.5 (Aflatoxin M_1)[a]	Milk	Humans
20.0	Any food except milk	Humans
20.0	Feed	All species
Exceptions		
300.0	Cottonseed meal used in feed	All species
300.0	Corn	Finishing beef cattle
200.0	Corn	Finishing swine (>100 lbs.)
100.0	Corn	Breeding cattle, breeding swine, and mature poultry

[a] Specifically for aflatoxin M_1, a toxic metabolite of aflatoxin B_1, that occurs in milk.

3. The mycotoxin is extracted from the subsample and quantified after separation from analytical interferences.

Dickens and Whitaker (1982) and Park and Pohland (1989) published reviews of accepted procedures for sampling and subsampling various agricultural commodities. Nesheim (1979), Schuller et al. (1976), and Scott (1989) published reviews of accepted analytical procedures to analyze various products for aflatoxins. Even when using accepted procedures, there are errors (the term "error" will be used to denote variability) associated with each of the above steps of the testing procedure. Because of these errors, the true mycotoxin concentration in a lot cannot be determined with 100% certainty by measuring the concentration in the sample taken from the lot.

The Iowa Corn Growers Association (1989) has published a task force report concerned with sampling and testing corn in the marketing system for aflatoxins. They proposed several alternate strategies for sampling and testing of corn in the marketing system because current sampling technologies are inadequate when applied to aflatoxins in corn. Most published information concerning the testing of agricultural products for mycotoxins deals specifically with aflatoxin. There is no evidence to indicate that testing food products for other mycotoxins would differ from testing for aflatoxins. Information presented here concerning aflatoxins should, therefore, apply to other mycotoxins.

Variation Among Test Results

Ten aflatoxin test results from each of 15 contaminated lots of shelled peanuts are shown in Table 6.2. Each test was made by comminuting a 5.45 kg sample in a subsampling mill developed by Dickens (Dickens and Satterwhite, 1969; Dickens et al., 1979), extracting aflatoxins from a 280 g subsample with the Association of Official Analytical Chemists (AOAC) Method II (BF method), and quantifying the aflatoxins densitometrically using thin layer chromatography (TLC) (AOAC, 1984). The 10 test results from each lot are ranked from low to high to demonstrate several important characteristics about replicated aflatoxin test results taken from a contaminated lot.

The distribution of the test results for each lot in Table 6.2 are not symmetrical about their mean (Whitaker et al., 1972). The distributions are positively skewed, meaning that more than one-half of the test results are below the mean. This skewness can be observed by counting the number of test results above and below the mean in Table 6.2. The mean of the ten test results is the best estimate of the lot concentration. If a single sample is tested from a contaminated lot, there is more than a 50% probability that the test result will be lower than the true lot concentration. The skewness is greater for small sample sizes, and the distribution becomes more symmetrical as sample size increases (Remington and Schrok, 1970). Also, for a given sample size, the distribution of test results becomes more symmetrical as the lot aflatoxin concentration increases (Table 6.2).

The wide range among replicated test results from the same lot is reflected in the large variance values and coefficients of variation (CV) shown in Table 6.2. The variance appears to be a function of aflatoxin concentration in the lot. As the lot concentration increases, the variance among test results increases, but the relative variance, as measured by the CV, decreases as the lot concentration increases. The variability shown in Table 6.2 is the sum of errors

Table 6.2 Replicated test results for ten 5.45 kg samples from each of twelve contaminated lots of shelled peanuts

Lot number	Observed aflatoxin test results (ppb)										Mean (ppb)	Variance	CV[a] (%)
1	0	0	0	0	0	0	0	6	10	14	3.0	26.9	172.9
2	0	0	0	0	2	4	8	14	28	43	9.9	214.8	148.0
3	0	0	0	0	0	0	0	16	40	69	12.5	561.6	189.6
4	0	0	0	0	0	3	8	26	52	70	15.9	647.2	160.0
5	0	0	0	0	3	13	19	41	43	69	18.8	588.4	129.0
6	0	0	3	12	12	12	12	25	63	103	24.2	1093.7	136.7
7	0	0	3	4	4	5	15	60	106	165	36.2	3249.7	157.5
8	0	0	32	32	34	37	55	67	77	134	46.8	1563.3	84.5
9	0	3	5	19	32	49	87	91	127	168	58.1	3353.4	99.7
10	4	7	40	41	55	60	75	95	99	230	70.6	4177.2	91.5
11	0	4	6	17	36	80	133	148	192	216	83.2	6871.7	99.6
12	18	50	53	72	82	108	112	127	182	191	99.5	3168.8	56.6

[a]Coefficient of variation.

associated with each step of the testing procedure. The total variance is equal to the sum of the sampling variance, subsampling variance, and analytical variance.

Sampling Variability

Studies by Whitaker and coworkers (1974a, 1976b, and 1979) on three granular products, peanuts, cottonseed, and shelled corn, indicate that sampling, especially for small sample sizes, is the largest source of the three errors. Sampling error should be small to negligible for small particulate processed products, such as flour or liquid products. Sampling error is high in the larger granular products, because aflatoxins may be found in only a small percentage of the kernels in the lot (less than 0.1%) (Whitaker and Wiser, 1969), but the concentration in a single kernel may be extremely high. Cucullu and coworkers (1966, 1977) reported aflatoxin concentrations in excess of one million ppb for individual peanut kernels, and five million ppb for individual cottonseeds. Shotwell et al. (1974) reported finding over 400,000 ppb of aflatoxins in a corn kernel. Because of this extreme range in aflatoxin concentrations among individual kernels in a contaminated lot, variation among replicated samples tends to be large. The sampling variance associated with raw peanut kernels, cottonseed, and shelled corn for a given sample size was estimated empirically by Whitaker and colleagues (1974a, 1976b, 1979). The above studies indicated that the sampling variance for all three products is a function of the lot concentration. They developed equations to predict the sampling variance as a function of the lot concentration and any size sample for the three products listed above. These three variance equations mentioned above are specific for the type product and the average kernel size (count per gram) of the product used in the study.

Subsampling Variability

Once the sample has been taken from the lot, the sample must be prepared for aflatoxin extraction. Because it is not practical to extract the aflatoxins from a large sample, the aflatoxins are usually extracted from a much smaller portion of product (subsample) taken from the sample. If the material is a granular product such as shelled corn, it is essential that the entire sample be comminuted in a suitable mill before the subsample is removed from the sample (Dickens and Whitaker, 1982). Removing a subsample from the sample before the comminution process would eliminate the benefits associated with the larger size sample of granular product.

After the sample has been comminuted, a subsample is removed for aflatoxin extraction. Presumably, the distribution of contaminated particles in the comminuted sample is similar to the distribution of contaminated kernels found in the lot. As a result, there is also variability among replicated subsamples taken from the same sample. However, if the number of particles in the subsample, as determined by degree of comminution and sample size, has been increased over the number of particles in the sample and the comminuted sample has been well mixed, the subsampling variance will not be as large as the sampling variance.

The subsampling variance for peanuts, cottonseed, and shelled corn, for a given subsample size and milling procedure, was estimated empirically (Whitaker et al., 1974a, 1976a, 1979). The above studies indicated that the subsampling variance for all 3 products is a function of the aflatoxin concentration in the sample. Equations were developed to predict the subsampling variance for the specific milling procedure as a function of the aflatoxin concentration and any size subsample for the 3 products listed above. The 3 subsampling variance equations are specific for the type product and for the particular mill used in the study to comminute the samples. The Dickens mill with a 3.18 and 1.59 mm screen were used to comminute the peanuts and cottonseed, respectively, while a Willey mill with a 1 mm screen was used to comminute shelled corn. Increasing the degree of comminution (more particles per unit mass) will decrease the subsampling variance for a given subsample size.

Analytical Variability

Once the subsample is removed from the comminuted sample, the aflatoxins are measured by accepted methods (AOAC, 1984; Nesheim, 1979; Schuller et al., 1976). These methods are fairly complicated, involving several steps such as solvent extraction, centrifugations, drying, dilutions, and quantification. As a result of errors associated with each step, there is considerable variation among replicated analyses on the same subsample extract. Whitaker and coworkers (1974b, 1976b, 1979) determined empirically the analytical variance associated with the BF method (AOAC Method II) used to extract aflatoxin from peanuts, with the CB method (AOAC Method I) used to extract aflatoxins from corn, and with the Velasco method used to extract aflatoxins from cottonseed. The above studies indicated

that the analytical variance for all three methods is a function of the aflatoxin concentration of the subsample. Whitaker and associates (1974a, 1976b, 1979) also developed equations to predict the analytical variance, as a function of the subsample concentration, and any number of analyses for the three methods listed above.

Additional studies by Whitaker and Dickens (1981) on the BF method indicate that the TLC quantification step is the major source of variability in the analytical process associated with testing peanuts for aflatoxins. While studies have not been conducted on other commodities, the above results with peanuts probably apply to corn and cottonseed also. Improved analytical methods, such as HPLC, can significantly reduce the analytical variance within an individual laboratory (Dorner and Cole, 1988), but have no effect on variance among laboratories.

Reducing Variability of Test Results

It is more difficult to estimate with a high degree of confidence the true concentration of aflatoxins in a lot than it is to classify a lot as being good or bad, depending on whether or not the lot concentration is below or above a defined tolerance. (The only exception to the above statement exists when the aflatoxin concentration approaches the value of the tolerance.) For example, it is easier to determine if a lot with 100 ppb is less than or greater than the tolerance of 20 ppb than it is to determine if the lot concentration is 100 ppb. The only way to achieve a more precise estimate of the true lot concentration is to reduce the total variance associated with test results. The sampling variance can be reduced by increasing the size of the sample. The subsampling variance can be reduced either by increasing the size of the subsample or by increasing the degree of comminution (increasing the number of particles per unit mass in the subsample). The analytical variance can be reduced by increasing the number of analyses. For example, if the sample size is doubled, then the sampling variance is cut in half (Walpole, 1974). This relationship is also true for reducing the subsampling and analytical variance components. Doubling sample size has a greater effect on decreasing the variability at small sample sizes than on large sample sizes. This characteristic suggests that increasing sample size beyond a certain point may not be the best use of resources, and that increasing subsample size or number of analyses may be a better use of resources in reducing the total variability of test results. Because different costs are associated with each step of the aflatoxin testing procedures, careful study is needed to determine the optimum balance in the sample size, the degree of sample comminution, the size of the subsample, and number of analyses needed to achieve a given precision. All of these factors must be considered, and they each contribute to variances among laboratories conducting tests.

Designing Aflatoxin Testing Programs

In a regulatory environment, if the estimated lot concentration exceeds a defined contamination level, then the lot is removed from the food chain. Because of the large variability among test results, two types of mistakes are associated with any aflatoxin testing program. First, good lots (lots with a concentration less than or equal to the tolerance) will test bad and be rejected by the testing program. This type of mistake is often called the processor's risk, since these lots will be rejected at an unnecessary cost to the processor. Secondly, bad lots (lots with a concentration greater than the tolerance) will test good and be accepted by the testing program. This type of mistake is called the consumer's risk, since contaminated lots will go into the food chain posing a possible health hazard to the consumer. To maintain an effective quality control program, the above risks associated with a testing program must be evaluated. Based upon these evaluations, the costs and benefits (benefits refers to removal of aflatoxin-contaminated lots) associated with testing programs can be evaluated.

The magnitude of the processor's and consumer's risk is uniquely defined for a particular testing program with designated values of a sample size, subsample size, and number of analyses and the accept level. As a result, increasing the sample size decreases both the processor's and consumer's risk. The same effect can be obtained by increasing either the degree of sample comminution, subsample size, or number of analyses. Changing the accept level also affects the magnitude of the processor's and consumer's risks. Reducing the accept level relative to the control level decreases the consumer's risk, but increases the processor's risk. Increasing the accept level relative to the control level decreases the processor's risk, but increases the consumer's risk. Only one of the two risks can be reduced by changing the accept level of the testing program relative to the control level, because reducing one risk will automatically increase the other risk. Whitaker and associates (1974b, 1976b) developed methods to predict the processor's and consumer's risk, the total number of lots accepted and rejected, the

amount of aflatoxins in the accepted and rejected lots, and the costs associated with an aflatoxin testing plan for shelled peanuts, cottonseed, and shelled corn. These methods have been used by the peanut industry to design aflatoxin testing programs for shelled peanuts (Whitaker and Dickens, 1979).

Selecting Samples

All of the above discussions assumed that there are no selection biases associated with drawing the sample from the lot. If the lot has been blended thoroughly from the various material handling operations, then the contaminated particles are probably distributed uniformly throughout the lot. In this situation, it is probably not too important from what location in the lot the sample is drawn. However, if the lot has been attacked by mycotoxin-producing fungi because of moisture leaks or for other reasons, then mycotoxin-contaminated particles may be located in isolated pockets in the lot (Shotwell et al., 1975). If the sample is drawn from a single location, the contaminated particles may be missed or too many contaminated particles may be collected. Because the contaminated particles may not be distributed uniformly throughout the lot, the sample should be an accumulation of small portions taken from many different locations throughout the lot (Bauwin and Ryan, 1982; Hurburgh and Bern, 1983).

It is generally more difficult to obtain a representative sample from a lot at rest (static lot) than from a moving stream of the product. Examples of static lots are products contained in storage bins, railcars, or many small containers such as sacks. When drawing a sample from a static lot, a probing pattern should be developed so that product can be collected from different locations in the lot. The sampling probe should be driven to the bottom of the container when possible. As a general rule, about 0.5 kg of sample should be drawn per 1,000 kg of product.

When sampling a static lot contained in separate containers such as sacks, the sample should be taken from many containers dispersed throughout the lot. The recommended number of sacks sampled can vary from one-fourth of the sacks in small lots to the square root of the number of sacks for large lots (U.S. Department of Agriculture, 1975).

If the lot is in a container where access is limited, the sample should be drawn when the lot is either being removed from or being placed into the container. If the accumulated sample for any sampling method is larger than required, the sample should be thoroughly blended and reduced to the required size with a suitable device such as a riffle divider.

Sampling a moving stream of product often reduces the selection biases associated with sampling a static lot. The sample should be taken as the material leaves the conveyance system, such as at the end of the conveyer belt. The quantity of material removed each time should be small, so as not to accumulate too large a sample. Commercial samplers are available that cut through the entire stream of feed at predetermined intervals. Cross-cut type samples should:

1. Have a sampling cup that moves through the entire stream at a constant velocity.
2. Have a sampling cup with an opening that is perpendicular to the stream flow.
3. Have a sampling cup with an opening that is 1.5 to 3 times the size of the largest particle in the lot.

If more sample is collected than desired, the sample should be thoroughly blended and the required amount of sample removed.

Analysis

Laboratory Procedures

Because little can be done to control many of the biological and climatic factors that contribute to mycotoxin appearance in agricultural commodities, detection, removal, and diversion are prudent means for preventing the entry of mycotoxins into the food chain (Figure 6.1). Mycotoxin detection in foods and feeds is a difficult problem, because of the complexity of agricultural commodities that are affected (Bullerman, 1978). Research efforts on mycotoxin analysis typically involve cooperative efforts between government agencies such as the USDA, FDA, and various state laboratories and are coordinated by societies such as the Association of Official Analytical Chemists, Association of Cereal Chemists, American Oil Chemist's Society, and the International Union for Pure and Applied Chemistry. These latter societies judge the suitability and reproducibility of a method by conducting collaborative studies whereby a number of different analysts carry out the specific procedure on identical samples. Generally, mycotoxin analytical methods involve the sampling, extraction, cleanup, and quantitation (Figure 6.2). Although most approaches are laboratory oriented, a number of qualitative field-practical methods of analyses have been utilized.

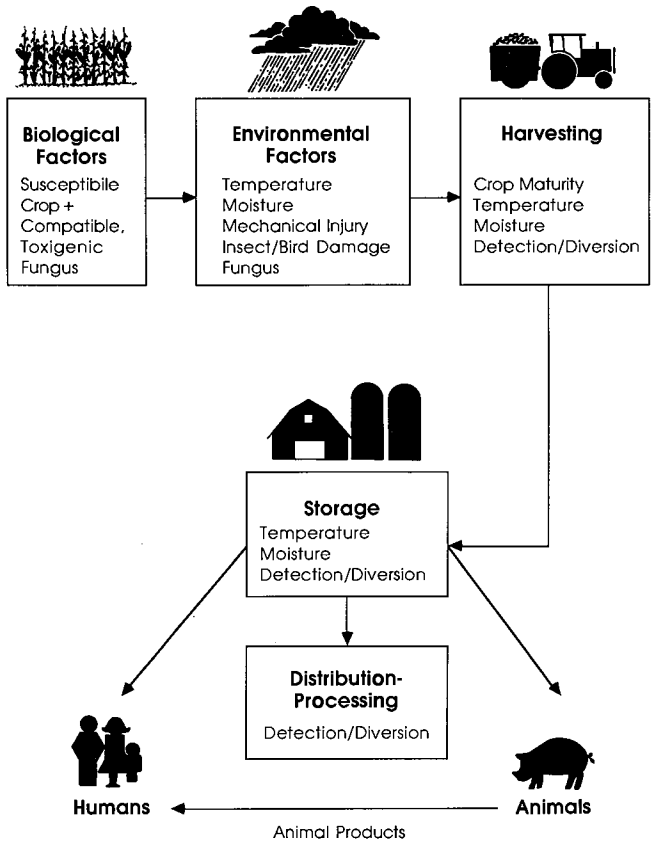


Figure 6.1. Factors affecting mycotoxin occurrence in the food chain (Pestka and Casale, 1989).

Extraction and Cleanup

Extraction methods differ for each commodity and mycotoxin that needs to be tested and they generally involve the use of one or more solvents. Because interfering materials, such as lipids and pigments, may be extracted by the solvent, these must be separated from the analyte before quantification. This can be achieved by reextraction with another solvent, precipitation, or chromatography. The cleanup step may not be required with the recently developed immunoassays.

Quantitation in the Laboratory

Initial screening for suspect mycotoxins may be carried out by animal challenge studies. Besides experimental farm and laboratory animals, various other live test systems such as brine shrimp and cell culture have been used successfully. Generally, these biological methods are confined to the research laboratory, because they generally lack specificity for identifying a specific mycotoxin. Physico-chemical methods are preferred for positive quantitation and confirmation of identity (Stoloff and Scott, 1984).

The most widely used physico-chemical method employed in the laboratory for final isolation and quantitation of the toxin is TLC. Here, a concentrated extract is applied to a thin layer of silica gel or similar material, and the components of the applied extract are separated by allowing a suitable solvent mixture to migrate along the plate carrying the different components with it at different rates. Mycotoxins can be detected visually by their inherent fluorescence or by color development after spraying with a chromogenic reagent. Resolution and quantitation can be greatly improved, in some instances, by employing instrumental methods such as GLC and HPLC. In the past two decades, advances have been made whereby TLC, GLC, and HPLC can be coupled with mass spectrometry and, thus, enable an analyst to obtain a fragmentation pattern of a putative mycotoxin for confirmation of identify. This confirmation of identity of a mycotoxin is needed when the commodity contains analytical interferences or the mycotoxin is being encountered under unique circumstances, and should

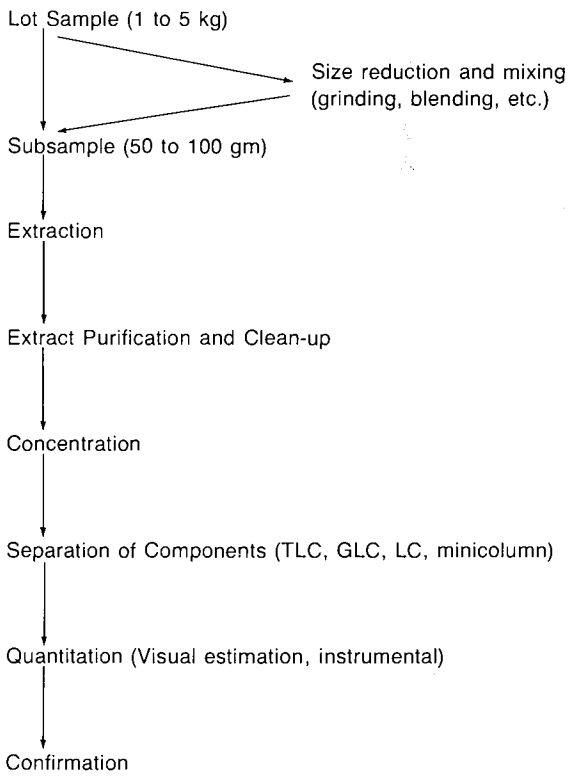


Figure 6.2. Diagram of general steps involved in analysis and quantitation of mycotoxins from agricultural commodities (Bullerman, 1978).

be useful during litigation procedures.

Rapid, Field-practical Procedures

The above described physico-chemical laboratory methods have certain limitations for the routine analysis of mycotoxins. As mentioned above, because of interfering chemicals in diverse commodities such as corn, wheat, cottonseed, and milk, time-consuming cleanup steps are required. Instrumentation requirements can range from several thousand dollars for TLC to hundreds of thousands of dollars for mass spectrometry. The end result is that lengthy and costly procedures can severely limit the number of samples that can be analyzed routinely. In many situations, rapid methods of analysis are more appropriate. For example, buyers of agricultural commodities (such as grain elevators) require screening tests that can detect aflatoxins within 15 to 30 minutes. Three available types of rapid methods for aflatoxins that approach this problem are long wave UV light (black light), small glass column (minicolumn), and immunoassay.

The black light screening method for aflatoxins in corn is based on bright greenish-yellow fluorescence produced by a corn metabolite of kojic acid. The kojic acid is produced by the same fungi that produce aflatoxins (Bullerman, 1978). Exposure of the commodity to long-wave ultraviolet light (black light) induces this fluorescence and, thus, indirectly indicates the potential presence of aflatoxins. Because this technique is non-specific for aflatoxins, it is best to use one of the more recently developed immunological screening methods (see later) or to use the black light test as a pre-screen for subsequent analytical confirmation procedures.

Other practical field methods for aflatoxins are based on minicolumns that contain various absorbent materials (Stoloff and Scott, 1984). In the classical minicolumn procedure, a solvent extract of the sample is applied to the column, the mycotoxin is moved onto the column with solvent, and mycotoxins such as aflatoxin can be detected as a narrow fluorescent band when viewed under UV light. The method is semi-quantitative, and can detect aflatoxins in the low ppb range, making it adequate for screening at the FDA action level. The minicolumn is only applicable to the detection of fluorescent mycotoxins. A new approach designated as SAM (selectively absorbed mycotoxins) has been described, whereby multimycotoxin mixtures (e.g., aflatoxins and zearalenone) can be resolved, selectively immobilized at target interfaces on a tapered glass column, and simultaneously detected as separate fluorescent bands (Phillips, 1987). Also, the SAM procedure is capable of detecting nonfluorescent mycotoxins, such as the trichothecenes, and has recently been configured for detection of deoxynivalenol.

It is now possible to use immunoassays, procedures common to the clinical laboratory, for the screening of mycotoxins in agriculture commodities (Pestka, 1988). The basis for most mycotoxin immunoassays involves competition between a sample or standard mycotoxin and a labeled mycotoxin (usually an enzyme label) for an antibody-binding site. In some cases, antibodies can be coupled in immunoaffinity columns and used to purify mycotoxins for subsequent analysis. Antibodies are proteins produced in an animal as a specific response to challenge with a foreign material, such as a mycotoxin-protein conjugate. Antibodies exhibit varying degrees of specificity and, thus, may not be able to discriminate minor differences in chemical structure. Libraries of polyclonal and monoclonal antibodies to aflatoxins, zearalenone, trichothecenes, ochratoxins, and other mycotoxins are available to directly analyze liquids, such as milk or solid samples, after a sample solvent extraction. A number of commercial kits (including minicolumns, SAM, and immunoassays) are currently on the market that enable an analyst to rapidly assay a sample for aflatoxins (Table 6.3) or selected mycotoxins.

Although many different techniques are available for analysis of specific trichothecenes, simultaneous analysis of all trichothecenes and confirmation of exposure is not an easy task. Because there is a multiplicity of trichothecenes that could occur in commodities and animal tissues and fluids, an approach to their analysis is to chemically convert the trichothecenes and their metabolites to four basic parent alcohol skeletons (Swanson, 1989, unpublished data). Thus, only four standards are necessary in the analysis of total trichothecenes within a sample.

Prevention

Production

Cultural Practices

Crop Residues. Crop residues often provide the primary inoculum for mycotoxigenic fungi when susceptible crops are grown. The fungi that are present in the crop residues may produce spores early in the season. In the case of ergot, the sclerotia in crop residues may germinate and provide the primary inoculum. *Aspergillus flavus* sclerotia or mycelium may be present in the soil or crop residue, and the sclerotia may germinate and provide early *A. flavus* inoculum, increasing the probability of aflatoxin

Table 6.3. Commercially available aflatoxin test kits (Lee, 1989)

Test kit	Analysis	Type of test	Level of Detection (ug/kg or ppb)	Analysis time[a] (min/sample)	Application	Manufacturer
Aflatest-P	B_1,B_2,G_1,G_2, M_1	Affinity column[b]	2 0.1 (M_1)	7	Instrumental, quantitative, fluorometer, HPLC	VICAM 29 Mystic Ave. Somerville, MA 02145 (617) 623-0030 (800) 338-4381
AflaQuick	B_1,B_2	Affinity column[b]	2	5		
AgriScreen[a,c]	B_1,B_2,G_1,M_1	ELISA[b], microtiter wells	1 0.2 (M_1)	12	Visual and Instrumental, semiquantitative, quantitative	Neogen Corp. 620 Lesher Place Lansing, MI 48912 (517) 372-9200
Afla-20[d]	B_1,B_2,G_1	ELISA[b], cup	20	4	Visual pass/fail	International Diagnostic System Corp. P.O. Box 799 St. Joseph, MI 49085 (616) 983-3122
Afla-10			10			
IDEXX-AFB	B_1,B_2,G_1	ELISA[b], micro-titer wells	3	45	Instrumental, semiquantitative	IDEXX 100 Fore Street Portland, ME 04101 (207) 774-4384 (800) 548-6733
CITE-Probe-aflatoxin	B_1,B_1	ELISA[b], probe	20	3	Visual, pass/fail	
EZ-SCREEN: aflatoxin	B_1,B_2,G_1	ELISA[b], Card[e]	20	7	Visual, pass/fail	Environmental Diagnostic Systems Corp. P.O. Box 908 2990 Anthony Road Burlington, NC 27215 (919) 226-6311 (800) 334-1116
Total aflatoxins	B_1,B_2,G_1,G_2	Affinity column[b]	1	30	Visual (with UV viewer), semiquantitative	Oxoid U.S.A., Inc. 9017 Red Branch Rd. Columbia, MD 21045 (301) 997-2216 (800) 638-7638
Aflatoxin M_1	M_1	Affinity column[b]	<0.1	30	Visual (with UV viewer), semiquantitative	
Aflatoxin test	B_1,B_2,G_1,G_2	ELISA[b], microtiter	1	30	Semiquantitative	Transia 8, rue Saint-Jeau-de-Dien 69007 Lyon, France 72-73-03-81
Aflatoxin M_1 test	M_1	ELISA[b], microtiter	0.01	40	Semiquantitative	
SAM-A	B_1,B_2,G_1,G_2	Selective adsorption[f]	10	10	Pass/fail	Rialdon Diagnostics 3609 E. 29th St. Bryan, TX 77802 (409) 846-6202 (800) 888-5688
SAM-AZ	B_1,B_2,G_1,G_2 (zearalenone)	Selective adsorption[f]	10 500	10	Pass/fail	
HV Minicolumn	B_1,B_2,G_1,G_2	Minicolumn	20	10	Pass/fail	Romer Labs, Inc. P.O. Box 2095 Washington, MO 63090 (314) 239-3009

[a]Does not include sample preparation and extraction.
[b]Immunochemical methods; affinity column or ELISA (enzyme-linked immunosorbent assay).
[c]Adopted AOAC Official 1st Action for screening for aflatoxin B_1 in cottonseed products and mixed feed; adopted AOAC interim Official 1st Action for screening for aflatoxin B_1 in corn and peanut butter.
[d]Adopted AOAC interim Official 1st Action for screening for aflatoxins B_1, B_2 and G_1 in corn, peanut butter, poultry feed, cottonseed, and raw peanuts.
[e]Three card system available: one sample/card or five sample/card at 20 ppb or one sample/card at 5 ppb.
[f]Modified Holaday-Velasco minicolumn (AOAC method 26.020-26.025).

contamination (Wicklow and Wilson, 1986). The primary inoculum of many *Fusarium* species and other mycotoxigenic fungi may come from crop residues. Thus, management of crop residues may be an important factor in lowering mycotoxin contamination in the field. In the case of *A. flavus* and peanuts, the fungus accumulates in the soil during the growing season, so that at harvest large numbers of propagules can be found in the soil rhizosphere.

Irrigation and Mineral Nutrition. Preharvest aflatoxin contamination of both corn and peanuts is associated with severe and prolonged late season drought. A series of studies utilizing environmental control plots has shown that undamaged peanuts grown in the absence of drought stress do not contain significant amounts of aflatoxins (less than 1 ppb). Field studies have shown that adequate calcium nutrition in peanuts can minimize aflatoxin contamination (Cole et al., 1985b). However, calcium uptake requires soil moisture and, therefore, is severely impeded during drought conditions. A highly controlled plot experiment demonstrated that application and uptake of calcium by the peanut seed could not totally prevent preharvest aflatoxin contamination when peanuts were subjected to stress conditions ideal for aflatoxin contamination.

Crop Rotation. Crop rotation historically has been a useful method of diminishing the impact of plant disease on crops. The use of crop rotations to lower primary inoculum of mycotoxigenic fungi, whether they are *Claviceps, Fusarium, Alternaria,* or *Aspergillus* species, can be of benefit, but crop rotations only supplement other management, production, and control practices.

Resistant Varieties

Genetic resistance to mycotoxigenic fungi could be based on resistance to fungal infection or resistance to growth after invasion, based on inhibition of mycotoxin synthesis. There are differences in host susceptibility to ergot, as well as to many *Fusarium* diseases, and resistant varieties should be used whenever possible (Frederiksen, 1986; Shurtleff, 1980; Wiese, 1977). However, little is known about possible genetic resistance to some *Fusarium* spp., *A. flavus,* or the fungi that produce ochratoxins, so research efforts in these areas are critically needed. Heritable differences in *A. flavus* susceptibility in corn and peanuts have been reported, but genotype development is only in the very early stages. In peanuts, the so-called "resistant" varieties have not been effective under field conditions conducive for preharvest contamination. Presently, there are no genotypes known to inhibit mycotoxin biosynthesis.

Environmental Factors Favoring Preharvest Mycotoxin Contamination

Infection of grain flowers by *Claviceps purpurea* is relatively easy. The fungus can infect the host only during the flowering stage. Sclerotia are stimulated to germinate and produce conidia by low soil temperatures and wet climatic conditions. To establish infection, the wind-dispersed conidia must be produced during the flowering season. Ergot occurs worldwide, and many different grains and grasses are susceptible. Cool wet spring seasons that prolong the flowering period increase the probability of infection, and warm summers favor the maturation of sclerotia. Insects and animals are often vectors of the conidia that cause secondary infections (Marasas and Nelson, 1987).

Preharvest infection by the *A. flavus* group and aflatoxin contamination occur worldwide. *Aspergillus flavus* may survive by colonizing organic matter in the soil or by producing sclerotia. The sclerotia germinate, the organism grows, and conidia are produced. *Aspergillus flavus* conidia may reside in the soil or be dispersed by wind or insects; infection may take place anytime after flowering. Infection by *A. flavus* does not necessarily mean that aflatoxin contamination will occur, because many factors contribute to aflatoxin contamination. The most important factor is water availability to the plant (Dorner et al., 1989).

Aflatoxin contamination of cottonseed, walnuts, and almonds in the western United States is favored by insect damage and extended periods with temperatures above 32°C (90°F). Both pink bollworm and stinkbug damage predispose cottonseed to aflatoxin contamination. Late irrigation of cotton increases bollworm damage and *A. flavus* infection. Insect-damaged almonds and walnuts in California may be colonized by *A. flavus,* and may contain aflatoxins.

In peanuts, two types of preharvest aflatoxin contamination occur. One is associated with insect damage and is easy to explain; insect damage provides a portal of entry for contamination by the aflatoxin-producing fungus. However, infection and contamination also occurs when the kernels are not visibly damaged, and obviously these contaminated kernels are more difficult to detect and remove during the milling process. In the latter situation, the fungi apparently gain entrance into the peanut kernel prior to drought stress, but remain dormant or inactive as the result of resistance mechanisms (phytoalexins) of the peanut. However, during times of drought stress, there is moisture loss from the peanut plant which

reduces the water activity of the peanut to the point that it no longer is capable of phytoalexin production. Thus, the defense mechanism is reduced or lost, and the opportunistic fungus grows and produces aflatoxins until moisture becomes a limiting factor for the fungus. Although irrigation of the crop is beneficial (Wilson and Stansell, 1983), it is not possible to produce peanuts exclusively under irrigation.

Corn grown in the U.S. corn belt is infrequently colonized by *A. flavus,* whereas corn grown in the southeastern United States is frequently heavily colonized and often contaminated with aflatoxins in the field. However, when the corn belt weather is excessively dry and warm during the latter part of the growing season (e.g., the 1988 growing season), *A. flavus* infection and aflatoxin contamination occurs. Corn silks can be invaded directly by *A. flavus* when temperatures are about 30°C (Payne, 1983), resulting in aflatoxin contamination of undamaged kernels, but *A. flavus* is more likely to colonize damaged kernels. Damage by the corn ear worm creates favorable habitats for *A. flavus* to thrive in corn ears, but damage by the maize weevil in the field is more likely to enhance aflatoxin concentrations.

Corn is especially prone to contamination during extended periods of drought conditions. Studies over a six-year period in Georgia revealed positive correlations between insect damage, aflatoxin amounts, and visible sporulation of *A. flavus* on corn ears, as well as positive correlations between aflatoxins, air temperature, and net evaporation. Moisture is the single most important factor influencing preharvest *A. flavus* infection and aflatoxin contamination in crops, but there is also a moisture/temperature interaction (Wilson, 1988).

Infection of crops by *Fusarium* species generally is favored by cool, wet conditions during flowering through maturation. Because there are many different *Fusarium* species capable of producing several mycotoxins in the field, it is difficult to predict contamination based on environmental factors. *Fusarium sporotrichioides* and *F. poae* are weakly pathogenic. These fungi develop saprophytically, and produce mycotoxins when crops are overwintered in the field or during storage of excessively wet grain in cold climates.

Fusarium graminearum causes a pink or red ear rot of corn in the field, and the fungus can also grow during storage. Infection by *F. graminearum* is enhanced by cool wet conditions during corn silking. Most field outbreaks of estrogenic syndrome and/or feed refusal in swine are associated with corn harvested wet and stored at moderate or low temperatures.

Infection of grain by *F. moniliforme* is common worldwide, and there is much concern about possible mycotoxin problems that may be associated with this fungus. However, taxonomy of *Fusarium* is difficult, and other *Fusarium* species are sometimes incorrectly identified as *F. moniliforme*. *Fusarium moniliforme* can cause ear rots of corn, head blight of grain sorghum, and seed rots of many grains. Rots caused by *F. moniliforme* are favored by prolonged periods of wet weather before flowering. *Fusarium* diseases are generally aggravated by insect damage (Marasas and Nelson, 1987).

Invasion of grains by *Alternaria* species is favored by the same environmental conditions that favor *Fusarium* invasion (Frederiksen, 1986; Shurtleff, 1980; Wiese, 1977).

Harvesting

Timeliness

Grain and oilseed crops that are susceptible to fungal deterioration should be harvested at their optimum maturity when the moisture content is lowest. Peanuts are indeterminant plants, and should be harvested when the greatest percentage of peanuts are mature. Many crops are subject to deterioration in the field if harvest is delayed, and delay may influence mycotoxin contamination.

Peanuts under drought stress left in the field waiting for precipitation become increasingly more contaminated if precipitation does not occur. If precipitation occurs, most contaminated peanuts will be left in the soil at harvest, because the stem connecting the peanut to the plant becomes deteriorated.

Cleanup

Damaged portions of crops should be removed during the harvesting process, whether the damage is caused by physical or biological phenomenon. Combines should be adjusted to remove broken and damaged material, as well as crop and weed residues. Care should be taken to remove foreign material and high moisture plant parts during harvesting. The risks of further mycotoxin contamination can be lowered with careful harvesting procedures. Seed broken by malfunctioning or improperly adjusted harvesting equipment allows for mold infection; besides, the producer is penalized when such grain is sold.

Drying

Grain and oilseed crops should be dried, as soon as possible, to moisture levels that do not allow fungal

growth. The final safe moisture content depends on the crop and the climatic conditions where the commodity is stored.

Storage

Moisture and Insect Control

Avoiding mycotoxin accumulation in stored grains and oilseeds depends primarily on moisture control. If the product is too dry to allow fungal growth, and if it is kept dry, no further deterioration will occur. However, if there is insect activity, rodent activity, moisture migration, condensation or water leaks in storage, then fungal growth that could lead to mycotoxin contamination will occur. Insect control is important, because insect activity in stored products creates favorable microclimates for fungal growth. Once fungal growth starts, the water of metabolism from the fungus will provide sufficient water for further growth and mycotoxin development (Marasas and Nelson, 1987).

Mycotoxin management in fermented products like sausages and cheese may depend upon refrigeration. Controlled atmosphere storage also could prevent fungal growth and mycotoxin formation in many products.

Antifungal Agents

Antifungal agents of low toxicity should be used to supplement good management practices, rather than as a substitute for safe clean handling practices of mixed feeds. Fungal growth and mycotoxin contamination of high moisture grains can be prevented with propionic acid or mixtures of propionic and acetic acids. Grain treated with these acids is somewhat corrosive, and this can create problems when handling treated grains or feeds. Liquid sprays are generally more effective than dry formulations of propionic acid, but the dry formulations are easier to use. Products such as gentian violet (GV11) and sorbic acid may be useful in controlling mycotoxin buildup in mixed feeds in large animal or egg production facilities (Hamilton, 1985; Tabib and Hamilton, 1988). However, antifungal agents should be used only where there is also control of moisture in the feeds, frequent movement of feed to avoid deterioration in the feed delivery system, and frequent cleaning to remove deteriorated feed.

Processing and Manufacturing

Damaged Product and Risk Categories Removed

Good Manufacturing Practices (GMPs) in the processing of agricultural products into human foods and animal feeds involve careful selection of the commodities and good quality control practices through each step from the source to final preparation of the food or feed. Warm and humid environments raise the likelihood of fungal growth, creating a visible sign that further investigation of the situation is warranted.

Commodities should be checked for visible signs of damage, because damage to a nut, kernel, or oilseed indicates a high probability of mold growth and possible mycotoxin contamination. Because of this, damaged product or damaged separable portions of a lot should be removed during processing as a normal result of good management practice to minimize mycotoxin residues.

For example, GMPs for peanuts require the removal of damaged off color kernels and shrivelled nuts, because they are most likely to contain aflatoxins. Kernels removed during processing are frequently crushed for oil, because any aflatoxins in the oil are destroyed or removed during the refining process. Similarly, shellers and peanut product manufacturers use electronic color sorting devices after roasting to remove additional discolored peanuts that may contain aflatoxins. Some manufacturers also remove the skins, a process called blanching, followed by electronic sorting to facilitate the detection of off color kernels and making electronic sorting more effective.

Strict Quality Control

The commodities that are susceptible to mold growth and mycotoxin contamination are monitored in varying degrees, based on experience with a given crop and its environment/geography. Periodic general surveys of food and feeds by manufacturers and government bodies have evaluated the adequacy of the present controls. In some commodities, such as peanuts, processors and manufacturers follow strict quality control standards. For raw peanuts, analyses are performed to confirm that the nuts meet the standards of the Peanut Advisory Council (PAC), the administrator for the aflatoxin control program for U.S. peanuts for the USDA under provisions of the USDA Peanut Marketing Agreement. Additional analyses are frequently

performed to confirm that the levels of aflatoxin contamination are below those required by the FDA aflatoxin action level before the product is used for food and feed purposes.

Detoxification Strategies

Although guidelines have been long established to prevent mycotoxin contamination in crops, i.e., recommended practices for growing, harvesting, handling, and storage, significant contamination may still occur (Anderson, 1983; Goldblatt, 1971; Goldblatt and Dollear, 1969; U.S. Department of Agriculture, 1968a, 1968b). The recent developments of rapid and inexpensive tests to detect mycotoxins (as previously discussed) have allowed for monitoring programs that facilitate the diversion of contaminated crops and animal feeds from the food and feed supply. However, good crop management techniques and practical methods of mycotoxin detection do not provide a complete solution, since contamination often is unavoidable and sampling problems may bias detection. Consequently, safe, effective, and practical methods to control mycotoxins are needed.

Numerous strategies for the detoxification of mycotoxin containing food and feed have been reported. The reader is referred to several excellent reviews on the subject for details (Anderson, 1983; Diener et al., 1979; Goldblatt and Dollear, 1977, 1979; Marth and Doyle, 1979; Palmgren and Hayes, 1987). There is considerable information, both theoretical and practical, on the detoxification of aflatoxins; however, information on detoxification of other mycotoxins is limited. Representative studies on aflatoxin contaminated food and feed are outlined below. These studies have been categorized as either physical, biological, or chemical in their basic approach to detoxification.

Physical Methods of Separation

Mechanical Separation

Electronic sorting and hand sorting of contaminated peanuts resulted in a reduction in the levels of aflatoxins (Dickens and Whitaker, 1975; Natarajan et al., 1975). However, complete removal of all contaminated particles cannot be expected with physical methods of separation. Thus, residual contamination may be present in the final product, but at very low concentrations.

Dry Cleaning, Wet Cleaning, Density Separation, and Preferential Fragmentation of Contaminated Corn

These techniques have proved to be largely ineffective for diminishing the aflatoxin content of corn (Brekke et al., 1975a).

Density Segregation of Contaminated Corn

Aflatoxin-contaminated corn was buoyant in water and sucrose solution. Separation of this fraction yielded a significant decrease in the total aflatoxin content (Huff, 1980). This process also promises to be an important adjunct to the existing techniques in peanuts (Cole, 1989).

Dry Milling of Corn

The distribution of aflatoxins was apparently low in grits and high in the germ, hull, or degermer fines of dry milled corn (Brekke et al., 1975b).

Wet Milling of Corn

Aflatoxin occurred mainly in the steepwater and fiber of wet milled corn with smaller amounts present in the gluten and germ (Yahl et al., 1971).

Physical Methods of Detoxification

Thermal Inactivation

Aflatoxins are heat-stable, and were not totally destroyed by boiling water, autoclaving, and a variety of food and feed processing procedures (Christensen et al., 1977). Roasting resulted in a reduction in the aflatoxin content of nuts, oilseed meals (Marth and Doyle, 1979), and corn (Conway et al., 1978). Baking temperatures did not significantly alter aflatoxin levels in dough (Reiss, 1978). The degradation of aflatoxins was a direct function of temperature, heating time, and moisture content (Mann et al., 1967). Aflatoxin M1 was apparently stable in raw milk and resistant to pasteurization and processing (Stoloff, 1980; Stoloff et al., 1975).

Irradiation

Gamma irradiation (2.5 Mrad) did not degrade aflatoxin in contaminated peanut meal, and UV light produced no observable change in fluorescence or toxicity of the treated sample (Feuell, 1977). However,

exposure of contaminated peanut oil to shortwave and long wave UV light resulted in a significant reduction of aflatoxin levels (Shantha and Sreenivasa, 1977).

Solvent Extraction

A variety of solvents were capable of extracting aflatoxins from different commodities with minimal effects on protein content or nutritional quality (Goldblatt and Dollear, 1979; Rayner et al., 1977). However, these methods for the removal of aflatoxins via solvent extraction (Shanta, 1987) appear to be impractical and expensive when compared with other methods of physical separation.

Adsorption from Solution

A variety of adsorbent materials, including activated carbon (Decker, 1980) and clays (Masimanco et al., 1973), have been shown to bind aflatoxins in aqueous solutions (e.g., water, Sorensen buffer, Czapek's medium, Pilzen beer, sorghum beer, whole milk, and skimmed milk). A recent report indicated that certain aluminosilicates were capable of binding aflatoxins in peanut oil (Machen et al., 1988). One of these compounds, a hydrated sodium calcium aluminosilicate, effectively removed aflatoxins from contaminated oil and prevented its mutagenicity. The adsorption of aflatoxins to bentonite clay, used for removing pigments from oils, is one of the factors resulting in aflatoxin removal from peanut and corn oils.

Biological Methods of Detoxification

Microbial Inactivation

Microorganisms, including yeast, molds, and bacteria, have been screened for their ability to modify and/or inactivate aflatoxins. *Flavobacterium aurantiacum* (NRRL B-184) was reported to significantly remove aflatoxin from a liquid medium without producing toxic by-products (Ciegler et al., 1966). These same investigators also demonstrated that certain acid-producing molds could catalyze the hydration of aflatoxin B_1 to B_{2a} (a less toxic product). The applications of microbial detoxification of aflatoxins have been reviewed (Ciegler 1978; Marth and Doyle, 1979). Recently, Hao and colleagues (1987) used *F. aurantiacum* to remove aflatoxin B_1 from peanut milk. Preliminary work indicated that this bacterium grew in both nondefatted and partially defatted peanut milk, and was not inhibited by the presence of aflatoxin B_1.

Fermentation

Fermentation of contaminated grains resulted in degradation of aflatoxins (Dam et al., 1977). However, ensiling contaminated high-moisture corn did not adequately degrade aflatoxins. It was postulated that insufficient acid was produced by this procedure to catalyze the transformation of aflatoxin B_1 to aflatoxin B_{2a} (Lindenfelser and Ciegler, 1970).

Chemical Methods of Detoxification

Structural Degradation Following Chemical Treatment

Many chemicals have been tested for their ability to structurally degrade and/or inactivate aflatoxins, including numerous acids, bases, aldehydes, bisulfite, oxidizing agents, and various gases (Anderson, 1983; Goldblatt and Dollear, 1979). Ammoniation (under appropriate conditions) resulted in a significant reduction in the level of aflatoxins in contaminated peanut and cottonseed meals (Dollear et al., 1968; Gardner et al., 1971; Masri et al., 1969; Park et al., 1984) and corn (Brekke et al., 1977, 1979). The safety of ammoniated corn was evaluated in rainbow trout (Brekke et al., 1979), chickens (Hughes et al., 1979), and rats (Norred, 1979, 1981; Southern and Clawson, 1980). In a long term feeding study in rats, Norred and Morrissey (1983) reported that ammoniation of corn containing 750 ppb aflatoxins resulted in significant protection from toxicity and hepatic neoplasia in experimental animals. These studies provide evidence that chemical treatment via ammoniation may provide an effective method to detoxify aflatoxin-contaminated corn and other commodities. The mechanism for this action appears to involve a chemical conversion of parent aflatoxin B_1 to products that exhibit greatly decreased toxicity. The use of aqueous or gaseous ammonia, as well as other chemical treatments for the detoxification of aflatoxins have been thoroughly reviewed (Anderson, 1983; Goldblatt and Dollear, 1979; Palmgren and Hayes, 1987; Park et al., 1988). Also, commercial procedures for ammoniation of peanut cakes and meal are being used in some African countries and within Arizona and California for cottonseed.

Modification of Toxicity by Dietary Chemicals

The toxicity of mycotoxins may be strongly influenced by dietary chemicals that alter the normal responses of mammalian systems to these substances. A variable array of chemical factors, including nutri-

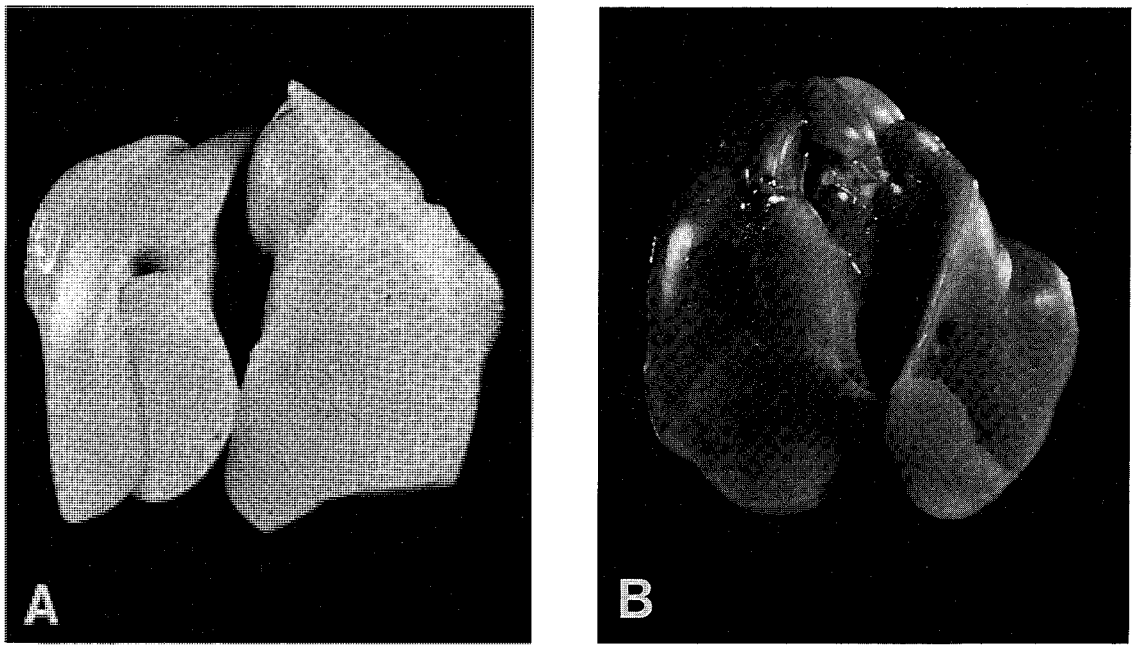


Figure 6.3. Representative livers from Leghorn chicks fed diets containing 7,500 ppb aflatoxin (A) or 7,500 ppb aflatoxin, and 0.5% HSCAS (B) (Phillips et al., 1988b).

tional components (e.g., dietary protein and fat, lipotropic agents, vitamins, and trace metals), food and feed additives (e.g., antibiotics and preservatives), as well as other chemical factors, may interact to modify the effects of aflatoxins in animals (Newberne, 1987). Recent findings (Monroe and Eaton, 1987) have indicated that butylated hydroxyanisole (BHA) increases the resistance of rats to the carcinogenic effect of aflatoxin by elevating glutathione S-transferase activity and, subsequently, reducing aflatoxin-DNA binding. In another study, Ramsdell and Eaton (1988) demonstrated that broccoli contains substances that result in a reduction in the binding of aflatoxin B_1 to DNA, apparently through an increase in glutathione transferase activity and gluthathione conjugation to aflatoxin B_1 epoxide.

Other than ammoniation, many of the techniques proposed to remove mycotoxins are currently perceived as impractical and/or ineffective and/or potentially unsafe for large-scale utilization. The development of safe and practical methods to remove and/or detoxify mycotoxins from a variety of contaminated sources is clearly warranted. A new approach to detoxification is the use of mycotoxin chemisorbents (inorganic sorbent materials) with the capacity to tightly bind and immobilize mycotoxins in the gastrointestinal tract of animals.

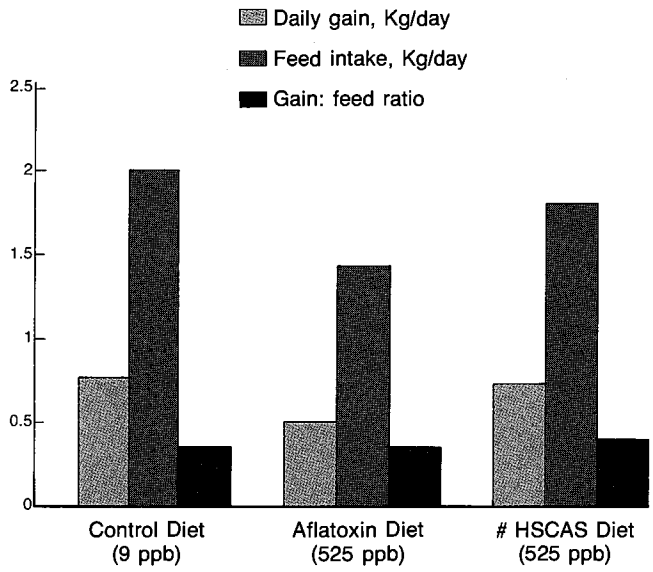


Figure 6.4. Effect of adding 0.5% hydrated sodium calcium aluminosilicate (HSCAS) to swine diets formulated with aflatoxin contaminated corn. Pigs fed the diet formulated with contaminated corn for 35 days had significantly decreased feed intake and daily gain, while feed intake and weight gains in the diet with added HSCAS were similar to pigs fed the control diet. Cellular changes associated with aflatoxin toxicity were detected in pigs fed the high aflatoxin diet and not in pigs fed the control or diet with added HSCAS (Colvin et al., 1989).

Table 6.4 Body weight (wt) gain (kg) and feed conversion (feed:gain) of barrows fed diets containing hydrated sodium calcium aluminosilicate (HSCAS) and aflatoxin (AF) for 28 days (Harvey et al., 1989)

Dietary treatment			Gain						
HSCAS (g/kg)	AF (mg/kg)	Initial wt.	0 to 1 wk	1 to 2 wk	2 to 3 wk	3 to 4 wk	0 to 4 wk	Final wt	Feed:gain
0	0	14.7 ± 0.79[a]	2.5 ± 0.51[a]	4.8 ± 0.26[a]	5.7 ± 0.56[a]	5.3 ± 0.35[a]	18.2 ± 1.22[a]	32.9 ± 1.95[a]	2.50
5	0	14.9 ± 0.87[a]	3.4 ± 0.44[a]	4.7 ± 0.46[a]	5.1 ± 0.21[a]	6.5 ± 0.30[a]	19.6 ± 0.90[a]	34.5 ± 1.71[a]	2.50
20	0	14.4 ± 0.73[a]	3.8 ± 0.35[a]	4.3 ± 0.51[a]	5.1 ± 0.43[a]	5.2 ± 0.24[a]	18.4 ± 1.12[a]	32.8 ± 1.60[a]	3.24
0	3	15.4 ± 0.78[a]	0.8 ± 0.58[b]	2.1 ± 0.61[b]	1.5 ± 0.60[b]	1.7 ± 1.06[b]	6.1 ± 3.05[b]	21.1 ± 3.45[b]	3.10
5	3	14.8 ± 0.54[a]	3.3 ± 0.17[a]	4.6 ± 0.43[a]	5.4 ± 0.48[a]	4.9 ± 0.51[a]	18.3 ± 0.45[a]	33.1 ± 0.78[a]	2.63
20	3	14.5 ± 0.78[a]	2.8 ± 0.37[a]	4.9 ± 0.32[a]	5.4 ± 0.24[a]	5.8 ± 0.27[a]	18.8 ± 0.67[a]	33.2 ± 1.29[a]	2.60

Values are expressed as group mean ± SEM. Values in columns for same measuring period with the same superscript do not differ significantly.

Alteration of Bioavailability by Mycotoxin Chemisorbents

Dietary additions of zeolite (Smith, 1980), bentonite (Carson, 1982), or spent bleaching clay from canola oil refining (Smith, 1984) have been shown to alter the effects to T-2 mycotoxin and zearalenone in rats. Phillips and coworkers (1987, 1988a, 1988b, 1989) evaluated the aflatoxin affinity of various inorganic sorbent materials in vitro and described the chemisorption of aflatoxins to a hydrated sodium calcium aluminosilicate (HSCAS or NovaSil™). *In vivo* studies demonstrated that HSCAS, when incorporated into the diet of Leghorn and broiler chicks at a level of 0.5%, significantly prevented the deleterious effects of purified aflatoxin B_1 and a crude mixture of aflatoxins B_1, B_2, G_1, and G_2 (Kubena et al., 1987, Phillips et al., 1988) (Figure 6.3). The dietary addition of activated charcoal at the same level (i.e., 0.5%) did not appear to have any protective properties (Kubena et al., 1988). Further studies with HSCAS indicated a protective action in swine (Figures 6.4, 6.5; Table 6.4) (Harvey et al., 1988a, 1989; Colvin et al., 1989) and the ability of this aluminosilicate to decrease

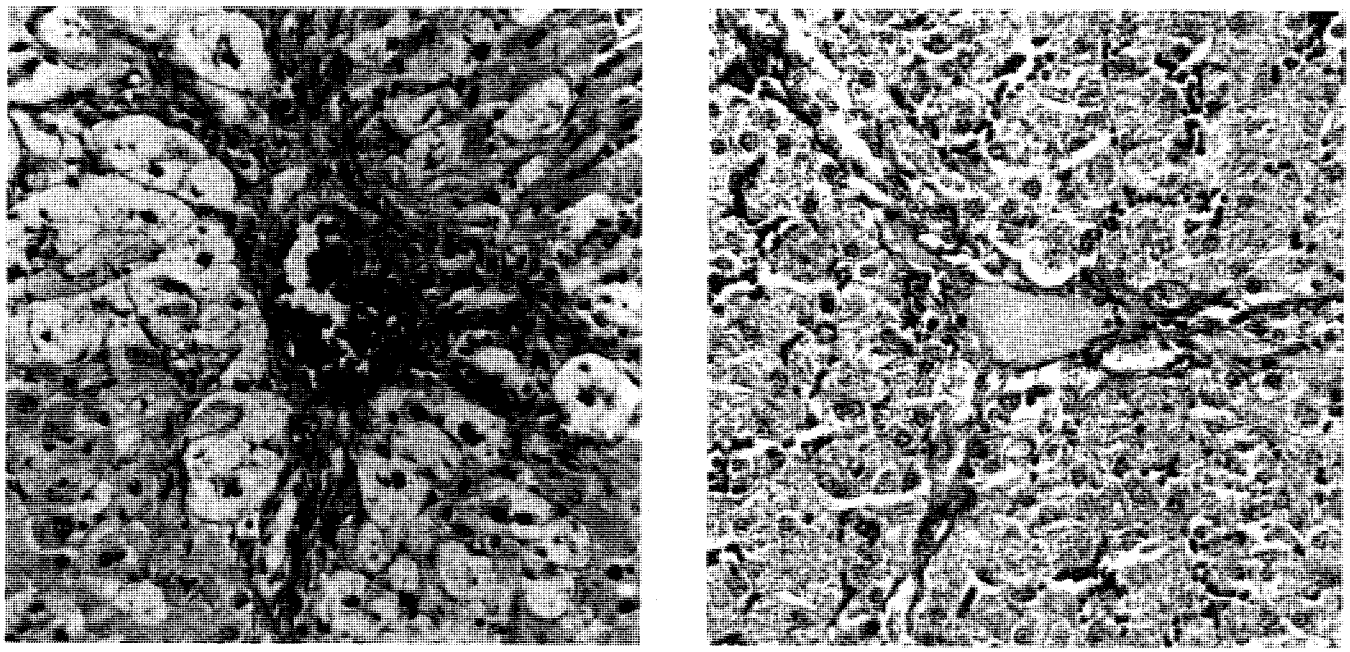


Figure 6.5. Photomicrographs of sections of porcine liver specimens. *Left:* Hepatic portal triad from a barrow fed a diet containing 3 ppm aflatoxins. Notice the peripheral lobular hepatocellular vacuolation, pyknotic nuclei, and periportal fibrosis. Hematoxylin and eosin stain; x 470. *Right:* Hepatic portal triad from a barrow fed a diet containing 3 ppm aflatoxins and 0.5% hydrated sodium calcium aluminosilicate (HSCAS). Notice the lack of lesions. Hematoxylin and eosin stain; x 470 (from Harvey et al., 1989).

the level of aflatoxin M_1 in the milk of lactating dairy cows (Harvey et al., 1988b). The basic mechanism for this action appears to involve aflatoxin chemisorption by HSCAS in the gastrointestinal tract of animals, resulting in a major reduction in aflatoxin bioavailability (Davidson et al., 1987).

Integrated Mycotoxin Control Management

Mycotoxins comprise a structurally diverse and chemically complex group of fungal-elaborated substances, many of which have been implicated as significant health hazards on a worldwide scale. Consequently, there is a critical need for practical and effective solutions to this problem. A comprehensive interdisciplinary effort on the part of academia, government, and industry to identify and implement state-of-the-art control and preventive management strategies to solve the mycotoxin problem is highly desirable. The development and utilization of essential components of a mycotoxin management system (e.g., innovative physical, chemical, and biotechnologies) for the prevention, detection, and detoxification of these important substances, along with optimal methods of food and feed production, harvesting, storage, and processing, will foster an integrated approach to the control of mycotoxins, with broad implications to agriculture and the health of animals and humans (Phillips, 1987; Phillips et al., 1988b) (Figure 6.6).

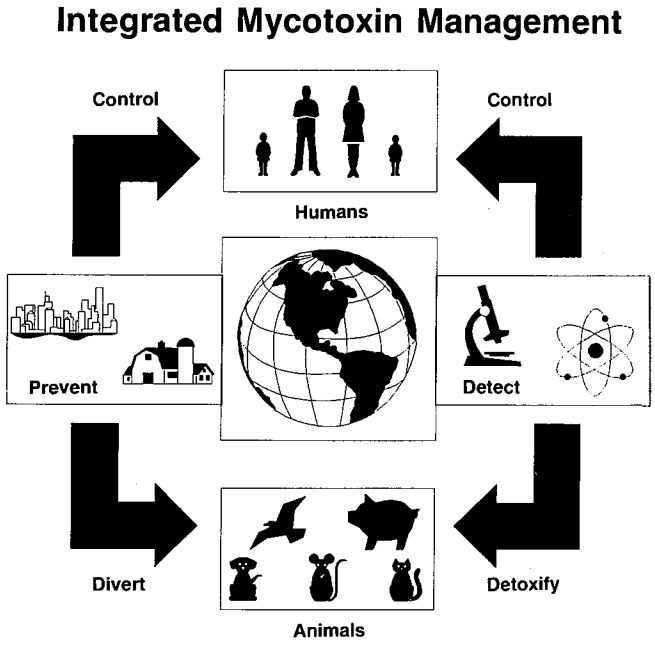


Figure 6.6. Chart depicting the components of an integrated approach to mycotoxin management (Phillips, 1987; Phillips et al., 1988b).

7. Research Needs

When the current status of knowledge of mycotoxins and mycotoxicoses is assessed and questions continue to arise because of the occurrence of various mycotoxins in field crops and raw food products, the gaps in our information on these topics become more apparent. From the information compiled herein and the questions continually being asked of investigators in the areas of mycotoxins and mycotoxicoses, the task force has determined the major needs for research in these areas. They are:

1. Surveillance of commodities, foods, and feeds for presence and quantity of mycotoxins.
2. Surveillance of human populations to assess the exposure level to selected mycotoxins.
3. Assessment of control methods of mycotoxins involving methods of decontamination/detoxification.
4. Development of consistent regulations controlling contamination of products in international trade.
5. Development of resistant plants to fungal and mycotoxin occurrence.
6. Development of biocompetitive agents for toxigenic fungi.
7. Improvement of sampling and analysis for mycotoxins.
8. Research on the effect of mycotoxins on animal disease through immunosuppression and interaction with other disease agents.
9. Toxicological evaluation of newly discovered mycotoxins.
10. Examination of the possible therapeutic and biocontrol nature of mycotoxins.
11. An integrated worldwide assessment of the economic aspects of mycotoxins.

Literature Cited

Alpert, M. E., M. S. R. Hutt, C. S. Davidson. 1968. Hepatoma in Uganda. *Lancet* i:1265-1267.

Alpert, M. E., M. S. R. Hutt, G. N. Wogan, and C. S. Davidson. 1971. Association between aflatoxin content of food and hepatoma frequency in Uganda. *Cancer* 28:253-260.

Ambrose, A. M. and F. DeEds. 1946. Some toxicological and pharmacological properties of citrinin. *J. Pharmacol. Exp. Ther.* 88:173-186.

Anderson, R. A. 1983. Detoxification of aflatoxin-contaminated corn. Pp. 87-90. In U. Diener, R. Asquith, and J. Dickens (Eds.). *Aflatoxin and Aspergillus flavus in corn.* Southern Cooperative Series Bulletin 279, Auburn University, Auburn, Alabama.

Applebaum, R. S., R. E. Brackett, D. W. Wiseman, and E. H. Marth. 1982. Responses of dairy cows to dietary aflatoxin: Feed intake and yield, toxin content, and quality of milk of cows treated with pure and impure aflatoxin. *J. Dairy Sci.* 65: 1503-1508.

Armbrecht, B. H., H. G. Wiseman, and W. T. Shalkop. 1972. Swine aflatoxicosis. II. The chronic response in brood sows fed sublethal amounts of aflatoxin and the reaction in their piglets. *Environ. Physiol. Biochem.* 2:77-85.

Armstrong, B. 1980. The epidemiology of cancer in the People's Republic of China. *Intl. J. Epidemiol.* 9:305-315.

Arp, L. H. and J. L. Richard. 1979. Intoxication of dogs with the mycotoxin, penitrem A. *J. Am. Vet. Med. Assoc.* 175:565-566.

Arp, L. H. and J. L. Richard. 1981. Experimental intoxication of guinea pigs with multiple doses of the mycotoxin penitrem A. *Mycopathologia* 73:109-113.

Association of Official Analytical Chemists. 1984. *Official methods of analysis.* S. Williams, Ed. 14th Ed. Association of Official Analytical Chemists, Arlington, VA. 1141 pp.

Aust, S. D. 1969. Evidence for the bioactivation of slaframine. *Biochem. Pharmacol.* 18:929-932.

Aust, S. D. 1974. *Rhizoctonia leguminicola*-slaframine. Pp. 97-109. In I. F. H. Purchase (Ed.). *Mycotoxins.* Elsevier Scientific Publishing Co., Amsterdam and New York.

Aust, S. D. and H. P. Broquist. 1965. Isolation of a parasympathomimetic alkaloid of fungal origin. *Nature* 205:204.

Autrup, H., K. A. Bradley, A. K. M. Shamsuddin, J. Wakhisi, and A. Wasunna. 1983. Detection of putative adduct with fluorescence characteristics identical to 2,3-dihydro-2-(7'-guanyl)-3-hydroxy-aflatoxin B_1 in human urine collected in Muranga district, Kenya. *Carcinogenesis* 4:1193-1195.

Autrup, H., T. Seremet, J. Wakhisi, and A. Wasunna. 1987. Aflatoxin exposure measured by urinary excretion of aflatoxin B_1-guanine adduct and hepatitis B virus infection in areas with different liver cancer incidence in Kenya. *Cancer Res.* 47:3430-3433.

Badiali, L., H. Mamdouh, H. Abou-Youssef, A. I. Radwan, F. M. Hamdy, and P. K. Hildebrandt. 1968. Moldy corn poisoning as the major cause of an encephalomalacia syndrome in Egyptian equidae. *Am. J. Vet. Res.* 29:2029-2035.

Bailey, J. A. E. Wrathall, and P. G. Mantle. 1973. The effect of feeding ergot to gilts during early pregnancy. *Br. Vet. J.* 129:127.

Baker, D. C. and R. A. Green. 1987. Coagulation defects of aflatoxin intoxicated rabbits. *Vet. Pathol.* 24:62-70.

Bamburg, J. R. 1972. The biological activities and detection of naturally occurring 12, 13-epoxy-delta 9-trichothecenes. *Clin. Toxicol.* 5:495.

Bamburg, J. R. 1983. Biological and biochemical actions of trichothecene mycotoxins. Pp. 41-110. In F. E. Hahn (Ed.). *Progress in molecular and subcellar biology.* Vol. 8. Springer-Verlag, Berlin.

Bamburg, J. R. 1983. Biological and biochemical actions of trichothecene mycotoxins. Pp. 41-110. In F. E. Hahn (Ed.). *Progress in molecular and subcellar biology.* Vol. 8. Springer-Verlag, Berlin.

Bamburg, J. R., W. F. Marasas, E. B. Smalley, and F. M. Strong. 1968. Toxic compounds from *Fusarium* and other *Hypomycetes.* *Biotech. Bioeng.* 10:445-455.

Barger, G. 1931. *Ergot and ergotism.* Garney and Jackson, London.

Bauwin, G. R. and H. L. Ryan. 1982. Sampling inspection and grading of grain. Pp. 115. In C. M. Christensen (Ed.). *Storage of cereal grains and their products.* Vol. 5, 3rd ed. Am. Assoc. Cereal Chem., St. Paul, Minnesota.

Beasley, R. P., L. Y. Hwang, C. C. Lin, and C. S. Chien. 1981. Hepatocellular carcinoma and hepatitis B virus. *Lancet* 2:1129-1132.

Beasley, V. R., S. P. Swanson, R. A. Corley, W. B. Buck, G. D. Karitz, and H. R. Burmeister. 1986. Pharmacokinetics of the trichothecene mycotoxin, T-2 toxin, in swine and cattle. *Toxicon* 24:13-23.

Bendele, A., W. W. Carlton, P. Krogh, E. B. Lillehoj. 1985. Ochratoxin A carcinogenesis in the (C57BL/6J x C3H)F_1 mouse. *J. Natl. Cancer Inst.* 75:733-742.

Berry, C. 1988. The pathology of mycotoxins. *J. Pathol.* 154:301-311.

Biester, H. E. and L. H. Schwarte. 1939. Moldy corn poisoning (Leucoencephalomalacia) in horses with history of previous attack as well as recovery from virus encephalomyelitis. *North Am. Vet.* 20:17-19.

Biester, H. E., L. H. Schwarte, and C. H. Reedy. 1940. Further studies on moldy corn poisoning (Leucoencephalomalacia) in horses. *Vet. Med.* 35:636-639.

Bodine, A. B. and D. R. Mertens. 1983. Toxicology, metabolism, and physiological effects of aflatoxin in the bovine. Pp. 46-50. In U. L. Diener, R. L. Asquith, and J. W. Dickens (Eds.). *Aflatoxin and Aspergillus flavus in corn.* Alabama Ag. Exp. Sta., Auburn University, Alabama.

Bodine, A. B., S. F. Fisher, and S. Gangjee. 1984. Effect of aflatoxin B_1 and major metabolites on phytohemagglutinin-stimulated lymphoblastogenesis of bovine lymphocytes. *J. Dairy Sci.* 67:110-114.

Boonchuvit, B., P. B. Hamilton, and H. R. Burmeister. 1975. Interaction of T-2 toxin with *Salmonella* infections in chickens. *Poultry Sci.* 54:1693-1696.

Boorman, G. 1988. NTP technical report on the toxicology and carcinogenesis studies of ochratoxin A (CAS No. 303-47-9) in F344/N rats (gavage studies). *NIH Publication No. 88-2813.* National Toxicology Program, Public Health Service, National Institute of Health, United States Department of Health and Human Services.

Brekke, O. L., A. J. Peplinski, and E. L. Griffin, Jr. 1975a. Cleaning trials for corn containing aflatoxin. *Cereal Chem.* 52:198-204.

Brekke, O. L., A. F. Peplinski, G. W. Nofsinger, H. F. Conway, A. C. Stringfellow, R. R. Montgomery, R. W. Silman, V. E. Sohns, and E. B. Bagley. 1975b. Pilot-plant dry milling of corn containing aflatoxin. *Cereal Chem.* 52:205-211.

Brekke, O. L., R. O. Sinnhuber, A. J. Peplinski, J. H. Wales, G. B. Putnam, D. J. Lee, and A. Ciegler. 1977. Aflatoxin in corn: Ammonia inactivation and bioassay with rainbow trout. *Appl. Environ. Microbiol.* 34:34-37.

Brekke, O. L., A. J. Peplinski, G. W. Nofsinger, H. F. Conway, A. C. Stringfellow, R. R. Montgomery, R. W. Silman, V. E. Sohns, and E. B. Bagley. 1979. Aflatoxin inactivation in corn by ammonia gas: A field trial. *Trans. Am. Soc. Ag. Engr.* 22:425-432.

Brown, J. M. M. and L. Abrams. 1965. Biochemical studies on aflatoxicosis. *Onderstepoort J. Vet. Res.* 32:119-146.

Brownie, C. F. and J. Cullen. 1986. Characterization of experimentally induced equine leukoencephalomalacia (ELEM) in ponies (*Equus caballus*): A preliminary report. *Vet. Hum. Toxicol.* 29:34-37.

Buchanan, J. R., N. F. Sommer, R. J. Fortlage, E. C. Maxie, F. Mitchell, and D. P. H. Hsieh. 1974. Patulin from *Penicillium expansum* in stone fruits and pears. *J. Am. Soc. Hort. Sci.* 99:262-265.

Buchi, G. H., P. J. Muller, B. D. Roebuck, and G. N. Wogan. 1974. Aflatoxin Q_1: A major metabolite of aflatoxin B_1 produced by human liver. *Res. Comm. Chem. Pathol. Pharmacol.* 8:585-591.

Buck, W. B., J. C. Haliburton, J. P. Thilsted, T. F. Lock, and B. S. Vesonder. 1979. *Equine leucoencephalomalacia: Comparative pathology of naturally occurring and experimental cases.* The 22nd Annual Proceedings of the American Association of Veterinary Laboratory Diagnosticians.

Buening, G. M., D. D. Mann, B. Hook, and G. D. Osweiler. 1982. The effect of T-2 toxin on the bovine immune system: Cellular factors. *Vet. Immunol. Immunopathol.* 3:411-417.

Bulatao-Jayme, E. M. Almero, C. A. Castro, T. R. Jardeleza, and L. Salamat. 1982. A case-control dietary study of primary liver cancer risk from aflatoxin exposure. *Intl. J. Epidemiol.* 11:112-119.

Bullerman, L. B. 1978. Methods for detecting mycotoxins in foods and beverages. Pp. 445-470. In L. R. Beuchat (Ed.). *Food and beverage mycology.* AVI Publication Co., Westport, Connecticut.

Bullerman, L. B. 1979. Significance of mycotoxins to food safety and human health. *J. Fd. Prot.* 42:65-86.

Bullerman, L. B. 1981. Public health significance of molds and mycotoxins in fermented dairy products. *J. Dairy Sci.* 64:2439-2452.

Bullerman, L. B. 1986. *Mycotoxins and food safety.* A scientific status summary by the Institute of Food Technologists' expert panel on food safety and nutrition. Institute of Food Technologists, Chicago.

Burditt, S. J., W. M. Hagler, Jr., and P. B. Hamilton. 1984. Feed refusal during ochratoxicosis in turkeys. *Poultry Sci.* 63:2172-2174.

Burfening, P. J. 1973. Ergotism. *J. Am. Vet. Med. Assoc.* 163:1288-1290.

Burmeister, H. R., A. Ciegler, and R. F. Vesonder. 1979. Moniliformin, a metabolite of *Fusarium moniliforme* NRRL 6322: Purification and toxicity. *Appl. Environ. Microbiol.* 37:11-13.

Burns, R. B. and P. Dwivedi. 1986. The natural occurrence of ochratoxin A and its effects in poultry. A review. Part II. Pathology and immunology. *World's Poultry Sci. J.* 42:48-55.

Busby, W. F. and G. N. Wogan. 1981. *Mycotoxins and N-nitroso compounds: Environmental risks. Vol. 2.* In R. C. Shank (Ed.). CRC Press, Boca Raton, FL.

Busby, W. F. and G. N. Wogan. 1984. Aflatoxins. Pp. 945-1136. In C. E. Searle (Ed.). *Chemical carcinogens.* American Chemical Society, Washington, D. C.

Butler, T. 1902. Notes on a feeding experiment to produce leucoencephalitis in a horse, with positive results. *Agric. Vet. Rev.* 26:748-751.

Butler, W. H. 1964. Acute toxicity of aflatoxin B_1 in rats. *Br. J. Cancer* 18:756.

Butler, W. H. 1974. Aflatoxin. Pp. 1-28. In I. F. H. Purchase (Ed.). *Mycotoxins.* Elsevier Scientific Publishing Co., Amsterdam, The Netherlands.

Campbell, T. C. and L. Salamut. 1970. Aflatoxin ingestion and excretion by humans. Pp. 271-280. In I. F. H. Purchase (Ed.).. *Mycotoxins in Human Health.* Macmillan Ltd., London.

Campbell, T. C. and L. Stoloff. 1974. Implications of mycotoxins for human health. *J. Agric. Fd. Chem.* 22:1006-1016.

Carlborg, F. W. 1979. Cancer, mathematical models, and aflatoxin. *Food Cosmet. Toxicol.* 17:159-166.

Carnaghan, R. B. A. 1965. Hepatic tumors in ducks fed a low level of toxic groundnut meal. *Nature* 208:308-312.

Carnaghan, R. B. A., G. Lewis, D. S. P. Patterson, and R. Allcroft. 1966. Biochemical and pathological aspects of groundnut poisoning in chickens. *Pathol. Vet.* 3:601-615.

Carson, M. S. 1982. The effect of dietary fiber and nonnutritive mineral additives on T-2 toxicosis in rats. M.Sc. Thesis. Department of Nutrition, University of Guelph, Guelph, Ontario, Canada.

Chan, P.K.-C. and P. A. Gentry. 1984. Inhibition of bovine platelet function by T-2 toxin, HT-2 toxin, diacetoxyscirpenol and deoxynivalenol. *Fd. Chem. Toxicol.* 22:643-647.

Chang, K., H. J. Kurtz, and C. J. Mirocha. 1979. Effects of the mycotoxin zearalenone on swine reproduction. *Am. J. Vet. Res.* 40:1260-1267.

Christensen, C. M., C. J. Mirocha, and R. A. Meronuck. 1977. *Mold, mycotoxins, and mycotoxicoses.* Agricultural Experiment Station Miscellaneous Report 142. University of Minnesota, St. Paul, Minnesota.

Ciegler, A. 1978. Trichothecenes: Occurrence and toxicoses. *J. Fd. Prot.* 41:399-403.

Ciegler, A., E. B. Lillehoj, R. E. Peterson, and H. H. Hall. 1966. Microbial detoxification of aflatoxin. *Appl. Microbiol.* 14:934-939.

Clifford, J. I. and K. R. Rees. 1966. Aflatoxin: A site of action in the rat liver cell. *Nature 209:313.*

Cole, R. J. 1973. Toxin from *Fusarium moniliforme*: Effects on plants and animals. *Science* 179:1324.

Cole, R. J. 1986. Etiology of turkey "X" disease in retrospect: A case for the involvement of cyclopiazonic acid. *Mycotoxin Res.* 2:3-7.

Cole, R. J. 1989. Technology of aflatoxin decontamination. *Mycotoxins and Phycotoxins '88.* In S. Natori, K. Hashimoto, and Y. Ueno (Eds.). Elsevier Scientific Publishing Co., Amsterdam.

Cole, R. J. and R. H. Cox (Eds.). 1981. *Handbook of toxic fungal metabolites.* Academic Press, Inc., New York. 937 pp.

Cole, R. J. and J. W. Dorner. 1986. Role of fungal tremorgens in animal disease. Pp. 501-511. In P. S. Steyn and R. Vleggaar (Eds.). *Mycotoxins and phycotoxins.* Elsevier Scientific Publishing Co., Amsterdam, The Netherlands.

Cole, R. J., J. W. Kirksey, J. H. Moore, B. P. Blankenship, U. L. Diener, and N. D. Davis. 1972. Tremorgenic toxin from *Penicillium verruculosum. Appl. Microbiol.* 24:248-256.

Cole, R. J., A. S. Csinos, P. D. Blankenship. T. H. Sanders, T. P. Gaines, and J. I. Davidson. 1985. Evaluation of soil calcium as methods of prevention of preharvest aflatoxin contamination of peanuts. *Proc. Am. Peanut Res. Educ. Soc.* 17:71.

Colvin, B. M., L. T. Sangster, K. D. Hayden, R. W. Bequer, and D. M. Wilson. 1989. Effect of high affinity aluminosilicate sorbent on prevention of aflatoxicosis in growing pigs. *Vet. Hum. Toxicol.* 31:46-48.

Conway, H. F., R. A. Anderson, and E. B. Bagley. 1978. Detoxification of aflatoxin-contaminated corn by roasting. *Cereal Chem.* 55:115-117.

Cook, W. O., J. L. Richard, G. D. Osweiler, and D. W. Trampel. 1986. Clinical and pathologic changes in acute bovine aflatoxicosis: Rumen motility and tissue and fluid concentrations of aflatoxin

B_1 and M_1. *Am. J. Vet. Res.* 47:1817-1825.

Cooray, R. 1984. Effects of some mycotoxins on mitogen-induced blastogenesis and SCE frequency in human lymphocytes. *Fd. Chem. Toxicol.* 22:529-534.

Corley, R. A., S. P. Swanson, and W. B. Buck. 1985. Glucuronide conjugates of T-2 toxin and metabolites in swine bile and urine. *J. Agric. Fd. Chem.* 33:1085-1089.

Corley, R. A., S. P. Swanson, G. J. Gullo, L. Johnson, V. R. Beasley, and W. B. Buck. 1986. Disposition of T-2 toxin, a trichothecene mycotoxin, in intravascularly dosed swine. *J. Agric. Fd. Chem.* 34:868-875.

Corrier, D. E. and R. L. Ziprin. 1986. Immunotoxic effects of T-2 toxin on cell-mediated immunity to listeriosis in mice: Comparison with cyclophosphamide. *Am. J. Vet. Res.* 47:1956-1960.

Corrier, D. E. and R. L. Ziprin. 1987. Immunotoxic effects of T-2 mycotoxin on cell-mediated resistance to *Listeria monocytogenes* infection. *Vet. Immunol. Immunopathol.* 14:11-21.

Crump, M. H., E. B. Smalley, R. E. Nichols, and D. P. Rainey. 1967. Pharmacologic properties of a slobber-inducing mycotoxin from *Rhizoctonia leguminicola*. *Am. J. Vet. Res.* 28:865-874.

Cucullu, A. F., L. S. Lee, R. Y. Mayne, and L. A. Goldblatt. 1966. Determination of aflatoxin in individual peanuts and peanut sections. *J. Am. Oil Chem. Soc.* 43:89.

Cucullu, A. F., L. S. Lee, and W. A. Pons. 1977. Relationship of physical appearance of individual mold damaged cottonseed to aflatoxin content. *J. Am. Oil Chem. Soc.* 54:235A.

Cullen, J. M., M. E. Wilson, W. M. Hagler, Jr., J. F. Ort, and R. J. Cole. 1988. Histologic lesions in broiler chicks given cyclopiazonic acid orally. *Am. J. Vet. Res.* 49:728-731.

Cundliffe, E. and J. Davis. 1977. Inhibition of initiation, elongation, and termination of eukaryotic protein synthesis by trichothecene fungal toxins. *Antimicrob. Agents Chemother.* 11:491-499.

Cysewski, S. J., A. C. Pier, A. L. Baetz, and N. F. Cheville. 1982. Experimental equine aflatoxicosis. *Toxicol. Appl. Pharmacol.* 65:354-365.

Dam, R., S. W. Tam, and L. D. Satterlee, 1977. Destruction of aflatoxins during fermentation and by-product isolation from artificially contaminated grain. *Cereal Chem.* 54:705-714.

Davidson, J. N., J. G. Babish, K. A. Delaney, D. R. Taylor, and T. D. Phillips. 1987. Hydrated sodium calcium aluminosilicate decreases the bioavailability of aflatoxin in the chicken. *Poultry Sci.* 66:89 (Abstract).

Davis, N. D. and U. L. Diener. 1987. Mycotoxins. Pp. 517-570. In L. R. Beuchat (Ed.). *Food and beverage mycology*. 2nd Ed. Van Nostrand Reinhold Company, Inc., New York.

Decker, W. J. 1980. Activated charcoal absorbs aflatoxin B_1. *Vet. Human Toxicol.* 22:388-389.

Derneke, T., Y. Kidane, and B. Weihib. 1979. Ergotism: A report of an epidemic. *Ethiopian Med. J.* 17:107-113.

DeNicola, D. B., A. H. Rebar, W. W. Carlton, and B. Yagen. 1978. T-2 toxin mycotoxicosis in the guinea pig. *Fd. Cosmet. Toxicol.* 16:601-609.

Demeke, T., Y. Kidane, and E. Wuhib. 1979. Ergotism: a report on an epidemic. *Ethiop. Med. J.* 17:107-113.

Dickens, F. and H. E. H. Jones. 1965. Further studies on the carcinogenic action of certain lactones and related substances in the rat and mouse. *Br. J. Cancer.* 19:392-403.

Dickens, J. W. and J. B. Satterwhite. 1969. Subsampling mill for peanut kernels. *Food Technol.* 23:90.

Dickens, J. W. and T. B. Whitaker. 1975. Efficacy of electronic color sorting and hand picking to remove aflatoxin contaminated kernels from contaminated lots of shelled peanuts. *Peanut Sci.* 2:45-50.

Dickens, J. W. and T. B. Whitaker. 1982. Sampling and sampling preparation. Pp. 17. In H. Egan, L. Stoloff, P. Scott, M. Costegnaro, I. K. O'Neill, and H. Bartsch (Eds.). *Environmental carcinogens-selected methods of analysis*: Some mycotoxins. Vol. 5. IARC, Lyon, France.

Dickens, J. W., T. B. Whitaker, R. J. Monroe, and J. N. Weaver. 1979. Accuracy of subsampling mill for granular material. *J. Am. Oil Chem.* 56:842.

Diener, U. L. 1981. Unwanted biological substances in foods: Aflatoxins. Pp. 122-150. In J. C. Ayres and J. C. Kirschman (Eds.). *Impact of toxicology on food processing*. AVI Publishing Company, Inc., Westport, Connecticut.

Diener, U. L., C. W. Hesseltine, J. C. Ayres, W. W. Carlton, R. J. Cole, L. A. Goldblatt, P. B. Hamilton, D. P. H. Hsieh, E. H. Marth, C. J. Mirocha, A. C. Pier, R. O. Sinnhuber, and J. R. Wallin. 1979. *Aflatoxin and other mycotoxins: An agricultural perspective*. CAST Task Force Report No. 80. Council for Agricultural Science and Technology, Ames, Iowa.

Diener, U. L., R. J. Cole, T. H. Sanders, G. A. Payne, L. S. Lee, and M. A. Klich. 1987. Epidemiology of aflatoxin formation by *Aspergillus flavus*. *Ann. Rev. Phytopathol.* 25:249-270.

Dietert, R. R., S. E. Bloom, M. A. Qureshi, and U. C. Nanna. 1983. Hematological toxicology following embryonic exposure to aflatoxin B_1. *Proc. Soc. Exp. Biol. Med.* 173:481-485.

Doerr, J. A., W. E. Huff, H. T. Tung, R. D. Wyatt, and P. B. Hamilton. 1974. A survey of T-2 toxin, ochratoxin, and aflatoxin for their effects on the coagulation of blood in young broiler chickens. *Poultry Sci.* 53:1728-1734.

Dollear, F. G., G. E. Mann, L. P. Codifer, Jr., J. K. Gardner, Jr., S. P. Koltun, and H. L. E. Vix. 1968. Elimination of aflatoxins from peanut meal. *J. Am. Oil Chem. Soc.* 45:862-865.

Donaldson, W. E., H. T. Tung, and P. B. Hamilton. 1972. Depression of fatty acid synthesis in chick liver (*Gallus domesticus*) by aflatoxin. *Comp. Biochem. Physiol.* 41B:843-847.

Dorner, J. W. and R. J. Cole. 1988. Rapid determination of aflatoxins in raw peanuts by liquid chromatography with postcolumn iodination and modified minicolumn cleanup. *J. Assoc. Off. Anal. Chem.* 71:43-47.

Dorner, J. W., R. J. Cole, L. G. Lomax, H. S. Gosser, and U. L. Diener. 1983. Cyclopiazonic acid production by *Aspergillus flavus* and its effects on broiler chickens. *Appl. Environ. Microbiol.* 46:698-703.

Dorner, J. W., R. J. Cole, T. H. Sanders, and P. D. Blankenship. 1989. Interrelationship of kernel water activity, soil temperature, maturity, and phytolexin production in preharvest aflatoxin contamination of drought-stressed peanuts. *Mycopathologia* 105:117-128.

Doster, R. C., R O. Sinnhuber, and N. E. Pawlowski. 1974. Acute intraperitoneal toxicity of ochratoxin A and B derivatives in rainbow trout (*Salmo gairdneri*). *Food Cosmet. Toxicol.* 12:499-505.

Dvorak, R., J. Jagos, J. Bouda, A. Piskac, and O. Zapletal. 1977. Changes in the clinico-biochemical indices in the rumen liquor and urine in cases of experimental aflatoxicosis in dairy cows. *Vet. Med.* (Prague) 22:161-169.

Edds, G. T. 1979. Aflatoxins. Pp. 80-164. *Conference on mycotoxins in animal feeds and grains related to animal health*. National Technical Information Service, Springfield, Virginia.

Edds, G. T. and R. A. Bortell. 1983. Biological effects of aflatoxins-poultry. Pp. 56-61. In U. L. Diener, R. L. Asquity, and J. W. Dickens (Eds.). *Aflatoxin and Aspergillosis flavus in corn*. Alabama Agricultural Experiment Station, Auburn University, Alabama.

Edgar, J. A. and C. C. J. Culvenor. 1985. Aspects of the structure of phomopsin A, the mycotoxin causing lupinosis. Pp. 589-594.

In A. A. Seawright, M. P. Hegarty, L. F. James, and R. F. Keeler (Eds.). *Plant toxicology*. Queensland Poisonous Plants Committee, Yeerongpilly.

Fehr, P. M. and J. Delage. 1970. Effet de l'aflatoxine sur les fermentations du rumen. *Can. Nutr. Diet.* 5:59-61.

Fernandez, J. M., W. J. Cromm, Jr., W. M. Hagler, Jr., and D. L. Mann. 1985. The effect of the parasympathomimetic slaframine on major metabolic hormones in steers. *J. Anim. Sci.* 61:371.

Feuell, A. J. 1977. Aflatoxin in groundnuts. IV. Problems of detoxification. *Trop. Sci.* 8:61.

Forgacs, J. and W. T. Carll. 1955. Preliminary mycotoxic studies on hemorrhagic disease in poultry. *Vet. Med.* 50:172.

Forgacs, J. and W. T. Carll. 1962. Mycotoxicoses. *Adv. Vet. Sci.* 7:273-383.

Forsell, J. H., J. R. Kateley, T. Yoshizawa, and J. J. Pestka. 1985. Inhibition of mitogen-induced blastogenesis in human lymphocytes by T-2 toxin and its metabolites. *Appl. Environ. Microbiol.* 49:1523-1526.

Frederiksen, R. A. (Ed.). 1986. *Compendium of sorghum diseases*. American Phytopathological Society and Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas. 82 pp.

Friend, S. C., L. A. Babiuk, and H. B. Schieffer. 1983. The effects of dietary T-2 toxin on the immunological function and herpes simplex reactivation in Swiss mice. *Toxicol. Appl. Pharmacol.* 69:234-244.

Frobish, R. A., B. D. Bradley, D. D. Wagner, P. E. Long-Bradley, and H. Hairston. 1986. Aflatoxin residues in milk of dairy cows after ingestion of naturally contaminated grain. *J. Food Prot.* 49:781-785.

Froetschel, M. A., W. M. Hagler, Jr., W. J. Croom, Jr., J. F. Ort, T. J. Lauterio, J. M. Fernandez, D. L. Mann, H. P. Broquist, and C. G. Scanes. 1985. Tolerance of broiler chicks to slaframine and its effects upon growth hormone. *J. Poultry Sci.* 64:20.

Froetschel, M. A., W. J. Croom, Jr., W. M. Hagler, Jr., L. P. Tate, Jr., and H. P. Broquist. 1986. Effects of slaframine on ruminant digestive function: Resting salivary flow and composition in cattle. *J. Anim. Sci.* 62:1404-1411.

Gallagher, R. T., J. L. Richard, H. M. Stahr, and R. J. Cole. 1978. Cyclopiazonic acid production by aflatoxigenic and nonaflatoxigenic strains of *Aspergillus flavus*. *Mycopathologia* 66:31-36.

Gan, L-S, P. L. Skipper, X. Peng, J. D. Groopman, J.-S. Chen, G. N. Wogan, and S. R. Tannenbaum. 1988. Serum albumin adducts in the molecular epidemiology of aflatoxin carcinogenesis: Correlation with aflatoxin B_1 intake and urinary excretion of aflatoxin M_2. *Carcinogenesis* 9:1323-1325.

Gardiner, R. A., K. L. Rinehart, Jr., J. Snyder, and H. P. Broquist. 1968. Slaframine: Absolute stereochemistry and a revised structure. *J. Am. Chem. Soc.* 90:5639-5640.

Gardner, H. K., Jr., S. P. Koltun, F. G. Dollear, and E. T. Rayner. 1971. Inactivation of aflatoxins in peanut and cotton seed meals by ammoniation. *J. Am. Oil Chem. Soc.* 48:70-73.

Garner, R. C. 1973a. Chemical evidence for the formation of a reactive aflatoxin B_1 metabolite by hamster liver microsomes. *FEBS Lett.* 36:261-264.

Garner, R. C. 1973b. Microsome-dependent binding of aflatoxin B_1 to DNA, RNA, polyribonucleotides and protein *in vitro*. *Chem. Biol. Interact.* 6:125-129.

Gelderblom, W. C. A., K. Jaskiewicz, W. F. O. Marasas, P. G. Thiel, R. M. Horak, R. Vleggaar, and N. P. J. Kriek. 1988. Fumonisins - Novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Appl. Environ. Microbiol.* 54:1806-1811.

Gentry, P. A. 1982. The effect of administration of a single dose of T-2 toxin on blood coagulation parameters in the rabbit. *Can. J. Comp. Med.*. 46:414-419.

Gentry, P. A. and M. L. Cooper. 1983. Effect of intravenous administration of T-2 toxin on blood coagulation in calves. *Am. J. Vet. Res.* 44:741-746.

Gentry, P. A., M. L. Ross, and G. S. Bondy. 1987. Inhibitory effect of trichothecene mycotoxins in bovine platelets stimulated by platelet activating factor. *Can. J. Vet. Res.* 51:490-494.

Gerberick, G. F. and W. G. Sorenson. 1983. Toxicity of T-2 toxin, a *Fusarium* mycotoxin, to alveolar macrophages *in vitro*. *Environ. Res.* 32:269-285.

Gerberick, G. F., W. G. Sorenson, and D. M. Lewis. 1984. The effects of T-2 toxin on alveolar macrophage function *in vitro*. *Environ. Res.* 32:246-260.

Gilgan, M. W., E. B. Smalley, and F. M. Strong. 1966. Isolation and partial characterization of a toxin from *Fusarium tricinctum* on moldy corn. *Arch. Biochem. Biophys.* 114:1-3.

Goldblatt, L. A. 1971. Control and removal of aflatoxin. *J. Am. Oil Chem. Soc.* 48:605-610.

Goldblatt, L. A. and F. G. Dollear. 1969. Progress on elimination of aflatoxins from agricultural products. FAO/WHO/UNICEF P. A. G. Meeting, Geneva, Switzerland.

Goldblatt, L. A. and F. G. Dollear. 1977. Detoxification of contaminated crops. Pp. 139-150. In J. V. Rodricks, C. W. Hesseltine, and M. A. Mehlman (Eds.). *Mycotoxins in human and animal health*. Pathotox Publishers, Inc., Park Forest South, Illinois.

Goldblatt, L. A. and F. G. Dollear. 1979. Modifying mycotoxin contamination in feeds - Use of mold inhibitors, ammoniation, roasting. Pp. 167-184. In *Interactions of mycotoxins in animal production*. National Academy of Sciences, Washington, DC.

Gough, F. J. and E. S. Elliot. 1956. Blackpatch of red clover and other legumes caused by *Rhizoctonia leguminicola*. *West Virginia Agric. Exp. Sta. Bull.* 387T:1-23.

Groopman, J. D., P. R. Donahue, J. Zhu, J. Chen, and G. N. Wogan. 1985. Aflatoxin metabolism in humans: Detection of metabolites and nucleic acid adducts in urine by affinity chromatography. *Proc. Natl. Acad. Sci., USA* 82:6492-6497.

Groopman, J. D., P. R. Donahue, J. Zhu, J. Chen, and G. N. Wogan. 1987. Temporal patterns of aflatoxin metabolites in urine of people living in Guangxi Province, P.R.C. *Proc. Am. Assoc. Cancer Res.* 28:36.

Grove, J. F. 1988. Non-macrocyclic trichothecenes. *Natural Products Reports* 5:187-209.

Guengerich, F. P. and S. D. Aust. 1977. Activation of the parasympathomimetic alkaloid slaframine by microsomal and photochemical oxidation. *Mol. Pharmacol.* 13:185-195.

Guthrie, L. D. 1979. Effects of aflatoxin in corn on production and reproduction in dairy cattle. *J. Dairy Sci.* 62:134.

Gyongyossy-Issa, M. I. C. and G. G. Khachatourians. 1984. Interaction of T-2 toxin with murine lymphocytes. *Biochim. Biophys. Acta* 803:197-202.

Gyongyossy-Issa, M. I. C. and G. G. Khachatourians. 1985. Interaction of T-2 toxin and murine lymphocytes and the demonstration of a threshold effect on macromolecular synthesis. *Biochim. Biophys. Acta* 844:167-173.

Gyongyossy-Issa, M. I. C., V. Khanna, and G. G. Khachatourians. 1985. Characterisation of hemolysis induced by T-2 toxin. *Biochim. Biophys. Acta* 838:252-256.

Gyongyossy-Issa, M. I. C., R. T. Card, D. J. Fergusso, and G. G. Khachato. 1986a. Prehemolytic erythrocyte deformability changes caused by trichothecene T-2 toxin -- an ektacytometer study. *Blood Cells* 11:393-403.

Gyongyossy-Issa, M. I. C., R. T. Card, D. J. Fergusso, and G. G. Khachato. 1986b. Prehemolytic erythrocyte deformability

changes caused by trichothecene T-2 toxin — an ektacytometer study. Reply to commentary. *Blood Cells* 11:407.

Gyongyossy-Issa, M. I. C., V. Khanna, and G. G. Khachatourians. 1986c. Changes induced by T-2 toxin in the erythrocyte membrane. *Fd. Chem. Toxicol.* 24:311-317.

Hamilton, P. B. 1971. A natural and extremely severe occurrence of aflatoxicosis in laying hems. *Poultry Sci.* 50:1880-1882.

Hamilton, P. B. 1985. Factors influencing activity of fungi and antifungal agents in poultry feed. Pp. 207-218. In J. Lacey (Ed.). *Trichothecenes and other mycotoxins.* John Wiley and Sons, Ltd., New York.

Hamilton, P. B., W. E. Huff, J. R. Harris, and R. D. Wyatt. 1982. Natural occurrences of ochratoxicosis in poultry. *Poultry Sci.* 61:1832-1841.

Hao, D. Y. Y., R. E. Brackett, and T. O. M. Nakayama. 1987. Removal of aflatoxin B_1 from peanut milk by *Flavobacterium aurantiacum.* Pp. 15. In *Summary and recommendations of the international workshop on aflatoxin contamination of groundnut.* ICRISAT Center, India.

Harvey, R. B., L. F. Kubena, T. D. Phillips, W. E. Huff, and D. E. Corrier. 1988a. *Approaches to the prevention of aflatoxicosis.* Pp. 102-107. Proc. Maryland Nutr. Conf.

Harvey, R. B., T. D. Phillips, L. F. Kubena, and W. E. Huff. 1988b. Dietary hydrated sodium calcium aluminosilicate and its impact on aflatoxin toxicity in pigs and mild residues in dairy cows. *Dairy Agent Update* 7:3-4. Department of Animal Science, Cornell University, Ithaca, New York.

Harvey, R. B., L. F. Kubena, T. D. Phillips, W. E. Huff, and D. E. Corrier. 1989. Prevention of aflatoxicosis by addition of hydrated sodium calcium aluminosilicate to the diets of growing barrows. *Am. J. Vet. Res.* 50:416-420.

Haschek, W. M. and J. C. Haliburton. 1986. *Fusarium moniliforme* and zearalenone toxicoses in domestic animals: A review. Pp. 213-235. In J. L. Richard and J. R. Thurston (Eds.). *Diagnosis of mycotoxicoses.* Martinus Nijhoff Publishers, Boston, Massachusetts.

Hayes, A. W. (Ed.). 1981. *Mycotoxin teratogenicity and mutagenicity.* CRC Press, Boca Raton, Florida.

Hayes, M. A., R. D. Hood, and H. L. Lee. 1974. Teratogenic effects of ochratoxin A in mice. *Teratology* 9:93-97.

Hesseltine, C. W. 1986. Global significance of mycotoxins. Pp. 1-18. In P. S. Steyn and R. Vleggaar (Eds.). *Mycotoxins and phycotoxins.* A collection of invited papers presented at the Sixth International IUPAC Symposium on Mycotoxins and Phycotoxins, Pretoria, Republic of South Africa, July 22 to 25, 1985. Elsevier Scientific Publishing Co., Amsterdam, The Netherlands.

Hesseltine, C. W. 1988. *Mycotoxins and alcohol production: A review.* Pp. 1-23. In press. Malhorta Publications, New Delhi, India.

Hintz, H. F., H. Heitman, Jr., A. N. Booth, and W. E. Gagne. 1967. Effects of aflatoxin on reproduction in swine. *Proc. Soc. Exp. Biol. Med.* 126:146-148.

Hocking, A. D., K. Holds, and N. F. Tobin. 1988. Intoxication by tremorgenic mycotoxin (Penitrem A) in a dog. *Aust. Vet. J.* 65:82-85.

Hoerr, F. J. and G. H. D'Andrea. 1983. Biological effects of aflatoxin in swine. Pp. 51-55. In U. L. Diener, R. L. Asquith, and J. W. Dickens (Eds.). *Aflatoxin and Aspergillus flavus in corn.* Alabama Agriculture Experiment Station, Auburn University, Alabama.

Holzapfel, C. W., I. F. H. Purchase, P. S. Steyn, and L. Gouws. 1966. The toxicity and chemical assay of sterigmatocystin, a carcinogenic mycotoxin, and its isolation from two new fungal sources. *S. Afr. Med. J.* 40:1100-1101.

Hoerr, F. J., G. H. D'Andrea, J. J. Giambrone, and V. S Panangala. 1986. Comparative histopathologic changes in aflatoxicosis. Pp. 179-189. In J. L. Richard and J. L. Thurston (Eds.). *Diagnosis of Mycotoxicoses.* Martinus Nijhoff Publishers, Dordrecht.

Howerth, E. W., R. D. Wyatt, and D. A. Hayes. 1989. Leukoencephalomalacia in a white-tailed deer from North Carolina. *J. Wildlife Dis.* 25:384-387.

Hsieh, D. P. H. 1989. Potential human health hazards of mycotoxins. Pp. 69-80. In S. Natori, K. Hashimoto, and Y. Ueno (Eds.). *Mycotoxins and phycotoxins '88.* Elsevier, Amsterdam.

Hsieh, D. P. H., M. T. Lin, and R. C. Yao. 1973. Conversion of sterigmatocystin to aflatoxin B_1. *Biochem. Biophys. Res. Comm.* 52:992-997.

Hsieh, D. P. H., Z. A. Wong, J. J. Wong, C. Michas, and B. H. Ruebner. 1977a. Comparative metabolism of aflatoxin. Pp. 37-50. In J. V. Rodricks, C. W. Hesseltine, and M. A. Mehlman (Eds.). *Mycotoxins in human and animal health.* Pathotox Publishers, Inc., Park Forest South, Illinois.

Hsieh, D. P. H., Z. A. Wong, J. J. Wong, C. Michas, and B. H. Ruebner. 1977b. Hepatic transformation of aflatoxin and its carcinogenicity. Pp. 697-707. In H. H. Hiatt, J. D. Watson, and J. D. Winsten (Eds.). *Origins of human cancer.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Huff, W. E. 1980. A physical method for the segregation of aflatoxin contaminated corn. *Cereal Chem.* 57:236-238.

Huff, W. E., R. D. Wyatt, and P. B. Hamilton. 1975. Effects of dietary aflatoxin on certain egg yolk parameters. *Poult. Sci.* 54:2014-2018.

Huff, W. E., J. A. Doerr, P. B. Hamilton, and R. F. Vesonder. 1981. Acute toxicity of vomitoxin (deoxynivalenol) in broiler chickens. *Poult. Sci.* 60:1412-1414.

Huff, W. E., J. A. Doerr, C. J. Wabeck, G. W. Chaloupka, J. D. May, and J. W. Merkley. 1984. The individual and combined effects of aflatoxin and ochratoxin A on various processing parameters of broiler chickens. *Poult. Sci.* 63:2153-2161.

Hughes, B. L., B. D. Barnett, J. E. Jones, and J. W. Dick. 1979. Safety of feeding aflatoxin-inactivated corn with the Leghorn layer-breeders. *Poultry Sci.* 58:1202-1209.

Hurburgh, C. R. and C. J. Bern. 1983. Sampling corn and soybeans. 1. Probing method. *Trans. Am. Soc. Agric. Engr.* 26:930.

Hurburgh, Jr., C. R. and H. M. Stahr. 1989. Aflatoxin in 1988 Iowa corn. Presented at NC151 Meeting, Marketing and Delivery of Quality Cereals and Oilseed in Domestic and Foreign Markets. St. Louis University, St. Louis, Missouri, February 17, 1989.

International Agency for Research on Cancer. 1986. Some halogenated hydrocarbons and pesticide exposures. *Evaluation of the carcinogenic risk of chemicals to humans* 41:22. World Health Organization, Lyon, France.

International Agency for Research on Cancer. 1987. IARC monograph on the evaluation of carcinogenic risk to humans. Suppl. 1, IARC, Lyon, France. Pp. 82-87.

Iowa Corn Growers Association. 1989. Aflatoxin: strategies for the futue. *Iowa Aflatoxin Task Force.* August 1989.

Jagadeesan, V., C. Rukmini, M. Vijayaraghavan, and P. G. Tulpule. 1982. Immune studies with T-2 toxin: Effect of feeding and withdrawal in monkeys. *Fd. Chem. Toxicol.* 20:83-87.

Jelinek, C. F. 1987. *Distribution of mycotoxin - an analysis of worldwide commodities data, including data from FAO/WHO/UNEP food contamination monitoring programme.* Joint FAO/WHO/UNEP Second International Conference on Mycotoxins. Bangkok, Thailand, September 28 to October 3, 1987.

Jelinek, C. F., A. E. Pohland, and G. E. Wood. 1989. Worldwide occurrence of mycotoxins in foods and feeds — an update. *J.*

Assoc. Off. Anal. Chem. 72:223-230.

Jemmali, M. 1987. *Trade and economic implications of mycotoxins: Need for greater uniformity.* Report No. 6. Working paper presented at the Second Joint FAO/WHO/UNEP International Conference on Mycotoxins. Bankok, Thailand, September 28 to October 3, 1987.

Joffe, A. Z. 1983. Foodborne diseases: Alimentary toxic aleukia. Pp. 351-495. In M. Rechcigle (Ed.). *Handbook of foodborne disease of biological origin.* CRC Press, Boca Raton, Florida.

Joffe, A. Z. 1986. *Fusarium* species: Their biology and toxicology. John Wiley and Sons, New York.

John, D. W. and L. L. Miller. 1969. Effect of aflatoxin B_1 on net synthesis of albumin, fibrinogen, and alpha-acid glycoprotein by isolated perfused rat liver. *Biochem. Pharmacol.* 18:1135-1146.

Johnson, A. D., M. E. Wilson, W. J. Croom, Jr., W. M. Hagler, Jr., J. F. Ort, J. M. Fernandez, and C. Scanes. 1986. Toxicity of slaframine on broiler chicks and its effects on circulating metabolites and hormones. *Poultry Sci.* 65:65.

Kanai, K. and E. Kondo. 1984. Decreased resistance to mycobacterial infection in mice fed a trichothecene compound (T-2 toxin). *Jpn. J. Med. Sci. Biol.* 37:97-104.

Kellerman, T. S., W. F. O. Marasas, J. G. Pienaar and T. W. Naude. 1972. A mycotoxicosis of equidae caused by *Fusarium moniliforme* Sheldon. A preliminary communication. *Oonderstepoort J. Vet. Res.* 39:205-208.

Kemppainen, B. W., R. T. Riley, and S. Biles-Thurlow. 1987a. Comparison of penetration and metabolism of [^{3}H]diacetoxyscirpenol, [^{3}H]verrucarin A and [^{3}H]T-2 toxin in skin. *Fd. Chem. Toxicol.* 25:379-386.

Kemppainen. G. W., J. G. Pace, and R. T. Riley. 1987b. Comparison of *in vivo* and *in vitro* percutaneous absorption of T-2 toxin in guinea pigs. *Toxicon.* 25:1153-1162.

Keogh, R. G. 1983. Ryegrass staggers: Management and control. *Proc. New Zeal. Grassland Assoc.* 44:248-250.

Kiermeier, F., G. Weiss, G. Behringer, M. Miller, and R. Ranfft. 1977. Vorkommen und Gehalt an Aflatoxin M, in Molkerei-Anlieferungsmilch. *Z. Lebens. Forsch.* 163:171-173.

King, B. 1979. Outbreaks of ergotism in Wollo, Ethiopia. *Lancet* 1:1411.

Kriek, N. P. J., T. S. Kellerman, and W. F. O. Marasas. 1981. Comparative study of the toxicity of *Fusarium verticillioides (=F. moniliforme)* to horses, primates, pigs, sheep and rats. *Oonderstepoort J. Vet. Res.* 43:129-131.

Krishnamachari, K. A. V. R. and R. V. Bhat. 1976. Poisoning of ergoty bajra (pearl millet) in man. *Indian J. Med. Res.* 64:1624-1628.

Krishuamachari, K. A. V. R., R. V. Bhat, V. Nagaraja, and T. B. G. Tilak. 1975a. Investigations into an outbreak of hepatitis in parts of Western India. *Indian J. Med. Res.* 63:1036-1049.

Krishuamachari, K. A. V. R., V. Nagarajan, R. V. Bhat, and T. B. G. Tilak. 1975b. Hepatitis due to aflatoxicosis. An outbreak in Western India. *Lancet* i:1061-1062.

Krogh, P. 1976. Epidemiology of mycotoxic porcine nephropathy. *Nord. Vet. Med.* 28:452-458.

Krogh, P. 1977. Ochratoxins. Pp. 489-498. In J. V. Rodricks, C. W. Hesseltine, and M. A. Mehlman (Eds.). *Mycotoxins in human and animal health.* Pathotox Publishers, Inc., Park Forest South, Illinois.

Krogh, P., B. Hald, and E. J. Pederson. 1973. Occurrence of ochratoxin A and citrinin in cereals associated with mycotoxic porcine nephropathy. *Acta Pathol. Microbiol. Scand. Sect. B. Microbiol.* 81:681-683.

Krogh, P., B. Hald, P. England, L. Rutquist, and O. Swohn. 1974. Contamination of Swedish cereals with ochratoxin A. *Acta Pathol. Microbiol. Scand. Sect. B Microbiol.* 82:701-702.

Kubena, L. F., R. B. Harvey, T. D. Phillips, and N. D Heidelbaugh. 1987. *Novel approach to the preventive management of aflatoxicosis poultry.* Pp. 302-304. Proc. U.S. Anim. Health Assoc.

Kubena, L. F., R. B. Harvey, T. D. Phillips, and W. E. Huff. 1988. Modulation of aflatoxins in growing chickens by dietary addition of a hydrated sodium calcium aluminosilicate. *Poultry Sci.* 67:106 (Abstract).

Kuiper-Goodman, T., P. M. Scott, and H. Watanabe. 1987. Risk assessment of the mycotoxin zearalenone. Reg. Toxicol. Pharmacol. 7:253-306.

Kuiper-Goodman, T. and P.M. Scott. 1989. Risk assessment of the mycotoxin, ochratoxin A. *Biomed. Environ. Sci.* 2:179-248.

Kurtz, H. J. and C. J. Mirocha. 1978. Zearalenone (F2) induced estrogenic syndrome in swine. Pp. 1256-1264. In T. D. Wyllie and L. G. Morehouse (Eds.). *Mycotoxic fungi, mycotoxins, mycotoxicoses.* Vol. 2. Marcel Dekker, New York.

Kurtzman, C. P., B. W. Horn, and C. W. Hesseltine. 1987. *Aspergillus nomius,* a new aflatoxin-producing species related to *Aspergillus flavus* and *Aspergillus tamarii. Anton v. Leeuwenhoek* 53:147-158.

Lacey, J. 1986. Factors affecting mycotoxin production. Pp. 65-76. In P. S. Steyn and R. Vleggaar (Eds.). *Mycotoxins and phycotoxins.* Elsevier Scientific Publishing Co., Amsterdam, The Netherlands.

LaFarge-Frayssinet, C., G. Lespinats, P. Lafont, F. Loisillier, S. Mousset, Y. Rosenstein, and C. Frayssinet. 1979. Immunosuppressive effects of *Fusarium* extracts and trichothecenes: Blastogenic response of murine splenic and thymic cells to mitogens (40439). *Proc. Soc. Exp. Biol. Med.* 160:302-311.

Lafont, P. and J. Lafont. 1970. Production de nidulotoxine par des *Aspergillus* appartenant a differentes especes. *Experientia* 26:807-808.

Lam, K. C., M. C. Tu, J. W. C. Leung, and B. E. Henderson. 1982. Hepatitis B virus and cigarette smoking: Risk factors for hepatocellular carcinoma in Hong Kong. *Cancer Res.* 42:5246-5248.

Lancaster, M. C. 1968. Comparative aspects of aflatoxin-induced hepatic tumors. *Cancer Res.* 28:2288-2292.

Lanier, A. P., T. R. Bender, W. J. Blot, J. F. Fraumeni, and W. B. Hurlburt. 1976. Cancer incidence in Alaska Natives. *Intl. J. Cancer* 18:409-412.

Lansden, J. A. and J. I. Davidson. 1983. Occurrence of cyclopiazonic acid in peanuts. *Appl. Environ. Microbiol.* 45:766-769.

Lanza, G. M., K. W. Washburn, and R. D. Wyatt. 1980. Strain variation in hematological response of broilers to dietary aflatoxin. *Poultry Sci.* 59:2686-2691.

LeBars, J. 1979. Cyclopiazonic acid production by *Penicillium camemberti* Thom and natural occurrence of this mycotoxin in cheese. *Appl. Environ. Microbiol.* 38:1052-1055.

Lee, L. S. 1989. Aflatoxin. *J. Am. Oil Chem. Soc.* 66:1398-1413.

Leistner, L. 1984. Toxigenic Penicillia occurring in feeds and foods. In H. Kurata and Y. Ueno (Eds.). *Toxigenic fungi – their toxins and health hazard.* Elsevier Science Publishing Company, Inc. New York. Pp. 162-171.

Lewis, G., L. M. Mackson, and R. Allcroft. 1967. The effect of feeding toxic groundnut meal to sheep over a period of 5 years. *Vet. Rec.* 80:312-314.

Lewis, W. H. (Ed.). 1977. *Medical botany.* Pp. 416-418. John Wiley & Sons, New York.

Lijinsky, W. and W. H. Butler. 1966. Purification and toxicity of aflatoxin G_1. *Proc. Soc. Exp. Biol. Med.* 123:151.

Lin, J. K., J. A. Miller, and C. E. Miller. 1977. 2,3-dihydro2(guan-7-yl)-3-hydroxy aflatoxin B_1: A major acid hydrolysis product of aflatoxin B_1-DNA or ribosomal RNA adducts formed in

hepatic microsome-mediated reactions and in rat liver *in vivo*. Cancer Res. 37:4430-4438.

Lindenfelser, L. A. and A. Ciegler. 1970. Studies on aflatoxin detoxification in shelled corn by ensiling. *J. Agric. Fd. Chem.* 18:640-643.

Linsell, A. 1982. Carcinogenicity of mycotoxins. Pp. 3-14. In H. Egan et al. (Eds.). *Environmental carcinogenesis, Selected methods of analysis. Vol. 5, Some mycotoxins.* IARC, Lyon, France.

Linsell, C. A. and F. G. Peers. 1977. *Trans. Royal Soc. Trop. Med.* Hyg. 71:471-473.

Lomax, L. G., R. J. Cole, and J. W. Dorner. 1984. The toxicity of cyclopiazonic acid in weaned pigs. *Vet. Pathol.* 21:418-424.

Loveless, A. R. 1967. *Claviceps fusiformis* Sp. Nov., the causal agent of an aglactia of sows. *Trans. Brit. Myco. Soc.* 50:15-18.

Lutsky, I., N. Mor, B. Yagen, and A. Z. Joffe. 1978. The role of T-2 toxin in experimental alimentary toxic aleukia: A toxicity study in cats. *Toxicol. Appl. Pharmacol.* 43:111-124.

Machen, M. D., B. A. Clement, E. C. Shepherd, A. B. Sarr, R. E. Pettit, and T. D. Phillips. 1988. Sorption of aflatoxins from peanut oil by aluminosilicates. *Toxicologist* 8:265 (Abstract).

Mann, G. E., L. P. Codifer, Jr., and F. G. Dollear. 1967. Effect of heat on aflatoxins in oilseed meals. *J. Agric. Fd. Chem.* 15:1090.

Mannon, J. and E. Johnson. 1985. Fungi down on the farm. *New Scientist.* 105 (1446):12-16.

Marasas, W. F. O. 1974. *Phomopsis leptospromiformis.* Pp. 111-127. In I. F. H. Purchase (Ed.). *Mycotoxins.* Elsevier Scientific Publishing Co., New York.

Marasas, W. F. O. and P. E. Nelson (Eds.). 1987. *Mycotoxicology.* Pennsylvania State University Press, University Park, Pennsylvania. 102 pp.

Marasas, W. F. O., T. S. Kellerman, J. G. Pienaar, and T. W. Naude. 1976. Leukoencephalomalacia: A mycotoxicosis of equidae caused by *Fusarium moniliforme* Sheldon. *Oonderstepoort J. Vet. Res.* 43:113-122.

Marasas, W. F. O., T. S. Kellerman, W. C. A. Gelderblom, J. A. W. Coetzer, P. G. Thiel, and J. J. Van Der Lugt. 1988. Leucoencephalomalacia in a horse induced by fumonisin B_1 isolated from *Fusarium moniliforme. Ondersteport J. Vet. Res.* 55:197-203.

Marquardt, R. R., A. Frohlich, and D. Abramson. 1989. Ochratoxin A — an important Western Canadian storage mycotoxin. Can. J. Physiol. Pharmacol. In press.

Marsh, C. D., A. B. Clawson, and M. Marsh. 1916. *United States Department of Agriculture Bulletin* 405:1.

Marth, E. H. and M. P. Doyle. 1979. Update on molds: Degradation aflatoxin. *Fd. Technol.* 33:81-87.

Masimanco, N., J. Remacle, and J. Ramaut. 1973. Elimination of aflatoxin B_1 by absorbent clays in contaminated substrates. *Ann. de Nutr. et Alimen.* 23:137-147.

Masri, M. S., H. L. E. Vix, and L. A. Goldblatt. 1969. Process for detoxifying substances contaminated with aflatoxin. United States Patent 3,429,709.

Mayer, C. F. 1953a. Endemic panmyelotoxicosis in the Russian grain belt. Part one: The clinical aspects of alimentary toxic aleukia (ATA). A comprehensive review. *Mil. Surg.* 113:173-189.

Mayer, C. F. 1953b. Endemic panmyelotoxicosis in the Russian grain belt. Part two: The botany, phytopathology, and toxicology of Russian cereal food. *Mil. Surg.* 113:295-315.

McLean, A. E. M. and E. K. McLean. 1967. Protein depletion and toxic liver injury due to carbon tetrachloride and aflatoxin. *Nutr. Soc. Proc.* 26:13.

McMillan, W. W., D. M. Wilson, and N. W. Widstrom. 1985. Aflatoxin contamination of preharvest corn in Georgia: A six year study of insect damage and visible *Aspergillus flavus. J. Environ. Qual.* 14:200-202.

Mertens, D. R. 1979. Biological effects of mycotoxins upon rumen function and lactating dairy cows. Pp. 118-136. Proceedings of a symposium, July 13, 1978, Michigan State University, Lansing. *Interaction of mycotoxins in animal production.* National Academy of Sciences, Washington, D. C.

Miller, J. K., A. Hacking, J. Harrison, and V. J. Gross. 1973. Stillbirths, neonatal mortality and small litters in pigs associated with the ingestion of *Fusarium* toxin by pregnant sows. *Vet. Rec.* 93:555-559.

Miller, D. M., B. P. Stuart, W. A. Crowell, R. J. Cole, A. J. Goven, and J. Brown. 1978. Aflatoxicosis in swine: Its effect of immunity and relationship to salmonellosis. *Am. Assoc. Vet. Lab Diag.* 21:135-146.

Miller, D. M., B. P. Stuart, and W. A. Crowell. 1981. Experimental aflatoxicosis in swine: Morphological and clinical pathological results. *Can. J. Comp. Med.* 45:343-351.

Miller, D. M., W. A. Crowell, and B. P. Stuart. 1982. Acute aflatoxicoses in swine: Clinical pathology, histopathology, and electron microscopy. *Am. J. Vet. Res.* 43:273-277.

Mislivec, P. B., M. W. Trucksess, L. Stoloff. 1988. The effect of other toxigenic mold species on aflatoxin production by *Aspergillus flavus* in sterile broth shake cultures. *J. Food Prot.* 51:449-451.

Monegue, H. J., C. E. Combs, G. T. Edds, and H. D. Wallace (Eds.). 1977. *The effects of various levels of aflatoxins on young swine.* Florida Agriculture Experiment Station Report. AL-1977-5.

Monroe, D. H. and D. L. Eaton. 1987. Comparative effects of butylated hydroxyanisole (BHA) on *in vivo* and *in vitro* metabolism of aflatoxin B_1 (AFB) in rat and mouse liver. *Toxicologist* 7:37 (Abstract).

Morehouse, L. G. 1985. Mycotoxins of veterinary importance in the United States. Pp. 383-410. In J. Lacey (Ed.). *Trichothecenes and other mycotoxins.* Proc. Intl. Mycotoxin Sympos., Sydney, Australia, 1984. John Wiley & Sons, New York.

Mortimer, P. H. 1978. Perennial ryegrass staggers in New Zealand. Pp. 353-361. In R. F. Keeler, K. R. van Kampen, and L. F. James (Eds.). *Proc. Joint U.S.-Aust. Sympos. Poisonous Plants.*

Mortimer, P. H. 1983. Ryegrass staggers: Clinical, pathological and seriological aspects. *Proc. New Zeal. Grassland Assoc.* 44:230-233.

Natarajan, K. R., K. C. Rhee, C. M. Cater, and K. F. Mattil. 1975. Distribution of aflatoxins in various fractions separated from raw peanuts and defatted peanut meal. *J. Am. Oil Chem. Soc.* 52:44-47.

National Peanut Council. Peanut Quality Task Force. 1988. *U. S. peanut quality: An industry commitment: Consensus report of the peanut quality task force.* National Peanut Council. 48 pp.

National Toxicology Program. 1989. Toxicology and carcinogenesis studies of ochratoxin A in F344N rats. Technical Report No. 358, U.S. Department of Human Health Services.

Neal, G. E. 1973. Inhibition of rat liver RNA synthesis by aflatoxin B_1. *Nature* (London) 244:432-435.

Neal, G. E., D. J. Judah, F. Stirpe, and D. S. P. Patterson. 1981. The formation of 2,3-dihydroxy-2,3-dihydro-aflatoxin B_1 by the metabolism of aflatoxin B_1 by liver microsomes isolated from certain avian and mammalian species and the possible role of this metabolite in the acute toxicity of aflatoxin B_1. *Toxicol. Appl. Pharmacol.* 58:431-437.

Nesheim, S. (Ed.). 1979. *Methods of aflatoxin analysis.* NBS Spec. Publ., (US) 519:355.

Newberne, P. M. 1987. Interaction of nutrients and other factors with mycotoxins. Pp. 177-216. In P. Krogh (Ed.). *Mycotoxins in food.* Academic Press, New York.

Newberne, P. M. and W. H. Butler. 1969. Acute and chronic effects of aflatoxin on the liver of domestic and laboratory animals: A review. *Cancer Res.* 29:239-250.

Newberne, P. M. and R. 1. Gross. 1977. The role of nutrition in aflatoxin injury. Pp. 51-65. In J. V. Rodricks, C. W. Hessletine, and M. A. Mehlman (Eds.). *Mycotoxins in human and animal health.* Pathotox Publishers, Inc., Park Forest South, Illinois.

Ngindu et al. 1982. Outbreak of acute hepatitis caused by aflatoxin poisoning in Kenya. *Lancet* i:1346-1348.

Nichols, T. E., Jr. 1983. Economic impact of aflatoxin in corn. Pp. 67-71. In U. L. Diener, R. L. Asquith, and J. W. Dickens (Eds.). *Aflatoxin and Aspergillus flavus in corn: Southern cooperative series bulletin 279.* Southern Regional Research Project S-132. Alabama Agricultural Experiment Station, Auburn University, Auburn, Alabama.

Nichols, T. E., Jr. 1987. Aflatoxin in the southeastern USA. Pp. 339-347. In M. S. Zuber, E. B. Lillehoj, and B. L. Renfro (Eds.). *Aflatoxins in maize: Proceedings of a workshop.* CIMMYT, Mexico.

Niyo, K. A. and J. L. Richard. 1986. Effect of T-2 mycotoxin on phagocytosis of *Aspergillus fumigatus* conidia by rabbit alveolar macrophages. Proc. 1st Intl. Vet. Immunol. Symp. University of Guelph, Ontario, Canada. Pp. 74.

Niyo, K. A., J. L. Richard, Y. Niyo, and L. H. Tiffany. 1988a. Effects of T-2 mycotoxin ingestion on phagocytosis of *Aspergillus fumigatus* conidia by rabbit alveolar macrophages and on hematologic, serum biochemical, and pathologic changes in rabbits. *Am. J. Vet. Res.* 49:1766-1773.

Niyo, K. A., J. L. Richard, Y. Niyo, and L. H. Tiffany. 1988b. Pathologic, hematologic, serologic, and mycologic changes in rabbits given T-2 mycotoxin orally and exposed to aerosols of *Aspergillus fumigatus* conidia. *Am. J. Vet. Res.* 49:2151-2160.

Norred, W. P. 1979. Effect of ammoniation on the toxicity of corn artificially contaminated with aflatoxin B_1. *Toxicol. Appl. Pharmacol.* 51:411-416.

Norred, W. P. 1981. Excretion and distribution of ammoniated ^{14}C-labeled aflatoxin B_1-contaminated corn. *Fed. Proc.* 40:280 (Abstract).

Norred, W. P. 1986. Occurrence and clinical manifestations of aflatoxicosis. Pp. 11-30. In J. L. Richard and J. R. Thurston (Eds.). *Diagnosis of mycotoxicoses.* Martinus Nijhoff Publishers, Dordrecht, The Netherlands.

Norred, W. P. and R. Morrissey. 1983. Effects of long-term feeding of ammoniated aflatoxin contaminated corn to Fisher 344 rats. *Toxicol. Appl. Pharmacol.* 70:96-104.

Norred, W. P., R. E. Morrissey, R. T. Riley, R. J. Cole, and J. W. Dorner. 1985. Distribution, excretion and skeletal muscle effects of the mycotoxin [C14]cyclopiazonic acid in rats. *Fd. Chem. Toxicol.* 23:1069-1076.

Norred, W. P., J. K. Porter, J. W. Dorner, and R. J. Cole. 1988. Occurrence of the mycotoxin, cyclopiazonic acid, in meat after oral administration to chickens. *J. Agric. Fd. Chem.* 36:113-116.

Norred, W. P., R. D. Plattner, K. A. Voss, C. W. Bacon, and J. K. Porter. 1989. Natural occurrence of fumonisins in corn associated with equine leukoencephalomalacia (ELEM). *Toxicologist* 9:258. (Abstract)

Northolt, M. D., H. P. van Egmond, P. Soentoro, and E. Deijll. 1980. Fungal growth and presence of sterigmatocystin in hard cheese. *J. Assoc. Off. Anal. Chem.* 63:115-117.

Nuehring, L. P., G. N. Rowland, L. R. Harrison, R. J. Cole, and J. W. Dorner. 1985. Cyclopiazonic acid mycotoxicosis in the dog. Am. J. Vet. Res. 46:1670-1676.

Okuda, K. and R. P. Beasley. 1982. Epidemiology of hepatocellular carcinoma. In K. Okuda and J. Mackay (Eds.). *VICC Report 17.* Geneva, Switzerland.

Osborne, D. J. and P. B. Hamilton. 1981. Decreased pancreatic digestive enzymes during aflatoxicosis. *Poultry Sci.* 60:1818-1821.

Palmgren, M. S. and A. W. Hayes. 1987. Aflatoxin in food. Pp. 65-95. In P. Krogh (Ed.). *Mycotoxins in food.* Academic Press, New York.

Palyusik, M. and E. Koplik-Kovacs. 1975. Effect on laying geese of feeds containing the fusariotoxins T2 and F2. *Acta Vet. Acad. Sci. Hung., Tomus* 25:363-368.

Pang, V. R., P. J. Felsburg, V. R. Beasley, W. B. Buck, and W. M. Haschek. 1987. The toxicity of T-2 toxin in swine following topical application. II. Effects on hematology, serum biochemistry, and immune response. *Fund. Appl. Toxicol.* 9:50-59.

Park, D. L. and A. E. Pohland. 1986. A rationale for the control of aflatoxin in animal feeds. Pp. 473-482. In P. S. Steyn and R. Vleggaar (Eds.). *Mycotoxins and phycotoxins.* A collection of invited papers presented at the Sixth International IUPAC Symposium on Mycotoxins and Phycotoxins, Pretoria, Republic of South Africa, July 22 to 25, 1985. Elsevier Scientific Publishing, Amsterdam, The Netherlands.

Park, D. L. and A. E. Pohland. 1989. Sampling and sample preparation for detection and quantitation of natural toxicants in food and feed. *J. Assoc. Off. Anal. Chem.* 72:399-404.

Park, D. L., L. S. Lee, and S. A. Kolton. 1984. Distribution of ammonia-related aflatoxin reaction products in cottonseed meal. *J. Am. Oil Chem. Soc.* 61:1071-1074.

Park, D. L., L. S. Lee, R. L Price, and A. E. Pohland. 1988. Review of the decontamination of aflatoxins by ammoniation: Current status and regulation. *J. Assoc. Off. Anal. Chem.* 71:685-703.

Patterson, D. S. P. 1973. Metabolism as a factor in determining the toxic action of the aflatoxins in different animal species. *Fd. Cosmet. Toxicol.* 11:287-294.

Patterson, D. S. P. and B. A. Roberts. 1970. The composition of liver microsomes and levels of detoxicating enzymes in 9 animal species. *Rev. Vet. Sci.* 11:399-401.

Payne, G. A. 1983. Nature of field infection of corn by *Aspergillus flavus.* Pp. 16-19. In U. L. Diener, R. L. Asquith, and J. W. Dickens (Eds.). *Aflatoxin and Aspergillus flavus in corn..* Southern Cooperative Series Bulletin 279, Auburn University, Auburn, Alabama.

Peace, D. M. and J. Harwig. 1982. Screening for ergot particles in grain products by light microscopy. *Can. Inst. Food Sci. Technol J.* 15:147-149.

Peers, F. G. and C. A. Linsell. 1973. Dietary aflatoxins and liver cancer — a population based study in Kenya. *Br. J. Cancer* 27:473-485.

Peers, F., X. Bosch, J. Kaldor, A. Linsell, and M. Pluijman. 1987. Aflatoxin exposure, hepatitis B virus infection and liver cancer in Swaziland. *Intl. J. Cancer* 39:545-553.

Pestka, J. J. 1988. Enhanced surveillance of foodborne mycotoxins by immunoassay. *J. Assoc. Off. Anal. Chem..* 71:1075-1081.

Pestka, J. J. and W. Casale. 1988. Naturally occurring fungal toxins. In M. S. Simmons and J. Nriagu (Eds.). *Food contamination from environmental sources.* John Wiley and Sons, Ltd. In press.

Pestka, J. J., M. A. Moorman, and R. Warner. 1989. Dysregulation of IgA production and IgA nephropathy induced by dietary vomitoxin. Food Chem. Toxicol. 27:361-368.

Pestka, J. J., J.-H. Tai, M. F. Witt, D. E. Dixon, and J. H. Forsell. 1987. Suppression of immune response in the B6C3F_1 mouse after dietary exposure to the *Fusarium* mycotoxins deoxynivalenol and zearalenone. *Fd. Chem. Toxicol.* 25:297-304.

Phillips, T. D. 1987. Novel approaches to detection and detoxification of mycotoxins. Pp. C3-C11. In *Recent developments in the study of mycotoxins.* Kaiser Chemicals, Cleveland, Ohio.

Phillips, T. D., L. F. Kubena, R. B. Harvey, D. R. Taylor, and N. D. Heidelbaugh. 1987. Mycotoxin hazards in agriculture: New approach to control. *J. Am. Vet. Med. Assoc.* 190:1617 (Abstract).

Phillips, T. D., L. F. Kubena, R. B. Harvey, D. R. Taylor, and N. D. Heidelbaugh. 1988a. Hydrated sodium calcium aluminosilicate: A high affinity sorbent for aflatoxin. *Poultry Sci.* 67:243-247.

Phillips, T. D., B. A. Clement, L. F. Kubena, and R. B. Harvey. 1988b. *Mycotoxins: Detection and detoxification.* Proc. 23rd Natl. Meet. Poultry Health and Condemnations, October 20 and 21, Ocean City, Maryland.

Phillips, T. D., B. A. Clement, L. F. Kubena, and R. B. Harvey. 1989. Prevention of aflatoxicosis in animals and aflatoxin residues in food of animal origin with hydrated sodium calcium aluminosilicate. *Proc. World Assoc. Vet. Food Hygienists*, Stockholm, Sweden, July 2-7, 1989.

Pienaar, H. G., T. S. Kellerman, and W. F. O. Marasas. 1981. Field outbreaks of leukoencephalomalacia in horses consuming maize infected by *Fusarium verticillioides (=F. moniliforme)* in South Africa. *J. So. Afr. Vet. Assoc.* 52:21-24.

Pier, A. C. 1981. Mycotoxins and animal health. *Adv. Vet. Sci. Comp. Med.* 25:185-243.

Pier, A. C. 1986. Immunomodulation in aflatoxicosis. Pp. 143-148. In J. L. Richard and J. L. Thurston (Eds.). *Diagnosis of mycotoxicoses.* Martinus Nijhoff Publishers, Dordrecht, The Netherlands.

Pier, A. C., S. J. Cysewski, J. L. Richard, A. L. Baetz, and L. Mitchell. 1976. Experimental mycotoxicoses in calves with aflatoxin, ochratoxin, rubratoxin, and T-2 toxin. *Proc. U.S. Anim. Health Assoc.* Pp. 130-148.

Pier, A. C., J. L. Richard, and J. R. Thurston. 1980a. Effects of mycotoxins on immunity and resistance of animals. Pp. 691-699. In D. Eaker and T. Wadstrom (Eds.). *Natural toxins.* Pergamon Press, New York.

Pier, A. C., J. L. Richard, and J. R. Thurston. 1980b. Effects of aflatoxin on the mechanisms of immunity and native resistance. Pp. 301-309. In H.-J. Preusser (Ed.). *Medical Mycology. Proc. Mycological Symposia of the XII Intl. Congr. of Microbiol.* Gustav Fischer Verlag, New York.

Pier, A. C., M. E. McLaughlin, J. L. Richard, A. Baetz, and R. R. Dahlgren. 1985. *In utero* transfer of aflatoxin and selected effects in neonatal pigs. Pp. 495-506. In J. Lacey (Ed.). *Trichothecenes and other mycotoxins.* Proc. Intl. Mycotoxin Sympos., Sydney, Australia, 1984. John Wiley & Sons, New York.

Pohland, A. E., S. Nesheim, and L. Friedman. 1990. Ochratoxin: a review. *Pure and Appl. Chem.* in press.

Pokrovsky, V. I. and V. A. Tutelyan. 1982. Ergotism: a companion of natural disaster. *Ter. Ark.* p. 108-110.

Polzhofer, K.-P. 1977. Thermal stability of aflatoxin M_1. *Zeitschrift fur Lebensmittel-Untersuchung und Forschung* 164:80-81.

Price, R. L., J. H. Paulson, O. G. Lough, C. Gingg, and A. G. Kurtz. 1984. Aflatoxin conversion by dairy cattle consuming naturally-contaminated whole cottonseed. *Bull. Dept. Nutr. Fd. Sci.*. University of Arizona, Tucson, Arizona. 16 pp.

Price, R. L., J. H. Paulson, O. G. Lough, C. Gingg, and A. G. Kurtz. 1985. Aflatoxin conversion by dairy cattle consuming naturally-contaminated whole cottonseed. *J. Fd. Prot.* 48:11-15.

Purchase, I. F. H. 1967. Acute toxicity of aflatoxins M_1 and M_2 in one-day-old ducklings. *Fd. Cosmet. Toxicol.* 5:339.

Purchase, I. F. H. 1971. The acute toxicity of the mycotoxin cyclopiazonic acid to rats. *Toxicol. Appl. Pharmacol.* 18:114-123.

Purchase, I. F. H. and J. J. Van der Watt. 1970. Carcinogenicity of sterigmatocystin. *Fd. Cosmet. Toxicol.* 8:289-295.

Rainey, D. P., E. B. Smalley, M. H. Crump, and F. M. Strong. 1965. Isolation of salivation factor from *Rhizoctonia leguminicola* on red clover hay. *Nature* (London) 205:203-204.

Ramsdell, H. S. and D. L. Eaton. 1988. Modification of aflatoxin B_1 biotransformation *in vitro* and DNA binding *in vivo by dietary broccoli in rats.* Toxicologist. 8:168 (Abstract).

Rao, B. L. and A. Husain. 1985. Presence of cyclopiazonic acid in kodo millet (*Paspalum scrobiculatum*) causing "kodua poisoning" in man and its production by associated fungi. *Mycopathology* 89:177-180.

Rayner, E. T., S. P. Koltun, and F. G. Dollear. 1977. Solvent extraction of aflatoxins from contaminated agricultural products. *J. Am. Oil Chem. Soc.* 54:242A-244A.

Reiss, J. 1978. Mycotoxins in foodstuffs. XI. Fate of aflatoxin B_1 during preparation and baking of whole wheat bread. *Cereal Chem.* 55:421-423.

Remington, R. D. and M. A. Schrok (Eds.). 1970. *Statistics and applications to the biological and health sciences.* Prentice-Hall, Englewood Cliffs, New Jersey. 105 pp.

Richard, J. L. and L. H. Arp. 1979. Natural occurrence of the mycotoxin penitrem A in moldy cream cheese. *Mycopathologia* 67:107-109.

Richard, J. L., J. R. Thurston, and C. K. Graham. 1974. Changes in complement activity, serum proteins, and prothrombin time in guinea pigs fed rubratoxin alone or in combination with aflatoxin. *Am. J. Vet. Res.* 35:957-959.

Richard, J. L., J. R. Thurston, B. L. Deyoe, and G. D. Booth. 1975. Effect of ochratoxin and aflatoxin on serum proteins, complement activity, and antibody production to *Brucella abortus* in guinea pigs. *Appl. Microbiol.* 29:27-29.

Richard, J. L., J. R. Thurston, E. B. Lillehoj, S. J. Cysewski, G. D. Booth. 1978a. Complement activity, serum protein, and hepatic changes in guinea pigs given sterigmatocystin or aflatoxin, alone or in combination. *Am. J. Vet. Res.* 39:163-166.

Richard, J. L., J. R. Thurston, and A. C. Pier. 1978b. Effects of mycotoxins on immunity. Pp. 801-817. In P. Rosenberg (Ed.). *Toxins: Animal, plant, and microbial.* Pergamon Press, New York.

Richard, J. L., P. Bocchetti, and L. H. Arp. 1981. Moldy walnut toxicosis in a dog, caused by the mycotoxin, penitrem A. *Mycopathologia* 76:55-58.

Richard, J. L., A. C. Pier, R. D. Stubblefield, O. L. Shotwell, R. L. Lyon, and R. C. Cutlip. 1983. Effect of feeding corn naturally contaminated with aflatoxin on feed efficiency, on physiologic, immunologic, and pathologic changes, and on tissue residues in steers. *Am. J. Vet. Res.* 44:1294-1299.

Richard, J. L., R. D. Stubblefield, R. L. Lyon, W. M. Peden, J. R. Thurston, and R. B. Rimler. 1986. Distribution and clearance of aflatoxins B_1 and M_1 in turkeys fed diets containing 50 or 150 ppb aflatoxins from naturally contaminated corn. *Avian Diseases* 30:788-793.

Richard, J. L., W. M. Peden, and J. R. Thurston. 1989. Combined cyclopiazonic acid and aflatoxin effects on serum bacteriostasis, glycocholic acid, complement activity, and enzymes and histopathologic changes in guinea pigs. In A. E. Pohland, V. R. Dowell, and J. L. Richard (Eds.). *Cellular and molecular mode of action of selected microbial toxins.* Plenum Press, New York. In Press.

Rodricks, J. V., and R. M. Eppley. 1974. Stachybotrys and stachybotryotoxicosis. Pp. 181-197. In I. F. Purchase (Ed.). *Mycotoxins.* Elsevier Scientific Publishing Co., New York.

Robbins, J. E., J. K. Porter, and C. W. Bacon. 1986. Occurrence and clinical manifestations of ergot and fescue toxicoses. Pp. 61-74. In J. L. Richard and J. L. Thurston (Eds.). *Diagnosis of Mycotoxicoses.* Martinus Nijhoff Publishers, Dordrecht.

Rosen, J. D. 1984. Presence of mycotoxins in a man-made material in a yellow rain sample. *Arch. Belg. Med. Soc. Hyg. Med. Trav.*

Med. Leg. Proc. 1st World Congr. Suppl. Pp. 173-176.

Rosenstein, Y. and C. LaFarge-Frayssinet. 1983. Inhibitory effect of *Fusarium* T-2 toxin on lymphoid DNA and protein synthesis. *Toxicol. Appl. Pharmacol.* 70:283-288.

Rosenstein, Y., C. LaFarge-Frayssinet, G. Lespinats, F. Loisillier, P. Lafont, and C. Frayssinet. 1979. Immunosuppressive activity of *Fusarium* toxins. Effects on antibody synthesis and skin grafts of crude extracts, T-2 toxin and diacetoxyscirpenol. *Immunology* 36:111-117.

Sabbioni, G., P. Skipper, G. Buchi, and S. R. Tannenbaum. 1987. Isolation and characterization of the major serum albumin adduct formed by Aflatoxin B_1 *in vivo* in rats. *Carcinogenesis* 8:819-824.

Sarasin, A. and Y. Moule. 1975. Translational step inhibited *in vivo* by aflatoxin B_1 in rat-liver polysomes. *Eur. J. Biochem.* 54:329-340.

Schuller, P. O., W. Horwitz, and L. Stoloff. 1976. A review of sampling and collaboratively studied methods of analysis for aflatoxin. *J. Assoc. Off. Anal. Chem.* 59:1315.

Schwarte, L. H., H. E. Biester, and C. Murray. 1937. A disease of horses caused by feeding moldy corn. *J. Am. Vet. Assoc.* 90:76-85.

Scott, P. M. 1981. Toxins of *Penicillium* species used in cheese manufacture. *J. Food Prot.* 44:702-710.

Scott, P. M. 1984. The occurrence of vomitoxin (deoxynivalenol, DON) in Canadian grains. Pp. 182-189. In H. Kurata and Y. Ueno (Eds.). *Toxic fungi - their toxins and health hazard.* Elsevier Scientific Publishing Company, Inc., New York.

Scott, P. M. 1985. Aflatoxins. Pp. 22-24. In P. M. Scott, H. L. Trenholm, and M. D. Sutton (Eds.). *Mycotoxins: A Canadian perspective.* NRCC Publication No. 22848. National Research Council of Canada, Ottawa.

Scott, P. M. 1989. Methods for determination of aflatoxin M_1 in milk and milk products - a review of performance characteristics. *Fd. Add. and Contam.* 6:283-305.

Scott, P. M., W. F. Miles, P. Tott, J. G. Dube. 1972. Occurrence of patulin in apple juice. *J. Agric. Food Chem.* 20:450-451.

Segal, R., I. Milo-Goldzweig, A. Z. Joffe, and B. Yagen. 1983. Trichothecene-induced hemolysis. I. The hemolytic activity to T-2 toxin. *Toxicol. Appl. Pharmacol.* 70:343-349.

Seiber, S. M., P. Correa, D. W. Dalgard, and R. H. Adamson. 1979. Induction of osteogenic sarcomas and tumors of the hepatobiliary system in non-human primates with aflatoxin B_1. *Cancer Res.* 39:4545-4554.

Shank, R. C. 1977. Environmental cancer. Pp. 291-318. In H. F. Kraybill and M. A. Mehlman (Eds.). John Wiley and Sons, Ltd., New York.

Shank. R. C. 1981. Mycotoxins and N-nitroso compounds: Environmental risks. Vol. I. Pp. 107-140. In R. C. Shank (Ed.). CRC Press, Inc., Boca Raton, Florida.

Shank, R. C., C. H. Bourgois, N. Keschamras, and P. Chandavimol. 1971. *Food Cosmet. Toxicol.* 9:501-507.

Shank, R. C., J. E. Gordon, G. N. Wogan, A. Nondasuta, and B. Subhamani. 1972a. *Food Cosmet. Toxicol.* 10:71-84.

Shank, R. C., G. N. Wogan, J. B. Gibson, and A. Nondasuta. 1972b. *Food Cosmet. Toxicol.* 10:61-69.

Shantha, T. 1987. Detoxification of groundnut seed and products in India. Pp. 16. In *Summary and recommendations of the international workshop on aflatoxin contamination of groundnut.* ICRISAT Center, India.

Shantha, T. and M. Sreenivasa. 1977. Photo-destruction of aflatoxin in groundnut oil. *Indian J. Technol.* 15:453.

Shirley, H. V. and S. H. Tohala. 1983. *Ochratoxicosis in laying hens.* University of Tennessee, Agriculture Experiment Station, 1982 Annual Science Progress Report 83-08:52-53.

Shotwell, O. L. and C. W. Hesseltine. 1983. Five-year study of mycotoxins in Virginia wheat and dent corn. *J. Assoc. Off. Anal. Chem.* 66:1466-1469.

Shotwell, O. L., C. W. Hesseltine, and M. L. Goulden. 1973. Incidence of aflatoxin in southern corn, 1969-1970. *Cereal Science Today* 18:102-195.

Shotwell, O. L., M. L. Goulden, and C. W. Hesseltine. 1974. Aflatoxin: Distribution in contaminated corn. *Cereal Chem.* 51:492.

Shotwell, O. L., M. L. Goulden, R. J. Bothast, and C. W. Hesseltine. 1975. Mycotoxins in hot spots in grains. 1. Aflatoxin and zearalenone occurrence in stored corn. *Cereal Chem.* 52:687.

Shurtleff, M. C. (Ed.). 1980. *Compendium of corn diseases.* American Phytopathological Society, Cooperative Extension Service, and Department of Plant Pathology, University of Illinois, Urbana, Illinois. 105 pp.

Siller, W. G. and D. C. Ostler. 1961. The histopathology of an enterohepatic syndrome of turkey poults. *Vet. Rec.* 73:134-138.

Sinnhuber, R. O., J. D. Hendricks, J. H. Wales, G. B. Putnam. 1977. Neoplasms in rainbow trout, a sensitive animal model for environmental carcinogenesis. *Ann. N.Y. Acad. Sci.* 298:389-408.

Sippel, W. L., J. E. Burnside, and M. B. Atwood. 1954. A diesease of swine and cattle caused by moldy corn. *Proc. 90th Ann. Mtg. Am. Vet. Med. Assoc.* Toronto. Pp. 174-181.

Smalley, E. B. 1977a. Chemistry and physiology of slaframine. Pp. 449-457. In T. D. Wyllie and L. G. Morehouse (Eds.). *Mycotoxic fungi, mycotoxins, mycotoxicoses.* Marcel Dekker, Inc., New York.

Smalley, E. B. 1977b. Salivary syndrome in cattle. Pp. 111-120. In T. D. Wyllie and L. G. Morehouse (Eds.). *Mycotoxic fungi, mycotoxins, mycotoxicoses.* Marcel Dekker, Inc., New York.

Smith, T. K. 1980. Influence of dietary fiber, protein and zeolite on zearalenone toxicosis in rats and swine. J. Anim. Sci. 50:278-285.

Smith, T. K. 1984. Spent canola oil bleaching clays: potential for treatment of T-2 toxicosis in rats and short-term inclusion in diets for immature swine. Can. J. Anim. Sci. 64:725-732.

Smith, J. E. and M. O. Moss. 1985. *Mycotoxins: Formation, analysis, and significance.* John Wiley and Sons, New York. 148 pp.

Southern, L. L. and A. J. Clawson. 1980. Ammoniation of corn contaminated with aflatoxin and its effects on growing rats. *J. Anim. Sci.* 50:459-466.

Stark, A. A. 1980. Mutagenicity and carcinogenicity of mycotoxins: DNA binding as a possible mode of action. *Ann. Rev. Microbiol.* 34:235-262.

Stoloff, L. 1975. Patulin, a contaminant of apple juice. *New York State Ag. Expt. Sta. Spec. Report* 19:51-54.

Stoloff, L. 1976. Occurrence of mycotoxins in foods and feeds. Pp. 23-50. In J. V. Rodricks (Ed.). *Mycotoxins and other fungal related food problems.* Adv. Chem. Ser. 149. American Chemical Society, Washington, DC.

Stoloff, L. 1980. Aflatoxin M_1 in perspective. *J. Food Prot.* 43:226-230.

Stoloff, L. 1983. Aflatoxin as a cause of primary liver-cell cancer in the United States: A probability study. *Nutr. Cancer* 5:165-186.

Stoloff, L. 1986. A rationale for the control of aflatoxin in human foods. Pp. 457-472. In P. S. Steyn and R. Vleggaar (Eds.). *Mycotoxins and phycotoxins.* A collection of invited papers presented at the Sixth International IUPAC Symposium on Mycotoxins and Phycotoxins, Pretoria, Republic of South Africa, July 22 to 25, 1985. Elsevier Scientific Publishing Co., Amsterdam, The Netherlands.

Stoloff, L. 1989. Personal communication to S. Appleton.

Stoloff, L. and P. M. Scott. 1984. Natural poisons. Pp. 477-500. In

S. Williams (Ed.). *Official methods of analysis of the association of official analytical chemists.* 14th Ed. Association Official Analytical Chemists, Arlington, Virginia.

Stoloff, L., M. Trucksess, N. Harding, O. J. Francis, J. R. Hayes, C. E. Polan, and T. C. Campbell. 1975. Stability of aflatoxin M in milk. *J. Dairy Sci.* 58:1789-1793.

Stubblefield, R. D., O. L. Shotwell, J. L. Richard, and A. C. Pier. 1981. Transmission and distribution of aflatoxin in contaminated beef liver and other tissues. *J. Am. Oil Chem. Soc.* 58:1015A-1017A.

Summers, J., J. M. Smolec, and R. Snyder. 1978. A virus similar to human hepatitis B virus associated with hepatitis and hepatoma in woodchucks. *Proc. Natl. Acad. Sci. USA* 75:4533-4537.

Sundloff, S. F. and C. Strickland. 1986. Zearalenone and zearanol: potential residue problems in livestock. *Vet. Hum. Toxicol.* 28:242-250.

Swanson, S. P. 1989. Unpublished data.

Swenson, D. H., E. C. Miller, and J. A. Miller. 1974. Aflatoxin B_1-2,3-oxide: Evidence for its formation in rat liver *in vivo* and by human liver microsomes *in vitro*. *Biochem. Biophys. Res. Commun.* 60:1036-1043.

Szczech, G. M., W. W. Carlton, and J. Tuite. 1973. Ochratoxicosis in beagle dogs. II. Pathology. *Vet. Pathol.* 10:219-231.

Szmuness, W. 1978. Hepatocellular carcinoma and the hepatitis B virus: evidence for a causal association. *Prog. Med. Virol.* 24:40-69.

Tabib, Z. and P. B. Hamilton. 1988. Factors influencing antifungal activity of gentian violet in poultry feed and ingredients. *Poultry Sci.* 67:58-63.

Tai, J.-H. and J. J. Pestka. 1988. Synergistic interaction between the trichothecene T-2 toxin and *Salmonella typhimurium* lipopolysaccharide in C3H/HeN and C3H/HeJ mice. *Toxicol. Lett.* 44:191-200.

Tai, J.-H. and J. J. Pestka. 1989. Impaired resistance to *Salmonella typhimurium* following oral exposure to the trichothecene T-2 toxin. *Fd. Chem. Toxicol.* 26:691-698.

Tanaka, T., A. Hasegawa, Y. Marsuki, and Y. Ueno. 1985. A survey of the occurrence of nivalenol, deoxynivalenol and zearalenone in food stuffs and health foods in Japan. *Food Add. Contam.* 2:259-265.

Terao, K. 1983. The target organella of trichothecenes in rodents and poultry. Pp. 147-162. In Y. Ueno (Ed.). *Trichothecenes - Chemical, biological and toxicological aspects, developments in food science.* Vol. 4. Elsevier, New York.

Terao, K. and Y. Ueno. 1978. Morphological and functional damage to cells and tissues. Pp. 189-238. In K. Uraguchi and M. Yamazaki (Eds.). *Toxicology, biochemistry and pathology of mycotoxins.* Halsted Press, New York.

Thurston, J. R., A. L. Baetz, N. R. Cheville, and J. L. Richard. 1980. Acute aflatoxicosis in guinea pigs: Sequential changes in serum proteins, complement, C4, and liver enzymes and histopathologic changes. *Am. J. Vet. Res.* 41:1272-1276.

Thurston, J. R., J. L. Richard, and W. M. Peden. 1986. Immunomodulation in mycotoxicoses other than aflatoxicosis. Pp. 149-161. In J. L. Richard and J. R. Thurston (Eds.). *Diagnosis of mycotoxicoses.* Martinus Nijhoff Publishers, Boston, Massachusetts.

Trenholm, H. L., B. K. Thompson, J. F. Standish, and W. L. Seaman. 1985. Occurrence of mycotoxins in Canada. Pp. 43-53. In P. M. Scott, H. L. Trenholm, and M. D. Sutton (Eds.). *Mycotoxins: A Canadian perspective.* NRCC Publication No. 22848. National Research Council of Canada, Ottawa, Canada.

Trucksess, M. W., G. E. Wood, R. M. Eppley, K. Young, C. K. Cohen, S. W. Page, and A. E. Pohland. 1986. *Occurrence of deoxynivalenol in grain and grain products.* In S. Barry, G. Llewellyn, and C. O'Rear (Eds.). *Biodeterioration* 6:243-247. The Canbrian News Ltd. Aberytwyth.

Trucksess, M. W., P. B. Mislivec, K. Young, V. R. Bruce, and S. W. Page. 1987. Cyclopiazonic acid production by cultures of *Aspergillus* and *Penicillium* species isolated from dried beans, corn meal, macadamia nuts and pecans. *J. Assoc. Off. Anal. Chem.* 70:123-126.

Trucksess, M. W., L. Stoloff, and P. B. Mislivec. 1988. Effect of temperature, water activity and other toxigenic mold species on growth of *Aspergillus flavus* and aflatoxin production on corn, pinto beans, and soybeans. *J. Food Prot.* 51:361-363.

Tsubouchi, H., H. Terada, K. Yamamoto, K. Hisada, and Y. Sakabe. 1988. Ochratoxin A found in commercial roast coffee. *J. Agric. Food Chem.* 36:540-542.

Tuite, J. 1979. Field and storage conditions for the production of mycotoxins and geographic distribution of some mycotoxin problems in the United States. Pp. 19-39. Proceedings of a symposium, July 13, 1978, Michigan State University. *Interactions of mycotoxins in animal production.* National Academy of Sciences, Washington, D. C.

Tung, T. C. and K. H. Ling. 1968. Study of aflatoxin of foodstuffs in Taiwan. *J. Nutr. Sci. Vitaminol.* 14:48-52.

Tung, H. T., W. E. Donaldson, and P. B. Hamilton. 1972. Altered lipid transport during aflatoxicosis. *Toxicol. Appl. Pharmacol.* 22:97-104.

Tung, H. T., F. W. Cook. R. D. Wyatt, and P. B. Hamilton. 1975a. The anemia caused by aflatoxin. *Poultry Sci.* 54:1962-1969.

Tung, H. T., R. D. Wyatt, J. P. Thaxton, and P. B. Hamilton. 1975b. Concentrations of serum proteins during aflatoxicosis. *Toxicol. Appl. Pharmacol.* 34:320-326.

Ueno, Y. 1974. Citreoviridin from *Penicillium citreoviridae* Biourge. Pp. 283-302. In I. F. H. Purchase (Ed.). *Mycotoxins.* Elsevier Scientific Publishing Co., New York.

Ueno, Y. 1984a. Toxicological features of T-2 toxin and related trichothecenes. *Fund. Appl. Toxicol.* 4:S124-S132.

Ueno, Y. 1984b. The toxicology of mycotoxins. *CRC Crit. Rev. Toxicol.* 14:99-132.

Ueno, Y. and I. Ueno. 1972. Isolation and acute toxicity of citreoviridin, a neurotoxic mycotoxin of *Penicillium citreoviride* Biourge. *Jpn. J. Exp. Med.* 42:91-105.

Ueno, U., K. Ishii, K. Sakai, S. Kanaeda, H. Tsunoda, T. Tanaka, and M. Enomoto. 1972. Toxicological approaches to metabolites of *Fusaria.* IV. Microbial survey on "bean-hulls poisoning of horses" with the isolation of toxic trichothecenes, neosolaniol, and T-2 toxin of *Fusarium solani. Jpn. J. Exp. Med.* 42:187-203.

Ueno, Y. M. Nakajima, K. Sakai, K. Ishii, N. Sato, and N. Shimada. 1973. Comparative toxicology of trichothecene mycotoxins: Inhibition of protein synthesis in animal cells. *J. Biochem.* (Tokyo) 74:285-296.

Uraguchi, K. 1978. Introduction. Pp. 1-11. In K. Uraguchi and M. Yamazaki (Eds.). *Toxicology, biochemistry and pathology of mycotoxins.* John Wylie and Sons, New York.

U.S. Department of Agriculture. 1968a. *Preventing mycotoxins in farm commodities.* Agricultural Research Service 20-16.

U.S. Department of Agriculture. 1968b. Guidelines for mold control in high moisture corn. *Farmers Bull.* p. 2238.

U.S. Department of Agriculture. 1975. *Inspectors Instructions.* Agricultural Marketing Service, Washington, DC. p. 22.

U.S. Department of Agriculture. 1979. Agricultural statistics. U.S. Government Printing Office. Washington, D.C.

U.S. Department of Agriculture. 1985. Agricultural statistics. U.S. Government Printing Office. Washington, D.C.

U.S. Department of Agriculture. 1986. Agricultural statistics. U.S.

Government Printing Office. Washington, D.C.
U.S. Department of Agriculture. 1987. Agricultural statistics. U.S. Government Printing Office. Washington, D.C.
U.S. Food and Drug Administration. 1988. Action levels for added poisonous or deleterious substances in food. Notice. *Federal Register*, Vol. 53, No. 33. Pp. 5043-5044.
van Egmond, H. P. 1988. Current situation on regulations for mycotoxins. Overview of tolerances and status of standard methods of sampling and analysis. *Fd. Add. Contam.* 5:(in press).
van Rensburg, S. J. 1977. Role of epidemiology in the elucidation of mycotoxin health risks. Pp. 699-712. In J. V. Rodricks, C. W. Hesseltine, and M. A. Mehlman (Eds.). *Mycotoxins in human and animal health*. Pathotox Publishers, Inc., Park Forest South, Illinois.
van Rensburg, S. J., P. Cook-Mozaffari, D. J. Van Schalkwyk, J. J. Van Der Watt, T. J. Vincent, and I. F. H. Purchase. 1985. Hepatocellular carcinoma and dietary aflatoxin in Mozambique and Transkei. *Br. J Cancer* 51:713-726.
van Walbeek, W., P. M. Scott, and F. S. Thatcher. 1968. Mycotoxins from food-borne fungi. *Can. J. Microbiol.* 14:131-137.
Vesonder, R. F. and C. W. Hesseltine. 1980. Vomitoxin: Natural occurrence on cereal grains and significance as a refusal and emetic factor to swine. *Process Biochem.* 16:12-16.
Vesonder, R. F. and B. W. Horn. 1985. Sterigmatocystin in dairy cattle feed contamination with *Aspergillus versicolor*. *Appl. Environ. Microbiol.* 49:234-235.
Visconti, A. and A. Bottalico. 1983. High levels of ochratoxin A and B in moldy bread responsible for mycotoxicosis in farm animals. *J. Agric. Food Chem.* 31:1122-1123.
Voss, K. A., W. P. Norred, R. D. Plattner, and C. W. Bacon. 1989. Hepato- and renal toxicity in rats of corn samples associated with field cases of equine leukoencephalomalacia. *Fd. Chem. Toxicol.* In press.
Wade, N. 1981. Yellow rain and the cloud of chemical war. *Science* 214:1008-1009.
Wagstaff, D. J. 1985. The use of epidemiology, scientific data, and regulatory authority to determine risk factors in cancer of some organs of the digestive system. *Reg. Toxicol. Pharmacol.* 5:384-404.
Wall Street Journal. September 30, 1988. *Spread of fungus-produced carcinogen in U.S. corn exposes regulatory gaps.*
Walpole, R. E. 1974. *Introduction to statistics*. Macmillan, New York. Pp. 156.
Watson, S. A., Mirocha, C. J., and Hayes, A. W. 1984. Analysis for trichothecenes in samples from Southeast Asia associated with "yellow rain". *Fund. Appl. Toxicol.* 4:700-706.
Weaver, G. A., H. J. Kurtz, F. Y. Bates, C. J. Mirocha, J. C. Behrens, and W. M. Hagler. 1981. Diacetoxyscirpenol toxicity in pigs. *Res. Vet. Sci.* 31:131-135.
Wei, R., P. E. Still, E. B. Smalley, H. K. Schroes, and F. M. Strong. 1973. Isolation and partial characterization of a mycotoxin from *Penicillium roqueforti*. *Appl. Microbiol.* 25:111-114.
Wells, J. M., H. G. Cutler, and R. J. Cole. 1976. Toxicity and plant growth regulator effects of cytochalasin H isolated from *Phomopsis* species. *Can. J. Microbiol.* 22:1137-1143.
Whitaker, T. B. and J. W. Dickens. 1979. Evaluation of the Peanut Administrative Committee testing program for aflatoxin in shelled peanuts. *Peanut Sci.* 6:7-9.
Whitaker, T. B. and J. W. Dickens. 1981. Errors in aflatoxin analyses of raw peanuts by thin layer chromatography. *Peanut Sci.* 8:92.
Whitaker, T. B. and E. H. Wiser. 1969. Theoretical investigation into the accuracy of sampling shelled peanuts for aflatoxin. *J. Am. Oil Chem. Soc.* 46:377.
Whitaker, T. B., J. W. Dickens, and R. J Monroe. 1972. Comparison of the observed distribution of aflatoxin in shelled peanuts to the negative binomial distribution. *J. Am. Oil Chem. Soc.* 49:590.
Whitaker, T. B., J. W. Dickens, and R. J. Monroe. 1974a. Variability of aflatoxin test results. *J. Am. Oil Chem. Soc.* 49:590.
Whitaker, T. B., J. W. Dickens, E. H. Wiser, and R. J. Monroe. 1974b. Development of a method to evaluate sampling plans used to estimate aflatoxin concentrations in lots of shelled peanuts. Information Bulletin, Technical Report Number 10. *Intl. Union of Pure and Applied Chem.* 7 pp.
Whitaker, T. B., J. W. Dickens, and E. H. Wiser. 1976a. Monte Carlo techniques to simulate aflatoxin testing programs for peanuts. *J. Am. Oil Chem. Soc.* 53:545.
Whitaker, T. B., M. E. Whitten, and R. J. Monroe. 1976b. Variability associated with testing cottonseed for aflatoxin. *J. Am. Oil Chem. Soc.* 53:502.
Whitaker, T. B., J. W. Dickens, and R. J. Monroe. 1979. Variability associated with testing corn for aflatoxin. *J. Am. Oil Chem. Soc.* 56:789.
Wicklow, D. T. and J. E. Donahue. 1984. Sporogenic germination of sclerotia in *Aspergillus flavus* and *Aspergillus parasiticus*. *Trans. Brit. Mycol. Soc.* 82:621-624.
Wicklow, D. T. and D. M. Wilson. 1986. Germination of *Aspergillus flavus* sclerotia in a Georgia maize field. *Trans. Br. Mycol. Soc.* 87:651-654.
Wieder, R., G. N. Wogan, and M. B. Shimkin. 1968. Pulmonary tumors in strain A mice given injections of aflatoxin B_1. *J. Natl. Cancer Inst.* 40:1195-1197.
Wild, C. P., D. Umbenhauer, B. Chapot, and R. Montesano. 1986. Monitoring of individual human exposure to aflatoxins (AF) and N-nitrosamines (NNO) by immuoassays. *J. Cell Biochem.* 30:171-179.
Wiese, M. V. (Ed.). 1977. *Compendium of wheat diseases*. American Phytopathological Society, Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan; and Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska. 106 pp.
Wilson, D. M. 1988. Potential for biological control of *Aspergillus flavus* and aflatoxin contamination. Pp. 55-56. In K. G. Mukerji and K. L. Garg (Eds.). *Biocontrol of plant diseases*. Vol. 1. CRC Press, Inc., Boca Raton, Florida.
Wilson, D. M. and J. R. Stansell. 1983. Effect of irrigation regimes on aflatoxin contamination of peanut pods. *Peanut Sci.* 10:54-56.
Wilson, D. M., L. T. Sangster, and D. M. Bedell. 1984. Recognizing the signs of porcine aflatoxicosis. *Vet. Med.* July:974-977.
Wilson, M. E., W. M. Hagler, Jr., J. M. Cullen, J. F. Ort, and R. J. Cole. 1989. Acute toxicity of cyclopiazonic acid in selected avian species. Pp. 371-381. In G. C. Llewellyn and C. E. O'Rear (Eds.). *Biodeterioration research 2*. Plenum Press, New York.
Wogan, G. N. 1965. Chemical nature and biological effects of the aflatoxins. *Bacteriol. Rev.* 30:460.
Wogan, G. N. 1973. Aflatoxin carcinogenesis. Pp. 309-344. In H. Busch (Ed.). *Methods in cancer research*. Academic Press, New York.
Wogan, G. N. and P. M. Newberne. 1967. Dose-response characteristics of aflatoxin B_1 carcinogenesis in the rat. *Cancer Res.* 27:2370-2376.
Wood, G. E. 1989. Aflatoxins in domestic and imported foods and feeds. *J. Assoc. Off. Anal. Chem.* 72:543-548.
World Health Organization. 1983. *Prevention of liver cancer*. WHO, Technical Report Series, Vol. 691. World Health Organization, Geneva, Switzerland.
Woronin, M. 1891. Uber das "Taumel-getreide" in Sud-Ussurien.

Bot. Z. 49:81-93.

Wyatt, R. D., D. M. Briggs, and P. B. Hamilton. 1973a. The effect of dietary aflatoxin on mature broiler breeder males. *Poultry Sci.* 52:1119-1123.

Wyatt, R. D., P. B. Hamilton, and H. R. Burmeister. 1973b. The effects of T-2 toxin on broiler chickens. *Poultry Sci.* 52:1853-1859.

Wyatt, R. D., W. M. Colwell, P. B. Hamilton, and H. R. Burmeister. 1973c. Neural disturbances in chickens caused by dietary T-2 toxin. *Appl. Microbiol.* 26:757-761.

Wyatt, R. D., P. B. Hamilton, and H. R. Burmeister. 1975a. Altered feathering of chicks caused by T-2 toxin. *Poultry Sci.* 54:1042-1045.

Wyatt, R. D., J. A. Doerr, P. B. Hamilton, and H. R. Burmeister. 1975b. Egg production, shell thickness, and other physiological parameters of laying hens affected by T-2 toxin. *Appl. Microbiol.* 29:641-645.

Yahl, K. R., S. A. Watson, R. J. Smith, and R. Barabolak. 1971. Laboratory wet-milling of corn containing high levels of aflatoxin and a survey of commercial wet-milling products. *Cereal Chem.* 48:385-391.

Yarom, R., Y. Sherman, R. More, I. Ginsburg, R. Borinski, and B. Yagen. 1984. T-2 toxin effect on bacterial infection and leukocyte functions. *Toxicol. Appl. Pharmacol.* 75:60-68.

Ybarra, C. L. and A. J. Webb. 1982. Economic significance of health and sanitary regulations for trade in agricultural products. Pp. 19-29. In H. Kurata and C. W. Hesseltine (Eds.). *Control of the microbial contamination of foods and feeds in international trade: Microbial standards and specifications.* Saikon Publishing Co., Tokyo.

Yeh, F. S., M. Yu, C.-C. Mo, S. Luo, M. J. Tong, and B. E. Henderson. 1989. Hepatitis B virus, aflatoxin and hepatocellular carcinoma in southern Guangxi, China. *Cancer Res.* 49:2506-2509.

Yoshizawa, T., C. J. Mirocha, J. C. Behrens, and S. P. Swanson. 1981. Metabolic fate of T-2 toxin in a lactating cow. *Fd. Cosmet. Toxicol.* 19:31-39.

Yu, F. L., I. H. Geronimo, W. Bender, and J. Permthamsin. 1988. Correlation studies between the binding of aflatoxin B_1 to chromatin components and the inhibition of RNA synthesis. *Carcinogenesis* 9:527-532.

Zhu, J.-Q., L.-S. Zhang, X. Hu, Y. Xiao, J.-S. Chen, Y.-C. Xu, J. Fremy, and F. S. Chu. 1987. Correlation of dietary aflatoxin B_1 levels with excretion of aflatoxin M_1 in human urine. *Cancer Res.* 47:1848-1852.

Ziprin, R. L. and D. E. Corrier. 1987. Listeriosis in diacetoxyscirpenol-treated mice. *Am. J. Vet. Res.* 48:1516-1519.

Ziprin, R. L., D. E. Corrier, and H. K. Ziegler. 1987. T-2 toxin-enhanced resistance against listeriosis in mice: Importance of gastrointestinal lesions. *Am. J. Vet. Res.* 48:998-1002.

Appendix 1: Selected Books on Mycotoxins and Mycotoxicoses

Betina, V. (Ed.). 1984. *Mycotoxins: Production, isolation, separation and purification.* Elsevier Scientific Publishing Co., New York. 528 pp.

Betina, V. (Ed.). 1989. *Mycotoxins - Chemical, Biological and Environmental Aspects.* Elsevier Scientific Publishing Co., New York. 438 pp.

Ciegler, A., S. Kadis, and S. J. Ajl (Eds.). 1971. *Microbial toxins.* Vol. 6. *Fungal Toxins.* Academic Press, New York. 563 pp.

Cole, R. J. (Ed.). 1986. *Modern methods in the analysis and structural elucidation of mycotoxins.* Academic Press, New York. 471 pp.

Cole, R. J. and R. H. Cox (Eds.). 1981. *Handbook of toxic fungal metabolites.* Academic Press, New York. 935 pp.

Goldblatt, L. A. (Ed.). 1969. *Aflatoxin, Scientific Background, Control, and Implications.* Academic Press, New York. 472 pp.

Hayes, A. W. (Ed.). 1981. *Mycotoxin teratogenicity and mutagenicity.* CRC Press, Inc., Boca Raton, Florida. 121 pp.

Heathcote, J. and J. Hibbert (Eds.). 1978. *Developments in Food Science 1: Aflatoxins: chemical and biological aspects.* Elsevier, Scientific Publishing Co., New York. 212 pp.

Herzberg, M. (Ed.). 1970. *Toxic micro-organisms - Mycotoxins, botulism.* UJNR Joint Panels on Toxic Microorganisms and the United States Department of the Interior, United States Government Printing Office, Washington, D. C. 490 pp

Interaction of mycotoxins in animal production: Proceedings of a symposium, 1979. National Academy of Sciences, Washington, D. C. 195 pp.

Kadis, S., A. Ciegler, and S. J. Ajl (Eds.). 1971. *Microbial Toxins.* Vol. 7. *Algal and Fungal Toxins.* Academic Press, New York. 401 pp.

Kadis, S., A. Ciegler, and S. J. Ajl (Eds.). 1972. *Microbial Toxins.* Vol. 8. *Fungal Toxins.* Academic Press, New York. 400 pp.

Keeler, R. F. and A. T. Tu (Eds.) 1983. *Handbook of natural toxins.* Vol. 1. Plant and fungal toxins. Marcel Dekker, Inc., New York. 934 pp.

Krogh, P. (Ed.). 1987. *Mycotoxins in food.* Academic Press, New York. 263 pp.

Kurata, H. and C. W. Hesseltine. 1982. *Control of the Microbial Contamination of Foods and Feeds in International Trade: Microbial Standards and Specifications.* Saikon Publishing Company, Tokyo. 342 pp.

Kurata, H. and Y. Ueno (Eds.). 1984. *Toxigenic fungi - Their toxins and health hazard.* Elsevier Scientific Publishing Co., New York. 363 pp.

Lacey, J. (Ed.). 1985. *Trichothecenes and other mycotoxins.* John Wiley and Sons, New York. 571 pp.

Marasas, W. F. O. and P. E. Nelson (Eds.). 1987. *Mycotoxicology: Introduction to the mycology, plant pathology, chemistry, toxicology, and pathology of naturally occurring mycotoxicoses in animal and man.* The Pennsylvania State University Press, University Park, Pennsylvania. 102 pp.

Marasas, W. F. O., P. E. Nelson, and T. A. Toussoun (Eds.). 1984. *Toxigenic fusarium species, identity and mycotoxicology.* The Pennsylvania State University Press, University Park, Pennsylvania. 328 pp.

Moreau, C. and M. Moss (Eds.). 1979. *Moulds, Toxins and Food.* John Wiley and Sons, New York. 477 pp.

Purchase, I. F. H. (Ed.). 1974. *Mycotoxins.* Elsevier Scientific Publishing Company, Amsterdam, The Netherlands. 443 pp.

Recheigl, Jr., M. (Ed.). 1983. *Handbook of foodborne diseases of biological origin.* CRC Press, Inc., Boca Raton, Florida.

Richard, J. L. and J. R. Thurston (Eds.). 1986. *Diagnosis of mycotoxicoses.* Martinus Nijhoff Publishers, Dordrecht. 411 pp.

Rodricks, J. V. (Ed.). 1976. Mycotoxins and other fungal related food problems. American Chemical Society, Washington, D. C. 409 pp.

Rodricks, J. V., C. W. Hesseltine, and M. A. Mehlman (Eds.). 1977. *Mycotoxins in human and animal health.* Pathotox Publishers, Inc., Park Forest South, Illinois. 807 pp.

Rosenberg, P. (Ed.). 1978. *Toxins: Animal, plant and microbial.* Pergamon Press, Elmsford, N.Y. 1134 pp.

Scotts, P. M., H. L. Trendolm, and M. D. Sutton. 1985. *Mycotoxins: Canadian perspective.* NRCC Publication, No. 22848, Ottawa, Canada. 185 pp.

Shank, R. C. (Ed.). 1981. *Mycotoxins and N-nitroso compounds: environmental risks.* CRC Press, Inc., Boca Raton, Florida. 235 pp.

Smith, J. E. and M. D. Moss. 1985. *Mycotoxins: Formation, analyses and significance.* John Wiley and Sons, New York. 148 pp.

Steyn, P. S. (Ed.). 1980. *The biosynthesis of mycotoxins: A study in secondary metabolism.* Academic Press, New York. 432 pp.

Steyn, P. S. and R. Vleggaar (Eds.). 1986. *Mycotoxins and phycotoxins.* Elsevier Scientific Publishing Co., Amsterdam, The Netherlands. 102 pp.

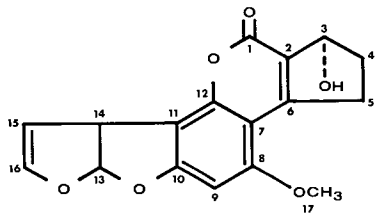
Ueno, Y. (Ed.). 1983. *Trichothecenes - Chemical, biological and toxicological aspects.* Elsevier Scientific Publishing Co., New York. 313 pp.

Uraguchi, K. and M. Yamazaki (Eds.). 1978. *Toxicology: Biochemistry and pathology of mycotoxins.* Halsted Press, New York. 288 pp.

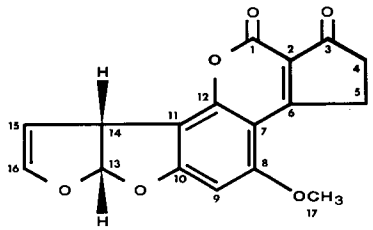
Van Edmond, H. (Ed.). 1989. *Mycotoxins in Dairy Products.* Elsevier Scientific Publishing Co., New York. 300 pp.

Wyllie, T. D. and L. G Morehouse (Eds.). 1977. *Mycotoxic fungi, mycotoxins, mycotoxicoses. An encyclopedic handbook.* 3 Vol. Marcel Dekker, Inc., New York. 538, 570, and 202 pp.

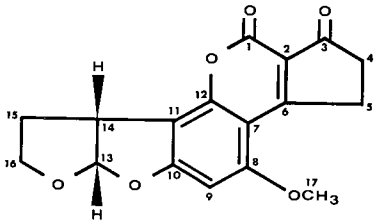
Appendix 2: Chemical Structures of Selected Mycotoxins



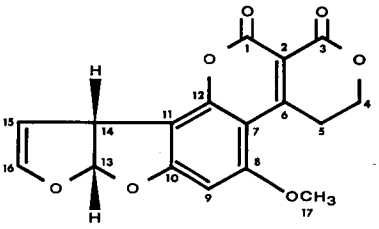
Aflatoxicol



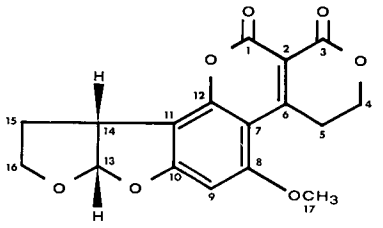
Aflatoxin B_1



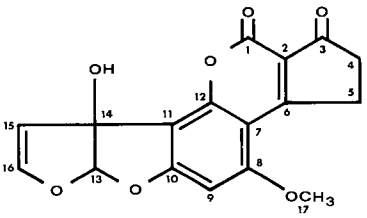
Aflatoxin B_2



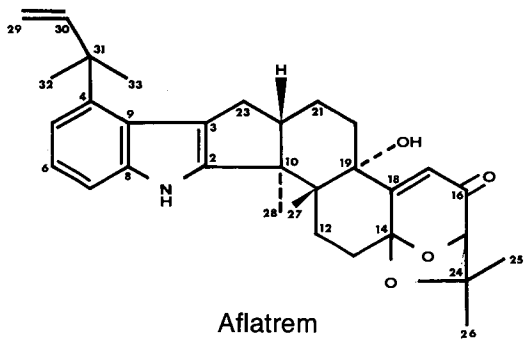
Aflatoxin G_1



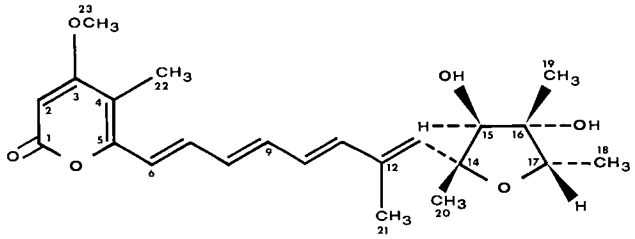
Aflatoxin G_2



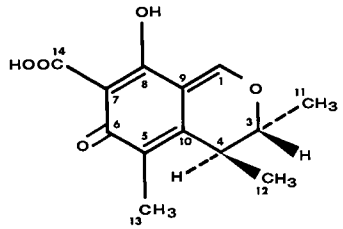
Aflatoxin M_1



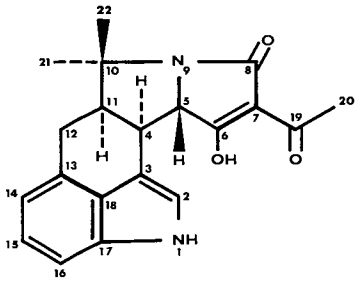
Aflatrem



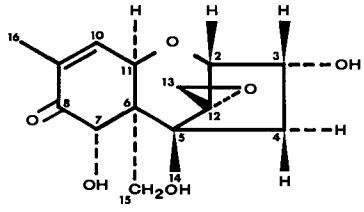
Citreoviridin



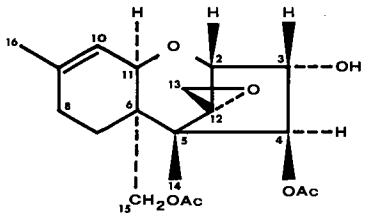
Citrinin



Cyclopiazonic Acid

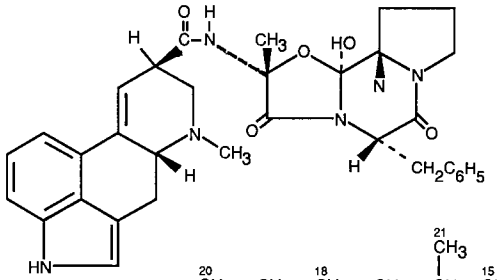


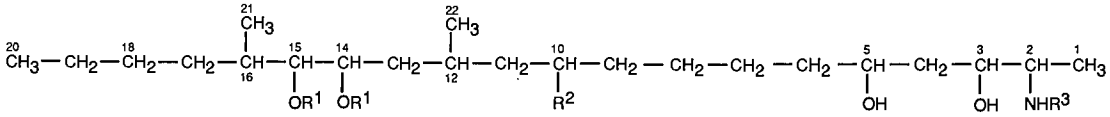
Deoxynivalenol



Diacetoxyscirpenol

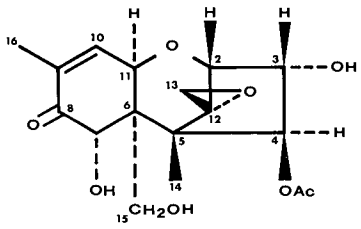
Ergotamine



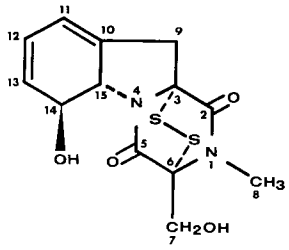


Fumonisins

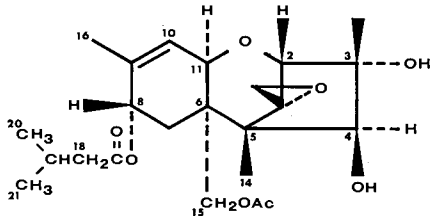
A_1: R^1 = $COCH_2CH(CO_2H)CH_2CO_2H$; R^2 = OH; R^3 = $C^{23}OC^{24}H_3$
A_2: R^1 = $COCH_2CH(CO_2H)CH_2CO_2H$; R^2 = H; R^3 = $COCH_3$
B_1: R^1 = $COCH_2CH(CO_2H)CH_2CO_2H$; R^2 = OH; R^3 = H
B_2: R^1 = $COCH_2CH(CO_2H)CH_2CO_2H$; R^2 = R^3 = H



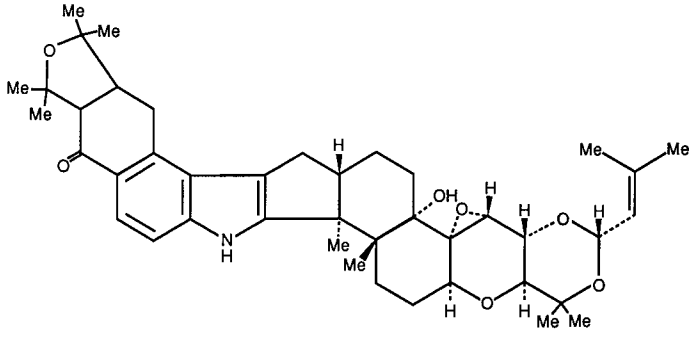
Fusarenon X



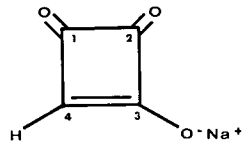
Gliotoxin



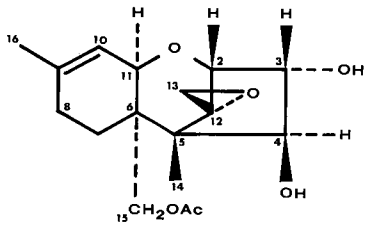
HT-2



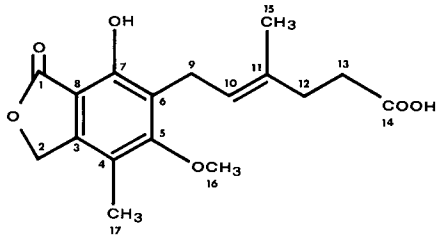
Lolitrem B



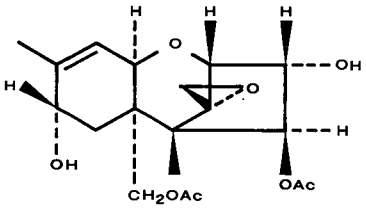
Moniliformin



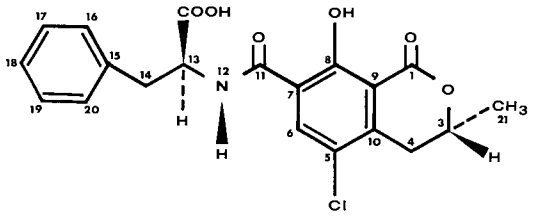
Monoacetoxyscirpenol



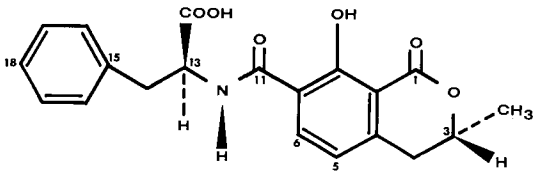
Mycophenolic Acid



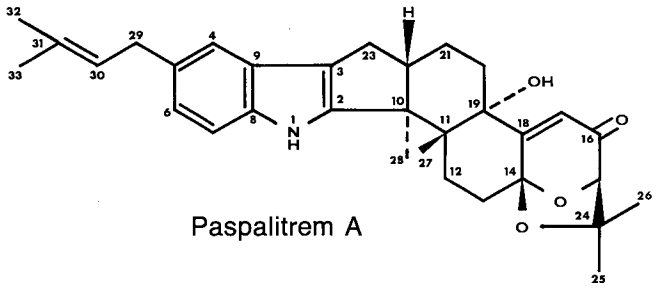
Neosolaniol



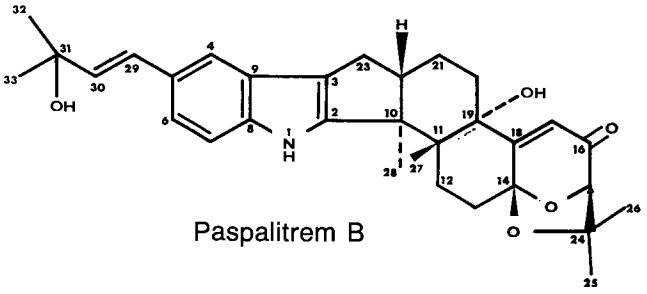
Ochratoxin A



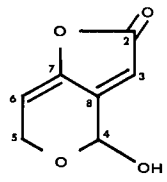
Ochratoxin B



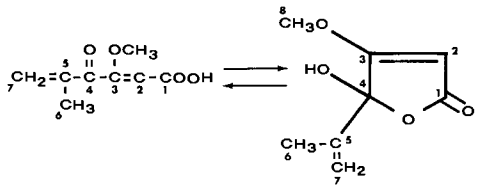
Paspalitrem A



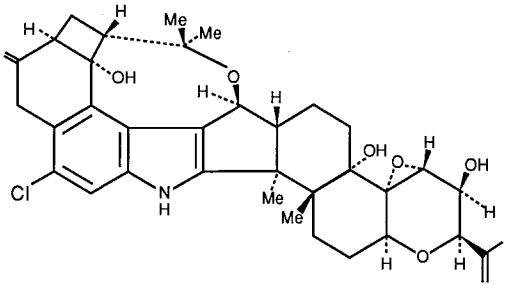
Paspalitrem B



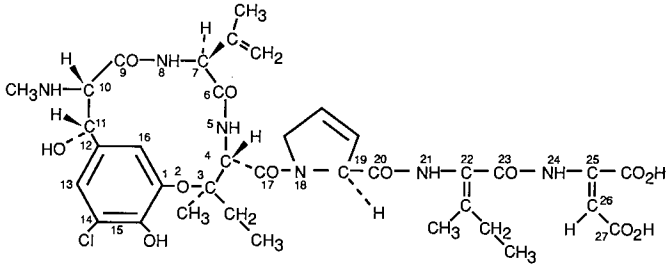
Patulin



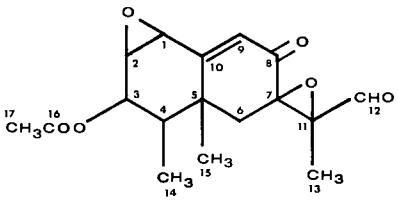
Penicillic Acid



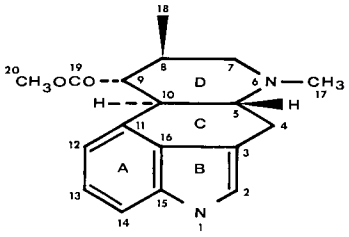
Penitrem A



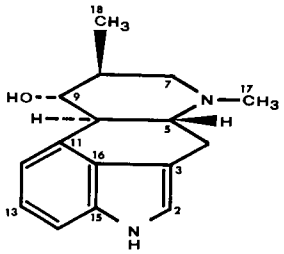
Phomopsin A



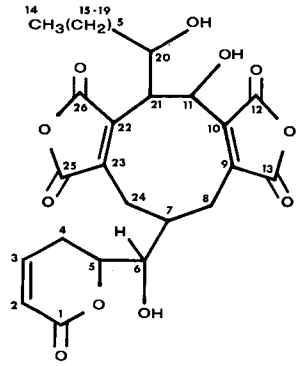
PR-Toxin



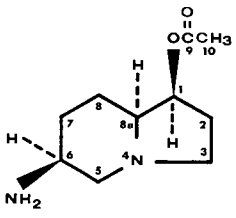
Roquefortine A



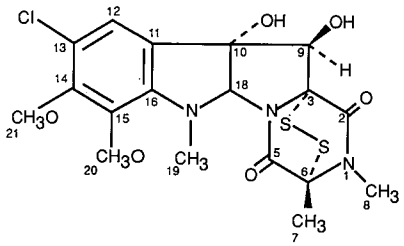
Roquefortine B



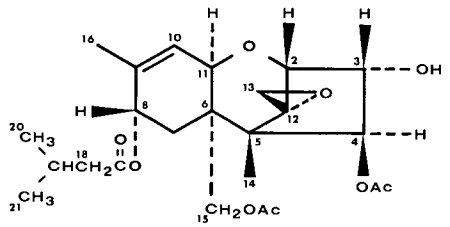
Rubratoxin B



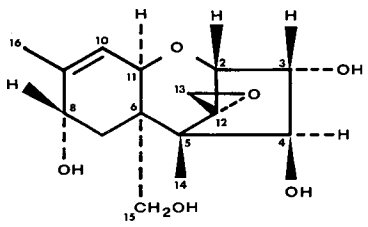
Slaframine



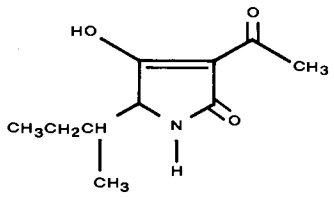
Sporidesmin



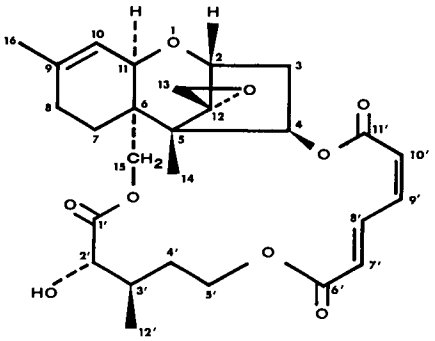
T-2



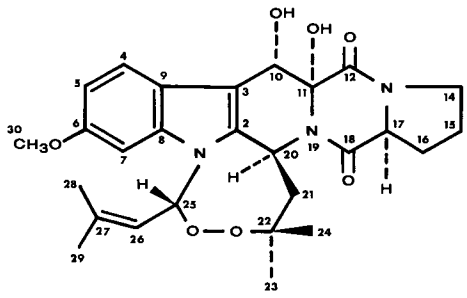
T-2 Tetraol



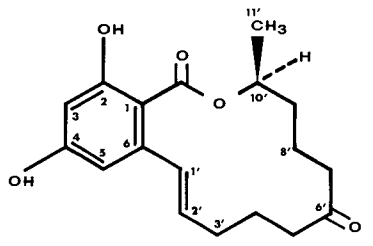
Tenuazonic Acid



Verrucarin A



Verruculogen



Zearalenone

Recent and Forthcoming CAST Publications

Task Force Reports

Task force reports are CAST's principal publications. Reports are prepared by groups of eminent scientists working in various disciplines related to the subject. Task forces write reports intended to be sound statements on the scientific aspects of subjects concerning food and agriculture, and do not take advocacy positions.

Ionizing Energy in Food Processing and Pest Control: I. Wholesomeness of Food Treated With Ionizing Energy
R109, July 1986, 50 pp., $4.00

Diet and Health
R111, March 1987, 62 pp., $5.00

Effective Use of Water in Irrigated Agriculture
R113, June 1988, 64 pp., $5.00

Long-Term Viability of U.S. Agriculture
R114, June 1988, 48 pp., $4.00

Ionizing Energy in Food Processing and Pest Control: II. Applications
R115, June 1989, 98 pp., $10.00

Mycotoxins: Economic and Health Risks
R116, November 1989, 91 pp., $15.00; Summary, 8 pp., $1.00

Ecological Impacts of Federal Conservation and Cropland Reduction Programs
R117, September 1990, 28 pp., $6.00; Summary, 8 pp., $1.00

Food Fats and Health
R118, December 1991, 96 pp., $12.00

Preparing U.S. Agriculture for Global Climate Change
R119, June 1992, 96 pp., $15.00; Summary, 7 pp., $3.00

Special Publications

***Alternative Agriculture*: Scientists' Review**
SP16, July 1990, 182 pp., $15.00; Summary, 8 pp., $3.00

Comments from CAST

Timely letters and other comments of broad interest, written by one or more authors.

Pesticides, Cancer, and the Delaney Clause
CC1987-2, May 1987, 15 pp., $2.00

Perspectives on the Safety of 2,4-D
CC1987-3, December 1987, 16 pp., $2.00

Reducing American Exposure to Nitrate, Nitrite, and Nitroso Compounds: The National Network to Prevent Birth Defects Proposal
CC1989-1, June 1989, 16 pp., $3.00

Antibiotics for Animals: The Antibiotic-Resistance Issue
CC1989-2, June 1989, 12 pp., $3.00

Pesticides and Safety of Fruits and Vegetables
CC1990-1, December 1990, 15 pp., $4.00

Herbicide-Resistant Crops
CC1991-1, May 1991, 28 pp., $8.00

Food Safety: The Interpretation of Risk
CC1992-1, March 1992, 23 pp., $8.00

Pesticides: Minor Uses/Major Issues
CC1992-2, June 1992, 19 pp., $8.00

Science of Food and Agriculture

Science of Food and Agriculture is a newsletter for high school science teachers and students. A four-issue subscription is $10.00.

AU Lean: low-fat hamburger, rhizotron, herbicide-resistant crops, soap as a bee killer, science briefs, food and nutrition news.
SFA3-2, July 1991, 16 pp., $2.50

Rolling beans cuts losses, low-palmitic acid soybeans, CO_2 levels affect plant growth, landscape contracting careers, science briefs, food and nutrition news.
SFA4-1, January 1992, 16 pp., $2.50

Forthcoming Reports

Animal Well-Being

Biological Pest Control in Agriculture: Opportunities and Challenges

Consequences for Food and Fiber (Plant and Animal) Production and Processing from Reduced or Less Reliable Western U.S. Water Resources

Contribution of Animal Products to Healthful Diets

Development of Host Plant Resistance to Pests

Household Waste Disposal through Agricultural Recycling

The Impact of Alternative Agricultural Practices on the Environment

Impact of Pests on Food Quality

Integrated Animal Waste Management

Naturally Occurring Antimicrobials in Food

Possible Geographical Redistribution of Food and Fiber (Plant and Animal) Production and Processing as a Consequence of More Free Trade

Public Land Grazing: Social, Economic, and Regulatory Issues

Public Perceptions of Agricultural Drugs and Chemicals

Quality of U.S. Agricultural Products

Relationship of Value-Added Activities on Agricultural Products and the U.S. Trade Balance

Risk/Benefit Assessment of Antibiotics Use in Animals

Risks Associated with Foodborne Pathogens

Waste Management and Utilization in Food Production and Processing

Water Quality: Agriculture's Role

Wetlands: Impact and Regulation

Individual Membership

Individual membership dues are $30 per calendar year. Members receive *NewsCAST*, *Science of Food and Agriculture*, and summaries of reports. They may request one free copy of each CAST report within one year of release.

Libraries and institutions may subscribe to all CAST publications for $35 per calendar year to U.S. addresses; $50, international. An international air mail subscription is $100 per calendar year.

Publication Orders

Please send a check or money order (in U.S. funds on a U.S. bank) to CAST. Orders from nonmembers outside the U.S. must be prepaid. Add $10 per publication for international air mail service.

Orders of 6 through 99 copies are discounted 25% off listed prices. Orders of 100 or more copies are discounted 35%.

Send to CAST, 137 Lynn Avenue, Ames, IA 50010-7197. Phone: (515) 292-2125.

CAST
COUNCIL FOR AGRICULTURAL SCIENCE AND TECHNOLOGY


CAST®
137 LYNN AVENUE, AMES, IOWA 50010-7120