



CAST

# MYCOTOXINS

Risks in Plant, Animal, and Human Systems

# Mycotoxins: Risks in Plant, Animal, and Human Systems

Council for Agricultural Science and Technology, Ames, Iowa, USA  
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**Task Force Report**  
No. 139 January 2003

**Council for Agricultural Science and Technology**  
Ames, Iowa, USA

# The Beggars

by Pieter Bruegel The Elder

The oldest recognized mycotoxicosis of humans is ergotism caused by the plant parasitic fungus, *Claviceps purpurea*. After periodic outbreaks in central Europe, the disease became epidemic in the Middle Ages, where it was known as St. Anthony's fire (Matossian 1989; van Rensburg and Altenkirk 1974). Gangrenous symptoms were described in medieval episodes of ergotism in humans, where early symptoms were hallucinations and swollen limbs with burning sensations, with subsequent necrosis leading to loss of appendages. Ergotism results from consumption of products made with grains contaminated with ergots. The ergots or sclerotia are often larger than the normal grain, are typically black in color, and may replace several grains in one spike or head of the respective grain.

Pieter Bruegel (ca. 1525–1569) was the first in a family of Flemish painters. His career spanned medieval times when many people were victims of gangrenous ergotism caused by *Claviceps purpurea*. This particular painting was likely a depiction of some of the victims of this tragic condition.

“Pieter Bruegel was usually known as Pieter Bruegel the Elder to distinguish him from his elder son. He spelled his name Brueghel until 1559, and his sons retained the ‘h’ in the spelling of their names. Pieter



Bruegel the Elder, generally considered the greatest Flemish painter of the sixteenth century, is by far the most important member of the family. He was probably born in Breda in the Duchy of Brabant, now in The Netherlands. Accepted as a master in the Antwerp painters' guild in 1551, he was apprenticed to Coecke van Aelst, a leading Antwerp artist, sculptor, architect, and designer of tapestry and stained glass. Bruegel traveled to Italy in 1551 or 1552,

completing a number of paintings, mostly landscapes, there. Returning home in 1553, he settled in Antwerp but ten years later moved permanently to Brussels. He married van Aelst's daughter, Mayken, in 1563. His association with the van Aelst family drew Bruegel to the artistic traditions of the Mechelen (now Malines) region in which allegorical and peasant themes run strongly. His paintings, including his landscapes and scenes of peasant life, stress the absurd and vulgar, yet are full of zest and fine detail. They also expose human weaknesses and follies. He was sometimes called the 'peasant Bruegel' from such works as *Peasant Wedding Feast* (1567)” (Pioch 2002).

*The Beggars* by Pieter Bruegel in 1568 is in The Louvre in Paris, France. Copyright Réunion des Musées Nationaux/Art Resource, NY, Louvre, Paris, France

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# Foreword

More than a decade has passed since a task force assembled by the Council for Agricultural Science and Technology (CAST) compiled information and published a report on mycotoxins in 1989. When that report was issued, it had been nearly a decade since the first CAST report on mycotoxins was published in 1979. This periodicity of publication is necessitated by the accumulation of new information on the occurrence, toxicology, analysis, and control of mycotoxins. The intent of this report — as with the two previous reports — is to compile the most complete current information on mycotoxins possible and to provide an understanding of their associated risks and impacts on plant, animal, and human systems. Preparation of the current report was recommended by the CAST National Concerns Committee and authorized by the CAST Board of Directors.

Dr. John L. Richard, Romer Labs, Inc., and Dr. Gary A. Payne, Department of Plant Pathology, North Carolina State University, served as cochairs for the report. A highly qualified group of international scientists served as task force members, contributors and reviewers. The group included individuals with expertise in agricultural utilization research, plant pathology, food science and human nutrition, public health, food safety, biological and agricultural engineering, animal science, and veterinary medicine.

The task force prepared an initial draft of the report, which was reviewed by the credited reviewers. The task force revised all subsequent drafts and reviewed the proofs. The CAST Executive Committee and Editorial and Publications Committee reviewed the final draft. The CAST staff provided editorial and structural suggestions and published the report. The

authors are responsible for the report's scientific content.

On behalf of CAST, we thank the cochairs, authors, contributors and reviewers who gave of their time and expertise to prepare this report as a contribution by the scientific community to public understanding of the issue. We also thank the employers of the scientists, who made the time of these individuals available at no cost to CAST. The members of CAST deserve special recognition because the unrestricted contributions they have made in support of CAST also have financed the preparation and publication of this report.

This report is being distributed widely; recipients include members of Congress, the White House, the U.S. Department of Agriculture, the Congressional Research Service, the Food and Drug Administration, the Environmental Protection Agency, and the Agency for International Development. Additional recipients include media personnel and institutional members of CAST. Individual members of CAST may receive a complimentary copy upon request for a \$3.00 postage and handling fee. The report may be reproduced in its entirety without permission. If copied in any manner, credit to the authors and to CAST would be appreciated.

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# Interpretive Summary

## Introduction

Modern mycotoxicology began with the discovery of the aflatoxins in the early 1960s. Disease due to ergot alkaloids (ergotism), however, has been known since the Middle Ages. Presently, toxic metabolites of fungi are considered to number in the thousands. Mycotoxins are of concern for human and animal diseases. And the plant pathogenic nature of many of the mycotoxin-producing fungi cause food-safety concerns and impact grain trade and marketing of food and feed.

## Major Classes of Mycotoxins

The major classes of mycotoxins include the aflatoxins, trichothecenes, fumonisins, zearalenone, ochratoxin A, and the ergot alkaloids. Toxins from the genus *Stachybotrys* also are candidates for potential inclusion in this list of major mycotoxins because they are considered by many to be involved in indoor air-quality problems throughout the world.

## Major Mycotoxin-Producing Fungi

Most mycotoxins of concern are produced by three genera of fungi, namely, *Aspergillus*, *Penicillium*, and *Fusarium*. The major mycotoxin-producing fungi are not aggressive pathogens in plants; however, mycotoxins are produced by several genera in plants during the growing season when portals of entry are provided and environmental conditions are appropriate.

## Mycotoxin Control

Control strategies are being developed around attempts to influence some of these conditions through management of agricultural practices prior to and at harvest. The occurrence of mycotoxins depends on favorable conditions being met for their production by fungi, and specific mycotoxins appear to be limited to

certain environmental loci and to specific crops. Other fungi may produce their toxic products in a wide variety of crops. Regional and other geographic distributions of the fungi and their toxins also may cause differences in the crops affected.

## Mycotoxins in Processed Foods

Mycotoxins can be present in processed foods made from mycotoxin-contaminated commodities, and they also can pass through animals and occur in meat, eggs, and milk. Mycotoxins may exist in food products as the parent compound or they may be changed to another toxic metabolite in the animal.

## Mycotoxicoses in Humans and Animals

While mycotoxins produce diseases (mycotoxicoses) in humans, as exemplified by aflatoxin, selected trichothecenes, and ergot alkaloids, the diseases caused by them in animals are more completely understood. The outcome of most intoxications in animals is loss of production and is dependent on species susceptibility to the individual mycotoxins. Most mycotoxins have specific effects on a given system in an animal, such as aflatoxin being primarily a hepatotoxin (liver toxin); however, many mycotoxins affect several systems simultaneously. Every system of the body is known to be affected by at least one mycotoxin.

## Diagnosis of Mycotoxicoses

Diagnosis of mycotoxicoses in animals is difficult as they may be similar to diseases with other causations. This is even more difficult in cases where more than one mycotoxin is involved because the toxins can produce additive, and sometimes synergistic, effects in animals.

## Analytical Testing for Mycotoxins

The presence of mycotoxins in commodities is presently unavoidable and, therefore, to avoid their occurrence in the food chain requires management strategies that would prevent contaminated commodities from entering food and feed processing facilities. Testing of the commodities is required to accomplish this process. This involves proper sampling, sample preparation, and analysis using a quality testing procedure, which may require processing samples in-house using test kits or sending samples to a qualified laboratory.

## Mycotoxin Control and Regulation

While controlling the occurrence of mycotoxins in finished products is possible, it may not be economically feasible. Therefore, regulatory bodies are continually assessing the levels of allowable exposure to humans by using a risk assessment process to establish tolerable daily intakes of selected mycotoxins. In some cases, biomarkers for certain mycotoxins are available to determine exposure in certain populations of individuals. These kinds of assessments are needed to establish problem areas for mycotoxin disease throughout the world.

Currently, worldwide regulations exist for mycotoxins but they need to be harmonized from country to country, especially for better trade negotiations. Approximately 77 countries are known to regulate mycotoxins, and the Food and Agriculture Organization (FAO) currently is updating information in this regard for a 2003 publication. Postharvest methods to decrease or eliminate mycotoxins are being studied and several approaches such as physical methods of separation and detoxification, biological and chemical inactivation, and decreasing bioavailability to host animals are being used and/or investigated.

## Economic Costs of Mycotoxins

The economic costs of mycotoxins are impossible to accurately determine, but the U.S. Food and Drug Administration (FDA) has utilized a computer model to estimate the losses due to selected mycotoxins. In the United States only, the mean economic annual costs of crop losses from the mycotoxins, aflatoxins, fumonisins, and deoxynivalenol, are estimated to be \$932 million. Sufficient information is not available for other mycotoxins to determine economic losses on

crops, livestock, and humans.

## Mechanisms of Mycotoxicity

Understanding the mechanisms of mycotoxin action on the host animals at the cellular and biochemical level is important in the overall goal to treat or inhibit the action of mycotoxins, thereby potentially controlling illnesses and deaths attributed to them. Obviously, this is a long-range process and immediate results are not to be expected. Regardless, new information and new technologies over the last 10 years have greatly facilitated our ability to detect mycotoxins and diagnose mycotoxicoses, and to decrease the content of mycotoxins in feeds through control and management practices.

## Research and Policy Needs

Listed below are areas of research and public policy that need to be addressed to provide a safer food and feed supply in the twenty-first century.

### 1. Public Policy

- Develop uniform standards and regulations for mycotoxin contamination.
- Support joint international cooperation (FAO/WHO/UNEP) to adopt standardized regulations.
- Develop a safe food supply for local populations.

### 2. Mycotoxin Detection

- Develop new technologies for mycotoxin analysis and improve detection (with specificity) of mycotoxins in prepared foods.
- Develop biomarkers for human and animal exposure to single and multiple mycotoxins.

### 3. Human and Animal Interactions

- Assess mycotoxins as virulence factors.
- Research the effect of mycotoxins as immunosuppressors.
- Evaluate toxicological interactions of toxins with the host.
- Examine population variation for sensitivity to mycotoxins.
- Assess interactions among mycotoxins and with drugs, diet, and nutrition.
- Assess role of fumonisins on humans and their involvement in esophageal cancer.
- Assess risks of ochratoxin exposure due to its oc-

currence in a variety of foods and environmental loci.

#### **4. Plant and Fungus Interactions**

- Establish a better understanding of the factors affecting mycotoxin formation in the field and in storage.
- Improve understanding of the ecology and epidemiology of mycotoxin-producing fungi.
- Develop sound agronomic-management practices to decrease mycotoxin contamination.
- Develop host-plant resistance to mycotoxin-producing fungi and to mycotoxin occurrence.
- Develop models to better forecast the potential of mycotoxin contamination.
- Research the genetic regulation and biosynthesis of mycotoxins by the producing organisms.

#### **5. Indoor Air Quality**

- Determine mycotoxins responsible for indoor air-quality problems.
- Develop sound sampling protocols for assessing fungal populations.
- Establish limits for respiratory exposure to mycotoxins.

#### **6. Economics of Mycotoxin Contamination**

- Develop accurate loss estimates for mycotoxin contamination.

#### **7. Bioterrorism**

- Assess potential for use of mycotoxins as bioterrorism agents.
- Assess mycotoxin-producing fungi as bioterrorism-agent candidates.

# Executive Summary

## Introduction

It has been nearly 40 years since modern mycotoxicology, as it might be termed, began with the discovery of the aflatoxins. Since that time, numerous other mycotoxins (toxic metabolites of fungi) have been discovered, many of which were later found to be causes of intoxications (mycotoxicoses) while others remained as laboratory curiosities. Studies directed at mycotoxins, including their detection, biosynthesis, and toxicology along with studies on the epidemiology and control of the producing fungi, are critical to maintaining a safe food supply.

The total number of mycotoxins is not known, but toxic metabolites of fungi potentially could number in the thousands. The number of mycotoxins actually known to be involved in disease is considerably less, but even this number is difficult to assess due to the diversity of effects of these unique compounds on animal systems. Not only are the mycotoxins of concern for human and animal diseases, but the plant pathogenic nature of many of the mycotoxin-producing fungi are of considerable economic concern in crops. The results of their plant pathogenic activities raise food safety concerns and impact grain trade and marketing of food and feed.

## Major Classes of Mycotoxins

The major classes of mycotoxins are aflatoxins, trichothecenes, fumonisins, zearalenone, ochratoxin A, and ergot alkaloids. The **aflatoxins** are produced primarily by *Aspergillus flavus* and *A. parasiticus* and they are important agents of disease; their effects range from acute death to chronic disease such as tumors.

The **trichothecenes** are a large class of mycotoxins produced by several fungal genera. *Fusarium* species are the most notable, but *Stachybotrys* is a significant producer of selected trichothecenes as well. Likely the most common occurring trichothecene is deoxynivalenol (DON or vomitoxin), which can be a significant contaminant of wheat, barley, and corn. T-

2 toxin is another trichothecene found more frequently in grains in Europe than in the United States.

The **fumonisins** occur primarily in corn and are produced by *F. verticillioides*, an almost-universal pathogen of corn. These toxins are capable of causing significant disease in horses and swine and have been shown to be carcinogenic in rats and mice.

**Zearalenone** is produced primarily by *F. graminearum* and causes vulvovaginitis and estrogenic responses in swine. It also may co-occur with DON in grains such as wheat, barley, oats, and corn.

The **ochratoxins** are produced primarily by *Penicillium verrucosum* and cause significant disease in animals, especially swine, and may be the causal agent of an endemic kidney disease in the Balkan countries.

**Ergot alkaloids** are produced primarily by several species of *Claviceps* that are plant pathogenic, and elaborate their toxins in specialized masses of fungal tissue called sclerotia. Ergotism is one of the oldest recognized mycotoxicoses.

## Minor Classes of Mycotoxins

The minor class of mycotoxins has representatives that occasionally are associated with mycotoxicoses of humans and other animals, or that occur frequently in selected substrates but have never been found associated with human or other animal disease.

## Mycotoxin Formation

In many cases, mycotoxins are formed in the field during the growing season; however, they also are formed or increased during harvest, drying, and storage. Most important in this process of mycotoxin production is the availability of water for growth of the producing fungus. Temperature, however, is an important factor as well. Thus, when the interaction of the plant and the fungus takes place, moisture and temperature greatly affect plant growth and health and the competitiveness of the mycotoxigenic fungus. In grain storage, the factors of water activity, sub-

strate aeration and temperature, inoculum concentrations, microbial interactions, mechanical damage, and insect infestation can play a role in mycotoxin contamination.

## Mycotoxin-Producing Fungi and Their Control

Mycotoxins are produced by a wide array of diverse fungal species that generally are not aggressive pathogens. They are adapted for colonization and growth on substrates with a wide range of moisture availability and nutritional content. Most of the mycotoxins that are considered to be important are produced primarily by three genera of fungi, namely, *Aspergillus*, *Penicillium*, and *Fusarium*. *Claviceps* and *Stachybotrys* also are important producers of mycotoxins.

Within the genus *Aspergillus*, the major class of mycotoxins, are the aflatoxins. The crops most usually affected are corn, cotton, peanuts, and certain tree nuts. *Aspergillus flavus* is a cause of ear rot in corn where conidia from the soil-inhabiting organism are carried to the silks of the corn plant and, under suitable environmental conditions, infections can occur. The production of aflatoxin can continue until the moisture in the kernels reaches about 15%. Peanuts are contaminated and infection occurs during high-temperature and low-moisture stress. In cotton, insects often play a role in the entry of the organism into the cotton bolls. In pistachios, a phenomenon whereby the hulls split prior to maturity allows for a portal of entry for the fungus. In both pistachios and almonds, however, contamination may involve damage by insect larvae. Although not conclusive in all crops, high temperatures seem to play a role in aflatoxin contamination. A number of control strategies for aflatoxin contamination in crops are being investigated and include controlling preharvest stress on the crop where possible, establishing breeding programs for resistance, and assessing potential biocontrol agents.

Within the genus *Fusarium*, there are a number of important mycotoxin-producing species. Some important plant pathogens are in this genus and are causes of wilts and scab or blight diseases of small grains. Ear rot also can be caused by *Fusarium*. *Fusarium graminearum* is the major causative agent; however, other species such as *F. verticillioides*, *F. proliferatum*, and *F. subglutinans* may cause ear rot. These latter agents may produce fumonisins during the pathogenic state in corn. *Fusarium graminearum* is a significant pathogen on wheat, barley, and oats

and is a major producer of DON in these grains. This organism also is capable of producing zearalenone in various commodities including corn. The organism survives in crop residue, which is a source of inoculum for the next year's crop. Control strategies for these infections and production of mycotoxins are being investigated and include elimination of the residue on the field soil through deep tillage, irrigation during drought stress, breeding for pathogen and insect-resistance, and genetic engineering.

*Penicillium spp.* are more typically associated with storage of crops and the production of mycotoxins such as ochratoxin. Ochratoxin usually is formed in storage or during drying of certain commodities for processing.

A number of fungi are capable of producing toxic alkaloids, and *Claviceps spp.* are the most notable in this regard. This organism is known as a replacement parasite in that it replaces plant structures with fungal tissue called ergots or sclerotia. These fungal bodies often contain toxic amounts of alkaloids leading to the disease known as ergotism in humans and other animals consuming them. Ergotism is one of the oldest known mycotoxicoses in humans and occurs following the incorporation, by several different processes, of the ergots in grain used in preparing food. Toxic alkaloids also are produced by the genera *Epichloe* and *Neotyphodium*, both of which can be endophytic in certain plant species such as fescue and ryegrass. Control of ergot is attempted by pasture management practices and, for endophytic relationships, the control efforts are aimed primarily at decreasing the toxicity of the endophytic fungus through selection.

*Stachybotrys* is a cellulolytic saprophyte that can be found in a variety of commodities and the trichothecene metabolites of this organism can produce disease similar to some of those produced by *Fusarium spp.* Recently, this organism seemed to be involved in human disease where building materials were contaminated with the organism and possibly its toxic metabolites.

Timely harvest, cleaning and drying of the crop, controlling temperature and moisture during storage, and using antifungal agents can assist in decreasing or eliminating mycotoxins in food and feed. Furthermore, research efforts to understand the genetic and biosynthetic aspects of mycotoxin development may lead to control strategies in grains. While the exact reasons that mycotoxins are produced by fungi are unknown, certain mycotoxins seem to function as potential virulence factors in producing disease in both plants and animals.

## Occurrence of Mycotoxins in Foods and Feeds

The occurrence of aflatoxins in foods and feeds has been fairly well studied; however, for the remainder of the more common mycotoxins, the occurrence in food is less well known. Ochratoxins and many of the *Fusarium* toxins have been given more attention recently. Also, more attention has been given to animal feed because mycotoxicoses were better recognized as occurring in livestock than in humans.

## Surveillance of Mycotoxin Occurrence

Surveillance programs exist in the United States and other countries to determine the mycotoxin occurrence in raw agricultural products, processed foods, and animal products such as meat, milk, and eggs. The U.S. Food and Drug Administration (FDA) analyzes products through a formal compliance program and an exploratory surveillance activity.

The occurrence of ergot in grain is limited and 0.05% ergots in grain have been suggested as a maximum acceptable level. The occurrence of aflatoxins is primarily in peanuts, corn, cottonseed, Brazil nuts, pistachios, various spices, figs, and copra. Concentrations are dependent on the environmental conditions during the growing season. DON is the most often reported mycotoxin other than aflatoxins. The other trichothecenes such as T-2 toxin and diacetoxiscirpenol (DAS) are less common. Zearalenone occurs in corn, wheat, barley, and grain sorghum but usually in low concentrations. Because *F. verticillioides* is almost ubiquitous in corn, low levels of fumonisins are common throughout the world in this commodity. Ochratoxin A is most often reported in corn, wheat, sorghum, oats, rice, wine, beer, and green coffee. Animal feeds in Canada and Europe may be highly contaminated with ochratoxin A, but apparently only low concentrations occur in U.S. grains. Of limited occurrence in agricultural commodities are sterigmatocystin, sporidesmins, rubratoxin B, cytochalasins, penitrems, and slaframine.

In processed foods, there is limited information about the occurrence of mycotoxins, most of which concerns the aflatoxins. Aflatoxins can occur in milk and in dairy products made from milk as a result of feeding contaminated rations to dairy cattle. The aflatoxins seem to be rapidly metabolized in most animal species, and residues in edible meat are rather limited

resulting in little concern for human health from this source of exposure. DON contamination of wheat has resulted in the discovery of this mycotoxin in wheat-based foods such as flour, bread, and baby foods. Ochratoxin A can be found in pork and poultry meat.

Monitoring for mycotoxins in foods imported into the United States is conducted by the FDA, and mycotoxin occurrence in foods worldwide is provided by data from the United Nations Food and Agriculture Organization (FAO).

Although commodities may be processed into various fractional components by wet or dry milling, the mycotoxins may still occur in selected fractions at different concentrations than that for the whole grain sample processed. Even in fermentation, mycotoxins are not in the ethanol fraction, but on a dry weight basis they actually may be increased in the spent grain product. In brewing processes, zearalenone, DON, ochratoxin, fumonisins, and aflatoxins can be found in the beer. Similarly, wine can contain ochratoxin A. Variable results, dependent on the mycotoxin and the commodity, have been obtained regarding the decrease in mycotoxins during food preparation techniques such as roasting, flaking, baking, cooking, and canning.

## Human Mycotoxicoses

Human disease resulting from mycotoxins is well established in some cases, but is more conjecturally associated in others. The **aflatoxins** are known to be causes of acute aflatoxicosis in humans, while more chronic disease such as carcinomas seem to be unclear because of co-occurrence of hepatitis B in the high-risk areas for aflatoxin contamination.

Presently, **ochratoxin** is the most probable mycotoxin involved in an endemic nephropathy in the Balkan countries. The risk for airborne exposure of humans to ochratoxin should be considered because disease has been demonstrated in animals following aerosol exposure.

**T-2 toxin** is conjecturally associated with a disease of past significance in Russia known as alimental toxic aleukia. It is of considerable interest that **DON** has been shown to produce disease in mice with histological changes nearly identical with human glomerulonephropathy. The disease known as **stachybotryotoxicosis** is considered to be caused by selected trichothecenes produced by *Stachybotrys chartarum*, but a true cause and effect relationship has not been established. Similarly, the **fumonisin**s seem to be the most likely cause of human esophageal cancer, but

this has not been conclusively demonstrated.

**Zearalenone** may be an important etiologic agent of intoxication in young children or fetuses exposed to this estrogenic compound, which results in premature thelarche, pubarche, and breast enlargement.

A number of mycotoxins are very immunosuppressive and are considered to be potential causes of immunosuppression in humans leading to secondary disease. This is difficult to prove due to the immunosuppression being overshadowed by the resulting secondary disease.

**Ergotism** is a known disease of humans causing both convulsive and gangrenous effects in recipients of ergot alkaloid contaminated food. Epidemiologic evidence also suggests mycotoxin involvement in other diseases of humans.

## Animal Mycotoxicoses

Mycotoxicoses in animals are more thoroughly understood than in humans because experimental studies seem to be more directly correlated with findings in animals after exposure. While there are deaths attributed to mycotoxins in animals, the economic effects of performance and productivity are measurable or considerably noticeable in many cases in animals. The general effects of mycotoxins on health and productivity of animals are dependent on dose, and generally the young of a species is more susceptible to the effects of mycotoxins than adults are.

### Major Mycotoxins Affecting Animals

The **aflatoxins** are potent liver toxins and most animal species exposed to these mycotoxins show signs of liver disease ranging from acute to chronic. Immunosuppression is an important consideration in aflatoxin-exposed animals. Aflatoxins are converted to another toxic metabolite that is excreted in milk and is important to consider in the economic aspects of aflatoxicosis in dairy cattle. Severe economic losses due to aflatoxins occur in poultry due to decreased productivity and disease.

The **trichothecenes** are potent inhibitors of protein biosynthesis and most effects in animals have this basic attribute. DON is the most common of this group causing animal disease and effects range from feed refusal and vomiting to immunosuppression and loss of productivity. Swine are considerably more sensitive to DON than poultry are, and cattle are quite insensitive.

The nephrotoxic effects of **ochratoxin A** in swine are a major disease in certain countries such as Denmark. The effects of ochratoxin in other animals are exhibited primarily as decreased productivity.

**Cyclopiazonic acid** is another mycotoxin that is found in several products and because of its co-occurrence with aflatoxins, it may be important in animal disease. It is capable of affecting egg-shell formation in poultry and has the potential for occurring in muscle tissue of affected animals.

In the United States, **fescue toxicosis** is a problem due to the extent of fescue grass in pastures. A toxic endophytic fungus is responsible for a number of syndromes, including a gangrenous condition of the extremities, primarily in cattle grazing fescue pastures.

### Mycotoxin Effects on Animal Systems

Because most of the mycotoxins have a primary effect on a specific body system, it is possible to use a systems approach to classify the mycotoxins. **Immunologic effects** are attributable to several mycotoxins. The predominant mycotoxins in this regard are the aflatoxins, the trichothecenes, and ochratoxin A. However, several other mycotoxins such as the fumonisins, zearalenone, patulin, citrinin, wortmannin, fusarochromanone, glitotoxin, and fescue and ergot alkaloids have been shown to produce some effects on the immune system.

The mycotoxins having primarily **hematopoietic effects** are the aflatoxins and the trichothecenes. Hemorrhagic anemia syndromes are attributable to each of these groups of mycotoxins.

Among those mycotoxins having primary **hepatotoxic effects** are the aflatoxins, ochratoxins, fumonisins, sporidesmin, rubratoxins, and phomopsins. All of them will produce significant liver damage when given to animals.

**Nephrotoxicity** can be demonstrated by ochratoxins, trichothecenes, and fumonisins.

Most notable mycotoxins having effects on the **reproductive system** are zearalenone and ergot alkaloids; however, some reproductive effects have been produced in selected animal species by aflatoxins and T-2 toxin.

**Teratogenic effects** have been demonstrated, at least experimentally, by aflatoxin B<sub>1</sub>, ochratoxin A, rubratoxin B, T-2 toxin, sterigmatocystin, and zearalenone.

The **neurotoxic effects** of mycotoxins are best demonstrated by vomiting and taste aversion pro-



duced by DON; seizures, focal malacia, and liquefaction of brain tissue mediated possibly through sphingolipid synthesis under the influence of fumonisins; staggers and tremors produced by a number of tremorgenic mycotoxins and exemplified by penitrem A; convulsive and other nervous effects of ergot alkaloids; and parasympathomimetic activity resulting from effects of a slaframine metabolite for selected receptors in the nervous system.

Several mycotoxins are classified as **carcinogens** and include aflatoxins, sterigmatocystin, ochratoxin A, fumonisins, and possibly patulin.

Several of the trichothecenes are classified as **dermonecrotic** because of their irritant and necrotizing activity.

Although the above classification is based on systems affected, it becomes more difficult to use this classification as we learn more of the mode of action of the mycotoxins. It is obvious that mycotoxins affect more than one system simultaneously and, therefore, produce a multiplicity of responses by the affected animal resulting in increased difficulty of attributing the response to a single body system. Natural intoxications by mycotoxins often are more complex than can be related to those experimental studies utilizing one mycotoxin. Therefore, natural responses may be the cause of two or more mycotoxins. A number of studies to determine the synergistic effects of mycotoxins have been conducted. Generally, the results of these studies have indicated that most mycotoxin interactions in experimental studies are additive or less than additive. In those few studies where there was synergism, aflatoxins were one of the toxins in the experimental design.

## Mycotoxin Diagnosis

The diagnosis of mycotoxicoses is difficult because the effects observed are not necessarily unique to a given mycotoxin, but may be shared by other toxins and pathogenic organisms as well. Furthermore, diagnosis is usually attempted based on information gathered in experimental studies where controlled criteria were used. In natural intoxications, however, a variety of factors such as environmental, nutritional, behavioral, and husbandry may influence the disease condition. To make the best possible diagnosis, information must be gathered from the animal(s), both living and postmortem, as well as conducting a thorough chemical examination of the feed involved in the intoxication.

## Mycotoxin Occurrence in the Food Chain

Avoiding mycotoxin occurrence in the food chain requires understanding the strategies to manage mycotoxins. Part of this strategy is to keep mycotoxin-contaminated commodities from entering into food and feed-processing facilities. This is accomplished by instituting a quality, overall testing program on incoming grain and supplies. It also involves maintaining valid records of all processes and having the ability to economically and safely remove any contaminated lots of material inadvertently received into a facility.

## Analytical Testing for Mycotoxins

The overall testing for mycotoxins involves obtaining an adequate sample (most important because of the distribution and low concentrations of mycotoxins in grains), preparing the sample, and finally conducting the analytical procedure. The selected sample must be submitted to extraction of the toxin and cleanup to rid the extract of as much extraneous, interfering substances as possible. Then the cleaned-up extract must be submitted to one of several possible detection systems to determine the presence, or most likely, the kind and quantity of mycotoxin present in the sample. Several quantitation procedures may be used including thin-layer chromatography, high-performance liquid chromatography, gas chromatography, fluorometry, or immunologically based tests such as enzyme-linked immunosorbent assay (ELISA). The methods must be validated to ensure that the methods employed are functional and accurate. This often is done by using check samples or certified reference material prepared with known concentrations of specific mycotoxins.

Regardless of the overall testing procedure used, including sampling, sample preparation, and analysis, there is considerable variability in each part. The greatest variability in mycotoxin testing occurs in the sampling step. Even during the sample preparation, which involves grinding and obtaining a suitable subsample for analysis, there is variability dependent on a number of factors. Finally, there is variability in the analytical procedure and the result obtained, as a quantity, is still only within a certain range of accuracy. Performance of analytical systems can be evaluated by participation in proficiency testing programs whereby individual laboratories receive certified reference material to test, and their performance

on testing a sample is evaluated relative to the actual concentration and to other participating laboratories.

## Mycotoxin Control and Regulation

Controlling mycotoxins in foods and feeds is an ever-evolving process, particularly as we learn more about their occurrence, environmental factors influencing their formation, and changes in agronomic practices. As noted earlier, mycotoxin contaminants are unavoidable and therefore small amounts of mycotoxins may be legally permitted if these amounts are known to be safe for human and animal health.

Monitoring programs assessing the occurrence of mycotoxins along with available toxicological data are used to make an assessment of exposure-risk to humans or animals. The result is the establishment of regulatory levels for selected mycotoxins where sufficient information as described can be obtained. In the United States, the FDA regulates mycotoxins under Section 402 or 406 of the Food, Drug and Cosmetic Act. Each risk assessment is based on the hazards or toxicity of a mycotoxin and the expected degree of exposure of individuals or populations. Included in the assessment is an uncertainty factor such that data from animal studies may be used to extrapolate to humans to achieve an acceptable or tolerable daily intake.

Examples of toxicological data may include acute toxicity information, teratogenicity and reproductive toxicity, effects on immunity, and chronic effects of the mycotoxin. These have been used in establishing a tolerable daily intake of 12  $\mu\text{g}/\text{kg}/\text{day}$  for DON in humans. For carcinogenic mycotoxins, such as aflatoxins, however, a multidisciplinary team has investigated all experimental evidence for carcinogenicity along with epidemiologic study evidence and conducted assessment of interactions, potencies, and exposure. In these investigations, it was determined that there is possible interaction of hepatitis B exposure with aflatoxin intake in the development of human hepatocellular cancer. From these results, recommendations for the control of aflatoxicosis have been proposed. Epidemiological evidence is important in many of these investigations for determining risk, and the assessment is easier if biomarkers are available to assess exposure of individuals or populations. Biomarkers are available for use in studies concerning aflatoxins, fumonisins, and ochratoxins. For other mycotoxins, however, the use of biomarkers is more problematic or unavailable.

The control programs for mycotoxins in the United States are within the objectives of the FDA mission, and aflatoxin is a mycotoxin of primary concern. One approach the FDA uses to control mycotoxin contamination of foods and feeds is to establish action levels, e.g., aflatoxins and patulin, as guides to be used when enforcement actions should be taken. Also, to minimize exposure, the FDA establishes advisory levels for certain mycotoxins such as has been done for DON and fumonisins. Other mycotoxins are of interest to the FDA and they are constantly evaluating the need for regulations based on exposure data and risk assessments.

Worldwide regulations exist for mycotoxins and generally are based on toxicological data, occurrence and distribution, and epidemiological data. There is a need for regulations to be harmonized from country to country especially where trade contracts exist. Also, it is important that regulations are not so strict to jeopardize the limited food supply of developing countries or to make the commodities excessively high priced. Throughout the world, new limits and regulations for mycotoxins have come into force, and because of acquisition of new data, the regulations are rapidly changing. Approximately 77 countries regulated mycotoxins in 1996, 13 had no regulations, and no data were available for 50 countries. A new update, scheduled for publication by the FAO in 2003, will show increased and more up-to-date information on mycotoxin regulations in foodstuffs and feedstuffs. It also will show the first results of regional harmonization of regulations for foodstuffs, e.g., in the European Union.

## Decontamination and Detoxification Strategies for Mycotoxins

Although agronomic and other practices are aimed at decreasing or eliminating mycotoxins in the field, there are still considerable reasons to look at postharvest means to eliminate or inactivate mycotoxins in grains and other commodities. The approaches to this are varied and may be categorized as physical means of separation, physical methods of detoxification, biological methods of inactivation, chemical methods of inactivation, and decreasing the bioavailability of mycotoxins to the host animal.

Physical means of separation include mechanical separation whereby contaminated particles of the substrate are removed from the lot to decrease the myc-

otoxins. This process is often impractical and incomplete. Density segregation has shown variable efficacy depending on the commodity and the mycotoxin of interest.

Physical methods of detoxification have been used with variable degrees of success. Thermal inactivation has been used for aflatoxins, but the aflatoxins are quite heat stable and limited success has been achieved. Irradiation with either gamma rays, microwaves, or ultraviolet light have been used, primarily with aflatoxins, and generally have decreased the concentrations in the substrates tested. Solvent extraction is effective for the aflatoxins but is cost prohibitive and impractical.

Biological methods of inactivation have been applied to field plots whereby nontoxigenic strains of the organism function as a biocontrol organism. This technique has achieved considerable success in decreasing aflatoxins in cotton, corn, and peanuts. Other organisms have been investigated to determine their efficacy in inactivating aflatoxins. A strain of *Eubacterium* has been used with considerable success in deactivating trichothecenes by biotransformation of the epoxide group of the molecule to a less toxic molecule. Dietary factors have shown some efficacy in influencing the toxicity of selected mycotoxins, especially aflatoxins.

Chemical methods of detoxification are most noted with the aflatoxins being degraded by ammoniation. This process is considerably effective in cottonseed and cottonseed meal and is utilized in several states for this purpose. Treatment of aflatoxins B<sub>1</sub>, G<sub>1</sub>, M<sub>1</sub>, and aflatoxicol with sodium bisulfite makes water soluble products, thereby decreasing or eliminating the toxicity of these aflatoxins. Ozonization has been used in degrading aflatoxins in corn and cottonseed meals. It also is efficacious in decreasing DON and moniliformin. It also has been used in vitro to detoxify and degrade cyclopiazonic acid, ochratoxin A, patulin, secalonic acid D, and zearalenone.

Probably the most interest in controlling the effects of mycotoxins in animals has been in the development of binders of mycotoxins to render them unavailable for absorption by the host animal. Calcium aluminosilicates seem to be effective in binding the aflatoxins, thereby decreasing or eliminating toxicity to study animals. Other mycotoxins did not seem to be bound effectively by this substance. There are a number of clay and zeolitic minerals manufactured for the purpose of binding mycotoxins, but their efficacy should be tested for specific mycotoxins before inclusion as dietary amendments. Cholestyramine seems to be somewhat effective in binding zearalenone, but

it also must be tested in vivo for efficacy.

## Economic Costs of Mycotoxins

It is not possible to estimate precisely the economic costs of mycotoxins. However, a computer based model is used in presenting an estimated sum of the value of food and feed losses, and mitigation efforts. From these studies, the potential economic costs of crop losses from mycotoxins, in the United States only, are large with a mean of \$932 million per year. Mean mitigation costs were estimated to be about \$466 million and the mean simulated livestock costs were about \$6 million per year. The major mycotoxins used in these assessments were aflatoxins, fumonisins, and deoxynivalenol. Human health costs from these mycotoxins are not presented here. All other mycotoxins likely having a potential effect on economic costs were not considered because of lack of sufficient information. However, even with the few mycotoxins used and the assumptions involved in the model, mycotoxins could potentially impose large costs on the economy.

## Mechanisms of Mycotoxicity

Understanding the mechanism of mycotoxin action on the host animal at the cellular and biochemical level is important in the overall goal to treat or inhibit the action of mycotoxins, thereby potentially controlling illnesses and deaths attributed to them. The difficulty of obtaining this understanding is the complexity of the interactions involved in the intoxications and the relationships with host factors such as nutrition and immune status. Furthermore, the mycotoxins may interact with other therapeutic or prophylactic measures of disease control, especially because many of the mycotoxins are immunosuppressive and some have been shown to interfere with animal vaccination programs. The potential number of biochemical targets for the diversity of mycotoxins, both chemically and biologically, offers a challenge in the overall understanding of their mode of action. Immediate results, therefore, would be unexpected in achieving adequate control measures for intoxications due to mycotoxins.

## Research and Policy Needs

Mycotoxins continue to present a threat to food safety. Changes in agricultural production practices and food processing, along with global changes in en-

vironmental and public policy, challenge us to develop and refine strategies and technologies to ensure safe food and a healthy environment. Increasing globalization of trade also adds a new dimension to the importance of mycotoxins not only as toxins, but also as impediments to free trade among countries.

Listed below are areas of research and public policy that need to be addressed to provide a safer food and feed supply in the twenty-first century.

### **1. Public Policy**

- Develop uniform standards and regulations for mycotoxin contamination.
- Support joint international cooperation (FAO/WHO/UNEP) to adopt standardized regulations.
- Develop a safe food supply for local populations.

### **2. Mycotoxin Detection**

- Develop new technologies for mycotoxin analysis and improve detection (with specificity) of mycotoxins in prepared foods.
- Develop biomarkers for human and animal exposure to single and multiple mycotoxins.

### **3. Human and Animal Interactions**

- Assess mycotoxins as virulence factors.
- Research the effect of mycotoxins as immunosuppressors.
- Evaluate toxicological interactions of toxins with the host.
- Examine population variation for sensitivity to mycotoxins.
- Assess interactions among mycotoxins and with drugs, diet, and nutrition.
- Assess role of fumonisins on humans and their involvement in esophageal cancer.

- Assess risks of ochratoxin exposure due to its occurrence in a variety of foods and environmental loci.

### **4. Plant and Fungus Interactions**

- Establish a better understanding of the factors affecting mycotoxin formation in the field and in storage.
- Improve understanding of the ecology and epidemiology of mycotoxin-producing fungi.
- Develop sound agronomic-management practices to decrease mycotoxin contamination.
- Develop host-plant resistance to mycotoxin-producing fungi and to mycotoxin occurrence.
- Develop models to better forecast the potential of mycotoxin contamination.
- Research the genetic regulation and biosynthesis of mycotoxins by the producing organisms.

### **5. Indoor Air Quality**

- Determine mycotoxins responsible for indoor air-quality problems.
- Develop sound sampling protocols for assessing fungal populations.
- Establish limits for respiratory exposure to mycotoxins.

### **6. Economics of Mycotoxin Contamination**

- Develop accurate loss estimates for mycotoxin contamination.

### **7. Bioterrorism**

- Assess potential for use of mycotoxins as bioterrorism agents.
- Assess mycotoxin-producing fungi as bioterrorism-agent candidates.

# 1 Objectives

Mycotoxins and mycotoxicoses studies ultimately are associated with improving food safety. A 100% safe food supply, i.e., one in which there is no risk, is unachievable. Therefore, we must base some 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. More than a decade has passed since the last task force compiled information and published a report on mycotoxins (Council for Agricultural Science and Technology 1989). When that report was issued, it had been nearly a decade since the first Council for Agricultural Science and Technology (CAST) report on mycotoxins was published (Council for Agricultural Science and Technology 1979). This periodicity of publication is necessitated by the accumulation of new information on the occurrence, toxicology, analysis, and control of mycotoxins. We have gained much insight into the field-related problems associated with mycotoxin formation; their mode of action; and their biosynthesis, occurrence, analysis, and control. The overall testing program for mycotoxins has improved as we learned more about sampling, sample preparation, and analytical procedures. Increased awareness by the medical community of the likely involvement of mycotoxins in human disease has engendered additional literature and understanding of the relationship of mycotoxins to public health.

The intent of this report — as with the two previous ones — was to compile the most complete information on mycotoxins possible, and to provide an understanding of their associated risks and impacts on plant, animal, and human systems. This compilation has two main goals: (1) to educate those making decisions that affect regulation and control of foods and feeds and (2) to illuminate the causes for mycotoxins to affect international trade of commodities and food products. It is our hope, however, that this report can be used by the entire scientific community and be adapted for educational purposes at all levels.

Originally, the major emphasis was mycotoxins' causation of animal diseases, *mycotoxicoses*. Although these diseases affect all aspects of the health status of animals, from production efficiency to lethality,

other effects are difficult to determine, e.g., immunosuppression, decreased resistance to other diseases. Human disease is even more problematic than animal disease. Many suspected mycotoxic effects in humans are extrapolations from experimental or known mycotoxicoses in animals. Although epidemiological studies continue to reveal possible relationships of mycotoxins to human disease, conclusive evidence often is lacking. Surveys of the occurrence of mycotoxins in foods and feeds are augmented by the diagnosis of mycotoxicoses in animals and epidemiological studies as well as plant pathology studies. Information from these events and studies leads to improved evaluation of the impacts of mycotoxins on plant and animal systems and the associated risks to food safety. We now have a better understanding of the mode of action of mycotoxins in plants, animals, and humans as well as the ecological/environmental relationships of the formation of mycotoxins in foods and feeds.

Current analytical procedures and the promise of new technologies will allow for improved measurement of the levels of mycotoxins in foods and feeds thereby creating a better understanding of the toxicity of the mycotoxins affecting human and animal health. These methods have become more rapid, precise, sensitive, and selective and less expensive for determining mycotoxins in a wide variety of complex matrices. Advances in economically feasible methods for controlling mycotoxins in foods continue to be made so that there is less consumption of toxic quantities of mycotoxins. Detoxification, decontamination, dilution, diversion, and destruction of mycotoxins are sometimes possible, allowing for control or management measures that are less wasteful and more economically and toxicologically acceptable.

Now that this information base has been collected in a single document, we, as consumers and scientists, can better evaluate the risks associated with mycotoxins. Furthermore, we can more accurately assess the needs for research involving the association of mycotoxins in plant, human, and animal disease and their overall implications in food safety and trade issues.

## 2 Introduction

### Overview and Definitions

Beginning in the late 1800s and continuing well into the 1900s, the concept of “secondary metabolism” among the fungi and other microbes was gaining acceptance. An important concept in the overall metabolism of these organisms is that the compounds were not utilized as components of the organism’s body but were often, and usually, elaborated into the “medium” on which the organism was growing. At the same time, the concept of antibiosis was becoming important, and the finding that fungal “antibiotics” could be toxic to animal species resulted in many proposed antibiotics never entering the marketplace because their toxicity precluded their clinical use. During this period came the first general recognition that fungal “secondary metabolites” could be important entities in diseases of animals and humans. Before this time, mushroom poisoning and ergotism (discussed later in this chapter) were the only known mycotoxicoses. Today, the word *mycotoxin* simply means a toxin produced by a fungus. The term was derived from “mycotoxicosis,” first used by Forgacs and Carll (1955), meaning a disease caused by a fungal toxin. The latter does not include mushroom poisonings, which result from intentional consumption of fungal thalli (bodies) as food; thus, will not be discussed further.

Mycotoxins are a relatively large, diverse group of naturally occurring, fungal toxins, many of which have been strongly implicated as chemical agents of toxic disease in humans and animals (Table 2.1). It is uncertain exactly how many toxic secondary metabolites or mycotoxins exist. Knowing the occurrence and distribution of toxins in foods and feeds is important because exposure to unknown bioactive agents could be an important confounding factor in attempts to explain the etiology of chronic disease in animals and humans. However, a rough estimate of the number of fungal metabolites and potential mycotoxins can be made. Turner (1978) catalogued approximately 1,200 secondary fungal metabolites produced by approximately 500 species of fungi. In 1983, Turner and Alderidge (1983) catalogued 2,000 more metabolites produced by approximately 1,100 species. Thus,

as an average estimate, there were approximately two unique secondary metabolites per fungal species.

In 1991, Hawksworth (1991) estimated that there were 69,000 known fungal species and that they represented 5% of the world’s total fungal species, which he estimated to be 1.5 million. Conservative estimates are on the order of 100,000 species (Esser and Lemke 1996). Based on the work of Hawksworth and the assumption of two unique secondary metabolites per fungal species, there may be as many as 3 million unique secondary fungal metabolites. A conservative estimate would be 200,000. Between 1971 and 1983, the number of known secondary fungal metabolites increased from 1,200 (Turner 1978) to 3,200 (Turner and Alderidge 1983). Assuming that the rate of discovery (accessible in the published literature) remained level, there would be approximately 6,000 described secondary fungal metabolites by 2002. This is less than 0.2% of the 3 million or 3% of the 200,000 total estimated above. Clearly, the number of secondary metabolites still undiscovered is quite large. Cole and Cox (1981) listed approximately 300 secondary fungal metabolites as mycotoxins. Approximately 10% of the secondary fungal metabolites described by Turner (1978) and Turner and Alderidge (1983) were classified as mycotoxins by Cole and Cox (1981). Thus, there are potentially 20,000 to 300,000 unique mycotoxins. The diversity of toxic mechanisms will be equally as great.

The usual route of mycotoxin exposure is ingestion as food or feed contaminants. However, dermal and inhalation also may be important routes of exposure. Direct effects of mycotoxins range from acute disease where severe conditions of altered health may exist prior to death as a result of exposure to the toxin. These conditions are more likely following exposure to high levels of a mycotoxin. Other, more insidious or occult conditions (e.g., growth retardation, impaired immunity, decreased disease resistance, decreased milk or egg production) or more chronic disease manifestations (e.g., tumor formation) may result from prolonged exposure to small quantities of toxin. The low level of exposure is of considerable concern where food and feeds are of a better quality

**Table 2.1. Commodities in which mycotoxins have 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 <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub> , M <sub>1</sub> , M <sub>2</sub> )	Peanuts, corn, wheat, rice, cottonseed, copra, nuts, various foods, milk, eggs, cheese, figs	Birds Duckling, turkey, poul, 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, coffee, raisins, grapes, dried fruits, wine	Swine, dog, duckling, chicken, rat, human	Nephrotoxicity (tubular necrosis of kidney) Porcine nephropathy Mild liver damage Enteritis Teratogenesis Carcinogenesis (kidney tumors) Urinary tract tumors
Patulin	Moldy feed, rotted apples, apple juice, wheat straw residue	Birds Chicken, chicken embryo, quail Mammals Cat, cattle, mouse, rabbit, rat, human 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
Penitrem	Moldy cream cheese, English walnuts, hamburger bun, beer	Dog, mouse, human	Tremors, death, incoordination, bloody diarrhea

Table 2.1. (continued)

Mycotoxin	Commodities found contaminated	Effects of mycotoxins	
		Affected species	Pathological effects
Sterigmatocystin	Green coffee, moldy wheat, grains, hard cheeses, peas, cottonseed	Mouse, rat	Carcinogenesis Hepatotoxin
Trichothecenes (T-2 toxin, diacetoxyscirpenol, neosolaniol, nivalenol, diacetylivalenol, deoxynivalenol, HT-2 toxin, fusarenon X)	Corn, wheat, commercial cattle feed, mixed feed, barley, oats	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

and thus would contain the lesser amounts of mycotoxin(s). Greater concern exists where populations depend on a single staple in the diet. If that staple is contaminated, consumers are exposed to greater amounts of the mycotoxin over a given period of time than populations that consume a wide variety of foods. There seems to be great diversity among animal species in susceptibility to a particular mycotoxin that is influenced by factors such as age, sex, strain, and nutritional status. Indirect exposure of humans to mycotoxins likely occurs when toxic residues or metabolites that persist in milk, eggs, or edible tissues are consumed.

Awareness is growing regarding the hazards of mycotoxins as contaminants of food and feed. The realization of the magnitude of these hazards began in the early 1960s and has led to a vast literature on the many facets of mycotoxins and mycotoxicoses. The risks associated with mycotoxins occurring in field crops, foods, feeds, and animal products raise food safety concerns and ultimately impact grain trade and marketing of food and feed.

## Major Classes of Mycotoxins

Many types of toxic metabolites have been obtained from fungal laboratory cultures, as noted earlier. Most of them are not known to be causes of human or

animal disease, so remain laboratory curiosities. The mycotoxins that pose the greatest potential risk to human and animal health as food and feed contaminants are **aflatoxins**, **trichothecenes**, **fumonisin**, **zearalenone**, **ochratoxin A**, and **ergot alkaloids**. However, other mycotoxins should be included because of their frequency of occurrence in commodities or their products or their co-occurrence with other important mycotoxins. This expanded list includes **cyclopiazonic acid**, **sterigmatocystin**, **gliotoxin**, **citrinin**, **penitrems** (perhaps other **tremorgenic mycotoxins**), **patulin**, and miscellaneous mycotoxins such as **fusarin C**, **fusaric acid**, **penicillic acid**, **mycophenolic acid**, **roquefortine**, **PR toxin**, and **isofumigaclavines A and B**.

**Aflatoxins** can be produced by four species of *Aspergillus*: *A. flavus*, *A. parasiticus*, *A. nomius*, and *A. pseudotamarii* (Ito et al. 2001; Kurtzman et al. 1987; Payne 1998). Four major aflatoxins —  $B_1$ ,  $B_2$ ,  $G_1$ , and  $G_2$  (B = blue and G = green fluorescence while the subscript designates relative chromatographic mobility), plus two additional metabolic products,  $M_1$  and  $M_2$  — are significant 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 **trichothecenes** are a family of nearly 150 structurally related compounds (Grove 1988) produced by several fungal genera, i.e., *Fusarium*, *Ceph-*



*alosporium*, *Myrothecium*, *Stachybotrys*, *Trichoderma*, and others. Several naturally occurring trichothecene mycotoxins are produced in foods and feeds by *Fusarium* species, e.g., deoxynivalenol (DON; sometimes referred to as vomitoxin), T-2 toxin, nivalenol, diacetoxyscirpenol. In some crop years, DON contamination of corn and wheat has been significant. Natural contamination of foods and feeds by T-2 toxin in the United States rarely has been reported but is more commonly detected in grains in Europe. The toxicology of T-2 toxin has been studied extensively.

In 1980 and 1981 in Canada and in 1982 in the United States, DON was found in wheat that had been severely infested with the wheat scab fungus, *Fusarium graminearum*. Soft winter wheats were the most severely affected. In Canada, dried corn was discovered that contained levels of DON slightly higher than those found in the wheat (Trenholm et al. 1985). In some parts of the United States, zearalenone occurred with DON in scabby wheat; in some cases of *Gibberella* ear rot of corn, zearalenone and DON are found together. In 1993 and subsequent years, widespread occurrence of DON in wheat and barley caused severe economic losses in the upper wheat growing regions of the United States and southern Canada (McMullen et al. 1997).

**Fumonisin**s are mycotoxins produced by *F. verticillioides* (syn., *moniliforme*) (Gelderblom et al. 1988b). This organism is involved in producing equine leukoencephalomalacia and pulmonary edema syndrome in swine. Fumonisin has been described as being capable of reproducing the disease in horses (Marasas et al. 1988b). There also is evidence linking *F. verticillioides* infected corn to the high incidence of human esophageal cancer in South Africa and China (Gelderblom et al. 1988b; Yoshizawa et al. 1994). One fumonisin ( $B_1$ ), isolated from culture material of *F. verticillioides*, was shown to have cancer-promoting activity in rats (Gelderblom et al. 1988a). Leukoencephalomalacia commonly occurs in horses in the United States; *F. verticillioides* is an almost-universal inhabitant of corn (Haliburton and Buck 1986).

**Zearalenone**, an estrogenic mycotoxin, causes vulvovaginitis and estrogenic responses in swine. Zearalenone is produced primarily by *F. graminearum*, which occurs naturally in high-moisture corn and has been found in moldy hay and pelleted feeds. Physiological responses in swine occur when the zearalenone level in feed corn exceeds about 1 milligram (mg)/kilogram (kg) (Kurtz and Mirocha 1978). Zearalenone can be transmitted to piglets in sows' milk, causing estrogenism in the young pigs. Zearalenone production is favored by high humidity

and low temperatures, conditions that often occur in the Midwest during autumn harvest (Christensen et al. 1977; Council for Agricultural Science and Technology 1979).

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

The **ergot alkaloids** are produced by several species of *Claviceps*, a genus of fungi that invades the female portion of the host plant and replaces the ovary with a mass of fungal tissue called sclerotium. The ergot alkaloids comprise the largest group of nitrogen-containing fungal compounds and are divided into four major groups based on their chemical similarities: (1) the clavines, (2) the lysergic acids, (3) the lysergic acid amides, and (4) the ergopeptines (Rehacek and Sajdl 1990). Selected members of these groups of compounds are involved in either nervous or gangrenous syndromes in humans and animals that consume grains or grain products contaminated with the sclerotia of the fungus. Ergotism is one of the oldest mycotoxicoses known, although occurrence of the disease has declined over time. Ergot alkaloids may be produced by fungi outside the genus *Claviceps* but the importance of their toxicity is less known.

## Minor Classes of Mycotoxins

**Cyclopiazonic acid** (CPA) originally was isolated from a culture identified as *Penicillium 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 either commonly found on agricultural commodities or used in fermented food production. These included *Aspergillus flavus*, *A. versicolor*, *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), peanuts (Lansden and Davidson 1983), and sunflower seeds (Ross et al. 1991). It was found in Kodo millet (Rao and Husain 1985) implicated in a human intoxication in India. There also is evidence

that CPA may have been involved, along with the aflatoxins, in the "Turkey X" syndrome in England in 1960 (Bradburn et al. 1994; Cole 1986a).

The mycotoxin **sterigmatocystin** is produced by several species of *Aspergillus* and *Bipolaris* and by *Penicillium luteum*. 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 cheese (Bullerman 1981; Scott 1985; Vesonder and Horn 1985).

**Gliotoxin** is a highly immunosuppressive compound produced by a wide variety of fungi. Interestingly, gliotoxin is produced by the organism *Aspergillus fumigatus* during its pathogenic state as a causative agent of the respiratory disease known as aspergillosis in turkeys (Richard et al. 1996a). This compound belongs to a group of fungal metabolites, some of them toxic, called epipolythiodioxopiperazines. It also seems to be involved in human yeast infections caused by *Candida albicans* (Shah and Larsen 1991). The importance of the involvement of this compound in mycotoxicoses following ingestion of contaminated foodstuffs is not known. However, camels have become intoxicated by eating hay contaminated with the organism *A. fumigatus* and containing the mycotoxin, gliotoxin (Gareis and Wernery 1994).

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

Fungi capable of producing **tremorgenic mycotoxins** belong to the genera *Penicillium*, *Aspergillus*, *Claviceps*, and *Acremonium*. These mycotoxins cause a disease in cattle called staggers. Clinical signs include muscle tremor, uncoordinated movements, general weakness in the hind legs, and stiff, stilted movements of the forelegs. Severely affected animals may not be able to stand. Other intoxications involving fungal tremorgens, most notably the **penitrem mycotoxins**, have been reported from moldy cream cheese, a hamburger bun (bread), and walnuts consumed by dogs (Richard and Arp 1979; Richard et al. 1981). Another case involving penitrems was in a human who consumed mold-contaminated beer (Cole 1986b).

The mycotoxin **patulin** is produced primarily by

species of *Penicillium* and *Aspergillus* but its production is not limited to these genera (Scott 1994). Its limited toxic properties are of major concern because it occurs in apples and, subsequently, applesauce and juice. Although it can be produced in other juices molded by toxigenic species of fungi, the natural occurrence in juices other than apple juice is not a significant problem.

The following paragraphs will review other, less-studied mycotoxins found at low frequency or under unusual circumstances.

**Citreoviridin** originally was isolated from cultures of molds obtained from rice associated with a disease called cardiac beriberi that has occurred for three centuries in Japan (Ueno and Ueno 1972). This mycotoxin also occurs in corn and other foods and feeds (Wicklow et al. 1988). Several species of *Penicillium* and one of *Aspergillus* have been reported to produce this mycotoxin. Interestingly, citreoviridin and aflatoxin have been found to occur simultaneously in corn, which allows for their possible interaction in producing animal disease (Wicklow et al. 1988). Citreoviridin causes paralysis, dyspnea, cardiovascular disturbances, and loss of eyesight in experimental animals (Ueno 1974).

*Penicillium roqueforti* and *P. caseicolum* (*P. camemberti*), used to make 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 to public health of the various toxins produced by *P. roqueforti* and *P. caseicolum* is not clear due to the lack of scientific research on compound stability, production in agricultural commodities, and toxicity.

In 1995, researchers in Italy reported a previously unknown toxin, **fusaproliferin**, produced by *Fusarium proliferatum* and *F. subglutinans* (Logrieco et al. 1996; Manetti et al. 1995; Ritieni et al. 1995). It is a sesquiterpene compound that is toxic to brine shrimp (*Artemia salina*) and insects and human cell cultures in laboratory assays. It also has teratogenic effects on chicken embryos (Ritieni et al. 1997a). Fusaproliferin has been detected in livestock feed samples associated with feed refusal (Munkvold et al. 1998) but there is no direct evidence that it causes feed refusal symptoms. No mammalian feeding studies have been conducted to date.

Fusaproliferin has been detected in corn samples from Italy (Ritieni et al. 1997b), Poland, and the United States. It sometimes occurs with **fumonisin**, **beauvericin**, and/or **fusaric acid** (Munkvold et al. 1998). Most strains of *F. proliferatum* and *F. subglu-*

*tinans* that have been tested are capable of fusaproliferin production in culture (Moretti et al. 1996). Although the significance of this compound in animal and human health remains to be determined, its acute toxicity to brine shrimp exceeds that of more-familiar compounds such as the fumonisins.

## Major Genera of Mycotoxigenic Fungi

Whether or not we accept that there are over 100,000 species of known fungi, the majority of the known toxigenic species fall into three recognized genera. These genera are *Aspergillus*, *Penicillium*, and *Fusarium*. Also, most of the known mycotoxins are elaborated by these genera.

The genus *Aspergillus* is within a large, very diverse family of fungi that are worldwide in distribution but primarily occupy subtropical and warm temperate climates. They generally are regarded as saprophytes that are important in nutrient cycling. Their growth at high temperatures and low water activity ( $a_w$ , ratio of the vapor pressure of the product to that of pure water) allows for their involvement in the colonization of a variety of crops, sometimes with limited parasitism especially under favorable conditions. Some of the most economically important toxigenic species of fungi belong to this genus.

Members of the genus *Penicillium* generally grow and can produce mycotoxins over a wider range of temperatures than those of the genus *Aspergillus* (Ominski et al. 1994). The *Penicillium* spp. are more abundant in temperate climates. Members of the genus are more commonly associated with storage than with preharvest contamination of grain (Sauer et al. 1992).

*Fusarium* is a large complex genus with species adapted to a wide range of habitats. They are worldwide in distribution and many are important plant pathogens. However, many species are soil borne and exist as saprophytes important in breaking down plant residues. A few species are significant mycotoxin producers, some of which are present preharvest in contaminated grains and other plants.

Two other mycotoxigenic genera that are important are *Claviceps* and *Stachybotrys*; both are of current and historical significance. Ergotism is one of the oldest known mycotoxicoses and is the result of humans and other animals consuming grain contaminated with the sclerotia of *Claviceps* spp. The ergots, hardened masses of fungal tissue, contain toxic alkaloids produced by selected members of this genus.

*Stachybotrys* is a genus in which there are certain saprophytic species that are highly cellulolytic and produce toxins contained in the conidia and other particulates that can become airborne resulting in illnesses likely through either the respiratory or digestive route of exposure. Illnesses have been described in animals and humans resulting from consumption of contaminated grains or their products, straw, or other plant materials that have become moist enough to support the growth and toxin production of this organism.

Although there are other genera of fungi that produce mycotoxins, these listed above are the most predominant and other genera will be discussed as the need arises later in this publication.

## Preharvest and Postharvest Occurrence of Mycotoxins

Mycotoxin contamination often is an additive process, beginning in the field and increasing during harvest, drying, and storage (Wilson and Abramson 1992). Christensen (1974) divided fungi that colonize grain into two groups, field fungi and storage fungi. Storage fungi were those that could grow at moisture contents in equilibrium with relative humidities of 70 to 90% where no free water was present.

In this classification, *Alternaria*, *Cladosporium*, *Fusarium*, and *Helminthosporium* were classified as field fungi; *Aspergillus* and *Penicillium* were classified as storage fungi. This classification was based on studies done in temperate climates. However, under warm, humid subtropical or tropical climates — or even in temperate climates in which the growing season is unusually hot and dry — species of *Aspergillus* and *Penicillium* can infect seeds in the field (Wilson and Abramson 1992). Perhaps the best example of a species that can infect seeds both in the field and in storage is *Aspergillus flavus*. In temperate climates, the fungus is predominately a storage fungus, but in the southern United States, corn is more likely to be colonized preharvest than in storage. Many species of *Fusarium*, as well as some species of *Penicillium*, also infect grain in the field as well as in storage.

Environmental conditions are extremely important in preharvest mycotoxin contamination of grain and oilseed crops. Plant growth and health, and the competitiveness of mycotoxigenic fungi, are greatly affected by temperature and moisture. Because of the diversity of mycotoxigenic fungi and the hosts that they colonize, no one set of conditions can be defined as

conducive for mycotoxin contamination. Aflatoxin contamination is favored in years with above average temperature and below average rainfall. Head scab of wheat, on the other hand, is favored by warm and wet spring conditions. Damage from insects and birds contributes to mycotoxin contamination in the field.

Several factors influence mycotoxin contamination of stored grain, including water activity, substrate aeration and temperature, inoculum concentrations, microbial interactions, mechanical damage, and insect infestation (Ominski et al. 1994). Temperature and the distribution and availability of water are the most important factors determining the colonization of stored grain by fungi (Wilson and Abramson 1992). Grain moisture may be expressed as percent moisture, or as  $a_w$ . *Water activity* is defined as the ratio of the vapor pressure of the product to that of pure water. Substrates with low water activity have little water available to support growth of fungi. Water activity roughly correlates to the equilibrium relative humidity in stored products (Wilson and Payne 1994).

An ecological succession of fungi often occurs as the grain's  $a_w$  and temperature change because mycotox-

igenic fungi grow within fairly strict  $a_w$  limits. Few fungi grow at  $a_w$  values below 0.70. As the content of water in the substrate increases above 0.70, the more xerophytic fungi are able to grow. The production of water by metabolic processes of these xerophytic fungi leads to a wetter substrate and one that can support growth of a broader range of fungal species. Therefore, in many cases, seeds may be contaminated with more than one fungus. For example, growth of *A. restrictus* begins at  $a_w$  of 0.70 or slightly higher, the *A. glaucus* group begins at an  $a_w$  of 0.80 to 0.85, and many *Penicillium* and *Aspergillus* species begin to grow at  $a_w$  above 0.85 (Wilson and Payne 1994). Often, mycotoxigenic fungi will invade the germ or embryo of seeds with little to no invasion of the adjacent endosperm (Payne 1998; Sauer et al. 1992).

Inoculum of postharvest fungi may come from a number of sources. Many mycotoxigenic fungi are common on a variety of plant materials, often growing saprophytically, and grain may be contaminated with these fungi through contact with soil or plant debris. Moreover, inoculum from seeds infected in the field can spread to other kernels in storage.

# 3 Fungal Growth and Mycotoxin Development by Major Mycotoxigenic Fungi

## Summary

Mycotoxins are produced by a diverse number of fungal species. In general, these fungi are not aggressive pathogens, but some species can invade and colonize plant tissue before harvest and in storage. The two primary factors influencing growth and mycotoxin production, both preharvest and postharvest, are temperature and moisture. These two factors have direct effects on the ecology and pathogenicity of the fungus and are often important in predisposing the plant to infection. While the most common exposure to mycotoxins is from the consumption of plant seeds, there are exceptions. *Neotyphodium* spp. establish an endophytic relationship with the plant and may produce indole alkaloids in all above-ground tissue. *Stachybotrys*, a fungus that has re-emerged as a health concern, is associated with damp building material in houses. Exposure to mycotoxins produced by this fungus presumably comes from inhalation of *Stachybotrys* conidia. It is not uncommon for a substrate to be contaminated with more than one fungus at the same time or for fungi to colonize a substrate in succession as the conditions favor one species over another.

No absolute controls are available for eliminating mycotoxins. Also, it is difficult to make general statements about control strategies because of the diversity of fungi producing mycotoxins, both pre- and postharvest, in a wide variety of crop species. Preharvest control strategies are based on use of good agronomic practices and resistant varieties. Harvesting and storage techniques are important in decreasing mycotoxin contamination of crops as well.

## Introduction

It is not possible to describe a single set of conditions that is favorable for the growth and production of mycotoxins by fungi. Mycotoxins are produced by a diverse group of fungi that differ in their morphology, biochemistry, and ecological niches. Even a single fungal genus, e.g. *Aspergillus*, contains species that differ greatly in their optimum temperature for

growth and their parasitic abilities.

In general, mycotoxigenic fungi are not aggressive pathogens but often are well adapted to growing on substrates with low moisture, and they can readily colonize improperly stored grain. Species from a number of genera, however, can colonize and produce mycotoxins in developing plant tissue.

This section focuses on species from six genera, *Aspergillus*, *Fusarium*, *Penicillium*, *Claviceps*, *Stachybotrys*, and *Neotyphodium* (sexual stage, *Epichloe*). These genera were chosen because they contain fungal species that produce mycotoxins of major concern and because they are representative of mycotoxigenic fungi that inhabit diverse ecological niches. The genera *Aspergillus*, *Fusarium*, and *Penicillium* represent the largest number of mycotoxigenic fungi and are often found on stored grain. Certain species of *Fusarium* and *Aspergillus* also can attack grain in the field. In contrast, infection by *Claviceps* spp. and *Neotyphodium* spp. occurs only in the field. *Neotyphodium* spp. also differ from other mycotoxigenic fungi in that they are endophytes and colonize vegetative tissue as well as reproductive tissue. *Stachybotrys* spp. represent yet another type of mycotoxin-producing fungus. Unlike most mycotoxin-producing fungi, *Stachybotrys* rarely colonizes grain but is of concern because it colonizes wet building material, particularly in houses. The fungus also differs because animals and humans apparently are exposed to the mycotoxin it produces by inhalation of mycotoxin-laden conidia rather than by ingestion.

It is difficult to make general statements about control strategies for mycotoxins that will apply universally because mycotoxins are produced pre- and postharvest by a diverse group of fungi on numerous crop species. Presently, no absolute controls are available for eliminating mycotoxin contamination. Mycotoxin occurrence in the field can be decreased by good agronomic practices and planting resistant varieties. Although heritable resistance to accumulation of some mycotoxins has been identified, adequate resistance is not yet available in commercial genotypes.

Harvesting and storage techniques also can be used to control the occurrence of mycotoxins in crops. The

two overriding factors that influence mycotoxin formation in storage are moisture or  $a_w$  and temperature. Other procedures such as cleaning, insect control, use of antifungal agents, and maintaining the integrity of the seed coat are important in decreasing or keeping mycotoxin levels to a minimum.

## *Aspergillus* Infection and Mycotoxin Development

The genus *Aspergillus* represents a large family of fungi that occupies very diverse ecological niches. Although members are distributed worldwide, *Aspergillus* spp. appear most abundant between latitudes 26° to 35° north or south of the equator (Klich et al. 1994). Thus, these fungi are more common in subtropical and warm temperate climates. Generally regarded as saprophytes, *Aspergillus* spp. grow on a large number of substrates and are very important in nutrient cycling. Their ability to thrive in high temperatures and with relatively low  $a_w$  makes them well suited to colonize a number of grain and nut crops. Under favorable conditions, some species have limited parasitic abilities and can colonize crops in the field.

Within this genus are some of the most important fermentation fungi, e.g., *A. niger*, *A. sojae*, *A. oryzae*, cultured for their ability to produce industrial enzymes and metabolites and to impart flavor to foods. Other members, however, are notorious for the mycotoxins they produce. Mycotoxins associated with *Aspergillus* species include aflatoxins, ochratoxins, versicolorins, sterigmatocystin, gliotoxin, citrinin, CPA, patulin, citreoviridin, and tremorgenic mycotoxins.

### *Aspergillus flavus*

Aflatoxins represent the major class of mycotoxins produced by *Aspergillus* spp. Only four species of fungi are known to produce aflatoxins and each belongs to *Aspergillus* section *Flavi* (Ito et al. 2001; Kurtzman et al. 1987; Payne 1998). These species are *A. flavus*, *A. parasiticus*, *A. nomius*, and *A. pseudotamarii*. Of these four species, only *A. flavus* and *A. parasiticus* are economically important. These two fungi have overlapping niches and can produce aflatoxin in developing seeds of corn, peanut, cotton, almond, and pistachio. Other tree nuts such as walnuts and Brazil nuts also are affected. Figs also may be infected, but the incidence is low. These fungi also can produce aflatoxin on most any poorly stored substrate. *As-*

*pergillus flavus* is the predominant species on all commodities (Payne 1992, 1998), although *A. parasiticus* is common on peanuts (Horn et al. 1994). Because these two species are similar, *A. flavus* will be used to refer to either *A. flavus* or *A. parasiticus*.

*Aspergillus flavus* was reported to cause an ear mold of corn as early as 1920 (Taubenhaus 1920), but the fungus was of little concern until the 1960s when it was shown to produce the factor (later identified as aflatoxin) associated with Turkey X disease. The significance of preharvest infection of corn by *A. flavus* (Barnsetetter 1927; Butler 1947; Eddins 1930) was largely discounted prior to 1971 because aflatoxin contamination was considered to be a storage problem only. The report of aflatoxin contamination in the southern and midwestern United States in the 1970s (Anderson et al. 1975; Lillehoj et al. 1975; Payne 1998; Rambo et al. 1974; Widstrom 1996) awakened the research community to the significance of preharvest contamination.

The occurrence of aflatoxin contamination is sporadic and highly dependent on environmental conditions. Large populations of *A. flavus* and aflatoxin contamination occur each year somewhere in the southern United States, but serious outbreaks are associated with above-average temperature and below-average rainfall (Payne 1998; Widstrom 1996). These two environmental conditions were associated with a high incidence of aflatoxin contamination in the U.S. corn belt in 1983 and 1988 (Hurburgh 1991). A high incidence of aflatoxin has been reported also in southern China, southeast Asia, and Africa (Hall and Wild 1994).

The infection process of *A. flavus* is best characterized in corn (Payne 1992, 1998). *Aspergillus flavus* is a soil-inhabiting fungus that reproduces by asexual conidia. Conidia carried to the corn silks by wind or insects can grow into the ear shortly after pollination and colonize kernel surfaces (Payne 1998; Widstrom 1996). If environmental conditions are favorable, the fungus may directly invade the seeds and cobs or it may enter through wounds created by insects. In either case, significant infection and aflatoxin contamination do not occur until the kernel moisture is below 32% (Payne 1998). Aflatoxin can continue to be produced in kernels until the moisture reaches 15% (Payne et al. 1988). Although insects are not required for aflatoxin contamination, their presence increases the level of contamination and high levels of aflatoxin are almost always associated with insect injury, especially by the European corn borer, *Ostrinia nubilalis* (Widstrom 1996).

There is evidence that peanut flowers also may be

infected with *A. flavus* (Griffin and Garren 1974), although this route of infection appears minor compared to infection of the pods (Cole et al. 1986). The exact route of infection into pods is not known but insects appear to play a major role. Both mites and lesser stalk borer larvae (*Elsomopalpus lingosellus*) are known vectors of the fungus. Even microscopic damage to the pods increases infection by the fungus (Lynch and Wilson 1991).

Even though there is evidence for direct infection of cotton by *A. flavus* (Klich and Chmielewski 1985; Klich et al. 1984), insects always are associated with high levels of aflatoxin in the field. Exit holes made by the pink boll worm larvae (*Pectinophora gossypiella* Saunders) appear to be the entry point for the fungus (Ashworth et al. 1971).

Infection of pistachios by *A. flavus* is associated with *early splits*, a condition in which the hull splits before the nut is mature (Doster and Michailides 1994a, b). In both pistachios and almonds, high aflatoxin contamination is associated with damage by the navel orange worm larvae (Doster and Michailides 1994a, b; Thomson and Mehdy 1978).

The two overriding conditions that influence aflatoxin contamination are temperature and moisture (Payne et al. 1998; Widstrom 1996). In corn and peanuts, high temperatures and drought stress lead to high levels of aflatoxin contamination (Payne 1998). Under field conditions where soil moisture and temperature were controlled, Cole et al. (1995) showed that neither by itself is sufficient. The researchers found that peanuts grown with adequate moisture had no aflatoxin. Similarly, peanuts grown under prolonged drought with temperatures less than 25°C or greater than 32°C were free of aflatoxin. Colonization by *A. flavus* and aflatoxin contamination were maximized at 30.5°C.

High temperature and drought conditions increase the airborne inoculum of the fungus (Jones et al. 1981; McGee et al. 1996). The increased growth and reproduction of the fungus at higher temperatures is presumably related to its relatively high optimum growth temperature. The fungus can grow over a wide range of temperatures (12 to 48°C), but its optimum for growth is 37°C (Klich et al. 1994). The higher temperatures and drought conditions also may favor *A. flavus* over other fungi because of its ability to grow on substrates with low water activity. The fungus can grow at an  $a_w$  as low as -35 megapascals (MPa) (Klich et al. 1994). Interestingly, the optimum temperature for aflatoxin production is 25 to 30°C (Ashworth et al. 1969a; Maggon et al. 1977). Temperature and drought stress also likely predispose the plant to in-

creased infection; however, little is known about the mechanisms.

The effect of temperature on aflatoxin contamination of cottonseed seems more complex and poorly understood (Payne 1998). Although aflatoxin contamination of cottonseed is rarely a problem in the southern United States, it can be a serious problem in western-grown cotton. Ashworth et al. (1969b) have argued that high night temperatures are important. High day and night temperatures also have been associated with higher aflatoxin levels in almonds (Doster and Michailides 1995).

The source of inoculum for *A. flavus* is the soil but the predominant survival structure is not known. The fungus produces sclerotia in culture and in cornfields in the southern United States (Wicklowsky et al. 1984; Zummo and Scott 1990); however, sclerotia have not been reported in the Midwest. The fungus probably survives as mycelium and, to some extent, as conidia and sclerotia (Payne 1998). Soil temperature (McGee et al. 1996) and moisture (Jones et al. 1981) greatly influence the number of conidia in the soil and the air.

### Preharvest Control Strategies

Any management practice to maximize plant performance and decrease plant stress will decrease aflatoxin contamination (Cole et al. 1995; Jones et al. 1981; Michailides 1996; Payne 1998; Widstrom 1996). This includes planting adapted varieties, proper fertilization, weed control, and necessary irrigation. Even the best management strategies will not eliminate aflatoxin contamination in years favorable for disease development. Previous cropping history has been shown to influence soil populations of the fungus (Horn et al. 1995) but the importance of initial inoculum has not been established. Further, *Aspergillus* molds grow on litter from pistachio trees but it is not known if practices to decrease this litter would decrease infection and aflatoxin contamination (Doster and Michailides 1994a, b). Decreasing damage by the navel orange worm can decrease aflatoxin contamination. Compared to plant stress, cropping history and plant debris likely play a minor role in aflatoxin contamination.

Breeding programs are underway for all major crops affected by aflatoxin contamination (Brown et al. 1998); however, no genotypes are available commercially with adequate resistance to aflatoxin accumulation. Inbred lines of corn (Brown et al. 1998; Campbell and White 1995a; Huang et al. 1997; Widstrom 1996) and genotypes of peanut (Holbrook et al. 1997; Waliyar et al. 1994) with some resistance to

aflatoxin accumulation have been identified. Evidence exists for resistance in corn (Campbell and White 1995b; Huang et al. 1997) and peanuts (Waliyar et al. 1994) to aflatoxin production per se. Breeding for resistance to aflatoxin contamination also is underway in almonds (Gradziel and Dandekar 1997).

Because chemical control procedures for mycotoxin contamination are not economically feasible for most grain crops, interest exists for developing effective biocontrol agents to decrease mycotoxin contamination. Recent research indicates the potential for a biocontrol agent to decrease aflatoxin contamination of cotton (Cotty 1994; Cotty and Sobek 1997), peanuts (Dorner et al. 1992), and corn (Dorner et al. 1997). Researchers have treated these crops with nonaflatoxigenic isolates of *A. flavus* or *A. parasiticus*. Because they occupy the same or similar ecological niche as the aflatoxigenic strains, the rationale for using nonaflatoxigenic isolates of the two fungi is that they are likely the best biocompetitors. So far, there is no evidence that the ability to produce aflatoxin confers a competitive advantage to *A. flavus* or *A. parasiticus*.

The most extensive testing of a biocontrol agent for prevention of aflatoxin contamination has been performed by Bock and Cotty (1999). They obtained a permit from the U.S. Environmental Protection Agency (EPA) to treat fields of cotton in Arizona with wheat seed colonized by a naturally occurring nonaflatoxigenic strain (AF36) of *A. flavus*. This treatment has increased the population of AF36 and decreased that of the toxigenic strains and the concentrations of aflatoxin in the cottonseed.

### *Aspergillus ochraceus* and Other *Aspergillus* Species

In addition to the *A. flavus* group, a number of other *Aspergillus* spp. can be found on commodities before harvest or, most commonly, in storage. One of the more common *Aspergillus* species is *A. ochraceus*. Recently, this species has been renamed as *A. alutaceus* var. *alutaceus* Berkley and Curtis 1875 (Kozakiewicz 1989), and both names appear in the literature. This fungus is best known for its ability to produce ochratoxin, and *A. ochraceus* and *Penicillium verrucosum* are the major producers of ochratoxin. However, other *Aspergillus* and *Penicillium* spp. also produce ochratoxin (Samson 2001; Pier and Richard 1992). The *Penicillium* spp. are probably a more important source of ochratoxin contamination of grain than the *Aspergillus* spp. Controversy exists over

which species of *Penicillium* is most important (Scott 1994). Ochratoxins are considered a potential cause of the human disease, Balkan endemic nephropathy (Krogh 1977; Smith and Moss 1985).

In Canada, the predominant mycotoxin produced by *Aspergillus* spp. is sterigmatocystin (Scott 1994). It is associated with grain contaminated with *A. versicolor* and *Eurotium* spp. In warmer climates, grain may be contaminated preharvest with *A. fumigatus*, *A. nidulans*, *A. sydowii*, *A. terreus*, and *A. versicolor*, species more commonly associated with storage (Lacey et al. 1991).

Control strategies for *A. ochraceus* and other *Aspergillus* species are covered in the harvest and storage section later in this chapter.

## *Fusarium* Infection and Mycotoxin Development

### *Fusarium*

*Fusarium* is a large and complex genus with species that are adapted to a wide range of habitats throughout the world (Summerell et al. 2001). *Fusarium* populations in agricultural field soils can be very high and include saprophytes that break down plant residues in the soil as well as pathogens that can cause rots, wilts, and other diseases. Because of its importance as a plant pathogen, a great deal is known about *Fusarium*. Present-day nomenclature has developed largely as a working tool of plant pathologists and others seeking usable, practical systems for correctly identifying *Fusarium* species. Practical identification has been based largely on morphological characters, such as colony structure and color and spore size and shape of strains of these fungi grown under carefully controlled conditions in the laboratory. But because some species are morphologically very similar, misidentifications occur, especially by those with limited experience in fungal identification (Burgess et al. 1998; Marasas et al. 1984b; Nelson et al. 1983). Recent advances in identifying and classifying *Fusarium* species include biochemical and genetic markers and vegetative and sexual compatibility among strains (Leslie 1995; O'Donnell and Cigelnik 1997).

Although there are dozens of *Fusarium* species, a rather limited number are responsible for most mycotoxin contamination of the food and feed supply (Marasas et al. 1984b). The *Fusarium* species of most concern are those that produce mycotoxins in wheat,



corn, rice, barley, oats, and other cereal grains that are human dietary staples and animal feed ingredients (Campbell et al. 2000). Many *Fusarium* species can infect heads of wheat and other small grain cereals under field conditions causing head scab or blight (Sutton 1982). In most regions of the world, *F. graminearum* (the sexual stage is called *Gibberella zeae*) is the major causal agent of head scab of small grains, including wheat, barley, rye, and oats (Table 3.1). Other species, e.g., *F. avenaceum*, *F. culmorum*, *F. poae*, *F. sporotrichioides*, can predominate in small grains in some regions and environmental conditions. *Fusarium graminearum* is the world's major causal agent of red ear rot of corn. *Fusarium graminearum* appears as a pink to red mold of the kernels and, in severe infections, of the cob, husks, and shank. Although *F. graminearum*, *F. fujikuroi*, and related species can cause ear blights of rice, the grain within the ears is not usually infected.

Pink and white ear rots of corn can be caused by a group of closely related *Fusarium* species, predominately *F. verticillioides* (syn., *moniliforme*), *F. proliferatum*, and *F. subglutinans* (Table 3.1). *Gibberella fujikuroi* is the teleomorph for these three anamorph species.

On suitable culture media, *F. graminearum* rapidly produces profuse mycelial growth, which usually becomes tinged with yellow or pink as the culture ages. The undersurface of the culture is often a brilliant carmine red. This species can be distinguished also by the long, straight, multicelled spores (macroconidia) produced in the mycelium. Many strains of *F. graminearum* are self-fertile and produce blue-black fruiting bodies (perithecia) of the *Gibberella* sexual stage on the surface of culture media and on infected plant tissues. Although cultures of *F. culmorum* and *F. crookwellense* may also exhibit *F. graminearum* in rapid mycelial growth and a red undersurface, only *F. graminearum* is sexually self-fertile. These *G. zeae* perithecia contain large numbers of sexual spores (ascospores), which are an important inoculum source in epidemics of wheat head scab. *Fusarium graminearum* produces trichothecenes, mainly DON and its acetylated derivatives, and zearalenone (Marasas et al. 1984b; Nelson et al. 1983). Strains of *F. graminearum* can be distinguished using deoxyribonucleic acid (DNA) markers (Ouellett and Seifert 1993).

Epidemics of wheat and barley head scab, also known as head blight, can occur worldwide wherever rainy or humid weather coincides with host flowering and early kernel-fill stages (Abramson 1998). In the United States, wheat head scab was first de-

scribed in the 1890s in Delaware, Indiana, and Ohio. Throughout the twentieth century, head scab epidemics occurred sporadically in most wheat- and barley-growing regions of the United States and Canada (Sutton 1982). From 1991 through 1997, head scab was a serious problem for wheat and barley growers in the eastern and midwestern United States and in the provinces of Ontario and Manitoba. For example, wheat and barley growers in the Red River Valley region of Minnesota, North Dakota, South Dakota, and Manitoba lost an estimated \$1 billion to scab in 1993 alone (McMullen et al. 1997). Recent epidemics in North America have been attributed to increases in conservation tillage practices and to cropping systems in which corn and wheat are rotated or wheat is grown each year (McMullen et al. 1997). Wheat scab affects more than 7 million hectares in the People's Republic of China, with yield losses of 20 to 40% in severe epidemics (Bai and Shaner 1994). All European wheat- and barley-growing regions are affected by scab, with recent severe epidemics in southern Russia, Romania, and Hungary during the 1990s. Scab incidence and severity also have increased in Argentina and Uruguay, with yield losses reaching 50% in some regions in 1993 (Dubin et al. 1997; Parry et al. 1995).

Wheat and barley head scab have been thoroughly studied in the greenhouse and other controlled environments as well as in the field. Premature loss of green color of the spikelets in the head is the first symptom of the disease and, under persistent wet conditions, bleached spikelets can be visible a few days after infection. This mixture of bright green and pale yellow spikelets makes infected heads easy to detect in the field. Infection of a spikelet during the early flowering stages can prevent kernel development. Spikelet infection during kernel fill yields kernels with various degrees of fungal growth, ranging from chalky "tombstone" kernels to kernels with varying degrees of shriveling and discoloration. If humid weather persists, *F. graminearum* can infect the entire head and produce blue-black perithecia and a pinkish mass of mycelium and macroconidia on the surface of the head. This pinkish crust on the grain's surface is the source of the name *scab* for this disease (Arthur 1891).

The major scab pathogen, *F. graminearum*, is a widespread colonizer of crop residues, where the pathogen survives during the winter. Wheat stubble and corn stalks are major inoculum sources in North America, while rice stubble is the major source of inoculum in the People's Republic of China (Bai and Shaner 1994). As temperatures increase in the

Table 3.1. Major mycotoxigenic *Fusarium* species in grain and mycotoxins produced

Host	Region	Fumonisin Trichothecenes Zearalenone	verticillioides	fujikuroi	proliferatum	subglutinans	poae	sporotrichioides	acuminatum	graminearum	culmorum	crookwellense	avenaceum	equiseti
			+	+	+	+	+	+	+	+	+	+	+	+
Wheat and small grains	Africa													
	Asia		+											
	Australia													
	Canada													
	Europe													
	Mexico													
	South America													
	United States													
Maize	Africa		+		+									
	Asia		+		+									
	Australia		+											
	Canada		+		+	+	+	+	+	+			+	+
	Europe		+		+	+	+	+	+	+	+	+	+	+
	Mexico		+			+								
	South America		+		+	+				+				+
	United States		+		+	+	+	+	+	+		+	+	+
Rice	Asia		+	+										
	United States			+										

spring, large numbers of perithecia form on the surface of infested residues. Spore trapping indicates that, at the time of wheat flowering, the majority of airborne *Fusarium* spores are *F. graminearum* ascospores, which can easily be distinguished by their curved shape and four-cell structure from the longer, straighter macroconidia. Ascospore release peaks each night, correlated with a rise in relative humidity (Paulitz 1996). Airborne release of ascospores from perithecia peaks during and after rainy periods. Recent scab epidemics in the midwestern United States have been associated with above-average July rainfall, while wheat heads are in flower (McMullen et al. 1997).

### Ear Rot of Corn Caused by *Fusarium graminearum*

*Fusarium graminearum* has a broad host range and can cause an ear disease of corn often called red ear rot or *Gibberella* ear rot. Red ear rot has been reported in corn-growing areas worldwide but is especially prevalent in temperate climates when relatively

cool temperatures and wet weather coincide with silk emergence. *Fusarium graminearum* ear rot epidemics have occurred sporadically throughout the midwestern United States, e.g., in 1965 and 1972 in Indiana, in 1986 in Minnesota, and regionwide during the unusually cool, wet summers of 1992 and 1993 (Abbas et al. 1988a; Munkvold and Yang 1995; Park et al. 1996; Tuite et al. 1974). Localized epidemics of *F. graminearum* ear rot are common in Ontario and eastern provinces, with outbreaks reported in years with wetter summers, e.g., 1972, 1975, 1976, 1977, 1986, 1987, 1990 (Miller 1994; Neish et al. 1983; Sutton 1982; Vigier et al. 1997). Red ear rot also affects corn grown in northern Italy, eastern Europe and the former Soviet Union, the People's Republic of China, and central and southern Africa (Bottalico et al. 1989; Chu and Li 1994; Marasas et al. 1981; Maric 1981; Sutton 1982). In some epidemics associated with persistent cool, wet weather, *F. sporotrichioides* has been recovered from corn with ear rot symptoms (Neish et al. 1983; Park et al. 1996; Vigier et al. 1997).

*Fusarium graminearum* ear rot of corn is a more complex and challenging disease than wheat head scab. Controlled environmental studies have been

limited because mature corn plants are so large and because it has been difficult to consistently establish infection in the greenhouse. Natural epidemics are often localized and sporadic and thus difficult to predict. During early stages of symptom development, infected ears are not easily detected in farm fields because husks may show few symptoms. In severe infections, however, red or pink mold can progress throughout the entire ear, colonizing kernels, cobs, and husks. If wet weather persists, blue-black perithecia and a pinkish mass of mycelium and macroconidia can form on the husk surface.

There are two routes of entry for *F. graminearum* infection of corn. First, spores landing on the emerged silks can infect the ear by the silk channel. Controlled field inoculations into the silk channel indicate that corn ears are most susceptible to *F. graminearum* within 4 to 7 days after silk emergence (Reid et al. 1992). Second, wounds caused by birds, insects, or extreme weather can provide an opportunity for fungal invasion; such ear damage is positively correlated with ear rot (Sutton 1982; Vigier et al. 1997). Infection through wounding must occur before the kernels have hardened significantly and have ceased to be an attractive substrate for spore germination.

Ascospores and macroconidia produced by *F. graminearum* growing on corn stalks and other crop residues are the major inocula for both wheat head scab and corn ear rot. In addition, studies in Ontario have shown a positive correlation between *F. graminearum* ear rot and rainfall in July and August, when corn is silking (Vigier et al. 1997). Epidemics also appear to be associated with wet weather late in the growing season (Miller 1994).

Both deoxynivalenol and zearalenone can be produced by many strains of *F. graminearum* on autoclaved grains under laboratory conditions. However, the technical difficulty and expense of mycotoxin analysis have limited quantitative analysis of these mycotoxins in large-scale field experiments. Antibodies raised against DON and other analytical tests have become increasingly available, which should facilitate field analyses. Studies of wheat and corn infected with *F. graminearum* in the field indicate that wheat can have high levels of DON but usually has little or no zearalenone, whereas corn can be contaminated with both classes of mycotoxins. Corn cob tissue often contains the highest concentrations of DON in the ear (Reid et al. 1996). Similarly, the rachis of field-inoculated wheat often was found to contain the highest average DON concentration. DON levels in kernels of wheat and corn are highly correlated with disease severity ratings and with various measures of

fungal load, including colony-forming units and ergosterol levels (Atanassov et al. 1994; Bennett et al. 1988; Miller et al. 1983; Reid et al. 1996; Schaafsma et al. 1993; Trigo-Stockli et al. 1995). However, even visibly healthy grain can contain significant amounts of DON. In an Ontario study, 52% of normal-appearing corn kernels collected from an infected field contained 0.28 to 5 micrograms ( $\mu\text{g}$ )/gram (g) DON by ELISA (enzyme-linked immunosorbent assay) detection (Sinha and Savard 1997).

### Ear Rot of Corn Caused by Other *Fusarium* Species

Farm animal disease outbreaks at the end of the nineteenth century drew the attention of agricultural scientists in Nebraska, who determined that "moldy corn poisoning" was associated with consumption of corn ears, stalks, and fermented silage heavily contaminated with a fungus described as *F. moniliforme* (Sheldon 1904). The fungi originally described as *F. moniliforme* currently are considered to comprise at least six different biological species, three of which are now recognized as *F. verticillioides*, *F. proliferatum*, and *F. subglutinans* (Leslie 1995). To help clarify the taxonomy of this complex genus and to develop a common taxonomic nomenclature, *G. fujikuroi* was organized into eight mating populations (A-H) (Klittich et al. 1997; Leslie 1996, 2001). Mating population A is most often associated with corn, and this prolific fumonisin producer has been renamed from *F. moniliforme* to *F. verticillioides* (Nirenberg 1976). Interestingly, members of the D population, which includes *F. proliferatum*, also are able to produce significant amounts of fumonisins. In contrast to mating population A, members of mating population F (*Fusarium* anamorph *verticillioides*, syn., *moniliforme*) do not produce fumonisins (Leslie 1996) and primarily are limited to sorghum as a host.

In scientific literature and disease reports from 1904 through the 1970s, it is often unclear whether fungi referred to as *F. verticillioides* are actually *F. verticillioides*, *F. proliferatum*, or *F. subglutinans*. During this period, *F. verticillioides* was considered to be the most common pathogen affecting ears of corn (Kommedahl and Windels 1981) but it is likely that many of these observations involved all three species collectively. Several reports from the northern corn belt states and from Canada indicate that *F. subglutinans* can be more prevalent than *F. verticillioides* and that *F. proliferatum* is also very common (Abbas et al. 1988b; Bullerman and Tsai 1994; Munkvold and

Stahr 1994; Park et al. 1996; Vigier et al. 1997). Disease symptoms caused by these three species are indistinguishable and are commonly called *Fusarium* ear rot. Other *Fusarium* species are isolated infrequently from corn ears with ear rot symptoms.

*Fusarium verticillioides*, *F. proliferatum*, *F. subglutinans*, and related species produce white mycelial growth, which may become tinged with purple as the culture ages. The undersurface can vary from colorless to orange or dark purple. All these species are characterized by the production of copious amounts of one-celled spores (microconidia) as well as long, multicelled macroconidia in the mycelium (Nelson et al. 1983). Unfortunately, for the nonspecialist, some of these species can be difficult to distinguish based on colony color, spore size, and shape. *Fusarium verticillioides* and closely related species can usually form *Gibberella* sexual stages but, unlike *F. graminearum*, these species are not self-fertile. To obtain the sexual stage, strains must be interbred or mated with another strain of the same species. The ability of strains to interbreed and produce perithecia-containing ascospores is a useful method to identify species of these morphologically similar fungi (Leslie 1995). Biochemical and genetic markers are being developed for this challenging group of *Fusarium* (O'Donnell and Cigelink 1997). Current evidence indicates that trichothecenes and zearalenone are not produced by *F. verticillioides* and related species. Fumonisin can be produced by all of these species but, in general, strains of *F. subglutinans* are poor fumonisin producers and strains of *F. proliferatum* vary from low to high production. *Fusarium verticillioides*, a consistent, high-level fumonisin producer, is the predominant fumonisin-producing *Fusarium* species in corn grown in the United States and many other regions of the world (Munkvold and Desjardins 1997).

Throughout the twentieth century, *Fusarium* ear rot has occurred widely in the United States and is now the most common ear disease of corn in the corn belt states. *Fusarium verticillioides* infects corn worldwide; the distributions of *F. proliferatum* and *F. subglutinans* are less well documented but these species have been reported from temperate regions on every continent. *Fusarium* ear rot is common in Canada but fumonisin levels generally have been low in naturally infected corn (Vigier et al. 1997). High levels of *Fusarium* ear rot and fumonisins, the major mycotoxin produced by *F. verticillioides* and *F. proliferatum*, have been reported in corn in Europe, the People's Republic of China, central and southern Africa, and South America (Kommedahl and Windels 1981; Munkvold and Desjardins 1997; Shephard et al.

1996).

*Fusarium verticillioides*, *F. proliferatum*, and *F. subglutinans* survive in corn residue, which is probably the most important source of inoculum for kernel infection. Macroconidia and microconidia from residue are splashed or carried by wind to above-ground infection sites on the plants. It is not certain whether root infection is caused by conidia or by mycelial growth from infested residue. These *Fusarium* species can be associated with every part of the corn plant: roots, stalks, leaves, cobs, and kernels. From 50 to 100% incidence of kernel infection is not uncommon, with the majority of kernels showing no visible damage. When ear rot appears, kernels can be visibly moldy, darkened, chalky, blistered, cracked, or streaked with white. In severe infections, white or pinkish mycelia can progress throughout the entire ear, colonizing husks and gluing them to the surface of the ear.

Because these *Fusarium* species are ubiquitous in corn plants and colonize corn residues in the soil, it is difficult to establish uninfected plants for controlled experiments in the field or greenhouse. Using strain-tracking approaches, several infection pathways have been established for *F. verticillioides* and related species. These species can be common in corn seed and can be transmitted to seedlings. There are many additional infection pathways, including root, leaf, and stalk infection, some of which can lead to systemic infection. The contribution of systemically infecting strains to mycotoxin contamination in kernels is not clear. The most important pathways for kernel infection are silk infection by airborne or rain-splashed microconidia and macroconidia and kernel damage by insects or birds (Munkvold and Desjardins 1997; Munkvold et al. 1997a). Larvae of the European corn borer (*Ostrinia nubilalis*) have been shown to act as vectors of *F. verticillioides*, transmitting the fungus from corn leaves to kernels (Sobek and Munkvold 1999). Other insects may act as vectors (Farrar and Davis 1991; Kommedahl and Windels 1981). In recent field tests, both visible ear rot symptoms and symptomless kernel infection were decreased in corn genetically engineered for resistance to the European corn borer (Munkvold et al. 1997b).

The weather conditions that favor *Fusarium* ear rot are not well understood. The three species in question may differ in optimum temperatures and moisture conditions for infection. There is evidence that *F. subglutinans* is more prevalent than *F. verticillioides* under cooler conditions (Logrieco et al. 1993; Vigier et al. 1997). Some studies have associated severe ear rot with dry weather during June and July early

in the growing season, followed by wet weather during silking and later in the growing season (Koehler 1959; Munkvold and Desjardins 1997; Schaafsma et al. 1993; Vigier et al. 1997). Silks are most susceptible to infection during the first week of silking (Schaafsma et al. 1993) and moisture on the silks favors infection (Munkvold et al. 1997a).

Fumonisin can be produced by almost all strains of *F. verticillioides* (*G. fujikori* mating population A) and by many strains of *F. proliferatum* on autoclaved grains under laboratory conditions. Analyses of shelled kernels have found higher fumonisin levels in visibly moldy corn than in good-quality corn (Shepherd et al. 1996). Due to the technical difficulty and expense of fumonisin analysis, the distribution of fumonisins in individual ears of corn infected with *F. verticillioides* has received little study. In one recent study (Desjardins et al. 1998), distribution of fumonisin B<sub>1</sub> in symptomatic and symptomless kernels from 116 individual ears of corn was determined. Over a wide range of fumonisin levels, 82 to 100% of the fumonisin B<sub>1</sub> in each ear was localized in symptomatic kernels.

### Preharvest Control Strategies

The major source of inoculum for the *Fusarium* spp. is colonized plant debris. Large numbers of perithecia are produced by *F. graminearum* on infested residues. Ascospores released from these perithecia in the spring infect wheat at flowering. Recent epidemics of wheat and barley scab in North America have been attributed to increases in conservation tillage practices and to cropping systems in which corn is rotated with wheat or in which wheat is grown each year in the same field (McMullen et al. 1997). Thus, avoiding rotation of corn with wheat may afford some control for wheat and barley head scab.

*Fusarium verticillioides*, *F. proliferatum*, and *F. subglutinans* survive in corn residue, which is probably the most important source of inoculum for kernel infection. Tillage, however, has not been shown to decrease *Fusarium* ear rot intensity, possibly because these fungi can survive for 21 months or more in infested residue (Cotten and Munkvold 1998).

Dry weather during grain fill and late-season rains have been associated with fumonisin production (Munkvold and Desjardins 1997). Therefore irrigation to avoid water stress during critical periods for the plant may decrease the risks of fumonisin contamination.

For head scab of wheat and ear rot of corn, very few

sources of resistance have been found. Scab resistance breeding programs in China have identified wheat lines with moderately high resistance to the spread of *F. graminearum* within the head (Bai and Shaner 1994). Chinese wheat germplasm is being used for scab resistance breeding programs worldwide (Dubin et al. 1997). Corn breeding programs in Canada have identified lines with *F. graminearum* ear rot resistance expressed in silks and in kernels (Chungu et al. 1996; Reid et al. 1994). Corn breeding programs have made little effort to screen for germplasm highly resistant to *F. verticillioides*. Furthermore, screening based on visible ear rot symptoms may not reflect symptomless kernel infection with *F. verticillioides* or the potential for fumonisin accumulation (Munkvold and Desjardins 1997).

Enhanced breeding strategies are exploiting advances in understanding the biosynthesis of *Fusarium* mycotoxins and their interactions with plant hosts. Efforts are underway to express mycotoxin-detoxifying enzymes in plants, with the goal of preventing mycotoxin accumulation and ear rot symptoms. A gene encoding a fumonisin ester hydrolase cloned from a saprophytic microbe present in corn ears is being expressed for fumonisin detoxification (Duvick et al. 1994). A *F. graminearum* gene encoding a trichothecene 3-O-acetyltransferase significantly decreases toxicity of trichothecenes and has potential for expression in corn and wheat (Kimura et al. 1998).

Another genetic engineering approach is altering the ribosomal protein that is the trichothecene target binding site in plant tissues. Harris and Gleddie (2001) have modified a rice gene to produce a trichothecene-tolerant ribosomal protein. When transformed into tobacco, the modified rice gene provided transgenic cell cultures with tolerance to the trichothecene DON. Research is underway to express this gene in corn and wheat plants, to evaluate its effect on disease symptoms and trichothecene accumulation.

Plants genetically modified for enhanced insect resistance have shown decreased contamination with fumonisins. Munkvold et al. (1999) found that *Bacillus thuringiensis* (Bt)-transgenic corn kernels expressing CryIA(b) or Cry9C protein had less damage from European corn borer, and therefore less *Fusarium verticillioides* infection, and lower fumonisin contamination than nontransgenic corn. A significant decrease was found in each year of the three-year study. However, infection of corn by *F. verticillioides* seems complex. Although insect damage clearly increases contamination by fumonisin, there appear to be other modes of fungal entry (Munkvold et al.

1997b). Thus, fumonisins can occur in Bt corn as a result of infection that is not related to insect injury.

## *Penicillium* Infection and Mycotoxin Development

*Penicillium* species are more commonly associated with storage than with preharvest contamination of grain (Sauer et al. 1992). Preharvest contamination of corn can occur, with *P. funiculosum* and *P. oxalicum* being the most common in the midwestern United States; *P. purpurogenum*, *P. funiculosum*, and *P. citrinum*, the most common in the southeastern United States; and *P. chrysogenum*, *P. steckii*, and *P. purpurogenum*, the most common in Spain (Lacey et al. 1991). *Penicillium oxalicum* can cause lesions on husks and kernels and may cause ear rot. *Penicillium funiculosum* can cause streaking of the pericarp but usually does not affect seed viability (Lacey et al. 1991). *Penicillium* spp. can occasionally be found on small grains, with infection favored by prolonged wet weather or lodging of grain (Lacey et al. 1991).

Control strategies for *Penicillium* species will be discussed in the harvest and storage section later in this chapter.

## Ergot and Endophytic Fungal Infection and Mycotoxin Development

### *Claviceps* Species

*Claviceps* spp. produce a variety of alkaloids. Ergot alkaloids refer specifically to those with the clavine or ergoline ring system. These include lysergic acid, lysergic acid amide (precursor for the illegal narcotic, lysergic acid diethylamide [LSD]), and ergopeptines such as ergotamine.

In addition to ergot alkaloids, some *Claviceps* spp. produce tremorgenic alkaloids of the indole diterpene class. The malady, paspalum staggers, is attributable to *Claviceps paspali* sclerotia on florets of *Paspalum* spp. grasses (Cole et al. 1977; Dorner et al. 1984). This species also produces ergot alkaloids, so that animals grazing on *Paspalum* that is seeding or in flower may suffer the combined effects of both toxic alkaloid classes.

Outbreaks of *Claviceps africanus* and/or *C. sorghi* in North America (Bandyopadhyay et al. 1990; Frederickson and Leuschner 1997) and Australia (Blakey

et al. 2000a, b) have become a major concern. Toxicoses in pigs and cattle resulting from the ingestion of contaminated feed have been reported (Blaney et al. 2000a, b).

Ergotism is one of the oldest known mycotoxicoses, and is the result of consuming grain contaminated with sclerotia of *Claviceps* spp. The *Claviceps* spp. are “replacement parasites” in that they replace the developing plant structures with fungal structures called ergots, or *sclerotia* (Tudzynski et al. 1995).

The early stage of infection involves production of honeydew, a sweet liquid suspension of asexual spores that are transferred by insects from floret to floret. The growing mycelium infects grass florets very close to the time of pollination, grows into the ovary, and surrounds (largely killing) the female structures of the florets. Fungal growth continues until the fungus produces a resting structure termed a sclerotium, or ergot. Ergots fall from the plant, overwinter in the ground, and then fruit sexually the following spring when grass hosts flower. Ergots give rise to ascospores, which are forcefully ejected so that they land on stigma (female part of the flower) of grass florets. These ascospores give rise to hyphae that invade the ovary, thus completing the infection cycle.

### *Epichloe* and *Neotyphodium* Species

Toxic alkaloids are also produced by group of fungi that establish an endophytic relationship with the host. Many cool-season grasses possess endophytic symbionts of the fungal genera *Neotyphodium* (if asexual) and *Epichloe* (if sexual) (Schardl and Phillips 1997). *Neotyphodium* was previously known as *Acremonium* until the name was changed in 1996–1997. *Neotyphodium* spp. and *Epichloe* spp. colonize most of the aerial tissues of the host plant, the floral meristems, the ovule, the embryo, and continue infection of new seedlings.

*Neotyphodium* and *Epichloe* species produce a diverse array of alkaloids, some with antimammalian activity and some with little or no antimammalian activity (Bush et al. 1997). All of these alkaloid classes are active against insects. The important antimammalian alkaloids are the ergot alkaloids and indole diterpenes. These alkaloid classes also are present in sclerotia produced by *Claviceps purpurea* and *C. fusiformis* (in the case of ergot alkaloids) as well as *C. paspali* (which has both ergot and indole diterpene alkaloids). Furthermore, the endophytes vary similarly to *Claviceps* spp. in the levels and chemical structures of ergot alkaloids and indole diterpenes expressed. Some grass-endophyte

associations accumulate ergopeptines, e.g., ergovaline, to levels that can cause symptoms to grazing livestock. In these instances, the amount of infected plant material that must be ingested to receive a toxic dose is much higher than for *Claviceps* spp. because the endophyte biomass is relatively low and the levels of ergot alkaloids in infected plants are much lower than in *Claviceps*-infected seed heads.

*Neotyphodium* and *Epichloe* species producing various levels of antiherbivore alkaloids are known from many cool-season grasses (Table 3.2). Reports of associated toxic effects are rare. Episodes of staggers-like toxicoses have been reported from the native New Zealand grass, *Echinopogon ovatus*, which was recently shown to possess a *Neotyphodium* spp. endophyte (Miles et al. 1998). The stupefying effects of lysergic acid amide and related alkaloids have been reported in horses grazing *Stipa robusta* in the southwestern United States (Petroski et al. 1992) and a related grass *Achnatherum inebrians* in northwestern China (Miles et al. 1996).

In the United States, most of the widely grown forage grass, tall fescue (*Festuca arundinacea*), possesses endophytic *Neotyphodium coenophialum*. This endophyte produces ergovaline and is implicated in episodic ergotism-like toxicosis of animals grazed on this grass (Lyons et al. 1986). Estimated losses to the U.S. beef cattle industry in 1990 were \$600 billion and substantial losses (undetermined) undoubtedly occur in the dairy and equine industries as well (Hoveland 1993).

Some endophytes produce sufficiently high levels of indole diterpenes, e.g., lolitrem B, to cause tremors in grazing livestock (Lacey 1991; Raisbeck et al. 1991; Rowan 1993). An example of this malady is ryegrass

staggers, suffered by livestock that graze perennial ryegrass (*Lolium perenne*) bearing the endophyte *Neotyphodium lolii*. The malady is usually not considered lethal. Case reports from South America, however, suggest an association of staggers and lethal toxicosis with certain endophyte-infected grasses.

Notably, not all endophytes of grasses produce potent antimammalian toxins. Many produce only loline alkaloids (saturated 1-aminopyrrolizidines), peramine (a pyrrolopyrazine alkaloid), or both. These two alkaloid classes exhibit anti-insectan activity but little or no antimammalian activity at physiological concentrations (Bush et al. 1997). The protection afforded by these anti-insectan alkaloids — as well as endophyte enhancements of drought tolerance, growth, nematode resistance, and resistance to some fungal diseases — makes endophyte infections of grasses desirable in many circumstances, including pasture and forage.

The *Neotyphodium* spp. have a simple life cycle that involves the colonization of most of the aerial tissues of the host plant, the floral meristems, the ovule, the embryo, and continued infection of new seedlings. In this manner, *Neotyphodium* spp. are transmitted vertically through the maternal lineages of infected grass plants. Typically, such seed transmission is nearly 100% efficient, meaning that virtually all seeds set by an endophyte-infected mother plant give rise to new infected plants in the next generation.

*Epichloe* spp. often exhibit the same life cycle of seed transmission as their *Neotyphodium* spp. relatives. However, the *Epichloe* spp. also can be transmitted horizontally by spores arising on infected plants to the florets of uninfected plants nearby (Chung and Schardl 1997; Schardl and Phillips 1997).

Table 3.2. Values reported for some endophyte-grass associations<sup>a</sup>

Host grass	Symbiont	Alkaloids ( $\mu\text{g g}^{-1}$ dry wt)			
		EV	SAP	LM	PM
<i>Lolium perenne</i>	<i>Epichloë typhina</i>	0	0	0	53
<i>Festuca gigantea</i>	<i>E. festucae</i>	0	300	0	4
<i>Festuca longifolia</i>	<i>E. festucae</i>	0.9	0	4.0	22
<i>Festuca rubra</i>	<i>E. festucae</i>	1.2	0	0	0
<i>Achnatherum inebrians</i>	<i>Neotyphodium</i> spp.	2,900	ND	ND	ND
<i>Echinopogon ovatus</i>	<i>Neotyphodium</i> spp.	0	1,060	0	0
<i>Festuca arundinacea</i>	<i>N. coenophialum</i>	0.5	1,100	0	2
<i>Festuca pratensis</i>	<i>N. uncinatum</i>	0	5,600	0	0
<i>L. perenne</i>	<i>N. lolii</i>	1.3	0	4.7	19

<sup>a</sup>Abbreviations: EV = ergovaline, SAP = saturated 1-amino-pyrrolizidines (lolines), LM = lolitrems, PM = peramine, ND = not determined. Data are from Miles et al. (1996, 1998) and Bush et al. (1997).

This process is coordinated with host flowering. In an infected plant, some or all of the flowering tillers may have fungal proliferation around the leaf sheaths surrounding the immature inflorescence. The proliferating fungal structure is termed a *stroma* and produces abundant spores of the type (conidiospores) that can serve as spermatia. A symbiotic fly, *Botanophila* spp. (formerly *Phorbia phrenione*), transfers spermatia between stromata, causing fertilization of each mating type with its opposite and thus initiating ascospore development. The ascospores are forcibly ejected, land on open florets of neighboring plants, and initiate new infections of the developing seeds. Those seeds then give rise to infected plants (Chung and Schardl 1997).

## Preharvest Control Strategies

### Ergot

Ergot poisoning is an ever present danger in livestock agriculture (Lacey 1991; Raisbek et al. 1991). Wild grasses in pasture, if permitted to produce seed heads, often will become infected with *Claviceps* spp. A partial solution is to maintain adequate grazing pressure to keep the pasture grasses in a vegetative state. However, grasses growing in fence rows may become accessible to the livestock after flowering, thereby serving as a source of ergot. Also, hay cut too late may bear ergot or *Claviceps* honeydew. Because the pasture grass, tall fescue, is both an excellent host of *Claviceps purpurea* and the host of an ergot-alkaloid producing endophyte (*Neotyphodium coenophialum*), potential confusion exists regarding the specific fungus responsible for episodes of ergot-like poisoning on tall fescue pastures. It is important to recognize that "fescue toxicosis," if caused mainly by *Claviceps* rather than the endophyte, cannot be ameliorated by replacing the pasture with endophyte-free tall fescue or with other pasture grasses that can host *Claviceps* spp. Thus, pasture management systems should be geared toward minimizing toxicosis of livestock from both endophytes and ergot fungi. Farmers should be watchful for ergotism-like signs in livestock, regardless of the endophyte status of their pasture grasses.

The nature of the infection process makes open-pollinated grains such as rye much more prone to *Claviceps* infection than close-pollinated grains such as wheat. However, the increased use of male-sterile wheat for breeding purposes increases ergot infections. Although this is not a major concern for the food supply, yields of hybrid wheat seed can be greatly

decreased by the pathogen. With the advent of more hybrid wheat, the fungus could become a problem.

### *Stachybotrys*

About 15 species of *Stachybotrys* are known (Samson et al. 1991). *Stachybotrys chartarum* can be commonly found in food, and *S. alternans* is a cellulose-degrading saprophyte that commonly colonizes straw and hay (Hintikka 1976). The fungus produces a sooty dark layer, especially around the nodes, on hay or straw (Hintikka 1976), which are substrates most commonly associated with stachybotryotoxicosis of animals. *Stachybotrys alternans* can be isolated from wheat, oats, barley, rye, peas, cotton, sugar cane roots, and soil (Hintikka 1976). *Stachybotrys atra* requires a minimum  $a_w$  of 0.94 for growth and toxin production (Frisvad and Samson 1991). Signs of disease in certain types of stachybotryotoxicoses resemble some diseases produced by certain *Fusarium* species.

Stachybotryotoxicosis was first reported about 1931 in the Ukraine (Forgacs 1972), and for a considerable time, it was considered a disease only of horses. The disease was found to occur in other animals, however, and became recognized in many parts of the world. In recent years, there has been concern for the association with *Stachybotrys* and illness in humans occupying buildings where there is proliferation of this fungus. Subsequent to a report that *S. chartarum* apparently was the cause of an illness that occurred for several years in a home in Chicago, Illinois (Croft et al. 1986), there was a report of 10 cases of acute idiopathic pulmonary hemorrhage in infants in Cleveland, Ohio (Etzel et al. 1996). *Stachybotrys chartarum* was isolated from the homes of some of the infants and was considered a potential cause of the syndrome. The association of growth of the fungus with water damage in the homes was evident. No true cause-and-effect relationship has been completely established in these cases, however. Further investigation is warranted relative to the toxins from the fungus and the disease occurring in individuals exposed to airborne fungal conidia.

## Harvest and Storage Techniques

### Time of Harvest

Many species of mycotoxigenic fungi that colonize grain and oil crops are well adapted to grow on substrates with low moisture. For this reason, delayed harvest can result in increased contamination with mycotoxins. For example, aflatoxin concentrations in



corn left in the field increases until the kernel moisture is 16 to 18% (Payne et al. 1988). Delayed harvest of contaminated grain can result in greater amounts of aflatoxin (Jones et al. 1981). Thus, early harvest of contaminated corn, followed by drying, may help avoid increased aflatoxin contamination. Such a strategy is not always economically feasible because of the added cost of drying the grain. The kinetics of fumonisin production in corn have not been determined but *F. verticillioides* can grow in grain until the moisture content reaches 18 to 20% (Munkvold and Desjardins 1997). Late-season rains after crop maturity have been associated with increased aflatoxin (Payne et al. 1988) and fumonisin contamination.

Peanuts are indeterminate plants that should be harvested when the greatest percentage of peanuts is mature. If peanuts grown under drought stress are left in the field and subjected to precipitation, they become increasingly contaminated.

In general, following the recommended practices of crop management and harvesting crops when they are mature helps to decrease aflatoxin contamination.

### Cleaning and Drying at Harvest

During harvest, it is important to properly adjust combines to prevent excess damage to kernels, which may predispose them to infection during storage. Furthermore, as the highest levels of mycotoxins often are associated with broken and insect-damaged kernels (Malone et al. 1998b; Munkvold and Desjardins 1997), careful adjustment of the combine may eliminate these contaminated kernels in the field, with minimum loss of sound kernels (Munkvold and Desjardins 1997; Widstrom 1996). Notably, sound kernels may contain high concentrations of fumonisin (Munkvold and Desjardins 1997) and aflatoxin (Jones et al. 1980), so removal of only damaged kernels will not eliminate mycotoxin contamination. An overall decrease of total fumonisins (FB<sub>1</sub> and FB<sub>2</sub>) of 60% was obtained by screening and gravity separating corn being discharged from a storage silo (Malone et al. 1998a).

Sclerotia of *C. purpurea* and *C. fusiformis* tend to be larger than host seeds, while those of *C. paspali* tend to be approximately the same size. The use of sieving techniques to rid rye and wheat seed of the larger ergots apparently has selected strains whose ergots more closely approximate the size and shape of the seeds (Tudzinsky et al. 1995).

Harvested grain, coffee beans, fruits, and oilseed crops should be dried immediately. The final safe moisture content depends on the crop and the climatic conditions where the commodity is stored. Allowing

corn contaminated with *A. flavus* to be held at kernel moistures above 18% for more than 4 to 6 hours can rapidly increase aflatoxin contamination (Widstrom 1996). Care also should be taken to clean storage bins and auger pits and to maintain clean trucks, trailers, and combines, to minimize future contamination of the crop (Widstrom 1996).

It is well accepted that grains should be dried to 15% or below before storing. Drying of coffee seems to be critical as well to avoid ochratoxin occurrence. Contamination of coffee with the ochratoxin-producing organism from the soil seems to occur in the drying process. Ochratoxin production seems to occur when the beans have an  $a_w$  activity of 0.94 to 0.80. If the time the beans are within this window of  $a_w$  is longer than three days, there is increased likelihood of the occurrence of ochratoxin in the coffee (Frank 1999).

### Temperature and Moisture Control in Storage

Growth of mycotoxigenic fungi on grain is influenced by  $a_w$ , substrate temperature, seed damage, aeration, fungal inoculum, microbial interactions, and insects (Ominski et al. 1994). Avoiding mycotoxin accumulation in stored grains and oilseeds depends primarily on moisture control. If the product is too dry to allow fungal growth and it is kept dry, no further deterioration will occur. However, if there is insect or rodent activity, moisture migration, condensation, or water leaks, fungal growth that could lead to mycotoxin contamination will occur.

If storage conditions are favorable for *A. flavus*, aflatoxin concentrations will increase in stored grain previously infected in the field. In addition, the fungus can directly infect stored grain. Germination and growth of *A. flavus* require  $a_w$  greater than 0.85 and temperatures greater than 10°C (Marin et al. 1998a). The lower limits for growth of *A. flavus* on seeds for a number of crops have been determined (Sauer et al. 1992) and are shown in Table 3.3. *Aspergillus ochraceus*, like *A. flavus*, can infect seeds in the field and in storage. *Aspergillus ochraceus* and *A. flavus*, however, differ in their limits for temperature and  $a_w$  (Table 3.3). *Aspergillus ochraceus* can germinate and grow at 10°C with an  $a_w$  of 0.85 to 0.87.

*Penicillium* spp. can grow and produce mycotoxins over a wider range of temperatures than *Aspergillus* spp. (Ominski et al. 1994). *Penicillium* species are more abundant in temperate climatic zones, whereas the *Aspergillus* spp. predominate in warmer cli-

mates. Patulin production by *P. expansum* can occur between 0°C and 24°C. Moisture requirements for *Penicillium* spp. vary widely among species. Some species can colonize substrates with no free water, whereas others can invade and destroy grains and their products with moisture contents in equilibrium with relative humidities of 90% or above at temperatures of -2% to +5% C (Sauer et al. 1992) (Table 3.3). Thus, at low temperatures and high moisture, some *Penicillium* species are very competitive with other fungi.

Table 3.3. Lower moisture limits for growth of *Aspergillus* spp. and *Penicillium* spp. on seeds of a number of plant species (Sauer et al. 1992)

Plant	<i>A. ochraceus</i>	<i>A. flavus</i>	<i>Penicillium</i> spp.
	Moisture content of grain (%)		
Starch cereal grains	15.5–16	17–18	16.5–20
Soybeans	14.5–15	17–17.5	17–20
Sunflower, safflower, peanuts, and copra	9.0–9.5	10–10.5	10–15

*Penicillium aurantiogriseum* Dierckx and *P. verrucosum* var. *verrucosum* require an  $a_w$  of only 0.80 to 0.82 to start growing (Wilson and Abramson 1992). Marin et al. (1998a) found that germination of conidia and growth of *P. aurantiogriseum* could occur at 5°C at an  $a_w$  of 0.9 to 0.95. At temperatures between 15°C and 25°C, it could grow at an  $a_w$  of 0.85. As the fungi continue to grow, moisture released during metabolism allows the substrate to be colonized by other species. *Penicillium* spp. are known to produce a number of mycotoxins on different commodities but only a few are found in naturally contaminated cereals (Wilson and Abramson 1992). These include ochratoxins, citrinin, penicillic acid, and xanthomegnin. Although these mycotoxins are attributed to *Penicillium* spp., most are also produced by the *Aspergillus* spp. (Wilson and Abramson 1992). The disease of corn known as blue eye can be caused by species of either *Penicillium* or *Aspergillus*. If the disease, whose symptoms are caused by the accumulation of fungal spores on the germ just under the pericarp, occurs in the field or shortly after storage, it is usually due to *Penicillium* spp. If the disease appears in corn stored for several months to a year at 14 to 14.5% moisture, it is most likely caused by *A. restrictus* or *A. glaucus* (Sauer et al. 1992).

Over 24 species of *Fusarium* have been associated with human or animal health problems (ApSimon 1994) and more than 100 active secondary metabolites

are known to be produced by *Fusarium* (Thrane 2001; Wilson and Abramson 1992). Species of *Fusarium* that may be found on stored grain include *F. culmorum*, *F. graminearum*, *F. verticillioides*, *F. tricinctum*, *F. poae*, *F. sporotrichioides*, *F. avenaceum*, *F. acuminatum*, and *F. sambucinum* (Lacey et al. 1991). Most of the contamination in storage comes from infections that began in the field. (Colonization of corn and wheat in the field with *Fusarium* species was described in an earlier section.) The fungus can continue to grow in stored grain if the moisture content is high. There have been no reports of fumonisin accumulation during storage (United Nations 2001). Zearalenone concentrations appear to be low in grain contaminated in the field but can increase in storage if the moisture is greater than 34% (Wilson and Abramson 1992). Zearalenone is not produced under anaerobic silage conditions but zearalenone present in corn before ensiling is not destroyed by the silage treatment (Wilson and Abramson 1992). In general, *Fusarium* species require a high  $a_w$  to colonize grain, usually above 0.90. *Fusarium culmorum* requires at least 0.90  $a_w$  for growth (Lacey et al. 1991).

Temperature and moisture environments can be altered by other factors such as rodent and insect activity. Therefore, insect and rodent control are important because their activity in stored products creates favorable microclimates for fungal growth. Once fungal growth starts, the water of metabolism is sufficient for further growth and mycotoxin development (Sauer et al. 1992).

### 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 (Sauer et al. 1992). Grain treated with these acids is somewhat corrosive, which can create problems when handling treated grains or feeds. Liquid sprays generally are more effective than dry formulations of propionic acid but the dry formulations are easier to use.

### Interaction Among Fungal Species

When conditions are favorable for fungal contamination of substrates it is not uncommon for more than

one fungus to be involved. In storage, grain is often colonized by a succession of fungi, the sequence of which is determined in part by the temperature and moisture of the grain. The most xerophytic fungi in stored grain are members of the *A. glaucus* group and *A. restrictus*, which can grow when the  $a_w$  is as low as 0.7 (Lacey et al. 1991). As the moisture of the grain increases through microbial activity, moisture migration, or insect activity, other fungal species begin to grow. Some species may colonize grain at conditions suboptimal for growth because they can out compete other species under these conditions. For example, *P. aurantiogriseum* and *P. viridicatum* are most abundant at 0°C and 1.0  $a_w$ , although they grow best in pure culture at 25°C and 1.0  $a_w$  (Lacey et al. 1991). Likewise, members of the *A. glaucus* group are most abundant in grain at 30°C and at 0.7  $a_w$  but grow best at 30°C and 0.90  $a_w$ .

Marin et al. (1998b) compared the effect of temperature (10 to 30°C) and water activity (0.92 to 0.994) on in vitro microbial interactions of *F. verticillioides*, *F. proliferatum*, *F. graminearum*, *A. flavus*, *A. niger*, *A. ochraceus*, *P. aurantiogriseum*, *P. griseofulvum*, and *P. citrinum*. Both temperature and  $a_w$  affected the relative competitiveness of each strain. The researchers found, however, that under the conditions examined, *F. verticillioides* and *F. proliferatum* were able to dominate several corn-contaminating fungi. *F. proliferatum* was more competitive than *F. verticillioides*; however, at 15°C, *F. graminearum* had a competitive advantage over the other two species. *Aspergillus* and *Penicillium* species became more competitive at lower  $a_w$ . *Aspergillus flavus*, for example, was dominant over *F. verticillioides* and *F. proliferatum* at 30°C and an  $a_w$  of 0.94. Similarly, *A. ochraceus*, and *A. niger* were dominant over *F. verticillioides* and *F. proliferatum* at 15°C and an  $a_w$  of 0.98. Because of the possible interaction of several fungal species, grain may be contaminated with a number of different mycotoxins.

## Genetic and Molecular Aspects of Mycotoxin Biosynthesis

### Aflatoxins

Aflatoxins are the most thoroughly studied mycotoxins. Their biosynthesis is the best characterized of any fungal secondary metabolite, with the possible

exception of penicillin. Aflatoxins are polyketides derived from acetate as the starter unit. Most of the biosynthetic steps in the pathway are known. Genetic analysis of the biosynthesis of aflatoxin by *Aspergillus flavus* and *A. parasiticus*, and sterigmatocystin production by *A. nidulans*, has led to the cloning of 17 genes responsible for 12 enzymatic conversions in the aflatoxin/sterigmatocystin pathway (Bhatnagar et al. 1992; Minto and Townsend 1997; Payne and Brown 1999; Woloshuk and Prieto 1998). Sterigmatocystin seems to be synthesized by a pathway similar to that for aflatoxin. The tractable genetic system of *A. nidulans* is adding to our understanding of the biosynthesis and regulation of these two mycotoxins. The genes for aflatoxin and sterigmatocystin biosynthesis and the pathway specific regulatory gene, *afIR*, are clustered in a 75 kb region of DNA (Brown et al. 1996; Payne and Brown 1999; Yu et al. 1995).

### Trichothecenes

Trichothecene biosynthesis begins with the formation of the sesquiterpene trichodiene, which is then oxygenated, esterified, and cyclized to yield a large family of sesquiterpene epoxides. The entire trichothecene biosynthetic pathway has been established for several *Fusarium* species (Desjardins et al. 1993). More than eleven pathway genes have been cloned for both *F. sporotrichioides* and *F. graminearum* (Brown et al. 2001). All of these genes, which encode enzymes, a transcription factor, and a transporter, are clustered in a region of DNA (Keller and Hohn 1997).

### Fumonisin

The fumonisin biosynthetic pathway is being investigated. Precursor feeding experiments have established that fumonisins originate by condensation of the amino acid alanine with an intermediate derived from acetate. Sexual genetic analysis has shown that several genes involved in fumonisin biosynthesis are clustered on chromosome 1 of *F. verticillioides* (Desjardins et al. 1996a). Recent studies have identified the first fumonisin biosynthetic gene, a polyketide synthase that is located in this gene cluster (Proctor et al. 1999).

Research also is focusing on using mycotoxin pathway genes to study *Fusarium* biology and the factors that regulate fungal growth and mycotoxin production in agricultural commodities. Using gene disruption to block trichothecene biosynthesis has been a

powerful tool for investigating the role of these mycotoxins in plant disease. In-field trials in the United States and Canada, trichothecene-nonproducing mutants of *F. graminearum* demonstrated decreased ability to cause wheat scab and corn ear rot (Desjardins et al. 1996b; Harris et al. 1999).

### Ergot Alkaloids

Considerable effort is underway to identify or generate genotypes of grass and endophyte that minimize expression of the antimammalian alkaloids while retaining beneficial characteristics. One approach is to use tall fescue genotypes associated with decreased ergot alkaloid levels in symbioses with *Neotyphodium coenophialum* (Agee and Hill 1994). Another approach is to identify endophyte genotypes that produce few or none of the major antimammalian alkaloids but still exhibit other plant protective characteristics (Christensen et al. 1993). An advantage to these approaches is that they make use of already-available biological materials. A disadvantage is that the benefits already well documented for *N. coenophialum* and *N. lolii* in existing grass cultivars need to be field tested for the new grass and endophyte genotypes.

Another approach to address tall fescue toxicosis is to specifically eliminate genes for biosynthesis of antimammalian alkaloids in the well-characterized endophytes (Schardl 1994). To this end, the gene for the first determinant step in ergot alkaloid biosynthesis has been cloned from the related fungus, *Claviceps purpurea* (Tsai et al. 1995). By eliminating the gene for the first determinant step in the pathway, the endophyte should be rendered incapable of producing ergot alkaloids. However, the endophyte should retain the capability to produce lolines and peramine because these anti-insect alkaloids appear chemically unrelated to ergot alkaloids. Thus, the potential exists to improve *N. coenophialum* for agricultural use through genetic engineering.

## Speculative Function of Mycotoxins to the Fungus

Many mycotoxin-producing fungal species can cause plant diseases under field conditions. Thus, it becomes logical to ask whether mycotoxins themselves play a role in plant diseases in addition to their role in animal diseases. Although it has been easy to demonstrate that some mycotoxins are highly toxic to plants, it has proven far more difficult to establish a causal role for mycotoxins in plant disease. Critical analysis of the role of mycotoxins in plant disease awaited the development of laboratory methods to specifically eliminate a toxin from a biological system. DNA-mediated transformation and other molecular biological methods are providing the essential tools to rigorously test the role of mycotoxins in plant disease. In principle, if production of a mycotoxin increases the ability of a fungus to cause a plant disease, then increasing plant resistance to the toxin should increase plant resistance to the disease itself.

Various strategies have been used to isolate and disrupt mycotoxin biosynthetic genes in plant pathogenic fungi. As of this writing, mycotoxin biosynthetic pathways that have been blocked by gene disruption include aflatoxin biosynthesis in *Aspergillus* species, ergot alkaloid biosynthesis in *Neotyphodium* species, fumonisin biosynthesis in *F. verticillioides*, and trichothecene biosynthesis in *Fusarium* species (Desjardins and Hohn 1997; Panaccione et al. 2000; Proctor et al. 1999). Laboratory and field tests of mycotoxin-nonproducing transformants have demonstrated that trichothecenes play an important role in wheat head blight and corn ear rot caused by *F. graminearum* (Desjardins and Hohn 1997; Harris et al. 1999), but that fumonisins are not required for corn ear rot caused by *F. verticillioides* (Desjardins et al. 2000). Similar efforts are underway to test the roles of aflatoxins, ergot alkaloids, and other mycotoxins in plant disease in the laboratory and in the field.

## 4 Occurrence of Mycotoxins in Food and Feed

### 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, e.g., 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, e.g., crop drying, or in storage. However, aflatoxins also can contaminate stored products in the absence of field contamination. Many other fungi that produce mycotoxins contaminate crops in much the same way.

Other than aflatoxins, mycotoxin contamination of feeds and foods is poorly studied. Aflatoxins have been detected in milk, cheese, corn, peanuts, cottonseed, Brazil nuts, copra, almonds, pecans, figs, spices, and a variety of other foods and feeds. Worldwide, corn, peanuts, and cottonseed are the most frequently analyzed crops, while copra, pistachio nuts, Brazil nuts, figs, and spices are of concern as well. Milk, eggs, and meat products sometimes become contaminated because the animal has consumed mycotoxin-contaminated feed.

Besides aflatoxins, ochratoxin and *Fusarium* mycotoxins have been given the greatest attention as food and feed contaminants. These mycotoxins tend to occur in the more-temperate regions of the United States. DON, zearalenone, and fumonisins are a greater economic concern than ochratoxin to U.S. animal producers. Mycotoxins produced by *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* species can contaminate products; however, the incidence and relative importance to animal and human health of these many different mycotoxins have not been established.

A truly mycotoxin-free food supply cannot 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

Published literature 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 these reports of natural occurrence and their implications into perspective.

After reviewing the literature on mycotoxin occurrence, several conclusions can be drawn.

1. Numerous reports of the fungal flora on foods and feeds document the frequent presence of potentially toxigenic fungi (Table 4.1). These reports define the conditions conducive to or restrictive of fungal growth and mycotoxin formation.
2. Mycotoxins can occur in a wide variety of agricultural commodities (Table 4.2).
3. Knowledge about the frequency of food and feed contamination still is rather limited. It is known, however, that mycotoxins can make their way into human foods (Table 4.3).
4. Humans are less exposed to mycotoxins in the developed countries than in developing countries where food resources are plentiful, food handling and preservation technology is well developed, and regulation and control of food quality restricts exposure to mycotoxins.
5. A major problem with mycotoxins in developed countries is associated with animal health, because animal feeds are most likely to contain mycotoxins and thus animal mycotoxicoses occur but are difficult to diagnose due to subtle or non-specific effects. The veterinary literature has been a rich source of information on known and potential mycotoxin problems.

Mycotoxins can be categorized as frequently occurring in (1) raw agricultural products; (2) processed foods; (3) imported products; and (4) animal products, e.g., milk, meat, and eggs.

Table 4.1. Summary of selected reports of potentially toxic molds from various food or agricultural commodities (adapted from Bullerman 1979, 1986)

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

— continued

Table 4.1. (continued)

Commodity	Potentially toxic genera/species found		Potential mycotoxins
Foods stored in homes, both refrigerated and nonrefrigerated	<i>Penicillium</i> spp.	<i>Aspergillus</i> spp.	Aflatoxins, kojic acid, ochratoxin A, penitrem, patulin, penicillic acid
Apples and apple products	<i>Penicillium expansum</i>		Patulin

Table 4.2. 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 <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub> , M <sub>1</sub> , M <sub>2</sub> )	Peanuts, corn, wheat, rice, cottonseed, copra, nuts, various foods, milk, eggs, cheese	Birds Duckling, turkey poul, 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, cracked eggshell
Fumonisin	Corn, polenta	Swine, horse, rat, mouse, humans	Pulmonary edema, leukoencephalomalacia, nephrotoxicity, hepatotoxicity
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)

Table 4.2. (continued)

Mycotoxin	Commodities found contaminated	Effects of mycotoxins	
		Affected species	Pathological effects
Patulin	Moldy feed, rotted apples, apple juice, applesauce, wheat straw residue	Birds Chicken, quail Mammals Cat, cattle, mouse, rabbit, rat	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, quail	Liver damage (fatty liver, cell necrosis) Kidney damage Digitalis-like action on heart Dilates blood vessels Antidiuretic Edema in rabbit skin Carcinogenesis Antibiotic
Penitrem	Moldy cream cheese, English walnuts, hamburger bun, beer	Dog, mouse, human	Tremors, death, incoordination, bloody diarrhea
Sterigmatocystin	Green coffee, moldy wheat, hard cheeses	Mouse, rat	Carcinogenesis Hepatotoxin
Trichothecenes (T-2 toxin, diacetoxyscirpenol, neosolaniol, nivalenol, diacetylivalenol, 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, 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



Table 4.3. Selected examples of natural occurrence of mycotoxins in processed foods

Mycotoxin	Food	Country	Average levels of contaminated samples ( $\mu\text{g}/\text{kg}$ )	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	Nonfat 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, The Netherlands	86	36	Scott 1984; Schothorst and Jekel 2001
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	1,000	—	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

## Natural Occurrence in Raw Agricultural Products

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

Ergotism is the oldest known mycotoxicosis of humans and animals. 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-contam-

inated 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, while death from gangrene could follow consumption of about 100 g of ergot over a few days. Canadian scientists have suggested a maximum acceptable level of 0.05% of ergot particles in flour (Peace and Harwig 1982).

Aflatoxins have been found to contaminate many crops, frequently at low nanogram (ng)/g levels, although occasionally they can be found at levels of tens to hundreds of ng/g. Commodities with a high risk of aflatoxin contamination include peanuts (Figure 4.1), corn, cottonseed, Brazil nuts, pistachios, spices, figs, and copra (Jelinek 1987). Commodities with a lower risk of aflatoxin contamination include figs, almonds, pecans, walnuts, and raisins. Soybeans, beans, puls-

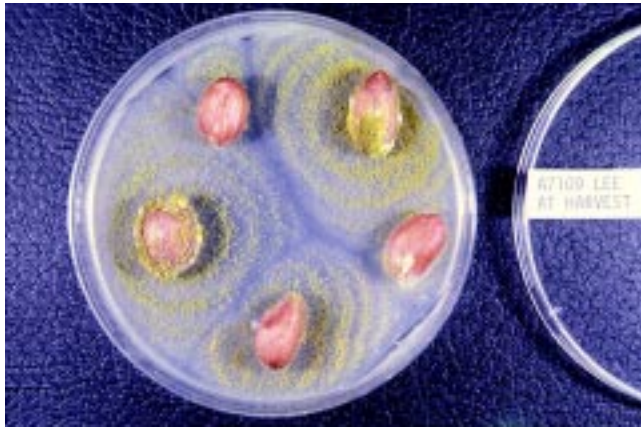


Figure 4.1. Growth of *Aspergillus flavus* (yellow-green fungus) from two of five surface sterilized peanuts placed on a nutrient culture medium. Photograph courtesy of R. J. Cole, USDA, ARS, National Peanut Research Laboratory, Dawson, Georgia.

es, cassava, grain sorghum, millet, wheat, oats, barley, and rice are resistant or only moderately susceptible to aflatoxin contamination in the field. However, all of these commodities are susceptible when stored under conditions of high moisture and temperature. Insect or rodent infestations can create microclimates that facilitate mold invasion of some stored commodities.

In the United States, the U.S. Food and Drug Ad-

ministration (FDA) analyzes raw agricultural products for selected mycotoxins through a formal compliance program and exploratory surveillance activities (Wood 1991; Wood and Trucksess 1998). The objectives of the compliance program are to (1) collect and analyze samples of foods and feeds to determine compliance with FDA regulatory levels; (2) remove from interstate commerce those foods that contain violative aflatoxin levels; and (3) determine awareness of potential problems and control measures employed by distributors, manufacturers, and processors. In contrast, the role of the exploratory surveillance program is to collect background exposure data for a particular mycotoxin, to be used in conjunction with toxicological data. Monitoring is aimed at regions and commodities that historically have a high level of contamination or as a response to new information on contamination problems in regions or commodities not normally affected.

Tables 4.4 and 4.5 summarize the FDA compliance data for peanuts and corn, respectively, from 1987 to 1997. In over 95% of peanut lots, the mean aflatoxin content is much less than the 20 parts per billion (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).

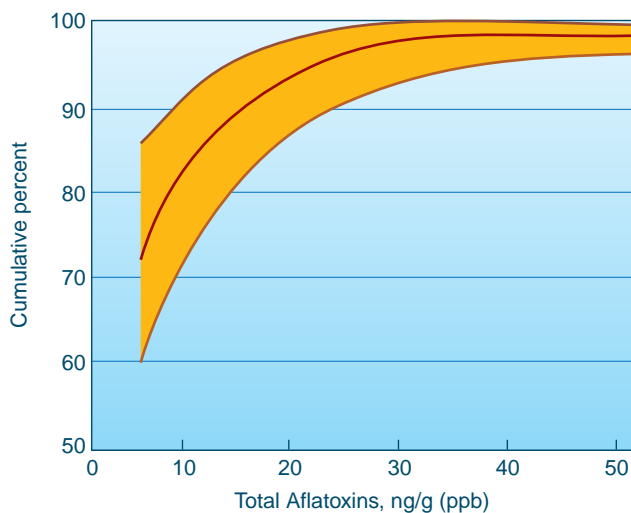


Figure 4.2. Frequency distribution (cumulative % less than indicated level) of total aflatoxin levels in raw shelled peanuts produced in the United States. Data are from the crop year reports of the Peanut Administrative Committee. Center plot is the average for the 12 crop years 1973–1984; boundary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

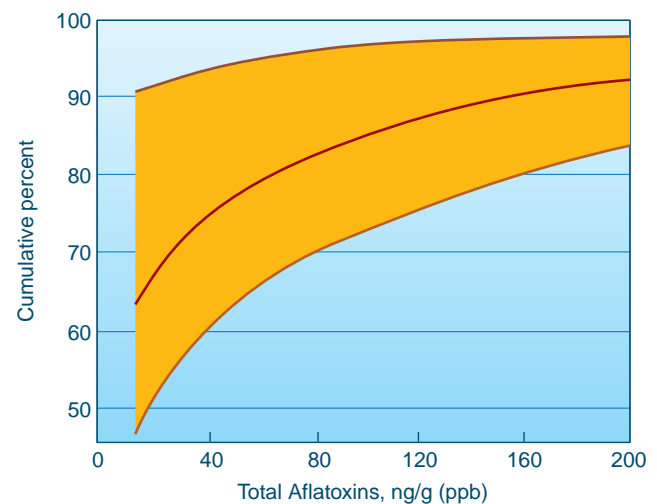


Figure 4.3. Frequency distribution (cumulative % less than indicated level) of total aflatoxin levels in shelled corn produced in the state of North Carolina. Data are from 8,653 farmer-submitted and elevator-survey samples assayed by the state analysts for the six crop years 1977–1978, 1980–1983. Center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

**Table 4.4. Peanut products examined for aflatoxins and levels<sup>a, b</sup>**

Year	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997
<b>Peanut butter</b>											
No. tested	146	372	158	105	116	82	64	77	70	62	88
No. positive	20	7	1	25	40	18	2	23	29	15	7
% positive	14	2	1	24	34	22	3	30	41	24	8
Minimum	3	3	17	1	1	1	4	2	1	2	3
Maximum	181	37	17	42	14	9	5	15	18	13	10
Mean	26.45	18.86	17.00	10.04	4.98	2.56	4.50	5.35	5.90	6.47	5.71
Median	12	18	17	5	3.5	1	4.5	5	6	6	5
<b>Shelled and roasted</b>											
No. tested	63	357	243	95	73	89	69	79	82	65	58
No. positive	2	9	0	20	12	10	2	4	6	0	2
% positive	3	3	0	21	16	11	3	5	7	0	3
Minimum	117	5	0	1	1	1	1	4	2	0	11
Maximum	134	34	0	250	34	31	2	44	5	0	13
Mean	125.5	14.33	—	30.85	8.67	10.80	1.50	18.25	2.83	—	12.00
Median	125.5	12	—	9	5.5	6.5	1.5	12.5	2.5	—	12
<b>In-shell and roasted</b>											
No. tested	15	80	52	14	16	19	13	19	5	14	20
No. positive	0	1	0	1	0	0	0	0	0	0	0
% positive	0	1	0	7	0	0	0	0	0	0	0
Minimum	0	6	0	12	0	0	0	0	0	0	0
Maximum	0	6	0	12	0	0	0	0	0	0	0
Mean	—	6	—	12	—	—	—	—	—	—	—
Median	—	6	—	12	—	—	—	—	—	—	—

<sup>a</sup>Data from FDA Compliance Programs, 1987–1997 provided by Dr. Garnett Wood, U.S. Food and Drug Administration.

<sup>b</sup>The peanut products were collected by FDA Inspectors during scheduled inspections of firms within the United States that had a history of Food and Drug Law violations; these results are therefore biased, but they give an indication of the effectiveness of industry control efforts.

After aflatoxins, the most-often reported mycotoxins in raw agricultural commodities are those produced by various *Fusarium* species. In the United States and Canada, grain contamination by DON and zearalenone frequently is encountered. In Japan, high concentrations of both DON and nivalenol have been found in corn, wheat, and barley; zearalenone sometimes occurs as a co-contaminant (Jelinek 1987). DON may be found in corn at levels greater than 1 mg/kg. DON is frequently found in U.S., Canadian, and European wheat, especially crops 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). DON was a major problem in the United States in the states of North Dakota, Minnesota, South Dakota, Ohio, Michigan, Indiana, and Illinois in the 1990s. DON-contaminated wheat and barley were greatly devalued by the U.S. marketing system. Because of a near-zero tolerance policy for DON, grain buyers and food processors refuse to purchase soft white wheat or malting barley from some midwestern states during years when scab is a problem.

The occurrence of other trichothecenes, T-2 toxin and diacetoxyscirpenol, has been reported less frequently in raw products, which may be related to inadequate analytical methodology. Data on diacetoxyscirpenol usually are qualitative only.



**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.

Table 4.5. Occurrence of aflatoxins in foods and feeds imported into the United States from 1987–1997<sup>a</sup>

Commodity	Samples examined (No.)	Samples contaminated (No.)	No. > 20 ng/g	Maximum (ng/g)
Human food				
Almonds	85	2	1	46
Apricot/kernals	49	7	7	310
Brazil nuts	158	29	16	1,118
Candy, assorted	363	50	22	555
Cookies/crackers	177	27	14	77
Corn flour/meal	172	45	10	100
Edible seeds, misc.	144	5	3	318
Figs	156	7	4	174
Filbert/hazelnuts	170	7	4	351
Lotus seeds	41	4	1	23
Marzipan	38	18	12	101
Melon seeds	252	46	15	276
Nutmeg	44	9	5	37
Nuts, assorted	316	22	8	60
Peanuts	329	70	25	901
Peanut products	434	106	46	422
Pecans	141	8	6	725
Pistachio nuts	107	20	14	442
Pumpkin seeds	262	28	14	150
Sesame seeds	432	6	1	41
Snack food	128	17	5	90
Sunflower seeds	20	3	2	125
Animal feed				
Corn	1,320	5	1	231
Cottonseed/meal	192	3	3	154

<sup>a</sup>Data from FDA Compliance Programs, 1987–1997, provided by Dr. Garnett Wood, U.S. Food and Drug Administration, Washington, D.C.

Zearalenone, produced by several *Fusarium* species, is a frequent contaminant of U.S. corn, wheat, barley, and grain sorghum. Generally, zearalenone concentrations are well below 1 mg/kg (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).

Several toxic metabolites are produced by *Fusarium verticillioides*, the most common fungus found on corn. An important group of mycotoxins from *F. verticillioides* (syn., *moniliforme*) was characterized in 1988 by South African investigators and named fumonisins (Gelderblom et al. 1988b). The fumonisins have been implicated in equine leukoencephalomalacia and porcine pulmonary edema and have been shown to be cancer promoters in rats. Because *F. verticillioides* is nearly ubiquitous in corn, low-level (less than 1 mg/kg) contamination with fumonisins is extremely common throughout the world. Some surveys have found these toxins at concentrations exceeding 5 mg/kg (Dutton 1996; Shephard et al. 1996). Other toxic *F. verticillioides* metabolites have been detected in corn or wheat (Jelinek 1987).

Although many *Fusarium* toxins have been found in commodities susceptible to contamination with aflatoxins, ochratoxins, or other mycotoxins, significant co-occurrence of mycotoxins from different mold species has not been found.

Ochratoxin A has been reported as naturally occurring in corn, wheat, sorghum, oats, rice, wine, beer, and green coffee (Jorgensen 1998; MacDonald et al. 1999; Otteneder and Majerus 2000; Patel et al. 1997; Scudamore et al. 1999; Stegen et al. 1997). Ochratoxin A contamination of coffee, corn, grapes, dried fruits, and wheat is generally less than 500 µg/kg. Barley and oats grown in Denmark and other Scandinavian countries are particularly susceptible to high levels of ochratoxin contamination (Krogh et al. 1973, 1974; van Egmond and Speijers 1994). Animal feeds in Canada and Europe may be highly contaminated with ochratoxin (over 5,000 µg/kg) (Jelinek 1987; Tsubouchi et al. 1988). The highest reported incidence and levels have been in barley, oats, wheat, and corn produced in northern European (e.g., United Kingdom, Denmark, Sweden) or Balkan (e.g., the former Yugoslavia) countries and in India. The reports in-

dicates that levels approaching the mg/kg range, at an incidence of over 20%, can be expected to occur in random samples of these grains in the affected areas. Surveys of U.S. barley, corn, oats, sorghum, and wheat over a number of years found some ochratoxin A in all but sorghum but at a low incidence (corn 0.5%, wheat 1%, oats 2%, barley 14%) and low level (all samples under 200 µg/kg) of contamination. Some insight into ochratoxin A frequency and levels of occurrence in commodities marketed in the EU have been published, together with estimates of human exposure to ochratoxin A (European Commission 1997).

The FDA Compliance Program studies have indicated the presence of mycotoxins other than aflatoxins. For example, in 1983, of the 27 samples of corn and sorghum analyzed, 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.

Citrinin can occur alone or with ochratoxin A. However, no suitable quantitative method for citrinin analysis exists, so current data are an estimation. 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 carrots in Europe and in tomatoes in the United States and Canada, while alternariol methyl ether and alternariol have been found in apples in West Germany (Jelinek 1987). *Alternaria* spp. frequently invade fruits and grains in the field and *Alternaria* metabolites could easily contaminate grains such as wheat, oats, barley, and grain sorghum. U.S. survey data are lacking to allow an accurate appraisal of the occurrence of these mycotoxins in foods and feeds.

Other mycotoxins, e.g., cyclopiazonic acid, stigmatocystin, sporidesmins, rubratoxin B, cytochalasins, penitrems, slaframine, have been reported in the literature as natural contaminants of agricultural commodities. Some are of limited occurrence. More surveys with suitable analytical methods to determine incidence and levels of mycotoxins in foods and feeds are sorely needed.

Patulin and penicillic acid are toxic under certain conditions but will not be considered in this chapter 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 deals with aflatoxins. There is a dearth of information for trichothecenes, zearalenone, and citrinin; however, there are considerably more data available on ochratoxin A (European Commission 1997). Because of the limited known occurrence of ochratoxin A, it does not seem to be as great a problem in the United States as it is in some European countries.

Foods processed from peanuts and corn 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). Wheat, barley, oats, apples, and tomatoes occasionally have been found to be contaminated with mycotoxins. Aflatoxins were found in one sample of spaghetti (van Walbeek et al. 1968).

Because it is grown in climates with perennial mycotoxin contamination and serves as a staple food in many countries, corn is probably the commodity of greatest worldwide concern. Procedures used to process corn help decrease aflatoxin contamination of resulting food products. Corn may be contaminated with *Fusarium* toxins and foods made from corn have been shown to be contaminated with deoxynivalenol (Davis and Diener 1987; Trucksess et al. 1986) and fumonisins (Dutton 1996; Kuiper-Goodman et al. 1996; Shephard et al. 1996). DON contamination of corn and corn products has been correlated with cool, wet conditions prior to harvest, primarily during flowering.

DON contamination of wheat can occur when pre-harvest weather conditions favor development of wheat scab. Wheat-based processed foods shown to be contaminated with DON include flour, bread, snack foods, baby foods, breakfast cereals, bran, and wheat germ (Abouzeid et al. 1991; 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). Ochratoxin A has been found in roasted coffee and various types of wine and beer.

In fruits and vegetables, mycotoxins have been found to naturally occur most frequently in apples, tomatoes, and their processed products. *Alternaria* tox-

ins — including tenuazonic acid, alternariol, and alternariol methyl ether — have been found in apple, carrot, and tomato products (Jelinek et al. 1987). Patulin is a common contaminant of apple juice, puree, and unfermented 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, the result of mold growth, has been reported. Sterigmatocystin has been detected in the rind of hard cheese (Northolt et al. 1980) and metabolites of *P. roqueforti* and *R. caseicola* (*camemberti*) in blue and Camembert-type cheeses have been reported (Bullerman 1981; Leistner 1984; Scott 1981).

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, e.g., aged hams and cured sausages (Pestka 1995). Ochratoxin A, the mycotoxin most commonly found as a residue in pork and poultry meat, is of the greatest concern. 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 less than 5% of the samples. Ochratoxin A has been detected in blood, kidneys, liver, and muscle tissue from slaughtered hogs in several European countries (European Commission 1977; Leistner 1984; van Egmond and Speijers 1994). 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).

While aflatoxin residues can be demonstrated experimentally in animal organs and tissues, these mycotoxins are metabolically decreased quite rapidly and do not accumulate at concentrations of concern to human health (Pestka 1995). Prelusky et al. (1996a) demonstrated that low-level fumonisin  $B_1$  accumulation occurred in kidney and liver in swine fed radio-labeled fumonisin  $B_1$ . In general, the carry-over of DON and other trichothecenes as well as zearalenone from feed to milk, meat, or eggs of food animals is negligible (Pestka 1995).

European-type sausages and country-cured hams frequently have intentional or adventitious fungal growth on their outer surfaces. Most 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.

## Occurrence in Foods Imported into the United States

A number of mycotoxins could be present in foods and feeds imported into the United States; 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 are shown in Table 4.5 for the period 1987–1997 (only samples positive for aflatoxin are shown). Jelinek (1987) has summarized information on the worldwide occurrence of mycotoxins, using data from the United Nations Food and Agriculture Organization (FAO) food contamination monitoring program and other sources.

## Fate of Mycotoxins During Grain Processing

Because mycotoxins occur in commodities that are subsequently incorporated into food, concern exists regarding the fate of these toxins undergoing certain processes, the possible concentration of mycotoxins or their derivatives in the end product, or distribution of the mycotoxins to the various components resulting from commodities submitted to a certain process. During most growing seasons, at least in the United States, crop damage by toxigenic fungi is insignificant for both the grower and processor. Grading and testing often stop contaminated lots of grain from entering a processing facility. However, inadvertent use of contaminated grains in food processing can pose a threat to animal or human health. These situations are likely when mycotoxin contamination occurs in certain crops prior to harvest. Therefore, fates of various mycotoxins during different types of food processing have been studied, to determine the likelihood of

contamination of different end products. This section will review studies describing the fate of mycotoxins during grain processing, rather than methods of destruction or decomposition of the mycotoxins.

### Wet Milling

Wet milling of mycotoxin-contaminated corn has been studied to determine possible contamination of corn products for human consumption. Generally, this process segregates the mycotoxins in the chemically diverse products resulting from the process (Bennett and Richard 1996). The major mycotoxins that have been studied are aflatoxins, zearalenone, DON, T-2 toxin, and fumonisins.

Aflatoxins, after wet milling of corn, were found in the steep water at the highest concentrations, with fiber concentrations almost as great, and lesser amounts in gluten and germ (Bennett and Anderson 1978). Starch contained only low amounts of aflatoxins. Similarly, zearalenone was not present in starch but occurred at the greatest concentration in gluten, with lesser amounts in the milling solubles (Bennett and Anderson 1978; Bennett et al. 1978). With fumonisin-contaminated corn subjected to wet milling, no fumonisins were found in any of the fractions from corn contaminated at 1 mg/kg, although there was some detectable fumonisin B<sub>1</sub> in the steep water. Wet-milled fractions from corn with an original concentration of 13.9 mg/kg fumonisin B<sub>1</sub> did contain fumonisin B<sub>1</sub> and B<sub>2</sub> but the starch fraction did not. The major portion of recoverable fumonisins was found in the steep water and process water. The amount of fumonisins in wet-milled fractions was gluten > fiber > germ (Bennett and Denhart 1996; Bennett and Richard 1996; Richard et al. 1993). As expected, due to DON's solubility in water, wet milling of DON-contaminated corn caused it to concentrate in the steep water, although measurable amounts of DON also were found in the starch (Scott 1984). In one study using T-2-contaminated corn, 67% of the T-2 was in the steep water and process water; only 4% was found in the starch but appeared to be concentrated in the germ (Collins and Rosen 1981). As with most mycotoxins, ochratoxin A was present in the steeped fraction and went into the process water and solubles, while 4% and 51% remained in the germ and grits, respectively (Wood 1982).

Generally, limited amounts of mycotoxins are found in the starch fraction from wet milling of corn. Most mycotoxins are removed by the steeping process, while varying amounts occur in the other fractions of fiber, gluten, and germ. If the mycotoxin concentra-

tion is sufficiently low in commodities used for these processes, it may be removed or decreased to concentrations below levels of concern through wet milling.

### Dry Milling

This process separates grain components into various particle sizes, resulting in fractions such as grits, germ, meal, and flour. Thus, corn and wheat are the major commodities subjected to this process and mycotoxins occurring in these commodities are of concern for their potential occurrence in the various fractions.

Both commercial- and laboratory-scale dry milling have been conducted with various concentrations of zearalenone-contaminated corn (Bennett et al. 1976). Unfortunately, zearalenone was present in all mill fractions, with the highest concentrations in the hull and high-fat fractions (Bennett and Richard 1996). Brekke and coworkers (1975) found that the highest levels of aflatoxin from naturally contaminated corn were in the germ and hull fractions and that aflatoxin distribution varied in the fractions according to its concentration in the starting material. Grits and low-fat meal and flour contained only 6 to 10% of the aflatoxin B<sub>1</sub>. Milling of ochratoxin-contaminated wheat and barley resulted in ochratoxin distributed equally between flour and bran (Chelkowski et al. 1981).

Numerous milling studies have been conducted with DON- and/or zearalenone-contaminated wheat (Trigo-Stockli et al. 1995). Generally, both mycotoxins were present in the bran, shorts, and flour, with the highest concentrations occurring in the bran and the lowest in the flour. Bennett et al. (1976) obtained similar results for corn. Flour made from wheat with high concentrations ( $\geq 10$  mg/kg) of DON can have levels above that recommended for wheat products destined for human consumption (Abouzied et al. 1991).

Because fumonisins are known to occur in corn-based foods (Bennett and Richard 1996; Richard et al. 1993) the fate of these mycotoxins in dry-milled, contaminated corn has been studied. Bennett and Denhart (1996) presented data from dry-milling studies of corn contaminated with 10 mg/kg of fumonisins showing that all dry-milled fractions were contaminated with fumonisins B<sub>1</sub> and B<sub>2</sub>. Pericarp fractions from yellow and white corn contained twice the concentrations of fumonisins B<sub>1</sub> and B<sub>2</sub> present in the original corn. Similar results were obtained in a later study by Katta and coworkers (1997) using blue, white, and yellow corn.

Ergot alkaloids in milled hard red spring wheat were followed by Fajardo and colleagues (1995); the highest concentrations were found in the late reduc-

tion streams and in the shorts derived from the process. The lowest concentrations of ergot alkaloids were in the high-quality patent flour.

Generally, when contaminated commodities are dry milled, mycotoxins are distributed in all fractions of significant proportions; therefore, testing of the fractions is necessary before incorporating them into human and animal food.

### Fermentation

Ethanol production from mycotoxin-contaminated commodities has been suggested as a way to utilize such grain when contamination is too high for incorporation into animal and human food. The major mycotoxins examined in fermentation studies include aflatoxins, ochratoxins, fumonisins, zearalenone, and DON. Generally, if the fermentation product is distilled into ethanol, it contains no mycotoxins.

Aflatoxin B<sub>1</sub> was decreased by 47% after cooking and fermenting the contaminated corn or wheat (Dam et al. 1977), although there was a higher concentration of aflatoxin B<sub>1</sub> in the solids after distillation than in the starting grain. Starting with zearalenone-contaminated corn (at 8 and 35.5 mg/kg levels), no zearalenone appeared in the ethanol; however, its concentration in the solids was about twice that of the original corn. Two lots of corn contaminated with 15 and 36 mg/kg of fumonisin B<sub>1</sub> were fermented for ethanol production (Bothast et al. 1992). Analysis of the various fermentation products showed that there was little fumonisin degradation during ethanol fermentation. No fumonisin B<sub>1</sub> was found in the distilled ethanol or the centrifuge solids. Most fumonisins were found in the distillers' dried grains, thin stillage, and distillers' solubles. Notably, a lower amount of ethanol was produced from the 36 parts per million (ppm) fumonisin B<sub>1</sub> corn than from the 15 mg/kg fumonisin B<sub>1</sub> corn, attributed to the corn's decreased quality of starch rather than the higher concentration of fumonisins.

During the brewing process, Schwartz et al. (1995)

found that both zearalenone and 15-acetyl DON increased during germination of the barley for malting. After brewing, 80% to 93% of the DON present in the malt grist was detected in the beer. Sixty percent of the zearalenone and 18% of the 15-acetyl DON were found in the spent grains. Scott et al. (1995) examined the losses of ochratoxin A and fumonisins B<sub>1</sub> and B<sub>2</sub> in fermentation of spiked wort. Some ochratoxin A (up to 21%) but negligible amounts of fumonisins were taken up by the yeast. These experiments indicated that ochratoxin A and fumonisins could contaminate commercially prepared beer. In this Canadian study, fumonisins were later detected in both domestic and imported beer (Scott and Lawrence 1995). Similarly, ochratoxins are regularly reported in beer and wine, likely attributed to the increasing sensitivity of test methods (Scott 1996). Aflatoxins have been recovered in finished beer made from malt or corn grits to which aflatoxins were added experimentally (Chu et al. 1975). Aflatoxins have been found in some beer, primarily native beers of Africa (Scott 1996). In the latter article, Scott reviewed the transmission of mycotoxins into beer and found that DON is the most successful survivor of the brewing process. Most mycotoxins can survive brewing and end up in the beer but usually as small percentages. Similarly, contamination of wine occurs through the use of ochratoxin A-contaminated grapes (Pietri et al. 2001).

### Other Processing

A number of studies dealing with destruction of mycotoxins during food preparation techniques, e.g., roasting, flaking, baking, cooking, canning, have been conducted (Scott 1984). Results have varied from one commodity to another and from one mycotoxin to another. Other researchers have looked at the fate of mycotoxins during preparation of indigenous foods. The use of these processes is limited and not reviewed here. Also, the processing of M<sub>1</sub>-contaminated milk is not reviewed here.



## 5 Mycotoxins and Human Disease

### Summary

Humans likely are exposed to mycotoxins through several routes such as ingestion (the most prominent means of exposure), contact, and inhalation. Historical accounts reveal that ergotism was one of the oldest recognized mycotoxicoses and, potentially, moldy grains were involved in other mycotoxicoses as well. The aflatoxins are known causes of acute aflatoxicosis in humans. But chronic forms of aflatoxicosis, especially carcinomas, are more problematic because epidemiological evidence is not as clearly defined due to other factors such as hepatitis B that may be interactive in the disease process.

Ochratoxin has been conjecturally associated with a disease known as Balkan endemic nephropathy. The rural populations in the Balkans have a high incidence of chronic kidney problems and tumors of the excretory organ system. Russian literature contains some evidence that *Fusarium* spp. were involved in human disease. The most likely toxic products of this genus, the trichothecenes, are conjecturally associated with the most prominent disease described, alimental toxic aleukia.

The trichothecene, DON, is capable of producing a disease in mice that is similar in histological descriptions to human glomerulonephropathy. Stachybotryotoxicosis is a disease that occurs in humans and other animals and is suspected to be caused by toxins of the organism *Stachybotrys chartarum*. The exact toxic product has not been identified in a cause-and-effect relationship in this disease. Some epidemiological evidence exists, however, for the involvement of the organism in disease.

The fumonisins have been associated with esophageal cancer in certain human populations, but again, no exact cause-and-effect relationship has been established. Epidemiological evidence indicates, however, that fumonisin-producing species of *Fusarium* on corn are involved. Of interest to many investigators is that a number of mycotoxins are immunosuppressive and likely could be involved in human disease. These are difficult to prove, however, because the underlying involvement of the mycotoxin probably is overshadowed

by disease resulting from the immunosuppressed state of the host.

There are other diseases that have been described in humans for which either analytic or epidemiologic evidence implicates a mycotoxin etiology.

### Introduction

Although ergotism in humans ("St. Anthony's fire") likely occurred in medieval times (see cover photograph) as being associated with ingestion of the plant parasitic fungus, *Claviceps purpurea*, little concern for toxic compounds produced by fungi was evident outside the veterinary and agricultural communities. Today, there is an apparent growing concern within the medical community regarding mycotoxin involvement in human diseases. Animal studies and epidemiological evidence indicate definite involvement of fungal metabolites as etiological agents of human disease. Furthermore, the potential for agents of mycoses to produce toxic metabolites that could be virulence factors involved in the pathogenesis of the mycotic disease is largely ignored. One must be careful, however, when blaming mycotoxins for human disease, as the literature includes incriminating statements without complete scientific evidence.

As in animal mycotoxicoses, the disease in the human recipient is the result of exposure by ingestion of contaminated food. However, other routes such as inhalation, contact, and passive exposure resulting from a mycotic infection by a toxigenic fungus must be considered.

*Mycotoxicosis*, the disease resulting from exposure to a mycotoxin, may be manifested as acute to chronic, and ranges from rapid death to tumor formation. More occult disease may occur when the mycotoxin interferes with immune processes, rendering the patient more susceptible to infectious diseases. The underlying mycotoxic event may be overshadowed by the infectious disease and thus not be considered in the overall syndrome. This may explain why little information is available on the natural occurrence of mycotoxins having immunosuppressive activities.

Table 5.1. Some human diseases in which analytic and/or epidemiologic data suggest or implicate mycotoxin involvement

Disease	Species	Substrate	Etiologic agent
Akakabio-byo	Human	Wheat, barley, oats, rice	<i>Fusarium</i> spp.
Alimentary toxic aleukia (ATA or septic angina)	Human	Cereal grains (toxic bread)	<i>Fusarium</i> spp.
Balkan nephropathy	Human	Cereal grains	<i>Penicillium</i>
Cardiac beriberi	Human	Rice	<i>Aspergillus</i> spp., <i>Penicillium</i> spp.
Celery harvester's disease	Human	Celery (Pink rot)	<i>Sclerotinia</i>
Dendrodochiotoxicosis	Horse, human	Fodder (skin contact, inhaled fodder particles)	<i>Dendrodochium toxicum</i>
Ergotism	Human	Rye, cereal grains	<i>Claviceps purpurea</i>
Esophageal tumors	Human	Corn	<i>Fusarium moniliforme</i>
Hepatocarcinoma (acute aflatoxicosis)	Human	Cereal grains, peanuts	<i>Aspergillus flavus</i> , <i>A. parasiticus</i>
Kashin Beck disease, "Urov disease"	Human	Cereal grains	<i>Fusarium</i>
Kwashiorkor	Human	Cereal grains	<i>Aspergillus flavus</i> , <i>A. parasiticus</i>
Onyalai	Human	Millet	<i>Phoma sorghina</i>
Reye's syndrome	Human	Cereal grains	<i>Aspergillus</i>
Stachybotryotoxicosis	Human, horse, other livestock	Hay, cereal grains, fodder (skin contact, inhaled haydust)	<i>Stachybotrys atra</i>

Therefore, we are largely dependent on results from experimental studies in animals.

Preharvest contamination of the commodity by toxigenic fungi can be carried over into and persist during storage. Historical accounts suggest that moldy grains and their toxic products have been responsible for major outbreaks of disease and are even considered events of the *Ten Plagues of Egypt* (Marr and Malloy 1996). Stored grains may be damaged by insects or moisture to the extent that a portal of entry is provided to the often-present toxigenic fungi in the storage environment.

Inhalation exposure can result from handling contaminated materials or from airborne fungal constituents, e.g., conidia. Industrial processes using fungi and the milling and other processing of grain for foods should be reviewed. Certainly, some environmental foci provide potential sources for airborne fungal inoculum of humans. Some mycotoxins are considerably dermanecrotic and exposure of skin or mucous membranes by contact can result in disease. Exposure of individuals to mycotoxins due to infection by a toxigenic fungus is problematic but is being investigated for the pathogenesis of certain infectious diseases.

The following section discusses mycotoxicoses for which there is considerable evidence for involvement of a specific mycotoxin(s). These and other human diseases where mycotoxin involvement is likely are presented in Table 5.1.

## Aflatoxicosis

The relationship of aflatoxins to disease in animals

has been extensively studied (Robens and Richard 1992). Although acute aflatoxicosis is well documented in humans (Krishnamachari et al. 1975a, b; Ngindu et al. 1982; Shank 1977), the relationship of aflatoxins to hepatocellular carcinoma and other human maladies is still being investigated. Several epidemiological studies have implicated aflatoxins in the increased incidence of human gastrointestinal (GI) and hepatic neoplasms in Africa, the Philippines, and China. Aflatoxin B<sub>1</sub> also has been implicated in human liver cell carcinoma (Berry 1988; Stark 1980).

### Acute Aflatoxicosis

Acute disease in humans resulting from aflatoxin ingestion has been manifested as an acute hepatitis (Krishnamachari et al. 1975a, 1975b; Ngindu et al. 1982; Shank 1977) usually associated with highly contaminated foodstuffs, especially corn. In some cases, exposure was sufficient to find aflatoxins in selected tissues and histopathologic evidence was convincingly adequate to allow for a diagnosis of aflatoxicosis. Typical but nonspecific changes in patients with acute aflatoxicosis include jaundice, low-grade fever, depression, anorexia, and diarrhea, with fatty degenerative changes in the liver evident, upon histopathologic examination, as centrilobular necrosis and fatty infiltration. Tenderness near the liver was evident in patients with acute, aflatoxin-caused hepatitis in Kenya; ascites may develop (Ngindu et al. 1982). Mortality reached 25% in outbreaks in India (Krishnamachari et al. 1975a, 1975b). Samples of liver obtained from patients that died contained detectable levels of aflatoxin B<sub>1</sub>.

Two human diseases of undefined etiology have

been linked to the consumption of aflatoxin-contaminated foods: kwashiorkor and Reye's syndrome. Kwashiorkor has been geographically associated with the seasonal occurrence and distribution of aflatoxin in food (Hendrickse et al. 1983). Animals given dietary aflatoxin possessed some of the same attributes of kwashiorkor, namely, hypoalbuminemia, fatty liver, and immunosuppression. Aflatoxins were detected in liver tissue taken at autopsy from 36 children with kwashiorkor (Hendrickse 1985), which has added to the credibility of establishing aflatoxin as the cause of this human disease with no other known etiology. Malnutrition may change the metabolism of dietary aflatoxin, resulting in its detection in some patients with kwashiorkor (DeVries 1989).

The etiology of Reye's syndrome, however, is more problematic. This disease, which includes an acute encephalopathy with fatty degeneration of the viscera, has been associated with aflatoxin because this mycotoxin has been found in Reye's syndrome patients in Thailand, New Zealand, the former Czechoslovakia, and the United States (Becroft and Webster 1972; Chaves-Carballo et al. 1976; Dvorackova et al. 1977; Ryan et al. 1979; Shank et al. 1971). Furthermore, aflatoxin B<sub>1</sub> produced a disease similar to Reye's syndrome in macaque monkeys (Bourgeois et al. 1971). However, Nelson et al. (1980) found no significant differences between matched controls and patients with Reye's syndrome relative to aflatoxins in serum and urine. Similar inconsistencies were found regarding the occurrence of aflatoxins in tissues and Reye's syndrome in patients (Rogan et al. 1985). Also, the U.S. cases seem to lack any geographical relationship to aflatoxin exposure (Hurwitz 1989). Thus, the cause-and-effect relationship of aflatoxins with Reye's syndrome has not been adequately established. Again, Reye's syndrome, which involves the liver, may change the metabolism of dietary aflatoxin in some patients.

### Chronic Aflatoxicosis

Chronic aflatoxicosis in humans usually implies the association of this mycotoxin with hepatocellular carcinoma. Several epidemiological studies in countries or localities with a high incidence of liver cancer examined the relevance of dietary aflatoxin and other factors with this disease (Henry et al. 1999). Most of the studies, which occurred mainly before 1980, sought to determine dietary levels of aflatoxin B<sub>1</sub> and correlate them with occurrence of hepatocellular carcinoma. These included studies by Shank et al. (1972) in Thailand, Peers and Linsell (1973) in

Kenya, van Rensburg et al. (1974) in Mozambique, Peers et al. (1976) in Swaziland, and van Rensburg et al. (1985) in Mozambique/Transkei. Some studies included measurement of aflatoxin M<sub>1</sub> (a metabolite of aflatoxin B<sub>1</sub>) in the urine (Campbell et al. 1970). After Essigmann et al. (1977) determined that aflatoxin B<sub>1</sub> can form a DNA adduct whose excision product, aflatoxin B<sub>1</sub>-guanine, occurs in urine, Autrup et al. (1983) found this product in urine from individuals from high liver cancer risk areas who were presumably exposed to aflatoxin. Sabbioni et al. (1987) discovered that aflatoxin B<sub>1</sub> binds to proteins and forms a lysine adduct that can be found in serum of individuals for a considerable period of time (half-life = 20 days) (Groopman et al. 1988) after consuming aflatoxin B<sub>1</sub>. Monoclonal antibodies developed to quantify aflatoxin B<sub>1</sub> adducts in humans (Groopman and Donahue 1988; Groopman et al. 1988) were used in attempts to measure exposure of populations to aflatoxins.

Some earlier studies were criticized for not considering exposure of the studied populations to hepatitis B virus (HBV). Most post-1980 research examined the hepatitis B surface antigen (HBsAg) as well as aflatoxin exposure in relation to incidence of hepatocellular carcinoma. Most found an aflatoxin effect independent of HBsAg prevalence (Peers et al. 1987; Sun and Chu 1984; Yeh et al. 1989). When all ethnic, social, and cultural groups were included, Autrup et al. (1987) found no aflatoxin effect on liver cancer but an independent assessment of the Bantu people found a positive correlation. A subsequent study by Campbell et al. (1990) in the People's Republic of China correlated liver cancer with HBsAg but not to aflatoxin exposure.

More recently, biomarkers have been used to understand the etiology of tumors, such as mutations of the tumor suppressor gene, p53, which is commonly mutated in human cancers. This development is reviewed by Scholl and Groopman (1995).

The outcome is that aflatoxin has been linked to specific p53 mutations where there is a G → T transversion in the third position of codon 249. These specific mutations in tumors can provide important evidence as to their cause. The armament of biomarkers substantially benefits epidemiological studies into the relationship of aflatoxins and human hepatocellular carcinoma. These markers were used by Ross et al. (1992) and Qian et al. (1994) in Shanghai, where results demonstrated that a specific biomarker for aflatoxin is related to human liver cancer and that HBV and aflatoxin B<sub>1</sub> interact as risk factors for liver cancer (Scholl and Groopman 1995).

Table 5.2. Ochratoxin in tissue and fluids of humans from various countries

Tissue or fluid	Country	No. of positive/no. of samples	Range or mean
Serum	Yugoslavia	42/639	1–57 ng/ml
Serum	Poland	77/1065	$\bar{x}$ = 0.27 ng/ml
Serum	Germany	173/306	$\bar{x}$ = 0.6 ng/ml
			0.1–14.4 ng/ml
Plasma	Denmark	46/96	0.1–9.2 ng/ml
Plasma	Bulgaria	45/312	$\bar{x}$ = 14 ng/ml
Serum	Bulgaria	110/576	$\bar{x}$ = 18 ng/g
Milk	Germany	4/36	0.017–0.3 ng/ml
Milk	Italy	9/50	1.7–6.6 ng/ml
Blood	Canada	63/159	0.27–35.33 ng/ml (mean conc. range)
Serum	France	≤ 22%	0.1–6 ng/ml

From Creppy et al. 1991; Frolich et al. 1991; Golinski et al. 1991; Hald 1991; Micco et al. 1991; Petkova-Bocharova and Castegnaro 1991.

## Ochratoxicosis

Ochratoxin A, produced primarily by *Aspergillus ochraceus* or *Penicillium verrucosum*, occurs on several commodities prevalent in human diets, including barley and green coffee beans. Known for its nephrotoxic effects, ochratoxin A can also impact the liver (Council for Agricultural Science and Technology 1989). A major renal disease of swine known as porcine nephropathy occurs in some European countries, especially Denmark, and is associated with consumption of ochratoxin-contaminated barley (Hald 1991). Most effects of ochratoxin A were discovered during experiments with swine that, when intoxicated, exhibited pain near the kidneys, drank excessive water, appeared depressed, urinated almost continuously, and ate less. The impaired renal function, which is characterized histopathologically as a tubular degeneration and atrophy with interstitial fibrosis and often hyalinization of the glomeruli, results in glucosuria and proteinuria, with casts evident in the urine (Hald 1991).

In 1956, the first clinical description of a human kidney disease of unknown etiology known as Balkan endemic nephropathy was published (Tanchev and Dorossiev 1991). With the recognition that mycotoxins can cause nephropathies and the epidemiological evidence of ochratoxin A in food of patients in the Balkan countries, ochratoxin A became a prime suspect in the causation of this disease. Furthermore, because ochratoxin A is carcinogenic in rats and mice and patients with Balkan endemic nephropathy frequently have kidney tumors, ochratoxin has been more strongly linked to this disease. However, researchers also have found the fungus (*P. aurantiogriseum*) to be common in food from endemic areas in the former Yugoslavia, Bulgaria, and Romania (Barnes et al. 1977). While this fungus caused nephrotox-

icity in rats, ochratoxin A was not a known metabolite of one of the major isolates examined (Yeulet et al. 1988). This has led to the search for another mycotoxin as a potential cause for Balkan endemic nephropathy (MacGeorge and Mantle 1991). Epidemiological evidence suggests that approximately half of the European population is exposed to ochratoxin A, and estimates of human exposure of Europeans have been published (European Commission 1997; Fink-Gremmels et al. 1995a, b). Table 5.2 compiles data illustrating levels of ochratoxin that can occur in human tissues and fluids from various countries.

The airborne nature of either ochratoxin or particles laden with this mycotoxin is now considered a potential risk for human exposure (Richard et al. 1999). Acute renal failure occurred in a female agriculture worker who had been exposed to granary and grain dust from wheat contaminated with *Aspergillus ochraceus* and its metabolite ochratoxin A. Although ochratoxin A was not demonstrated in granary air samples, guinea pigs and rabbits experimentally exposed to air passed through a layer of the ochratoxin-contaminated wheat experienced renal failure (DiPaolo et al. 1993). Interestingly, high levels of ochratoxin A ( $\geq 1,500 \mu\text{g}/\text{kg}$ ) were found in dust samples obtained from heating ducts in a household in which occupants (humans and animal) had complained or exhibited signs of recurring health problems (Richard et al. 1999). Subsequently ochratoxin A was found in dust collected from cow barns in Norway (Skaug et al. 2001).

Ochratoxin A biotransformation is cytochrome P450 dependent in animals and humans and results in the formation of metabolic intermediates active in the carcinogenic and other toxic activities of ochratoxin A (Fink-Gremmels et al. 1995a, b). Phenylalanine, a structural component of ochratoxin A, is likely involved in the complex toxicological activities of the

parent compound. The dihydroisocoumarin moiety is likely involved in as-yet-undetermined toxic activities of ochratoxin in humans and animals. Animal studies by Marquardt and Frolich (1992) indicate that ochratoxin is absorbed in the GI tract and in the proximal and distal tubules. It enters the enterohepatic circulation and can be excreted and reabsorbed. It can bind to the albumin fraction in blood and thus can persist in animal tissues for extended periods of time. Intestinal microflora can convert ochratoxin A into ochratoxin alpha (Pitout 1969), a nontoxic metabolite that can be measured in the urine and feces; small amounts of the parent compound are usually present also.

While most evidence for the causal relationship of Balkan endemic nephropathy points toward ochratoxin A, data are not conclusive and further work is needed to substantiate claims in this nephrotoxic disease.

## Trichothecene Toxicoses

The trichothecenes are the largest family of known mycotoxins, chemically called sesquiterpenoids. *Fusarium* species are the most notable fungi that produce trichothecenes, although species of other genera, e.g., *Trichothecium*, *Trichoderma*, *Myrothecium*, *Stachybotrys*, also are important producers of these compounds. The trichothecenes are potent protein inhibitors, a basic mechanism of their toxicity, and have been shown to produce a wide variety of effects in experimental animal studies (Richard 1998).

### Alimentary Toxic Aleukia

In Russia during the first half of the twentieth century, a disease known as alimentary toxic aleukia (ATA) occurred — particularly in the Orenburg District in 1944 — that was characterized by total atrophy of the bone marrow, agranulocytosis, necrotic angina, sepsis, hemorrhagic diathesis, and mortality reaching 80% (Joffe 1978). Clinical stages of the disease have been described, with progressive severity of signs and symptoms in the patients (Nelson et al. 1994). Patients experienced vomiting, diarrhea, abdominal pain, and burning in the upper GI tract; onset could occur shortly after consuming contaminated food. Later, petechial hemorrhages developed on the skin, often accompanied by hemorrhages in the oral cavity, followed by development of necrotic lesions and enlargement of the local lymph nodes. Joffe (1986) noted that treatments employed for ATA vic-

tims were blood transfusion, administering nucleic acid and calcium preparations, antibiotics, vitamins C and K, and dietary improvement. This disease was determined to be caused by consuming overwintered cereal grains or their products. Two common organisms isolated from the grains after over 20 years of storage were *Fusarium poae* and *Fusarium sporotrichioides*, which subsequently were shown capable of producing trichothecenes such as T-2 toxin, neosolanol, HT-2 toxin, and T-2 tetraol.

Some signs of ATA could be reproduced in cats given purified T-2 toxin orally. Due to a lack of proof of the involvement of trichothecenes in the original outbreak, they remain conjecturally associated with the disease. However, most signs of ATA have been well documented in animals given T-2 toxin, the major toxic component from the two fungi isolated from the overwintered grain (Joffe and Yagen 1977).

### Stachybotryotoxicoses

While this disease has been known for some time to occur in horses and cattle consuming *Stachybotrys*-contaminated hay (Forgacs 1972), more recently, involvement of toxins (most notably, macrocyclic trichothecenes) from this genus of fungi has become suspect in illnesses of humans occupying *Stachybotrys*-contaminated buildings (Dearborn et al. 1999). This cellulolytic fungus can grow on moist cellulose products in buildings. The consumption of contaminated hay by horses and cattle causes an acute manifestation of disease characterized by neurological signs such as tremors, incoordination, impairment or loss of vision, or, more chronically, dermonecrosis, leukopenia, and GI ulceration and hemorrhage. Humans handling contaminated hay exhibited dermatitis, while inhalation of hay dust caused inflammation of the nose, fever, chest pain, and leukopenia. Although macrocyclic trichothecenes were isolated from the hay in the human and animal intoxications, illnesses in humans occupying *Stachybotrys*-contaminated buildings have not been proved to be causally related to these toxic products (Jarvis 1990; Robbins et al. 2000). Individuals living in and personnel working in a house contaminated with *S. atra* developed pulmonary irritation and headaches, fatigue, malaise, and diarrhea (Croft et al. 1986). Samples of air duct dust and ceiling fiber board covered with *S. chartarum* from a problem residence yielded the trichothecenes verrucarol, verrucarins, satratoxin H, and trichoverrins (Hendry and Cole 1993). Airborne particles of contaminated materials can be collected on membrane filters

(polycarbonate) and trichothecenes can be identified from analysis of membrane-collected materials (Pasanen et al. 1993).

### Glomerulonephritis

Among the most frequently isolated trichothecenes from grains and foods is DON, a metabolite of the plant pathogenic fungi *Fusarium graminearum* and *F. culmorum*. This compound was shown to be indirectly associated with a nephropathy in a mouse model (Pestka et al. 1989). Investigators found that DON increased immunoglobulin A (IgA) levels in the sera of mice, resulting in mesangial accumulation of this immunoglobulin and a disease similar to human glomerulonephropathy. Involvement of DON in the latter disease is speculative. However, relative to this toxin and not to glomerulonephritis, an outbreak of intoxication in humans who consumed bread made from mold-damaged wheat in the Kashmir Valley, India, implicated DON as the etiologic agent (Bhat et al. 1989). Symptoms of the disease included nausea, GI distress or pain, vomiting, and throat irritation. Some patients had blood in their stools or developed a rash.

Symptoms in humans — including headaches, vomiting, and diarrhea — have been attributed to a variety of trichothecenes from cereal grains with red-mold disease (akakabi-byo) and black-spot disease (kokuten-byo). Multiple causation, including mycotoxins, has been suggested for these sporadic episodes of human disease (Ueno 1984a, b).

### Citreoviridin Toxicosis

Acute cardiac beriberi or “shoshin kakke,” a disease that occurred for centuries, including the early twentieth century in Japan and other Asian countries, was characterized by palpitations, nausea, vomiting, rapid and difficult breathing, rapid pulse, abnormal heart sounds, low blood pressure, restlessness, and violent mania leading to respiratory failure and death (Ueno 1974).

The disease was regarded as either an infection or avitaminosis until a fungus was isolated (Miyake and Igaku 1943, cited in Ueno 1974), identified (Naito 1964, cited in Ueno 1974), an extract component identified (Hirata 1949), and its structure elucidated (Sakabe et al. 1964). This dark yellow compound was called citreoviridin and the neurologic syndrome and respiratory failure were reproduced in laboratory

animals (Ueno and Ueno 1972). The Rice Act of 1921 passed by the Japanese government decreased the availability of moldy rice in the markets and resulted in rapid decline of the disease. Improved diet and inspection have made cardiac beriberi of little importance in modern times. However, it should be recognized that substantial quantities of citreoviridin can occur in corn infected with *Eupenicillium ochrosalmoneum* (Wicklow et al. 1988).

### Ergotism

The oldest recognized mycotoxicosis of humans is ergotism. After periodic outbreaks in central Europe, the disease became epidemic in the Middle Ages, where it was known as St. Anthony's fire (Matossian 1989; van Rensburg and Altenkirk 1974). Gangrenous symptoms were described in medieval episodes of ergotism in humans, where early symptoms were swollen limbs with burning sensations, with subsequent necrosis leading to loss of appendages (see cover photo). Ergotism results from consumption of products made with grains contaminated with ergots. The ergots or sclerotia are often larger than the normal grain, are typically black in color, and may replace several grains in one spike or head of the respective grain. Because the sclerotia may be inadvertently included in grains processed for human consumption, there is a potential threat for human disease and the need for continued surveillance of grains used for such purposes.

Two different manifestations of ergotism are likely due to different modes of action of the various ergot alkaloids produced by the different species of *Claviceps*. Convulsive and gangrenous ergotism are described in the literature (Beardall and Miller 1994). The gangrenous form likely results from the vasoconstrictive action of certain alkaloids belonging primarily to the ergotamine group, associated primarily with wheat and rye. Edema, pruritis, necrotic extremities, prickling sensations, and severe muscular pain are associated with the gangrenous form of ergotism (van Rensburg and Altenkirk 1974). The ergot of pearl millet involved in an outbreak of the convulsive form in India in 1975 contained alkaloids of the clavine group (Krisnamachari and Bhat 1976). No manifestations of circulatory effects were noted in the latter outbreak. Clinical evidence of convulsive ergotism includes tingling under the skin, pruritis, numbness of extremities, muscle cramps, convulsions, and hallucinations (van Rensburg and Altenkirk 1974).

Wheat and rye infections are usually caused by *C. purpurea*, while the 1975 outbreak in Indian pearl millet was caused by *C. fusiformis*. Apparently, different species produce different alkaloids. The classification of alkaloids has been reviewed by Ninomiya and Kiguchi (1990) and Rehacek and Sajdl (1990).

Attempts to associate disease severity with quantity of sclerotia were unsuccessful, as alkaloid concentration varies among sclerotia and only a chemical quantitation of total alkaloids is meaningful relative to form and severity of ergotism (Krisnamachari and Bhat 1976).

Two human outbreaks have occurred in recent times. In Africa, 93 cases of gangrenous ergotism were reported involving grain infected with *C. purpurea* (Demeke et al. 1979), while 78 cases of GI ergotism involving millet infected with *C. fusiformis* occurred in India (Krishnamachari and Bhat 1976).

## Zearalenone Toxicosis

Little conclusive information is available regarding the effects of zearalenone on humans. However, concern for ingestion of this estrogenic compound has increased due to popular press citations of studies where premature puberty occurred in 7- and 8-year-old children (Painter 1997). This precocious development included premature thelarche, premature pubarche, prepubertal breast enlargement in boys, and pseudopuberty in girls in epidemic proportions in Puerto Rico (Saenz de Rodriguez et al. 1985). Researchers examined local food and found high concentrations of an estradiol equivalent in some of the meat. There was concern that exposure could have been from mothers who ingested contaminated food during pregnancy. Exogenous food contamination was also suspected and considered the most likely source of exposure to the estrogen-like substance. Zearalanol (Ralgro®), a processed anabolic product made from zearalenone and approved for use as an anabolic agent in cattle and sheep, is used in Puerto Rico. It was discovered that FDA recommendations that animals implanted with this agent be kept from slaughter for at least 60 days postimplantation were not observed because farmers did not consider it as a hormonal product. Earlier, Hsieh (1989) suggested that zearalenone was possibly involved in human cervical cancer and premature thelarche. More recently, Szuets and colleagues (1997) measured zearalenone in 5 of 36 patients exhibiting thelarche in Hungary and found high concentrations of zearalenol in the cereal foods of the patients. They discovered that both zearale-

none and zearalenol bind to estrogen receptors of human myometrial tissue.

Evidence exists for the lasting effects of zearalenone on the endocrine system. Furthermore, toxicological responses to natural toxins such as zearalenone, aflatoxins, or certain trichothecenes can be modulated by endocrine metabolites, leading to increased responses to the toxin or perhaps protection against toxicity (Harvey et al. 1994c). Glucocorticosteroids can induce cytochrome P-450 enzymes, which are potentially active in certain mycotoxin metabolic schemes (Ortiz de Montellano 1986; Ruckpaul and Rein 1989; Timbrell 1991). Chentanez and coworkers (1988) found that the human glucocorticosteroid cortisol exacerbated the hepatotoxicity of aflatoxin B<sub>1</sub>. Further research is necessary to elucidate interactions of mycotoxins with the endocrine system and to find the true nature and source of causative compound(s) in precocious development.

## Fumonisin Toxicosis

An association between consumption of moldy corn and the high human esophageal cancer rates found in parts of southern Africa (Marasas 1993, 1996; Marasas et al. 1979, 1981) and the Linxian area of China (Li et al. 1980; Yang 1980) has been known for some time. Esophageal cancer rates and the consumption of corn products, which likely contain fumonisins (Doko and Visconti 1994), have simultaneously increased in northeastern Italy in recent years (Franceschi et al. 1995). Various *Fusarium* species are common on corn worldwide, but it is the consumption of food prepared from "home-grown" *F. verticillioides* (syn., *F. moniliforme*)-contaminated corn that has been correlated with esophageal dysplasia (suggesting a precancerous condition) and esophageal cancer in southern Africa and China (Jaskiewicz et al. 1987; Marasas 1993; Marasas et al. 1979, 1981, 1988a; Yang 1980).

Marasas et al. (1984a) found esophageal hyperplasia in rats fed diets supplemented with *F. verticillioides* culture material (ground, moldy corn produced in the laboratory under controlled conditions), while van Rensburg et al. (1982) showed that *F. verticillioides* culture material enhanced nitrosamine-induced tumorigenicity in the esophagus of rats. Others found epithelial hyperplasia, papillomas, and carcinomas in the forestomach of rats fed *F. verticillioides* culture material (Jaskiewicz et al. 1986) or *F. verticillioides*-inoculated corn bread containing nitrosamines, including N-methyl-N-benzyl nitrosamine (Li et al.

1982). Interestingly, nitrosamines and nitrosamine precursors are also present in foods from a high esophageal cancer area of China (Yang 1980), further suggesting that nitrosamines may contribute to esophageal cancer, perhaps as cancer initiators. *Fusarium verticillioides* culture materials of strains MRC 602 and 826 are hepatocarcinogenic in rats (Jaskiewicz et al. 1986; Marasas et al. 1984a). Not all fungi identified as *F. verticillioides*, however, elicit the same effects, as illustrated by strain MRC 1069 culture material, which was not hepatocarcinogenic in rat feeding studies (Jaskiewicz et al. 1986).

*Fusarium* spp. produce a number of mycotoxins. One of these, the highly mutagenic compound fusarin C (Gelderblom et al. 1988a; Lu et al. 1988), has received attention as a possible carcinogen. However, when *F. verticillioides* cultures containing high or low levels of fusarin C were fed to rats, only the "low fusarin C" culture material was hepatocarcinogenic (Jaskiewicz et al. 1986). Fusarin C's instability during cooking under neutral or slightly basic conditions (Zhu and Jeffrey 1992) and its rapid conjugation and excretion by the liver (Gelderblom et al. 1988a) further argue against fusarin C being a carcinogen. On the other hand, fusarin C is stable at the low pH encountered under some cooking conditions or in the stomach and, therefore, until the factor(s) causing esophageal cancer in southern Africa and China are positively identified, fusarin C should not be dismissed as a possible contributing factor (Zhu and Jeffrey 1992).

In 1988, Gelderblom et al. (1988b) discovered a class of mycotoxins called fumonisins. They are produced by *F. verticillioides*, *F. proliferatum*, and at least one strain of *F. nygamai* (reviewed by Marasas 1996) and have worldwide distribution on corn. Fumonisin B<sub>1</sub>, the most common homologue, is the most thoroughly studied. Unlike fusarin C, fumonisin B<sub>1</sub> does cause diseases attributed to *F. verticillioides* including leukoencephalomalacia in horses (Kellerman et al. 1990; Marasas et al. 1988b); pulmonary edema in swine (Colvin and Harrison 1992); and hepato- and nephrotoxicity in rats (Gelderblom et al. 1988b; Voss et al. 1993a, 1995), mice (Sharma et al. 1997; Voss et al. 1995), and rabbits (Gumprecht et al. 1995).

Fumonisin B<sub>1</sub> and other fumonisins with a primary amino group are liver cancer promoters in rats (Gelderblom et al. 1993), and fumonisin B<sub>1</sub> (50 ppm, ≥90% purity) caused hepatocarcinomas when fed to male BD IX rats for 26 months (Gelderblom et al. 1991). However, when fed to Fischer 344/N/Netr rats for two years, fumonisin B<sub>1</sub> (≥92% purity) did not cause liver tumors but rather was a kidney carcino-

gen, causing renal tubule adenomas and carcinomas in males fed 50 or 150 ppm. Focal tubular hyperplasia also was increased in males fed at least 50 ppm for two years. Apoptosis, a type of programmed cell death, was increased in males fed ≥15 ppm for up to 26 weeks. At that time, cell proliferation in the renal tubules was increased in males fed 50 or 150 ppm and was marginally increased in females fed 100 ppm (National Toxicology Program 1999). Fumonisin B<sub>1</sub> was not carcinogenic to female rats. In mice, the incidence of hepatocellular adenoma and carcinoma was increased in females fed 50 or 80 ppm fumonisin B<sub>1</sub>. Increased apoptosis was also seen in these groups and hepatocellular hypertrophy was found at two years. The latter occurred in males fed 15, 80, or 150 ppm and in females fed 50 or 80 ppm (National Toxicology Program 1999). Although fumonisin B<sub>1</sub> was clearly carcinogenic to rodents when fed at levels of 50 ppm or more, the reasons for the differences in sex-related response and target organs found in the two studies (Gelderblom et al. 1991; National Toxicology Program 1999) are unknown. Resolving this issue is important and may contribute significantly to assessing the relationship of fumonisins to human health (Gelderblom et al. 1996). There were no significant esophageal changes in either rats or mice after two years, and efforts to enhance nitrosamine-induced esophageal neoplasia in rats with fumonisin B<sub>1</sub> were unsuccessful (Wild et al. 1997).

In liver and kidney, apoptosis is the initial, and sometimes only, effect seen in fumonisin-exposed animals. Only at higher doses or after longer exposures do the other features of fumonisin toxicity, e.g., overt necrosis, mitosis and regeneration, fibrosis, become evident (reviewed by Voss et al. 1996a). This is important because apoptosis may play a key role in cancer promotion or carcinogenesis and is induced by alterations in cellular sphingoid bases or sphingolipids (Merrill et al. 1997). Because they inhibit the enzyme ceramide synthase (Wang et al. 1991; Yoo et al. 1992, 1996), fumonisins have pronounced effects on cellular sphingolipid metabolism that, in turn, may alter sphingolipid-mediated regulatory processes related to cell survival and replication. Tolleson et al. (1996) found that fumonisin B<sub>1</sub> (100 μM) inhibited growth and induced morphological features consistent with apoptosis in HET-1A (an SV-40 T-antigen-immortalized human esophageal epithelial cell line) and other human cells in vitro, suggesting that fumonisins may cause esophageal epithelial apoptosis in humans.

It has been suggested that fumonisins play a role in human diseases other than esophageal cancer. Fumonisins in *F. verticillioides* culture material disrupt



sphingolipid metabolism in nonhuman primates (Shephard et al. 1996) and, as in other species, this may be the mechanistic trigger for hepatotoxicity in vervet monkeys fed *F. verticillioides* culture material (Jaskiewicz et al. 1987). Interestingly, atherogenic serum lipid profiles, including increased cholesterol, were found in vervets fed *F. verticillioides* culture material, suggesting that fumonisins may, under some conditions, contribute to human atherosclerotic vascular disease, either directly or as a secondary effect of hepatotoxicity (Fincham et al. 1992).

Whether fumonisins play a role in human esophageal cancer or other diseases is far from being resolved. A number of factors must be considered, including the possibility that fumonisin derivatives may also be toxic. Under alkaline conditions used in nixtamalization, i.e., the process used to make masa flour from corn, fumonisins may be converted to their hydrolyzed forms. Nixtamalized culture materials containing hydrolyzed fumonisin B<sub>1</sub> but no measurable fumonisin B<sub>1</sub> elicited the same liver cancer-promoting activity (Hendrich et al. 1993), liver and kidney toxicities (Voss et al. 1996b), and in vivo sphingolipid effects (Voss et al. 1998) in rats as did untreated culture materials or purified fumonisin B<sub>1</sub>. Fumonisins also may exert effects by other, indirect mechanisms. It has been proposed that neural tube defects in newborns from southern Texas may have been mediated by a fumonisin or hydrolyzed fumonisin-induced deficiency in a critical folate receptor (Flynn et al. 1997; Stevens and Tang 1997). Fumonisin B<sub>1</sub> was nevertheless not teratogenic to rats (Collins et al. 1998a, 1998b), mice (Reddy et al. 1996), or rabbits (LaBorde et al. 1997).

As with *F. verticillioides*, epidemiological surveys of fumonisin B<sub>1</sub> suggest but do not prove a relationship with human cancer. Populations from "high-risk" (for esophageal cancer) areas in southern Africa and China have a relatively high fumonisin exposure, compared to populations from nearby "lower-risk" areas in which corn is also a dietary staple (Chu and Li 1994; Rheeder et al. 1992; Yoshizawa et al. 1994). In Italy, some corn-based foodstuffs have been found to contain relatively high fumonisin levels (Doko and Viconti 1994), suggesting that the ongoing exposure to fumonisins in polenta, a popular food in the northeastern part of the country, may contribute to the rising esophageal cancer rates in this region. In response to these concerns about known and suspected health effects of fumonisins, the U.S. FDA recently issued a draft guidance for fumonisin levels (total FB<sub>1</sub> + FB<sub>2</sub> + FB<sub>3</sub>) in foods and feeds (U.S. Food and Drug Administration 2000a). The recommendations for human

foods vary by product type and range from 2 ppm in some degermed, dry-milled products to 4 ppm in other dry-milled products and corn intended for masa production.

In conclusion, fumonisin B<sub>1</sub> is toxic and carcinogenic to rodents and there are data suggesting that fumonisins or *F. verticillioides* cause esophageal cancer or other human health problems. However, an International Agency for Research on Cancer (IARC) working group on fumonisins concluded that there is "inadequate evidence" for carcinogenicity in humans from oral exposure to fumonisin B<sub>1</sub> (International Agency for Research on Cancer 1993) and a role for fumonisins in any other human disease has likewise not been proven. Additional research on the relationships between fumonisins, sphingolipid metabolism disruption, and apoptosis is critical for understanding the experimental carcinogenicity of fumonisins and determining the relevance of these mycotoxins to human health.

## Gliotoxin Toxicosis

Gliotoxin is an immunosuppressive mycotoxin that first attracted the interest of investigators searching for new antibiotics. However, its toxicity precluded clinical use as an antibiotic. Interest in gliotoxin was revived when it was discovered to be produced by *Aspergillus fumigatus* and other fungi such as *Candida albicans* (Shah and Larsen 1991) and that it had unusual immunosuppressive activities (Mullbacher et al. 1985). Of significance to the medical and veterinary mycologist is gliotoxin's possible involvement in the pathogenesis of diseases such as aspergillosis. Gliotoxin is produced in mice experimentally infected with *A. fumigatus* (Eichner et al. 1988), in natural bovine udder infection (Bauer et al. 1989), and both experimentally and naturally infected turkeys (Richard and DeBey 1995; Richard et al. 1996a). This toxin has been found in vaginal secretions of women with *Candida vaginitis* (Shah et al. 1995). The involvement of gliotoxin in the pathogenesis of aspergillosis and candidiasis could be important because the immunosuppressive nature of this toxin could exacerbate the infection and possibly be a virulence factor. This suggestion has been made regarding human infections caused by *Fusarium* spp., as these are known to produce several immunosuppressive compounds. However, their production has not been demonstrated during the pathogenic state in humans or animals (Nelson et al. 1994).

The medical mycologist should consider this aspect of infections caused by any toxigenic fungus, especially those that produce immunosuppressive compounds (Richard 1991). The significance of gliotoxin in edible tissue is unknown, although a recent report found that contaminated hay caused intoxication in camels, gliotoxin does not appear to be important as an ingested mycotoxin (Gareis and Wernery 1994).

## Immunomodulation

Immunosuppression is a likely major economic effect of mycotoxins. Mycotoxins known to have this effect are aflatoxins, certain trichothecenes, ochratoxin A, and gliotoxin. Immunosuppression by mycotoxins, studied primarily in animal experiments, may involve specific classes of immunoglobulins, or antibodies if the amount of mycotoxin consumed is sufficient. However, the major effects appear to involve cellular immune phenomena and nonspecific humoral factors associated with immunity (Richard 1991). These mycotoxins can cause a variety of immune-related changes, including thymic aplasia and inhibition of phagocytosis by macrophages, delayed cutaneous hypersensitivity, lymphocyte proliferation, and leukocyte migration. The involvement of specific mycotoxins in infectious diseases depends on the agent of disease, the toxin dose and constitution, the animal species, and perhaps the sensitivity of the test employed.

One of the most consistent features of the effects of the aflatoxins on immunity is the decrease of cell-mediated immunity. Aflatoxins have been shown to affect the production of cytokines important in relating certain immune processes by a number of cell types. While aflatoxins are not notable in impacting antibody production, they have an important effect on levels of nonspecific humoral factors such as complement, interferon, and some bactericidal serum components. Increased susceptibility to yeast infections,

pasteurellosis, and salmonellosis has been demonstrated in poultry. Aflatoxins can move transplacentally in swine, immunocompromising neonatal piglets. Chicken embryos can exhibit depressed immune responses when exposed to aflatoxin B<sub>1</sub>. Trichothecenes generally decrease the serum proteins (other than the increase in IgA levels discussed earlier with deoxynivalenol in the mouse). The trichothecenes usually decrease phagocytosis and delayed hypersensitivity; exceptions do occur, as has been found with delayed hypersensitivity where small amounts of T-2 toxin were given to mice subcutaneously and in phagocytosis by macrophages if T-2 toxin is given prior to immunization (Richard 1991).

Trichothecenes administered *in vivo* and *in vitro* can decrease the response of lymphocytes to mitogens. However, in some cases, low levels of certain toxins appear to function as mitogens, evident with T-2 toxin and deoxynivalenol in that at low concentrations in *in vitro* assays, they increased lymphocyte proliferation. The trichothecenes, especially T-2 toxin, have resulted in decreased resistance to a variety of organisms. However, when intraperitoneal injections of T-2 toxin were given to mice a few days prior to inoculation with *Listeria monocytogenes*, an enhanced resistance to disease resulted. This did not occur when the toxin was given after inoculation with the organism. Notable among the effects of ochratoxin was decreased natural killer cell activity. This decrease was overcome if production of interferon, a component decreased by ochratoxin A, was stimulated with Poly I:C treatment. Ochratoxin A is an inhibitor of protein synthesis and likely contributes to the overall effect of decreased humoral factors, especially immunoglobulins, by this mycotoxin. Some discrepancies regarding the effect of ochratoxin on complement activity may be related to species differences or dietary factors such as phenylalanine.

For a thorough discussion of the immunotoxicity of mycotoxins, see Richard (1991) and Pestka and Bondy (1994a, b).

## 6 Mycotoxicoses of Animals

### Summary

Fungal toxins produce a wide range of injurious effects in animals, in addition to posing foodborne hazards to humans. The economic impact of decreased productivity, subtle but chronic damage to vital organs and tissues, increased disease incidence because of immune suppression, and interference with reproductive capacity is many times greater than that of acute livestock death. Mycotoxins can be classified according to the organ systems they affect. At least one mycotoxin affects each system in the animal body as a direct or indirect mechanism of toxicity. Several important mycotoxins affect the same system, e.g., the immune system, and a given mycotoxin may affect several systems concurrently.

Aflatoxins cause liver damage including cancer, decreased milk and egg production, and suppression of immunity in animals consuming low dietary concentrations. While the young are most susceptible, all ages are affected; clinical signs include GI dysfunction, decreased 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 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 anorexia, weight loss, poor feed utilization, vomiting, bloody diarrhea, abortion, and death. Dysregulated immune and neuroendocrine function are significant features of certain trichothecene intoxications.

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

Fumonisin affect sphingolipid metabolism and cause diseases in a species-specific fashion. Ingestion of fumonisin-contaminated feed causes neurotoxic ef-

fects in horses, pulmonary edema in swine, as well as liver and kidney damage including tumors and cancer in certain species.

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

Other effects have been attributed to mycotoxins. 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 toxin, zearalenone, and sterigmatocystin.

Nervous system functions are altered adversely by several mycotoxins, inducing such clinical signs as tremors, uncoordinated movements, limb weakness, 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, i.e., aflatoxins, ochratoxins, fumonisins, are known to induce or promote tumors in at least two species. Cancers have developed in liver, kidney, urinary system, digestive tract, and lung.

The range and potency of mycotoxins make this group of naturally occurring toxins an ongoing animal health hazard and a constant risk for contamination of the food supply. These problems have become increasingly complex because of the multiplicity of effects, the interaction of affected systems and more than one mycotoxin, the basic metabolic pathways affected, and the physiopathologic nature of the intoxications. While understanding individual mycotoxins is simplified by using a systems approach, the true nature of such intoxications requires a more holistic comprehension of the complexities of the chemical-

Table 6.1. LD<sub>50</sub><sup>a</sup> of selected mycotoxins

Toxin	Animal	Sex	Age/Size	Route	LD <sub>50</sub>	Reference
Aflatoxin B <sub>1</sub>	Duckling	M	Day-old	PO <sup>b</sup>	0.37 mg/kg	Butler 1964; Wogan 1965
	Rat	M-F	Day-old	PO	1.0 mg/kg	
	Rat	M	21 days	PO	5.5 mg/kg	
	Rat	F	21 days	PO	7.4 mg/kg	
	Rat	M	100 g	IP <sup>c</sup>	6.0 mg/kg	
	Rat	F	150 g	PO	17.9 mg/kg	
	Dog	M-F	Adult	IP	ca. 1.0 mg/kg <sup>d</sup>	
	Dog	M-F	Adult	PO	ca. 0.5 mg/kg	
	Hamster	M	30 days	PO	10.2 mg/kg	
Aflatoxin B <sub>2</sub>	?					
Aflatoxin G <sub>1</sub>	Duckling	M-F	Day-old	PO	0.79 mg/kg	Lijinsky and Butler 1966
Aflatoxin G <sub>2</sub>	Duckling	M-F	Day-old	PO	172.5 µg/duckling	Lijinsky and Butler 1966
Aflatoxin M <sub>1</sub>	Duckling	M-F	Day-old	PO	16.6 µg/duckling	Purchase 1967
Aflatoxicol	?					
Aflatrem	?					
Citreoviridin	ddys <sup>e</sup> mice	M	—	SC <sup>f</sup>	11.0 mg/kg	Ueno and Ueno 1972
	ddys mice	M	—	IP	7.2 mg/kg	
	ddys mice	M	—	PO	29.0 mg/kg	
Citrinin	Rat	—	—	SC/IP	67.0 mg/kg	Ambrose and DeEds 1946
	Mice	—	—	SC/IP	35.0 mg/kg	
	Guinea pig	—	—	SC	37.0 mg/kg	
	Rabbit	—	—	IV <sup>g</sup>	19.0 mg/kg	
Cyclopiazonic acid	Rat	M	—	IP	2.3 mg/kg	Purchase 1971; Wilson et al. 1989
	Rat	M	—	PO	36.0 mg/kg	
	Rat	F	—	PO	63.0 mg/kg	
	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. 1979; Cole 1973
	Mice	M	25 g	IP	29.1 mg/kg	
	Mice	F	25 g	IP	20.9 mg/kg	
Ochratoxin A	Rat	—	Weanling	PO	22.0 mg/kg	Doster et al. 1974
	Trout	—	6 mos	IP	5.53 mg/kg	
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	

—continued

Table 6.1. (continued)

Toxin	Animal	Sex	Age/Size	Route	LD <sub>50</sub>	Reference
Roquefortine A	Mice	—	—	IP	340.0 mg/kg	Cole and Cox 1981
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; Ueno et al. 1973
	Rat	M-F	—	PO	7.3 mg/kg	
	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
Monoacetoxyscirpenol	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; Ueno et al. 1973
	ddys mice	M-F	—	IP	5.2 mg/kg	
	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	

<sup>a</sup>LD = lethal dose

<sup>b</sup>PO = per os (by mouth)

<sup>c</sup>IP = intraperitoneal

<sup>d</sup>ca. = approximately

<sup>e</sup>ddys = recombinant congenic strain of mice used in the study of the major histocompatibility complex region of chromosomes

<sup>f</sup>SC = subcutaneous

<sup>g</sup>IV = intravenous

biochemical-metabolic-molecular-environmental basis for mycotoxicoses.

The diagnosis of mycotoxicoses is based on knowledge gained in experimental studies with specific toxins and specific animal species. Signs of disease caused by natural occurring mycotoxins can be changed by environmental and other factors such as nutrition, sex, and breed. Thus, diagnosis of mycotoxicoses is difficult but can be achieved by good observations of the signs in affected animals and analysis of the feed involved in the intoxication.

## Introduction

Mycotoxins cause illness and lethality in domestic animals fed moldy feedstuffs. These acute intoxications can have devastating effects and are difficult to diagnose and treat because the suspect feed may be consumed before it can be tested. Because of the large number of structurally unrelated mycotoxins produced by the various fungi, it is difficult to pinpoint which toxin(s) is responsible for a particular outbreak, even if a mycotoxicosis is strongly suspected. Some indication of mycotoxin potencies can be obtained

Table 6.2. Selected examples of documented mycotoxicoses associated with naturally contaminated food, feed, or environmental sources

Toxin	Fungus	Animal affected	Source	Syndrome	Reference
Aflatoxins	<i>Aspergillus flavus</i>	Dogs, humans	Moldy corn	Jaundice, abdominal pain, edema, anorexia, gastrointestinal bleeding, death	Ngindu et al. 1982
Aflatoxins	<i>Aspergillus flavus</i>	Feeder pigs	Moldy corn	Weight loss, rough hair coat, anorexia, ataxia, tremors, coma, death	Coppeck et al. 1989
Aflatoxins	ND <sup>a</sup>	Humans	Contaminated food	Liver failure, death; aflatoxin-DNA adducts detected in tissues	Harrison and Garner 1991
Aflatoxin B <sub>1</sub> , B <sub>2</sub>	ND	Dairy cattle	Moldy dairy concentrate lodged in feed chute	Tachycardia, tachypnoea, death	Cockcroft 1995
Clavine alkaloids	<i>Claviceps fusiformis</i>	Camels, humans	Pearl millet	Nausea, vomiting, giddiness, drowsiness	Krishnamachari and Bhat 1976
Cyclopiazonic acid	<i>Aspergillus flavus</i> , <i>A. tamarii</i>	Humans	Kodo millet seed	Giddiness, nausea	Rao and Husain 1985
Ergometrine	<i>Claviceps purpurea</i>	Humans	Barley infested with wild oats; 0.75% ergot	Weakness, fomication, burning sensation, nausea, vomiting, diarrhea, limb swelling, gangrene	Demeke et al. 1979
Fumonisin	<i>Fusarium verticillioides</i>	Horses, pigs	Corn-based feed	Equine leucoencephalomalacia, porcine pulmonary edema	Sydenham et al. 1992
Fumonisin	<i>Fusarium verticillioides</i> , <i>F. proliferatum</i>	Swine	Corn screenings	Dyspnea, weakness, cyanosis, death	Osweiler et al. 1992
Fusarochromanone	<i>Fusarium equiseti</i>	Chickens	Pelleted feed (corn, wheat, peas)	Tibial dyschondroplasia	Krogh et al. 1989
Ochratoxin A	ND	Turkeys, laying hens, broiler chickens	Corn-based feed	Nephrotoxicity, decreased feed consumption, mortality	Hamilton et al. 1982
Ochratoxin A	<i>Penicillium verrucosum</i>	Swine	Cereal feed grain	Ochratoxin-positive blood samples	Holmberg et al. 1991
Ochratoxin A	<i>Aspergillus ochraceus</i>	Humans	Inhaled dust from stored wheat	Respiratory irritation, edema, asthenia, reduced diuresis, acute renal failure	Di Paolo et al. 1993
Ochratoxin A	ND	Humans	Wheat, barley, mixed cereals, dried vegetables, meat and fish, olives	Chronic interstitial nephropathy	Maaroufi et al. 1995

—continued

Table 6.2. (continued)

Toxin	Fungus	Animal affected	Source	Syndrome	Reference
Ochratoxin	ND	Geese, broilers	Corn-based feed	Pale beaks, weakness, ataxia, lameness, tremor, diarrhea, mortality	Shlosberg et al. 1997
Slaframine; swainsonine	<i>Rhizoctonia leguminicola</i>	Cattle, horses, sheep	Red clover, wheat stubble, lespedeza grass	Profuse salivation, diarrhea, lacrimation, stiff joints, frequent urination, tremors, spontaneous abortion, labored breathing, bloat, death	Croom et al. 1995
Tremorgens	<i>Aspergillus fumigatus</i> , <i>A. niger</i> , <i>A. flavus</i> , <i>A. clavatus</i> , <i>Rhizopus</i> , <i>Mucor</i> , <i>Paecilomyces</i> , <i>Penicillium</i> , <i>Cephalosporum</i>	Humans	Dust from moldy silage, containing red clover, timothy grass, and alfalfa	Fatigue, headache, fever, chills, nausea, vomiting, tremor, dementia	Gordon et al. 1993
T-2 toxin	<i>Fusarium tricinctum</i>	Dairy cattle	Moldy corn	Inappetance, abortion, death, visceral hemorrhage	Hsu et al. 1972
Trichothecenes	<i>Stachybotrys</i> spp.	Humans	Airborne spores, mold growth in air ducts and insulation	Malaise, fatigue, headache, sore throat, diarrhea, alopecia	Croft et al. 1986
Trichothecenes	<i>Fusarium</i> spp.; <i>Aspergillus</i> spp.	Humans	Bread made from moldy wheat	Abdominal pain, vomiting, diarrhea, bloody stools, facial rash	Bhat et al. 1989
Zearalenone; alternariol	ND	Swine	Sorghum-based mixed feed	Hyperestrogenism	Sydenham et al. 1988

<sup>a</sup>ND = not determined

from LD<sub>50</sub> (the single dose of a substance required to cause 50% mortality in a population) studies, such as those shown in Table 6.1. However, the LD<sub>50</sub> has limited value in assessing toxicity of a compound or its potential risks. Of more practical use are reports of natural cases of intoxication caused by consumption of mycotoxin-contaminated food or feed. Whereas it is difficult to prove that a particular outbreak was caused by a mycotoxin, a number of reports in the literature are reasonably well documented. Selected examples are presented in Table 6.2.

The impact of fungal toxins on animals extends far beyond the obvious effect of causing death. The economic impact of lowered productivity, decreased weight gain (see Figure 6.1), decreased feed efficiency, decreased meat and egg production, increased 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 from mycotoxins in feeds



Figure 6.1. Effects of dietary T-2 toxin on growth of turkey poults. The turkey poult on the left was given a normal ration, and the poult on the right was given a ration containing 10 ppm of T-2 toxin for three weeks. Photograph courtesy of John L. Richard, USDA, ARS, National Animal Disease Center, Ames, Iowa; now at Romer Labs, Inc., Union, Missouri..

and human foods, along with the unknown subtle effects induced by these mycotoxins, are coupled to universal concerns about health risks.

Most mycotoxin toxicity data have come from experimental studies with purified compounds in otherwise healthy animals (reviewed by Prelusky et al. 1994b). In this section, toxicities will be characterized by a "systems approach" where the emphasis is on the body system(s) affected by the mycotoxin (Richard 1998). Specifically highlighted are the effects of important mycotoxins on productivity, liver, kidney, hematopoiesis, immune system, reproduction, birth defects, neuroendocrine system, and cancer.

The diagnosis of animal mycotoxicoses is based on experimental studies with specific toxins and specific animals. Diagnosis of naturally occurring mycotoxicoses, however, is difficult because there are a multiplicity of factors such as breed, sex, environment, nutritional status, and other toxic entities that can affect the intoxication. Diagnosis is dependent on receiving a sample of the food or feed that was involved in the intoxication, collecting data from the animals relative to the illness, and conducting a thorough postmortem examination if death is a result. Therefore, the best diagnosis is achieved when a toxin, which is referable to the kind of disease observed, is found in the sample of feed involved in the intoxication.

## General Effects of Mycotoxins on Health and Productivity

### Aflatoxins

Aflatoxins are potent liver toxins. Their effects in animals vary with dose, length of exposure, species, breed, and diet or nutritional status (Figure 6.2). 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 (Sinnhuber et al. 1977; Wogan and Newberne 1967), primarily of the liver, in some species (Busby and Wogan 1984; Wogan 1973). Generally, young animals are more susceptible than older ones to the toxic effects of aflatoxins. Aflatoxins have elicited the greatest public health concern because of their widespread occurrence in several dietary staples, e.g., peanuts, tree nuts, milk, corn, dried fruits, and their potential as human carcinogens.

One of the experimental animals used in the early studies of aflatoxicosis was trout. They seem to be quite sensitive to the effects of the aflatoxins and the

LD<sub>50</sub> was calculated to be equivalent to 0.5 to 1.0 mg/kg crystalline aflatoxins B<sub>1</sub> and G<sub>1</sub> in the same proportions (Lee et al. 1991). Eighty parts per billion total dietary aflatoxins produced hepatomas in the trout at a very high incidence. Rainbow trout in the early stages of development are quite sensitive to hepatogenicity. Immersion of fry or embryos in 0.5 ppm aflatoxin B<sub>1</sub> for 0.5 hr resulted in 30 to 40% incidence of hepatocellular carcinoma nine months later (Sinnhuber et al. 1977). According to the review of toxicity of mycotoxins in fish by Lee and associates (1991), epizootics of aflatoxicosis still occur in fish and were likely the cause of a trout liver cancer epizootic that occurred in California in hatcheries from 1939 to 1942. Aflatoxin-contaminated cottonseed meal was implicated as the causative agent in this outbreak.

Trout fed aflatoxins develop hepatic cancer (Sinnhuber et al. 1977). Aflatoxin toxicity has been reported in suckling piglets, growing and finishing swine, and breeder stock. Clinical and pathological signs include 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 vary, depending on age, diet, concentration, and length of exposure. Swine appear to be resistant to dietary levels of aflatoxins up to 300 ppb fed from time of weaning to marketing (Monegue et al. 1977).



Figure 6.2. 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 John L. Richard, USDA, ARS, National Animal Disease Center, Ames, Iowa; now at Romer Labs, Inc., Union, Missouri.



Acute aflatoxicosis in cattle has been thoroughly described. Clinical signs consist of decreased 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 due to decreased feed efficiency, immunosuppression, and lower reproductive (Bodine and Mertens 1983). Aflatoxins affect rumen function *in vitro* and *in vivo* by decreasing cellulose digestion, volatile fatty acid formation, and proteolysis (Dvorak et al. 1977; Fehr and Delage 1970). Cook and his associates (1986) demonstrated decreased rumen motility in steers given a single dose of aflatoxins.

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

Another characteristic of aflatoxin exposure in dairy cattle is the conversion of aflatoxin B<sub>1</sub> to the hydroxylated metabolite, aflatoxin M<sub>1</sub>, which is excreted in milk. Milk from Holstein cows given aflatoxin B<sub>1</sub> for seven days contains aflatoxin M<sub>1</sub>, although aflatoxin M<sub>1</sub> is not detected in milk four days after termination of aflatoxin B<sub>1</sub> administration (Applebaum et al. 1982; Price et al. 1984). The excreted amounts of aflatoxin M<sub>1</sub> as a percentage of aflatoxin B<sub>1</sub> average 1 to 2% (van Egmond 1989a), but values as high as 6% have been reported at &g daily intake levels of aflatoxin B<sub>1</sub> (Veldman et al. 1992).

Aflatoxicoses have produced severe economic losses in the poultry industry, affecting ducklings, broilers, layers, turkeys, and quail. Clinical signs of intoxication include anorexia, decreased weight gain, 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, are found in chickens given a high level (1.5 ppm) of dietary aflatoxins (Carnaghan et al. 1966). In chickens given half of this dose, clinical responses include hypoproteinemia; decreased hemoglobin; and decreased serum triglycerides, phospholipids, and cholesterol (Brown and Abrams 1965; Tung et al. 1972). Aflatoxins can decrease activities of several enzymes important to di-

gestion of starches, proteins, lipids, and nucleic acids in broiler chickens (Osborne and Hamilton 1981). The decreased activities of pancreatic amylase, trypsin, lipase, ribonucleic acid (RNA) enzyme RNase, and DNase could contribute to the malabsorption of nutrients associated with aflatoxicoses.

Hamilton (1971) reported an egg production decrease to 5% of normal in laying hens given near-LD<sub>50</sub> levels of aflatoxins in naturally contaminated corn. Aflatoxin-contaminated feed (up to 10 ppm) consumed by layers for 4 weeks decreases egg production and size (Huff et al. 1975). Total yolk weight and yolk as a percentage of total egg weight decreased, accompanied by greater yolk and plasma carotenoid concentrations (Huff et al. 1975).

### Trichothecenes

General signs of trichothecene toxicity in animals include weight loss, decreased feed conversion, feed refusal, vomiting, bloody diarrhea, severe dermatitis (Figure 6.3), hemorrhage, decreased egg production, abortion, and death (Morehouse 1985). Histologic lesions consist of necrosis and hemorrhage in proliferating tissues of the intestinal mucosa, bone marrow, spleen, testis, and ovary. Trichothecenes inhibit protein and DNA synthesis; many of their toxic effects stem from this mechanism. Because T-2 toxin was one of the first trichothecenes discovered, its effects on animals were characterized early.

Deoxynivalenol is common in North American and European cereal grains, and of the trichothecenes, poses the greatest problems to animal health (reviewed by Miller et al. 2001; Rotter et al. 1996). Al-



Figure 6.3. Severe oral lesion in a turkey poult that consumed a ration containing 10 ppm T-2 toxin for three weeks. Photograph courtesy of John L. Richard, USDA, ARS, National Animal Disease Center, Ames, Iowa; now at Romer Labs, Inc., Union, Missouri.

though DON can be acutely lethal when ingested in large quantities, moderate- to low-level ingestion of the toxin can cause poor performance and altered immune function (Pier et al. 1980a, b). Monogastric animals, particularly swine, exhibit the greatest sensitivity to DON, while chickens and turkeys, followed by ruminants, appear to have higher tolerance (Prelusky et al. 1994b).

Diminished feed consumption and lower weight gain are the principal clinical effects seen in pigs that have eaten DON in naturally contaminated feeds ( $\leq 2$  ppm feed) (Friend et al. 1982; Rotter et al. 1994b; Trenholm et al. 1984). At 1.3 ppm DON in diet, feed intake by growing pigs is significantly decreased, followed by complete feed refusal at 12 ppm and vomiting at 20 ppm (Abbas et al. 1986; Forsyth et al. 1977; Young et al. 1983). The most common signs of acute DON exposure are abdominal distress, increased salivation, and malaise; however, vomiting has been reported at higher dietary concentrations (Vesonder and Hesseltine 1981; Young et al. 1983). Extensive lesions are not typically documented in field cases, because pigs regulate toxin ingestion by adjusting their feed intake (Chavez and Rheume 1986; Friend et al. 1986; Harvey et al. 1989b). Although pigs fed DON exhibit altered blood parameters, these effects cannot be easily separated from nutritional status, i.e., weight loss as a result of significantly decreased feed intake (Lun et al. 1985; Young et al. 1983). Nevertheless, altered stomach condition and serum protein status do indicate a specific effect of *Fusarium* toxins/DON (Prelusky et al. 1994a; Rotter et al. 1994b, 1995b).

The extent of DON effects in pigs relates to age and sex as well as the contamination source (Trenholm et al. 1984; Foster et al. 1986; Prelusky et al. 1994b). Initial studies reported that detrimental effects can be observed when purified DON is added at a level of 5 ppm (Trenholm et al. 1984); however, the situation with naturally contaminated diets is more complex. Because *F. graminearum* produces many metabolites besides DON (Miller 1995), mycotoxicosis may be caused by multiple toxins. Unidentified/bound toxins, conjugated mycotoxins, or toxic agents of other origin might contribute substantially to the animal response (Foster et al. 1986; Prelusky et al. 1994b).

Poultry are much less sensitive than pigs to DON. Levels as high as 8 ppm in feeds have not been associated with impaired productivity (Hamilton et al. 1985a, b, 1986). Rapidly growing broilers are generally more sensitive than laying hens to feed refusal (Huff et al. 1986). In day-old broiler chicks given DON, the seven-day oral  $LD_{50}$  is approximately 140 mg/kg body weight (bw), with widespread hemorrhag-

ing, urate deposition, neural toxicity, and upper GI tract irritation being the primary signs (Huff et al. 1981).

Ruminants are relatively insensitive to DON because rumen microorganisms are able to metabolize/detoxify this toxin (King et al. 1984). Dairy cows fed 6.4 ppm for 6 weeks (Trenholm et al. 1985) or 66 ppm for 5 days (Côté et al. 1986) showed no signs of impaired performance or illness. Whitlow et al. (1994) suggested, however, from the results of epidemiological studies of milk production and DON-contaminated feed that there should be an investigation of the presence of other mycotoxins in DON-contaminated feeds.

### Ochratoxin A

Ochratoxin A, a nephrotoxic mycotoxin produced by several *Aspergillus* and *Penicillium* species, primarily affects the kidneys in animals exposed to naturally occurring levels (Krogh 1977). Changes in the renal function of pigs exposed to ochratoxin A include impairment of proximal tubular function, altered urine excretion, 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 (Szczecz et al. 1973).

Turkeys and chickens both exhibit decreased productivity during field outbreaks of ochratoxicosis. Symptoms include retarded growth, decreased feed conversion, nephropathy, and mortality. There is an apparent increased susceptibility to air sacculitis caused by *Escherichia coli* among affected birds (Hamilton et al. 1982). While feed refusal was demonstrated in turkeys exposed to ochratoxin A, it was not observed in chickens (Burditt et al. 1984).

Ochratoxin A also has detrimental effects on egg production in laying hens. There is 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 decreased eggshell quality and increased the percentage of eggs with blood and meat spots (Shirley and Tohala 1983).

### Cyclopiazonic Acid

The biological effects of CPA in rats, dogs, pigs, sheep, laying hens, and chickens have been thoroughly described (Bryden 1991). Clinical signs of intoxication include anorexia, diarrhea, pyrexia, dehydration, weight loss, ataxia, immobility, and extensor

spasm at 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. 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). The chelation of cations such as calcium, magnesium, and iron may be important mechanisms of CPA toxicity. In laying hens, it has been demonstrated that CPA perturbs normal egg-shell formation so that hens fed diets containing CPA lay eggs that have thin shells or visibly cracked shells (Bryden 1991). Approximately 50% of a dose of CPA administered orally or intraperitoneally is distributed to skeletal muscle of rats and chickens within three hours (Norred et al. 1985, 1988). Other studies have demonstrated that residues of CPA may be transferred into milk and eggs (Dorner et al. 1994). Cyclopiazonic acid also has been found to occur in corn, peanuts, cheese, and kodo millet (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), plus the toxicity of some feeds contaminated by *A. flavus* can be greater than expected from their levels of aflatoxins, the possible role of CPA in diagnosed aflatoxicoses should be explored.

### Fescue Alkaloids

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. Fescue toxicosis in cattle is associated with consumption of tall fescue grass containing the endophytic fungus, *Neotyphodium coenophialum*. A number of syndromes have been included in fescue toxicosis including "fescue foot," "fat necrosis," "agalactica," "summer slump," and "summer toxicosis" (Cheek 1998; Thompson and Stuedemann 1993). The underlying physiological mechanisms of these different syndromes have been described by Oliver

(1997) and largely revolve around vasoconstriction of peripheral blood flow by ergot or alkaloid toxins. Animals grazing tall fescue infected with the endophyte may have decreased weight gains, increased body temperature, rough hair coats, gangrenous necrosis of tissue of feet, tail and ears, and decreased conception rate. Clinical manifestation of the different syndromes is changed by environmental temperature. During summer months, hyperthermia is a major feature but during winter months fescue foot, which involves gangrene of extremities, is observed in animals grazing tall fescue (Bryden 1994). Reproductive abnormalities are the major features of horses grazing tall fescue pastures (Cross 1997). Various alkaloids isolated from the infected grass suggest that ergot-like alkaloids were involved in this intoxication (Bacon et al. 1986). Ergovaline is a primary ergot alkaloid found in many samples of infected tall fescue (Rottinghaus et al. 1991). Endophyte-infected fescue, however, contains many other alkaloids that probably contribute to the overall toxicoses (Porter 1995).

Approximately 22% of U.S. cattle graze fescue pastures. If body weight of weaned calves is decreased 25% by effects of the fungus, the annual loss exceeds \$500 million. Decreased calving rate contributes a further \$300 million yearly loss. Additional losses stem from fescue foot, a gangrenous loss of the extremities that occurs during 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, 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. Further research is needed to identify agents that will combat the toxicosis and alter potency of the fungus.

### Immunologic Effects

The immune system is a key target of several important mycotoxins (Bondy and Pestka 2000). One type of adverse effect occurs when a mycotoxin suppresses one or more functions of the immune system, with the net result being impaired resistance to infectious agents. Mycotoxins might thus predispose livestock to infectious disease, possibly resulting in feed refusal and decreased productivity. Increased infections in food-producing animals might result in more animal-to-human transmission of pathogens such as *Salmonella* and *Listeria*. Veterinary clini-

cians have long recognized immunosuppression in food-producing animals that have ingested mycotoxin-contaminated grains (Richard et al. 1978b). Although the entire episode may be primarily a mycotoxin-facilitated disease, such recognition 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 often difficult or impossible to accomplish. Only when mycotoxicoses are acute manifestations of disease is there a chance at such an evaluation.

Substantial experimental evidence exists that mycotoxins can be immunotoxic (Pestka and Bondy 1994a, b; Pier 1981; Richard 1991; Thurston et al. 1986; Vidal 1990). The difficulty of conducting immunological studies in the field means that mycotoxin-induced immune alterations have been examined primarily in experimental animals. Several mycotoxins produced by the *Fusarium* and the *Aspergillus-Penicillium* groups have notable immunotoxic effects.

### Aflatoxins

Although they are primarily known as hepatotoxins and hepatocarcinogens, aflatoxins seem to have been 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 regional corn crop (Miller et al. 1978). Extensive experimental evidence shows that aflatoxins are very capable of lowering the resistance of several animal species to bacterial, fungal, and parasitic infections (Pier 1981, 1986; Pier et al. 1980a; Richard 1991; Richard et al. 1978b).

Table 6.3 summarizes the general features of immunosuppression by aflatoxins. Interpretation of results from studies of aflatoxins and immunity requires particular care, because some used mixtures of aflatoxins, while others used purified aflatoxin B<sub>1</sub>. Differences have been demonstrated in this regard with aflatoxin B<sub>1</sub> and its metabolites (Bodine et al. 1984). In general, evidence from experimental models supports the contention that aflatoxin B<sub>1</sub> particularly suppresses the cell-mediated immune response. Aflatoxin-induced immune modulation has been discussed in several reviews (Pestka and Bondy 1994a, b; Pier

Table 6.3. Effects of aflatoxins on immunity

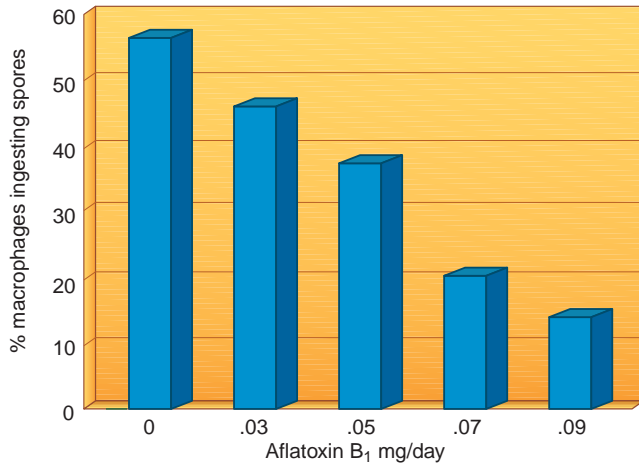
Effects of cellular responses
Phagocytosis by macrophages reduced
Delayed cutaneous hypersensitivity reduced
Lymphoblastogenesis reduced (response to mitogens)
Graft versus host response reduced
Effects on humoral factors
Immunoglobulins (IgG and IgA) concentrations in serum may be reduced
Complement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

1986; Pier et al. 1985, 1986; Richard et al. 1978b).

Because aflatoxin is an economic threat to the poultry industry, its effects on avian immunity have been extensively studied. Cell-mediated responses are particularly sensitive, as reflected by decreased thymus weight and lower peripheral T lymphocyte numbers in chickens fed aflatoxin B<sub>1</sub> (Ghosh et al. 1990, 1991; Viridi et al. 1989). Graft versus host response is suppressed in chickens given 300 ppm aflatoxin B<sub>1</sub> (Kadian et al. 1988). Delayed hypersensitivity response to dinitrofluorobenzene is decreased in broiler chicks given 1 ppm aflatoxin B<sub>1</sub> feed (Ghosh et al. 1991). Oral administration of aflatoxin B<sub>1</sub> to chicks at 0.1 and 0.5 mg/kg body weight lowers peripheral blood lymphocyte proliferation responses to the T cell mitogen concanavalin A (Con A) (Scott et al. 1991).

Phagocytic functions of macrophages and the reticuloendothelial system are consistently inhibited by aflatoxin B<sub>1</sub>. In chicks, the percentage of nitroblue tetrazolium-positive cells in spleen tissue is depressed by aflatoxin B<sub>1</sub> (0.3 to 1.0 ppm), indicating depressed macrophage function (Ghosh et al. 1991). Oral administration of aflatoxin B<sub>1</sub> (0.35 to 0.7 mg/kg bw) in rats depresses both macrophage number and function (Raisuddin-Singh et al. 1990). Clearance of circulating colloidal carbon is decreased in chicks given aflatoxin B<sub>1</sub> (0.3 mg/kg feed), suggesting decreased phagocytic status of the reticuloendothelial system (Kadian et al. 1988). In vitro data also indicate suppression of phagocytic activity in vivo in chickens and rats. Phagocytosis, intracellular killing of *Candida albicans*, and spontaneous superoxide anion (O<sup>2-</sup>) production are suppressed in rat peritoneal macrophages exposed to aflatoxins in vitro (Cusumano et al. 1990). Activation of aflatoxin B<sub>1</sub> by mixed function oxidases is apparently required for macrophage toxicity (Neldon-Ortiz and Qureshi 1991, 1992).

Aflatoxins impair the function of more than one cell type of the mononuclear phagocyte system. This ac-



**Figure 6.4.** Decreased phagocytosis of macrophages with increased doses of aflatoxins in rabbits (Pier et al. 1980b).

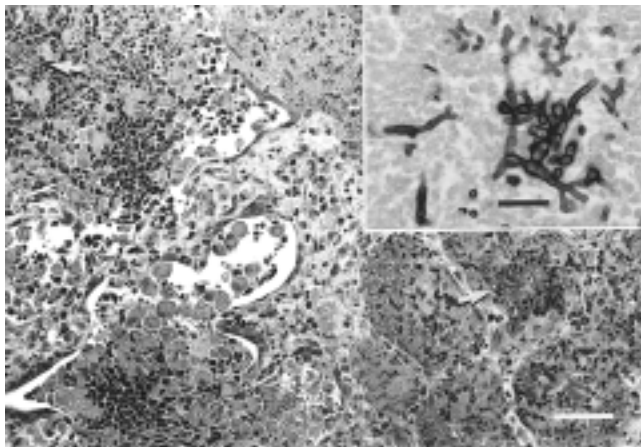
tivity decrease seems to be related to effects on phagocytic cells (Figure 6.4.) but, perhaps more importantly, to a heat-stable substance in the serum necessary for phagocyte activity (Richard et al. 1978a).

Complement, a serum constituent produced by the liver, plays an important role in several immune reactions. Impairment of this activity means decrease of an integral part of the host's immunologic potential. Serum complement activity is decreased in pigs given feed containing 500 ppm aflatoxin B<sub>1</sub>; howev-

er, complement activity is unaffected in pigs given 300 ppm aflatoxin B<sub>1</sub> in feed and in rabbits given 95 ppm aflatoxin B<sub>1</sub> in feed (Panangala et al. 1986; Singh et al. 1987). Aflatoxins decrease the hemolytic complement activity in guinea pigs (Richard et al. 1978a; Thurston et al. 1980) and other species (Pier 1986).

Modulatory effects of aflatoxin B<sub>1</sub> on humoral immunity are not as apparent as effects on cell-mediated immunity, particularly in cross-species comparisons. In swine given up to 500 ppm aflatoxin B<sub>1</sub> in feed and inoculated with *Erysipelothrix rhusiopathiae* bacteria, there is no significant difference in antibody titers compared to inoculated swine given uncontaminated feed (Panaganaga et al. 1986). The ability of guinea pigs to produce antibodies to *Brucella abortus* is unaffected by aflatoxin (0.045 mg/kg bw) administered orally (Pier et al. 1989). In rabbits given aflatoxin (approximately 24 ppm feed), the antibody-forming response to sheep red blood cells (SRBCs), a T cell-dependent antigen, is unchanged compared to animals given aflatoxin B<sub>1</sub>-free diets (Singh et al. 1987).

To summarize, aflatoxin B<sub>1</sub> suppresses cell-mediated immunity to a greater extent than humoral immunity. Some aspects of innate immunity, especially phagocytic responses, are also inhibited by aflatoxin B<sub>1</sub>. It is clear that aflatoxins in the low ppm range would be immunomodulatory.



**Figure 6.5.** Photomicrograph of a section of the lung from a rabbit given T-2 toxin orally for 17 days, exposed to aerosols of *Aspergillus 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  $\mu$ m. Inset reveals tangled masses of *A. fumigatus* hyphae in this lung specimen. Gridley stain; bar = 25  $\mu$ m. (Niyo et al. 1988b)

## Trichothecenes

Trichothecenes may be as important as aflatoxins in causing immunosuppression. The capacity of trichothecene mycotoxins to inhibit protein synthesis apparently contributes to their potential to modulate immune function. Acute exposure to trichothecenes results in severe damage to actively dividing cells in tissues such as bone marrow, lymph nodes, spleen, thymus, and intestinal mucosa. General effects on function of immunocompetent cells, host resistance, and immunoglobulin production at lower doses using oral and other routes of exposure have been reported and reviewed extensively (Pestka and Bondy 1994a, b; Richard 1991; Rotter et al. 1996; Thurston et al. 1986; Vidal 1990).

Repeated exposure of experimental animals to trichothecenes results in markedly increased susceptibility to *Candida* (Salazar et al. 1980), *Cryptococcus* (Fromentin et al. 1981), *Listeria* (Corrier and Ziprin 1986a, 1986b; Pestka et al. 1987; Tryphonas et al. 1986), *Salmonella* (Boonchuvit et al. 1975; Tai and Pestka 1988a, 1990; Vidal and Mavet 1989), *Mycobacterium* (Kanai and Kondo 1984), and herpes simplex

**Table 6.4. 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 1986a; DiNicola et al. 1978; Yarom et al. 1984; Jagadeesan et al. 1982; Lutsky et al. 1978
Increased sensitivity to bacterial endotoxin	Tai and Pestka 1988a, b
Depressed delayed-type hypersensitivity	Pestka et al. 1987a
Effects on infectious diseases	
Resistance to mycobacterial infection in mice decreased	Kanai and Kondo 1984
Mortalities of chickens and mice to <i>Salmonella</i> spp. increased	Boonchuvit et al. 1975; Tai and Pestka 1989
Susceptibility to herpes simplex virus in mice increased	Friend et al. 1983
Mortality of mice with listeriosis increased (dependent upon time of exposure to toxin)	Corrier and Ziprin 1986a, b, 1987; Ziprin et al. 1987; Ziprin and Corrier 1987
Infectious response in rabbits with aspergillosis increased	Niyo et al. 1988b

virus type 1 (Friend et al. 1983). There is a marked increase in susceptibility to lipopolysaccharide (LPS) when T-2 toxin is coadministered, suggesting that impaired resistance to LPS might be one mechanism for increased susceptibility to gram-negative bacteria (Tai and Pestka 1988b).

Interaction of trichothecenes with infectious disease agents occurs when the resistance mechanisms appear to depend on cellular immunity (Table 6.4) (Corrier and Ziprin 1986a; DiNicola et al. 1978; Tai and Pestka 1988b). An example of this was demonstrated by Niyo et al. (1988a, b), who reported a decrease in phagocytosis by alveolar macrophages (Table 6.5) and increased severity of experimental aspergillosis in rabbits treated with T-2 toxin (Figure 6.5). Depression of host resistance by trichothecenes seems to involve suppression of several cellular functions, when evaluated using assays such as allograft rejection time, neutrophil migration, macroph-

**Table 6.5. 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 <sup>a</sup>
0.5	2.1	ND	1.1
	2.3 <sup>b</sup> (0.12)	1.5 <sup>b</sup> (0.9)	1.1 <sup>b, c</sup> (0.11)

<sup>a</sup>Not determined.

<sup>b</sup>Mean (SEM).

<sup>c</sup>Significant at P < 0.01.

age phagocytosis, and delayed-type hypersensitivity (Buening et al. 1982; Corrier et al. 1987a, 1987b; Gerberick and Sorenson 1983; Gerberick et al. 1984; Niyo et al. 1988a, 1988b; Paucod et al. 1990; Pestka et al. 1987; Rosenstein et al. 1979; Sorenson et al. 1986).

Corrier et al. (1986b) determined that short-term, intraperitoneal pre-inoculation treatment with T-2 toxin can be immunostimulatory and actually enhance resistance to *Listeria*, whereas post-inoculation T-2 toxin treatment is markedly immunosuppressive. Cooray and Jonsson (1990) noted a similarly enhanced resistance to mastitis pathogens by pre-inoculation gavage of T-2 toxin in mice.

Dose-dependent decreases or increases in B cell and T cell mitogen responses can be found in lymphocytes obtained from animals exposed to DON, T-2 toxin, or various macrocyclic trichothecenes (Friend et al. 1983; Hughes et al. 1988, 1989; LaFarge-Frayssinet et al. 1979; Paucod et al. 1990; Robbanna-Barnat et al. 1988; Taylor et al. 1985; Tryphonas et al. 1986). For example, ingestion of DON at 2 ppm for 5 weeks (Tryphonas et al. 1986) and 5 ppm for one week (Robbanna-Barnat et al. 1988) depresses mitogen responses. Trichothecene exposure *in vitro* impairs or, at lower doses, enhances mitogen-induced lymphocyte proliferation (Atkinson and Miller 1984; Bondy et al. 1991; Cooray 1984; Hughes et al. 1988, 1990; Miller and Atkinson 1986, 1987; Tomar et al. 1986, 1987, 1988).

Trichothecenes can both suppress and stimulate immunoglobulin production. Repeated exposure to trichothecenes impairs murine antibody responses to challenge with sheep red blood cells (Pestka et al. 1987; Robbanna-Barnat et al. 1988; Rosenstein et al. 1979). Dietary DON causes marked elevation of serum IgA, with concurrent decreases in immunoglobulin M (IgM) and immunoglobulin G (IgG) in the mouse (Forsell et al. 1986). IgA is polyspecific and reacts with bacterial and self antigens (Rasooly and Pestka 1992). Concurrent with serum IgA elevation are immunopathologic effects such as increased polymeric IgA and IgA immune complexes, kidney mesangial IgA accumulation, and hematuria. These effects are persistent (Dong and Pestka 1993) and highly analogous to the common human glomerulonephritis, IgA nephropathy (Dong et al. 1991). IgA nephropathy occurs to a greater extent in males than females and, recently, Greene et al. (1995) determined that, in the mouse, males exhibit a predilection for DON-induced IgA nephropathy in terms of threshold toxin dose as well as onset and magnitude of response. Another common feature of the human disease and the murine model is the involvement of polyspecific

“natural” IgA that may be associated with immune complex formation and subsequent glomerulonephritis (Rasooly and Pestka 1992, 1994).

The suppressive effects of trichothecenes on immune function can be explained by their potent capacity to inhibit protein synthesis; however, their immunostimulatory effects at low doses are problematic. Superinduction of cytokines has been demonstrated using trichothecenes and other protein synthesis inhibitors (Azcona-Olivera et al. 1995a, 1995b; Dong et al. 1994; Efrat et al. 1984; Holt et al. 1988; Miller and Atkinson 1986, 1987). Proposed mechanisms for these effects include interference with synthesis of high-turnover proteins that limit transcription or half-life of interleukin messenger RNA (mRNA); analogous mechanisms could be proposed at the level of translation. Dysregulation of IgA production is apparently brought about by macrophage- and T cell-mediated polyclonal differentiation of B cells to IgA secretion at the level of the Peyer's patch (Bondy and Pestka 1991; Pestka et al. 1990a, 1990b; Warner et al. 1994; Yan et al. 1997, 1998).

### Ochratoxin A

Ochratoxin A immunomodulation has been reviewed by Thurston et al. (1986). General indicators of immunosuppression following ochratoxin A ingestion include lymphocytopenia and depletion of lymphoid cells, particularly in the thymus, bursa of Fabricius, and Peyer's patches (Boorman et al. 1984; Chang et al. 1979). Immunosuppression by ochratoxin A has been studied mainly in poultry, with evidence suggesting that this mycotoxin has an effect on immunoglobulins and phagocytic cells (Burns and Dwivedi 1986). 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.

Although several studies indicate that intraperitoneal injection of ochratoxin A suppresses antibody responses to sheep red blood cells or *Brucella abortus* (Creppy et al. 1983; Haubeck et al. 1981; Prior and Sisodia 1982), depressed T-independent or T-depend-

dent antigen responses were not seen when ochratoxin A was administered in the diet (Campbell et al. 1983; Prior and Sisodia 1982; Richard et al. 1975). Because phenylalanine has been shown to attenuate ochratoxin A-induced immunosuppression in mice and to decrease mortality in broiler chicks fed ochratoxin A-contaminated diets, dietary factors could account for some of the discrepancies in studies of ochratoxin A immunotoxicity using different administration routes (Gibson et al. 1990; Haubeck et al. 1981). The complement system is unaffected by dietary ochratoxin A (0.45 mg/ml) in guinea pigs (Richard et al. 1975); however, chickens fed ochratoxin A (2 ppm) have significantly decreased complement activity (Campbell et al. 1983). Perhaps species or other experimental factors are responsible for these differences.

### Fumonisin

Fumonisin B<sub>1</sub> is the etiologic agent of equine leukoencephalomalacia, porcine pulmonary edema, and is hepatotoxic and carcinogenic to rats (Colvin and Harrison 1992; Gelderblom et al. 1991; Kellerman et al. 1990; Marasas et al. 2001; National Toxicology Program 1999). Haschek et al. (1992) observed that pigs dosed intravenously with fumonisin over the period of several days (total of 4.6 to 7.9 mg/kg) or fed 48 to 166 ppm of the toxin exhibited membranous material in pulmonary macrophages. Fumonisin B<sub>1</sub> and B<sub>2</sub> were cytotoxic to turkey lymphocytes with 50% cytotoxic doses ranging between 0.3 to 2 µg/ml (Dombrink-Kurtzman et al. 1992a, b, 1994). Chicken peritoneal macrophages treated with fumonisin B<sub>1</sub> (10 to 100 µg) show morphological alterations, small but significant decreases in cell viability, and decreased phagocytic potential (Qureshi and Hagler 1992). Tumoricidal factor production in a chicken macrophage cell line is unaffected (Qureshi and Hagler 1992).

### Zearalenone

Although there have been few investigations of potential zearalenone immunotoxicity, other estrogens have been shown to decrease host resistance to pathogens, increase tumor susceptibility, and impair cell-mediated immunity and inflammatory responses (Luster et al. 1980). Zearalenone and its analogues are capable of inhibiting mitogen-stimulated lymphocyte proliferation (Forsell and Pestka 1985). Dietary exposure to 10 ppm zearalenone in the mouse for 2 weeks decreases resistance to *Listeria* but has no ef-

fects on antibody or delayed-type hypersensitivity responses (Pestka et al. 1987b). The same study indicated that zearalenone and deoxynivalenol do not act synergistically when the same immune functions are monitored. Zearalenone and zearalenol can induce thymic atrophy and macrophage activation (Luster et al. 1984).

### Patulin

Several studies indicate that patulin inhibits multiple aspects of macrophage function in vitro. Sorenson et al. (1985) found that protein synthesis was inhibited in rat alveolar macrophages exposed to patulin in vitro and that cell membrane function was compromised. In rat and rabbit peritoneal macrophages in vitro, patulin suppressed the oxidative burst (Escoula et al. 1988b). In mouse peritoneal macrophages, patulin significantly decreased O<sub>2</sub><sup>-</sup> production, phagosome-lysosome fusion, and lysosomal enzyme activity (Bourdiol et al. 1990). Oral administration of patulin (10 mg/kg for 4 days) decreased the mortality of mice experimentally infected with *Candida albicans* from 80% in control mice to 50% in mice treated with patulin (Escoula et al. 1988a). Patulin administration also resulted in increased circulating neutrophils, which could have contributed to increased resistance. Neutrophilia in rats administered patulin orally was attributed to GI inflammation by McKinley et al. (1982).

### Citrinin

Citrinin is primarily known as a nephrotoxin; few studies have addressed its potential for immunotoxicity. Campbell et al. (1981) administered citrinin (125 to 500 ppm for three weeks) in feed to broiler chicks and found that cellular and humoral immune responses were not affected. In mice given citrinin intraperitoneally (0.12 to 3.0 mg/kg bw), decreased peripheral leukocytes were observed (Reddy et al. 1988). Antibody response to sheep red blood cells was depressed at the lowest citrinin dose but stimulated at higher doses. Splenic lymphocyte blastogenesis with or without mitogens was stimulated by citrinin administration. From this study, citrinin appeared to be immunostimulatory rather than immunosuppressive; however, it remains to be determined whether ingestion of feed contaminated with low levels of citrinin produces responses similar to intraperitoneal exposure.



### Wortmannin

Wortmannin is a steroid metabolite first isolated from *Penicillium wortmanni* and later found to be produced by other fungi, including *Myrothecium roridum*, *Fusarium oxysporum*, and *F. sambucinum* (Abbas and Mirocha 1988). This toxin has been used in metabolic studies to increase our understanding of insulin action and glucose homeostasis (Bryden et al. 2001). The potential for immunosuppression by wortmannin was suggested by the anti-inflammatory activity of structurally related fungal metabolites. 11-desacetoxy-wortmannin inhibits carrageenan-induced rat paw edema and decreases symptoms of arthritis (Wiesinger et al. 1974). Exposure of human neutrophils or mouse bone marrow-derived macrophages to wortmannin or its 11-desacetoxy analogue in vitro inhibits induction of the respiratory burst during phagocytosis (Baggiolini et al. 1987). Gastric and myocardial hemorrhages are prominent in rats given crude wortmannin in feed or purified wortmannin by gavage (Gunther et al. 1989a). Accompanying the hemorrhagic lesions are necrosis of the spleen, lymph nodes, and gut-associated lymphoid tissue. Rats given crude wortmannin in feed display depletion and mild necrosis of thymus lymphoid tissue, whereas in wortmannin-gavaged rats there is severe necrosis of the thymus cortex accompanied by hemorrhage (Gunther et al. 1989a, 1989b). There is a possibility that wortmannin contributed to the mycotoxicosis known as alimentary toxic aleukia; however, this has not been proven experimentally (Mirocha and Abbas 1989).

### Fusarochromanone

Fusarochromanone (TDP-1) is a mycotoxin produced by *Fusarium equiseti* that has been associated with tibial dyschondroplasia in poultry. Avian tibial dyschondroplasia is an abnormality of bone growth plates in which there is an accumulation of uncalcified and avascular cartilage in the proximal head of the tibiotarsal bone. This lesion can be induced by fusarochromanone and the toxin has been used by a number of groups to study bone calcification (Bryden et al. 2001). Several studies indicate that fusarochromanone has some influence on immune function, particularly suppression of humoral immunity (Chu et al. 1988). The compound is both inhibitory and stimulatory (at lower levels) in the mitogen-induced lymphocyte proliferation assay (Minervini et al. 1992). Deacetylation to its metabolite, TDP-2, strongly decreases these immunotoxic effects. Additional stud-

ies are needed to verify whether immunotoxicity is similarly evident in vivo.

### Gliotoxin

Gliotoxin, a mycotoxin with antimicrobial and immunosuppressive capabilities, is produced by several genera of fungi including the pathogenic fungi, *Aspergillus fumigatus* and *Candida albicans* (Richard et al. 1994a). This mycotoxin is suspected of being involved in the pathogenesis of these agents and belongs to the class of fungal metabolites called epipolythiodioxopiperazines (Jordan and Cordiner 1987; Taylor 1971). The immunosuppressive activity of this compound was first discovered when workers found that gliotoxin secreted by *A. fumigatus* inhibited the phagocytic phenomenon of macrophage adherence to plastic (Mullbacher and Eichner 1984; Mullbacher et al. 1985). Since that time other immunosuppressive activities of this mycotoxin have been described, including inhibition of mitogenic stimulation of lymphocytes in vitro (Mullbacher et al. 1987; Richard et al. 1994b), cytotoxicity to avian lymphocytes (Richard et al. 1994b), and inhibition of allograft rejection (Mullbacher et al. 1988).

The possibility of involvement of gliotoxin in the pathogenesis of aspergillosis is strengthened by the fact that it is produced in infected animal tissue. Eichner and coworkers (1988) determined that gliotoxin and its dimethylthioether derivative were detectable in the peritoneal lavages from mice inoculated and infected with *A. fumigatus*. Gliotoxin also has been isolated and identified in naturally infected bovine udder tissue while other known toxins of this agent were apparently absent or were undetectable (Bauer et al. 1989). Richard and DeBey (1995) demonstrated the presence of gliotoxin in infected tissues of turkey poultts experimentally infected with known gliotoxin-producing strains of *A. fumigatus*. Gliotoxin subsequently was found to occur naturally in turkeys infected with *A. fumigatus* (Richard et al. 1996a).

The status of gliotoxin as an agent involved in mycotoxicoses established through ingestion of contaminated feeds is relatively unknown.

### Fescue and Ergot Alkaloids

It has been demonstrated that cattle grazing tall fescue pastures infected with endophyte have a suppressed antibody response when immunized with tetanus toxoid (Dawe et al. 1997). The immunosuppressing effect of the grass could be due to a number of different alkaloids produced by the endophyte. The

decrease in immune function in cattle with fescue toxicosis may be due, in part, to the effect of ergot alkaloids on decreasing serum prolactin levels. Prolactin has been reported to function as a regulatory factor in immune responses (Reber 1993).

## Hematopoietic Effects

### Aflatoxins

Hemorrhagic anemia syndrome, caused by the consumption of moldy feed by poultry, has been linked to the presence of aflatoxin-producing fungi (Forgacs and Carll 1962). The syndrome is characterized by massive hemorrhagic lesions in the major organs and musculature. A possible hemolytic anemia with bone marrow hyperplasia and an increase in bone marrow nucleic acid occurred in broiler chicks consuming aflatoxin. Hemoglobin, packed cell volume, and circulating erythrocytes significantly decreased (Tung et al. 1975). Aflatoxins also caused significant time increases in whole blood clotting, recalcification, and prothrombin 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. 1973). 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 will be discussed in the following section.

Exposure to aflatoxins also may affect hemostasis in developing embryos. Hatched chicks, following embryonic exposure to aflatoxin B<sub>1</sub>, had significantly decreased cell counts, hematocrits, and hemoglobin concentrations (Dietert et al. 1983). However, there were no differences in erythrocytes of treated versus control groups.

### Trichothecenes

Trichothecenes, e.g., DON, T-2 toxin, cause 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, macrophages, lymphocytes, and erythrocytes may be decreased with prolonged exposure to trichothecenes. Erythrocyte numbers also can be decreased by trichothecene-induced hemolysis (Segal et al. 1983). T-2 toxin caused a complete hemoly-

sis of rat erythrocytes following a lag period dependent on the concentration of T-2 toxin. Hemolysis was thought to occur by the formation of free radicals because darkness and free radical scavengers — including vitamin E, mannitol, and histidine — inhibit erythrocyte hemolysis by T-2 toxin (Segal et al. 1983).

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

## Hepatotoxicity

### Aflatoxins

Diseases resulting from consumption of grains contaminated with aflatoxins range from acute liver disease, including necrosis and hemorrhage, to more chronic forms with extensive fibrosis and bile duct proliferation (Wogan and Newberne 1967). Clinical manifestations of hepatotoxicity include changes in clinical chemistry values associated with liver function (Norred 1986; Thurston et al. 1980). Jaundice, hemorrhage, and diarrhea, along with decreased performance, may be evident in affected animals with acute disease (Edds 1979; Norred 1986).

Ingested aflatoxins typically are absorbed in the GI tract, where they begin to be metabolically activated or detoxified in the mucosal cells of the intestinal tract and subsequently in the liver (or perhaps the kidney) after hematogenous delivery (Chhabra et al. 1974; Hartiala 1977). Similar pathways likely exist for the other aflatoxins. A major metabolite resulting from cytochrome P450-dependent epoxidation of either aflatoxin B<sub>1</sub> or G<sub>1</sub> is the -8,9-epoxide. This product interacts with DNA, forming an adduct presumably involved in the carcinogenic activity of aflatoxins. Binding of the epoxide intermediate with proteins could be important in the acute hepatic toxicity of the aflatoxins (Sabbioni et al. 1987). Generally, formation of other derivatives of aflatoxin B<sub>1</sub> is a detoxification mechanism, as most of these metabolites are less toxic than the parent compound. Notably, epoxides of some of these intermediates of lower toxicity, e.g., aflatoxins M<sub>1</sub> and P<sub>1</sub>, can occur (Essigmann

et al. 1983). An important detoxification mechanism is the formation of the glutathione conjugate of aflatoxin B<sub>1</sub>-8,9-epoxide. The enzyme for this conjugation is known as glutathione S-transferase. Its level of activity in different species may explain their relative susceptibility to aflatoxins (Eaton et al. 1994). Other factors, e.g., diet, stress, sex, immune state, drug treatment, may be involved in the susceptibility of a given species at a given time to aflatoxins. The involvement of these factors plus differences in the biotransformation of aflatoxins relative to animal susceptibility has not been fully elucidated.

Eliminating aflatoxins from the body must include passage of some of the dosage through the intestinal tract unabsorbed, while biliary excretion (major aflatoxin B<sub>1</sub> metabolite is aflatoxin B<sub>1</sub>-glutathione) and urinary excretion (major aflatoxin B<sub>1</sub> metabolites are aflatoxins M<sub>1</sub> and P<sub>1</sub> and aflatoxin B<sub>1</sub>-N<sup>7</sup>-guanine) function as major routes of elimination. Milk is also a route of excretion in lactating animals (major aflatoxin B<sub>1</sub> metabolite is aflatoxin M<sub>1</sub>) and a major route of exposure of the suckling young. Further information on aflatoxin biotransformation can be found in Eaton et al. (1994).

### Ochratoxin

Although ochratoxins are primarily nephrotoxic, they can cause liver damage, 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 an LD<sub>50</sub> (20 mg/kg bw) dose of ochratoxin A (Terao and Ueno 1978). Ochratoxin effects on liver seem to be 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).

### Fumonisin

Fumonisin are hepatotoxic (Gelderblom et al. 1988) but liver disease may be coincident with other manifestations. Even in horses, when the dosage is sufficiently high, there is liver disease, along with clinical evidence of elevated system disorders. In rats dosed with fumonisins, the usual manifestation is acute hepatitis and even hepatocellular carcinoma (Gelderblom et al. 1992). Fumonisin involvement in diseases of systems, e.g., brain, lungs, kidney, other

than the hepatic system is likely based on their mode of action. Fumonisin B<sub>1</sub> inhibits a key enzyme in sphingolipid metabolism known as N-acyltransferase. This enzyme is involved in the conversion of sphingosine and sphinganine to ceramide, which is subsequently converted to complex sphingolipid. Disruption of this pathway can produce several outcomes because the basic process is involved in cellular regulation and other important biochemical events (Wang et al. 1991).

### Sporidesmin

Sporidesmin is a hepatotoxic mycotoxin produced by *Pithomyces chartarum* on certain grasses. When consumed by animals, sporidesmin can cause a photosensitization disease known as facial eczema (Richard 1998). This classic mycotoxicosis, which occurs primarily in New Zealand, results from destruction of bile duct epithelial cells, with blocking of the bile ducts causing phylloerythrin (a breakdown product of chlorophyll) to accumulate in the circulating blood. Photosensitization occurs when these high energy-absorbing compounds in the peripheral blood circulation absorb energy from sunlight. Photosensitization particularly affects light-skinned or nonwooled areas, thus the name, facial eczema. Sheep, cattle, horses, and deer are known to be affected following consumption of the dead grasses on which the organism thrives.

### Rubratoxin

Rubratoxin has been suspected as the cause of hepatotoxic, hemorrhagic disease of cattle and pigs fed moldy corn (Sippel et al. 1954). Rubratoxin B produces hepatic degeneration, centrilobular necrosis, and hemorrhage of the liver and intestine in experimental animals given the compound. Rubratoxin B is known to be produced by *Penicillium rubrum* and *P. purpurogenum* on corn and both organisms have been found in moldy grains that have intoxicated domestic animals. Analytical methods are not sensitive enough to detect rubratoxin in grains, especially at low concentrations. Rubratoxin apparently exerts a synergistic effect with aflatoxin in guinea pigs (Richard et al. 1974).

### Phomopsins

Lupinosis is a hepatotoxic condition characterized by severe liver damage and icterus in animals ingesting *Lupinus* species infected with the fungus *Phomop-*

*sis leptostromiformis* (Edgar and Culvenor 1985). The mycotoxin phomopsin A is the major fungal metabolite responsible for lupinosis; phomopsin B probably is involved as well. The disease is most common in sheep in Australia, New Zealand, and South Africa but apparently also occurs in the United States. While the disease occurs primarily in sheep grazing *Lupinus* species, it has been found in cattle, horses, and pigs and has been produced experimentally in goats, rabbits, dogs, and mice (Marasas 1974).

## Nephrotoxicity

### Ochratoxin A

Ochratoxin A, a mycotoxin produced primarily by *Aspergillus ochraceus* or *Penicillium verrucosum*, is nephrotoxic. Dogs, rats, and swine are known to be affected with kidney problems due to ochratoxin A. A major renal disease in swine known as porcine nephropathy that occurs in certain European countries, particularly Denmark, is associated with the consumption of ochratoxin-contaminated barley (Hald 1991). Affected pigs show signs of pain in the kidney area, consume excessive amounts of water, appear depressed, urinate almost continuously, and have decreased feed consumption. The impaired renal function results in glucosuria and proteinuria, with casts evident in the urine. Pathologically, the kidneys are bilaterally affected, showing enlargement and paleness with large amounts of connective tissue evident in the cortex when viewed on a cut surface. Histopathologically, there is tubular degeneration and atrophy, interstitial fibrosis, and, perhaps, hyalinization of the glomeruli (Hald 1991).

Ochratoxin biotransformation is cytochrome P450 dependent in animals and humans and forms metabolic intermediates active in the carcinogenic and other toxic activities of ochratoxin A (Fink-Gremmels et al. 1995b). The toxic activity of ochratoxin A may relate to one of its structural components, phenylalanine, which likely is involved in inhibition of enzyme reactions where it is known to function. Generally, ochratoxin A is passively absorbed in the GI tract in a non-ionized form and, through enterohepatic circulation, can undergo secretion and reabsorption (Frolich et al. 1991; Marquardt and Frolich 1992). Absorption of this mycotoxin also occurs in the proximal and distal tubules of the kidney. Using the regression equation proposed from the data of Krogh (1976) (see Marquardt and Frolich 1992), approximately 4.6 ng ochratoxin A/g of kidney tissue would

occur in swine consuming feed containing 200 ng of ochratoxin A/g. In the blood, which contains higher concentrations of ochratoxin A than other tissues, ochratoxin A is bound to the albumin fraction and thus persists for extended periods (Marquardt and Frolich 1992). A major mechanism of detoxification is conversion by ruminal and intestinal microflora into the nontoxic ochratoxin alpha, which is then excreted in the urine and feces along with some amounts of the parent compound. Several reviews on ochratoxin metabolism have been published (Creppy 1995; Fink-Gremmels et al. 1995b; Marquardt and Frolich 1992).

### Trichothecenes

DON and nivalenol, trichothecenes produced primarily by *Fusarium graminearum*, are associated indirectly with a nephropathy that has been studied in mice (Dong and Pestka 1993; Dong et al. 1991; Greene et al. 1994a, 1994b, 1995; Pestka et al. 1989). These toxins increase IgA levels in the sera of mice, resulting in mesangial accumulation of this immunoglobulin and a disease similar to that found in the human IgA nephropathy — the most common human glomerulonephritis worldwide.

### Fumonisin

Experimental animals fed fumonisin B<sub>1</sub> exhibit altered renal histopathology as well as alterations in kidney weight, urine volume, proteinuria, enzymuria, and ion transport (Bondy et al. 1995; Voss et al. 1995). These effects occur in several species and appear to be mediated by altered sphingolipid biosynthesis.

## Reproductive Effects

### Zearalenone

The major effects of zearalenone are estrogenic (see Figure 6.6) and primarily involve the urogenital system. Swine are the most commonly affected animals, although cattle, poultry, and laboratory rodents also are affected (Hagler et al. 2001). 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, swol-



**Figure 6.6.** Enlarged uterus in mouse (left) given an intraperitoneal injection of zearalenone. Compare with uterus of control mouse on the right. Photograph courtesy of John L. Richard, USDA, ARS, National Animal Disease Center, Ames, Iowa; now at Romer Labs, Inc., Union, Missouri.

len prepuce, and mammary gland enlargement; decreased libido may be a variable sequela but mature boars apparently have enough testicular reserve to avoid decreased spermatogenesis. In severe cases, this syndrome may progress to rectal and vaginal prolapse. 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.

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 also may be disrupted, resulting in decreased litter size and diminished viability of neonates (Chang et al. 1979; Miller et al. 1973; Sundloff and Strickland 1986). Zearalenone also decreased conception rates in dairy heifers. Zearalenone caused embryonic death, inhibition of fetal development, and decreased numbers of fetuses present in exposed swine. The majority of cases of zearalenone intoxication occur after animals consume cereal grains that have been contaminated by the toxin. It has been demonstrated in New Zealand and Europe, however, that sheep and cattle grazing pastures in which grass is contaminated with zearalenone can subsequently have reproductive problems (Hagler et al. 2001).

The mode of action of zearalenone and its derivatives involves displacement of estradiol from its uter-

ine binding protein (Hidy et al. 1977), elucidating an estrogenic response (Hagler et al. 2001). The metabolism of zearalenone in several animal species has demonstrated that the major products of the parent compound are two metabolites, alpha- and beta-zearalenol, that can be found as the glucuronide conjugate in urine and feces (Hagler et al. 2001). The major compounds found in plasma and urine samples, treated with beta-glucuronidase, from pigs dosed with zearalenone were zearalenone and alpha-zearalenol (Farnworth and Trenholm 1983). Zearalenone can be converted to a compound known as zeranol (Ralgro(r)) or alpha-zearalanol that is used as an anabolic agent in cattle and sheep.

### Ergot Alkaloids

Abortion may be a variable and controversial sequela to ergot ingestion, depending on species affected and alkaloid content of sclerotia. Ergot-contaminated diets of pregnant swine are associated with decreased piglet birth weights and increased stillborn rates; gestation time may be shorter or longer than normal. Ergot also inhibits prolactin secretion in pregnant swine, resulting in diminished udder development and agalactia at farrowing. Severity of effects is directly related to dietary concentrations; feeds containing at least 0.3% ergot sclerotia have definite detrimental effects on overall reproductive performance (Burfening 1973; Loveless 1967). Altered reproduction patterns and reproductive failures in swine are associated with ergot (Bailey et al. 1973). In horses the endophyte of tall fescue is the causative agent for reproductive abnormalities in pregnant mares. Increased gestational length, agalactia, foal and mare mortality, toughened and thickened placentas, weak and dysmature foals, and decreased serum, prolactin, and progesterone levels occur in mares consuming endophyte-infected tall fescue pastures (Cross 1997).

### Other Mycotoxins

Other mycotoxins also have reproductive effects (Council for Agricultural Science and Technology 1989). Aflatoxin B<sub>1</sub> 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 toxin elicited embryo-toxic effects, whereas that fed in the diet did not. Clinical signs of T-2 toxin and diacetoxyscirpenol exposure in domestic animals usually are manifested as decreased egg production or rate of weight gain. The T-2 toxin consumption by breeding

sows has caused drastically decreased conception rates and weak piglets, with decreased litter sizes in those sows conceiving. Ochratoxin A, rubratoxin B, secalonin acid D, and sterigmatocystin all are reported to affect embryonic survival.

## Teratogenic Effects

Experimentally, aflatoxin B<sub>1</sub>, ochratoxin A, rubratoxin B, T-2 toxin, sterigmatocystin, and zearalenone are teratogenic in at least one mammalian species (Hayes 1981). When administered in combination during organogenesis, ochratoxin A enhanced T-2 toxin-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).

## Neurotoxic Effects

### Trichothecenes

DON and other trichothecenes have two characteristics that suggest neurotoxicity in animals. DON induces vomiting at high acute doses, while, at lower doses, it induces feed refusal (Forsyth et al. 1977; Friend et al. 1992; Pestka et al. 1987a; Prelusky and Trenholm 1993; Vesonder and Hesseltine 1981). DON is considered one of the least toxic trichothecenes with regard to mortality. However, it has anorexic and emetic potencies that are equivalent to or higher than those reported for more acutely toxic trichothecenes. For example, although T-2 toxin is at least 10 times more lethal (Ueno et al. 1973; Yoshizawa and Morooka 1973), it has 10-fold less emetic potency than DON. The oral minimum effective dose for DON-induced vomiting in swine is approximately 50 µg/kg (Pestka et al. 1987a; Prelusky and Trenholm 1993; Young et al. 1983), whereas Robison et al. (1979) observed the oral minimum effective dose for T-2 in pigs to be greater than 500 µg/kg.

The threshold for DON-mediated anorexia is similar to that of the much more lethal T-2 toxin. At levels as low as 2 to 3 ppm toxin in the diet, both DON and T-2 depress porcine feed consumption and weight gains. However, effects at this lower concentration are transitory, with animals quickly compensating for any initial losses (Friend et al. 1992; Prelusky et al. 1994a; Weaver et al. 1978). At higher levels of contamination (over 8 ppm DON or T-2), pronounced effects on feeding behavior become more long term (Har-

vey et al. 1990b; Karavaev 1986). While T-2 can produce severe pathological and biochemical alterations at these higher intakes (8 to 20 ppm) (Harvey et al. 1990a, 1990b; Hayes and Schiefer 1982), direct clinical changes due to DON at the same intakes unrelated to anorexia and resultant weight losses are not apparent (Lun et al. 1985; Rotter et al. 1994b).

Sensitivity to the anorectic effects of DON differ considerably in the three species most commonly studied, with the order of tolerance being rat > mouse > pig. Neurochemical alterations in laying hens dosed with DON are not apparent, given the high tolerance of poultry to trichothecenes (Fitzpatrick et al. 1988b).

The serotonergic system mediates both an anorectic response (Blundell 1984; Pollock and Rowland 1981; Shor-Posner et al. 1986) and an emetic response (Andrews et al. 1988). DON has been shown to modulate serotonin activity (Fitzpatrick et al. 1988a; Prelusky 1993), suggesting a possible link between DON-induced emesis and the serotonergic mechanism. DON-induced vomiting is blocked in swine by pretreatment with selective 5HT<sub>3</sub> receptor blockers (Prelusky and Trenholm 1993). DON also inhibits small-intestinal motility in rodents, mediated through 5HT<sub>3</sub> receptors (Fioramonti et al. 1993). Relatedly, gastric relaxation and/or delayed gastric emptying are important components of both emesis (Andrews and Hawthorn 1988) and food intake (Hunt 1980).

Even though previous studies (Fitzpatrick et al. 1988a; Prelusky 1993) demonstrated a central nervous system (CNS) effect, it was not clear whether DON acts directly at the central (brain) level. However, research (Fioramonti et al. 1993; Prelusky and Trenholm 1993) suggests that at least part of DON's mechanism of action is mediated through action on the peripheral 5HT<sub>3</sub> receptors found in the GI tract. It is unclear which substances activate these receptors.

DON-induced conditioned taste aversion also has been investigated (Clark et al. 1987; Ossenkopp et al. 1994; Osweiler et al. 1990). Combined taste aversion to a novel taste (saccharin) could be established by concurrent treatment with DON. However this effect was transitory, suggesting that once conditioning was established, the response to pretreatment with DON diminished with time relative to the level of toxin used. The same result has been observed with T-2 toxin (Wellman et al. 1989). Ossenkopp et al. (1994) have found that DON-induced combined taste aversion is mediated by the area postrema (located in the brainstem), which is thought to mediate the emetic action of DON (and T-2 toxin) (Borison and Goodheart

1989; Prelusky and Trenholm 1993).

Tolerance to DON may provide some clues about the toxin's mode of action. At lower dietary DON concentrations, reduction in food intake is transitory in several species, e.g., pigs, mice, lasting only a few days before animals begin to compensate for initial losses (Côté et al. 1985; Friend et al. 1982; Rotter et al. 1992, 1994a). With increased DON levels in feed, animals may not return fully to control intake but the extent of feed refusal diminishes with time. Several lines of evidence suggest that tolerance development occurs with most anorexic compounds that rely on a central serotonergic mechanism (Silverstone 1992).

### Fumonisin

A relationship between moldy corn and sporadic outbreaks of equine leukoencephalomalacia (ELEM), an 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). ELEM was shown to be caused by fumonisins (Dutton 1996; Marasas et al. 1988b; Norred and Voss 1994). Neurotoxic symptoms — including loss of feed consumption, lameness, ataxia, oral and facial paralysis, head pressing, and recumbency — may begin within days after exposure to fumonisin B<sub>1</sub>. Seizures may be present and morbidity can occur within hours after the onset of clinical signs. Focal malacia and liquefaction of cerebral white matter with peripheral hemorrhage is the pathognomonic necropsy finding. Microscopically, there are liquifactive necrosis and gliosis. Edema, hemorrhage, and perivascular cuffing of leukocytes are often present in the surrounding neuropil. The exact mechanisms by which fumonisins promote this disease are not known but are apparently related to their ability

to inhibit sphingolipid synthesis.

### Tremorgens

Fungi represent a rich source of secondary metabolites capable of eliciting a tremorgenic response in animals (Richard 1998). Fungal tremorgens have been implicated in the etiology of several tremorgenic syndromes (Table 6.6). Some tremorgenic syndromes that occur in pastures involving cattle are referred to as “staggers syndromes.” Typically, these are characterized by muscle tremor, uncoordinated movements, a general weakness in the hind legs, and stiff, stilted movements of the forelegs. More severely affected animals cannot stand. These effects are aggravated when the animals are moved or excited. Some deaths can occur; these are indirect, usually from dehydration, pneumonia, or drowning while attempting to drink. The animals generally recover, with no apparent damage, 24 to 48 hours after the toxic feed source is removed. Syndromes include paspalum staggers, ryegrass staggers, corn staggers, and Bermuda grass staggers (Table 6.6). With the exception of Bermuda grass staggers, fungal tremorgens have been implicated in the etiology of these diseases. Ryegrass staggers occur following the production of tremorgenic mycotoxins or lolitremes by the ryegrass endophyte, *Neotyphodium lolii*, formerly called *Acremonium lolii*.

Another tremorgenic syndrome in cattle occurs when corn silage from a trench-type silo is improperly unloaded with a front-end loader. This aerates the silage, causing fermentation involving toxigenic strains of *A. fumigatus*. These fungi can produce alkaloids and tremorgens of the verruculogen/fumitremorgen group. The disease is characterized by a general deterioration typical of protein deficiency and

**Table 6.6.** Listing of intoxication, species involved, suspected or incriminated etiological agent, and their fungal source (Bryden 1974; Cole and Dorner 1986; Hocking et al. 1988)

Intoxication	Animal	Suspected or incriminated mycotoxin	Fungal source
Paspalum staggers	Cattle	Paspalitrems	<i>Claviceps paspali</i>
Ryegrass staggers	Sheep, cattle	Lolitrems	<i>Neotyphodium lolii</i> <sup>a</sup>
Bermuda grass tremors	Cattle	Unknown	Unknown
Corn staggers	Cattle	Aflatrem; paspalinine	<i>Aspergillus flavus</i>
Moldy silage	Cattle	Verruculogen/fumitremorgens	<i>Aspergillus fumigatus</i>
Moldy cream cheese	Dog	Penitrem A	<i>Penicillium crustosum</i>
Moldy walnuts	Dog	Penitrem A	<i>Penicillium crustosum</i>
Moldy hamburger bun	Dog	Penitrem A	<i>Penicillium crustosum</i>
Moldy beer	Human	Penitrems	<i>Penicillium crustosum</i>

<sup>a</sup>Plant endophyte.

malnutrition, even though ample pasture and supplemental feed may be available. Other prominent clinical signs are diarrhea, irritability, and abnormal behavior.

One of the most characterized tremorgenic intoxications involves a naturally occurring mycotoxin called penitrem A. This compound has been involved in three episodes of intoxication in dogs consuming either moldy cream cheese, English walnuts, or a hamburger bun (bread) (Arp and Richard 1979, 1981; Hocking et al. 1988; Richard and Arp 1979; Richard et al. 1981). Penitrem A and *P. crustosum* also have been implicated in a human intoxication involving moldy beer (Table 6.6). The individual involved was a physician, who became acutely ill approximately four hours after consuming about 30 ml 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, with no apparent residual effects noted after 30 hours.

The mode of action of fungal tremorgens is through release of neurotransmitters from synaptosomes in the CNS and in peripheral nerves at the neuromuscular junction (Knaus et al. 1994). Although many tremorgens affect the high-conductance, calcium ( $\text{Ca}^{2+}$ )-activated potassium ( $\text{K}^+$ ) channels in the release of neurotransmitters, this may not be the mechanism for the tremorgenic activity as some nontremorgenic mycotoxins have similar activity (Knaus et al. 1994). Tremorgens likely are eliminated quickly by biliary excretion; penitrems can be transformed to more polar compounds by bile (Laws and Mantle 1987).

### 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 the ovary with a mass of fungal tissue called a sclerotium. These sclerotia (ergots) (Figure 6.7) contain alkaloids produced by the fungus in varying amounts, depending on the host plant and environmental factors (Robbins et al. 1986). When the sclerotia are ingested, two major signs of disease (gangrene or convulsions and GI disorders) may occur. Additionally, production losses have been noted in animal feeding studies. Lameness and necrosis of ears, tails, and feet have occurred in animals pastured on infected grasses or fed grains containing ergot sclerotia. Nervous signs, predomi-

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

Ergotism occurs in the United States, particularly in areas where rye, barley, and other susceptible small grains or grasses are grown, i.e., midwestern to northwestern states. Effects of ergot on animal production include decreased weight gains, milk production, reproductive efficiency, and agalactia (Robbins et al. 1986). 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 (less than 0.3%) of sclerotia in graded grains (Marasas and Nelson 1987).

### Slaframine

Slobbers or salivary syndrome, characterized by excessive salivation in horses and cattle, has been caused by 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; Smalley 1977a, b). Outbreaks of salivary syndrome have led to decreased 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* (Gardiner et al. 1968; Rainey et al. 1965).

Slaframine is a parasympathomimetic metabolite. Its active ketoimine liver metabolite has an affinity



Figure 6.7. Black sclerotia (ergots) caused by *Claviceps purpurea* infection in barley. Photograph courtesy of John L. Richard, USDA, ARS, National Animal Disease Center, Ames, Iowa; now at Romer Labs, Inc., Union, Missouri.



for muscarinic receptors of the nervous system (Aust 1969; Aust and Broquist 1965; Guengerich and Aust 1977). Slaframine has a particularly high affinity for receptors in the GI 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). Thus, slaframine may be an important therapeutic agent in treating digestive disorders.

## Carcinogenesis

### Aflatoxins

Induction of neoplasms (abnormal growth of tissue) by aflatoxins has been extensively studied (Eaton and Groupman 1994). Aflatoxin B<sub>1</sub> — as well as the secondary aflatoxin B<sub>1</sub> metabolites, aflatoxin M<sub>1</sub> (present in milk of cows ingesting high levels of aflatoxin-contaminated feed), aflatoxicol, and aflatoxin G<sub>1</sub> — have caused hepatic, renal, and colonic neoplasms in rats (0.2 to 0.12 µg/day) (Wogan and Newberne 1967) and hepatic neoplasms in trout (0.4 ppb for 9 months), 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), and monkeys (Berry 1988). Nasal neoplasms were induced in sheep fed 1.75 ppm aflatoxin in the diet for 3-1/2 years (Lewis et al. 1967). Pulmonary neoplasms were produced in mice injected with 0.2 µg of aflatoxin B<sub>1</sub>/day for 4 weeks (Wieder et al. 1968).

In *in vitro* mutagenicity tests, aflatoxin B<sub>1</sub> activated by rat liver microsomal preparations has caused point mutations and frame shift mutations in host cell DNA (Berry 1988). Aflatoxin B<sub>1</sub> also chemically (covalently) binds to DNA *in vitro* (see Hepatotoxicity in this chapter). This tight (covalent) binding of aflatoxin B<sub>1</sub> to DNA causes structural alterations in the DNA. Similar covalent interactions between chemicals and DNA and the resultant structural alterations have caused genomic mutations in *in vitro* and *in vivo* systems.

### Sterigmatocystin

Sterigmatocystin, a precursor of aflatoxin, has lower toxicity. However, its carcinogenic potential, which experimentally is about one tenth that of aflatoxin B<sub>1</sub>, is not trivial. Sterigmatocystin has caused liver cell carcinoma in laboratory rats (Purchase and Van der Watt 1970). It was also mutagenic by several *in vitro* tests, e.g., Ames test, Rec assay, and *Bacillus sub-*

*tilis* assay (Berry 1988). Tumorigenicity of sterigmatocystin was considerably less than aflatoxin B<sub>1</sub> to rats (0.1 to 0.01) (Berry 1988). Sterigmatocystin covalently bonds to DNA at approximately 20 to 30% of the level observed with aflatoxin B<sub>1</sub>.

### Ochratoxin A

Although mutagenicity of ochratoxin A has not been established during several *in vitro* assays (e.g., Ames test, 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) reported a 40% increase in urinary tract cancer in eastern Europeans with Balkan endemic nephropathy, a condition now suspected by many to result from ingestion of local grains contaminated with ochratoxin.

### Fumonisin

Fumonisin with a free primary amino group have been shown to be liver cancer promoters in rats (Gelderblom et al. 1993). Gelderblom et al. (1991) found hepatocarcinomas in male rats fed 50 ppm fumonisin B<sub>1</sub> (≥90% purity) for 26 months. The National Toxicology Program has recently completed two-year carcinogenicity studies of fumonisin B<sub>1</sub> in rats and mice (National Toxicology Program 1999). The results of these studies are discussed in Chapter 6 of this report. In essence, fumonisin B<sub>1</sub> was confirmed to be carcinogenic in female mice and male rats.

## Dermal Toxicity

### Trichothecenes

Several trichothecene mycotoxins are skin irritants. Skin tests have been used to determine qualitatively the biologic activity of this class of compounds (Bamburg et al. 1968; Gilgan et al. 1966). Ueno (1984a) determined the minimum effective dose for dermal toxicity of 18 trichothecenes and compared their structure-activity relationships. T-2 toxin, the most toxic compound tested, caused erythema on the shaved backs of guinea pigs at a dose of 5 ng. T-2 toxin and macrocyclic trichothecenes caused an edema ap-

proximately 24 hours after application. Trichothecenes, e.g., nivalenol, DON, exhibited very low dermal toxicity (Ueno 1984a).

The cutaneous penetration rate and the metabolism of diacetoxyscirpenol, verrucarins A, and T-2 toxin varied in human and guinea pig skin, depending on structure, dosage, and carrier (Kemppainen et al. 1987a). In vivo cutaneous absorption of T-2 toxin in guinea pigs was compared with in vitro cutaneous penetration in static and flow through diffusion cells (Kemppainen et al. 1987b). Penetration data from static studies may underestimate the rate and amount of absorption through intact skin. No T-2 toxin 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).

## Limitations of Systems Approach for Classification of Mycotoxins

Because studies have advanced from large-dose, complete-animal responses to small-dose, subcellular/molecular responses, the systems approach for classification of mycotoxins becomes increasingly difficult. Also, as we learn more about responses in different species, the variation or multiplicity in systems affected become evident. This multiplicity of effects is readily exemplified by the trichothecene mycotoxins, which impact productivity, liver, kidney, hematopoietic system, CNS, and immune system. Similar problems are found with fumonisins, which affect brain, kidney, liver, and lung.

In addition to the multiplicity of effects, the study of animal mycotoxicoses has become increasingly complex because of the interaction of systems affected, the basic metabolic pathways affected, and the physiopathologic nature of the intoxications.

## Synergistic Effects

The scientific literature is replete with information on the effects of individual mycotoxins in various livestock species. Multiple mycotoxin contamination is an area that recently has become a greater concern to the livestock industry. The concern arises from the fact that concentrations of individual mycotoxins associated with poor livestock performance and/or disease syndromes in commercial operations usually are lower than those reported to cause toxic effects in controlled laboratory studies. Additionally, in some of

these reports the feed contained more than one mycotoxin. For example, aflatoxin and fumonisin B<sub>1</sub>, and vomitoxin and zearalenone commonly occur together in the same grain. Many fungal species also are capable of simultaneously producing several mycotoxins. Therefore, an individual grain may be naturally contaminated with more than one mycotoxin (Trenholm et al. 1989), or the incorporation of numerous grain sources, which are each contaminated with a different mycotoxin(s), into a single feed may result in a diet that contains a number of different mycotoxins.

Poor livestock performance and/or disease syndromes, reported in commercial operations, may be due to synergistic interactions between multiple mycotoxins. When mycotoxins are fed in combination, interactive effects can be classified as additive, less than additive, synergistic, potentiative, or antagonistic (Klaassen and Eaton 1991; Kubena et al. 1988). Additive effects occur when the combined effects of two mycotoxins are equal to the sum of the effect of each toxin given alone (example: 2 + 4 = 6). Less than additive effects occur when the combined effects of two mycotoxins are less than the sum of the effect of each toxin given alone (example: 2 + 4 = 5). Synergistic effects occur when the combined effects of two mycotoxins are much greater than the individual effects of each toxin alone (example: 2 + 2 = 10). Potentiative effects occur when one mycotoxin does not cause a toxic effect on a certain organ or system, but when fed with another mycotoxin it makes the latter much more toxic (example: 0 + 2 = 10). Antagonistic effects occur when one mycotoxin interferes with the effects of another (example: 4 + 0 = 1). Klaassen and Eaton (1991) listed several mechanisms by which these interactions may occur including alteration in absorption, protein binding, and biotransformation, or excretion of one or both of the interacting toxins.

Table 6.7 contains a summary of mycotoxin interaction studies published in refereed journals. In all cases, the interactive effects of only two mycotoxins were studied. For purposes of brevity, and due to the uncertainty of the biological and economic significance of statistical changes in other physiological response variables, the summary was restricted to production effects. The majority of these studies was conducted with poultry with a few studies with pigs and one with sheep. Results of the review indicated that additive or less than additive effects (26/33; 78%) were the predominant interactions observed, with synergistic interactions occurring in 19% (6/33) of the studies, and antagonistic interactions occurring in the remaining study (1/33; 3%). Aflatoxin was one of the two myc-

Table 6.7. Mycotoxin combinations in livestock

Mycotoxins	Species tested	Production effects	References
AFB and OA	Chicks	Synergism <sup>a</sup> Less than additive <sup>b</sup>	Huff and Doerr <sup>a</sup> 1981; Huff et al. <sup>a</sup> 1984; Huff et al. <sup>b</sup> 1992
AFB and OA	Swine	Additive <sup>a</sup> Less than additive <sup>b</sup>	Harvey et al. <sup>a</sup> 1989a; Tapia and Seawright <sup>b</sup> 1985
AFB and T-2 toxin	Chicks	Synergism	Huff et al. 1988; Kubena et al. 1990c
AFB and T-2 toxin	Swine	Less than additive	Harvey et al. 1990a; Harvey et al. 1990b
AFB and DAS	Chicks	Synergism	Kubena et al. 1993
AFB and DAS	Swine	Additive	Harvey et al. 1991a
AFB and DAS	Sheep	Synergism	Harvey et al. 1995a
AFB and DON	Chicks	Additive	Huff et al. 1986
AFB and DON	Swine	Less than additive	Harvey et al. 1989b
AFB and FB1	Poult	Additive <sup>a</sup> AFB effect only <sup>b</sup>	Kubena et al. <sup>a</sup> 1995a; Weibking et al. <sup>b</sup> 1994
AFB and FB1	Swine	Synergism	Harvey et al. 1995b
AFB and CPA	Chicks	Less than additive	Smith et al. 1992
AFB and Kojic acid	Chicks	Additive	Giroir et al. 1991
AFB and MON	Chicks	Less than additive	Kubena et al. 1997a
OA and T-2 toxin	Swine	Less than additive	Harvey et al. 1994a
OA and T-2 toxin	Chicks	Additive	Kubena et al. 1989a
FB1 and DON	Chicks	FB1 effect only	Kubena et al. 1997b
FB1 and DON	Swine	Synergism	Harvey et al. 1996
DON and MON	Chicks	Less than additive	Harvey et al. 1997a
DON and MON	Poult	MON effect only	Morris et al. 1999
DON and T-2 toxin	Chicks	Additive	Kubena et al. 1989b
FB1 and MON	Laying hens	FB1 effect only	Kubena et al. 1999
FB1 and MON	Poult	MON effect only	Li et al. 2000
FB1 and MON	Swine	Additive	Harvey et al. 1997b
FB1 and T-2 toxin	Poult	Additive	Kubena et al. 1995b
FB1 and T-2 toxin	Chicks	Less than additive	Kubena et al. 1997b
FB1 and DAS	Poult	Additive	Kubena et al. 1997c
FB1 and OA	Poult	Additive	Kubena et al. 1997c
OA and PCA	Chicks	OA effect only	Kubena et al. 1984
OA and Citrinin	Chicks	Antagonism	Manning et al. 1985
OA and DON	Chicks	Additive	Kubena et al. 1988
OA and DAS	Chicks	Less than additive	Kubena et al. 1994a
T-2 toxin and CPA	Chicks	Less than additive	Kubena et al. 1994b

<sup>a</sup>, <sup>b</sup> Superscripts in Production Effects column refer to the study conducted in the reference in that same row.

Legends: AFB = Aflatoxin; OA = Ochratoxin A; DAS = Diacetoxyscirpenol; DON = Deoxynivalenol; FB1 = Fumonisin B<sub>1</sub>; CPA = Cyclopiazonic acid; MON = Moniliformin; PCA = Penicillic acid.

otoxins involved in five of the six instances of synergism.

Results of these studies should be interpreted with some caution. Many of the studies involved acute or subacute levels of mycotoxins, whereas syndromes reported in the field occur with much lower concentrations of mycotoxins. It is plausible that with these high mycotoxin doses the occurrence of interactive effects may not be detectable because one mycotoxin singly has already produced a maximum deleterious response. Furthermore, the studies cited do not consider the more subtle changes that may be occurring within the immune system. Multiple mycotoxin-induced immunosuppressions may make livestock species more susceptible to disease agents present in commercial production systems. Also, only pairs of

mycotoxins were evaluated in these studies, whereas under commercial conditions contaminated feeds may contain two or more mycotoxins. Finally, studies were conducted under laboratory conditions where animals generally are not exposed to many of the environmental stressors (heat, ammonia, disease, etc.) that occur under commercial conditions.

Future research in the area of mycotoxin interactions should include combinations of two or more mycotoxins at concentrations that commonly occur in the field. Two such studies where chicks and poult were fed a combination of six toxins that included 1 mg deoxynivalenol/kg, 5 mg moniliformin/kg, 5 mg fumonisin B<sub>1</sub>/kg, 100 mg aflatoxin/kg, 1 mg zearalenone/kg, and 0.5 mg ochratoxin A/kg in the diet, were re-

cently reported (Morris et al. 1998a, b). Results indicated that the combination of mycotoxins at these concentrations depressed chick performance but did not affect poult performance. However, several serum biochemical values were altered in poult fed the combination of mycotoxins. To be determined is if the presence of all six mycotoxins was required to cause the effects observed. Additionally, response variables such as performance, serum chemistry, organ weights, hematology, and histopathology, immune function also should be evaluated in future studies to determine if a compromised immune system may be contributing to previously reported disease syndromes. Both humoral and cell-mediated immunity should be evaluated and the evaluation also should include the effects of mycotoxins on vaccine efficacy and the ability of animals to respond to a disease challenge. The effects of heat stress, a common stressor in livestock operations worldwide, on immune function of livestock fed multiple mycotoxins also should be evaluated.

In summary, a review of the literature on mycotoxin interactions indicated that additive or less than additive effects were the predominant interactions observed. Synergistic interactions were only observed in six of the 33 reported studies, and in five of the six studies aflatoxin was one of the two toxins evaluated. These data suggest that mycotoxin synergism may be less of a concern than had been suggested by field outbreaks where multiple mycotoxins had been implicated as possible intoxicants. Further research that evaluates lower concentrations of multiple mycotoxins under commercial environmental conditions is warranted.

## Diagnosis of Mycotoxicoses of Animals

Many individuals in the field of veterinary medicine long ago recognized that mycotoxins were involved in the diseases of animals. The recognition that fungi or perhaps their toxins were involved in sporadic outbreaks of disease in humans, however, met with only limited means to diagnose these diseases in humans. The difficulties of diagnosing mycotoxicoses in humans likely relate to the variety of foods consumed causing unrecognized intoxications because the overall concentration of mycotoxins in the diet was quite low. Most human intoxications were reported in localized groups of individuals where there was consumption of a primary staple in the diet. When contamination of that dietary staple occurred,

there was an outbreak of disease and the cause could be easily traced to the toxin in a single staple in the diet. Therefore, criteria for diagnosis of mycotoxicoses in humans is based on those established in the veterinary literature and will not be addressed specifically in this report.

Diagnosis of mycotoxicoses in animals often is attempted long after the incriminating feedstuff is consumed and other possible causes of disease have been eliminated. This presents the diagnostician with the difficult task of trying to determine whether a mycotoxin(s) is the culprit. An accurate diagnosis depends on several factors, not the least of which is availability of an adequate sample of the feed involved in the intoxication. However, the disease manifestation in the animal must be considered first, because it can often give the analytical chemist insight into the mycotoxin(s) for which to look. Examining a feed sample for any mycotoxin could represent a major expense and lead to misdiagnosis, as there are hundreds of mycotoxins and only some are likely important animal disease agents.

The impact of mycotoxins on economic aspects such as animal health and productivity is difficult to assess because of the following factors associated with their occurrence, consumption, and disease-causing potential: (1) chemical diversity, (2) agronomic practices, (3) climate, (4) harvest and storage methods, (5) food processing, (6) dietary diversity, (7) exposure routes, (8) age and species susceptibility, (9) indiscriminate disease manifestation, (10) varied production by fungal isolates, (11) co-occurrence with other toxins (interactions), and (12) overshadowing by secondary disease.

Because mycotoxins generally have a specific target organ in their disease-producing capacity, only a single body system is often considered to be involved. However, a true understanding of disease, and specifically mycotoxicoses, includes the awareness that most mycotoxins affect more than one body system. This is important for making an accurate diagnosis of mycotoxicoses.

The single target organ is valid for a general discussion of mycotoxin effects. This "systems approach" can help reach an accurate diagnosis, as categories of mycotoxins may be suspected when it is recognized that an individual body system is affected. Mycotoxins exhibit the following primary disease effects: hepatotoxic, GI toxic, hematotoxic, nephrotoxic, reproductive toxic, neurotoxic, immunotoxic, endocrine toxic, teratogenic, mutagenic, and carcinogenic.

Most data about mycotoxin effects on animals have come from well-defined toxicological laboratory stud-

ies. Thus, considerable information about a single purified mycotoxin on a specific animal under well-controlled experimental conditions exists. The characteristics of these studies often include the following:

1. Pure toxin is used.
2. Calculated dosages are used.
3. Dosages are administered at the same time each day.
4. Sufficient numbers of animals are given similar doses to establish significance.
5. Dosages usually are given as a separate entity, to ensure the dose was administered.
6. If the dosage is given with food, food intake is measured.
7. Healthy animals are used.
8. Animals are kept in a controlled environment.
9. Animals are fed and watered adequately.
10. Animals have ample room and are often housed individually.
11. The light/dark cycle is usually controlled.
12. Animals are kept under constant surveillance or observed frequently.
13. Careful measurement of effects/changes is conducted.

However, results of these controlled studies can be far from the “real-life” or natural situation shown in

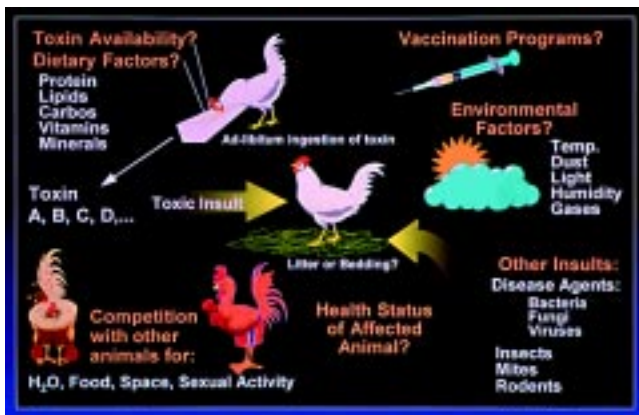


Figure 6.8. Cartoon depicting the potential for interactive factors involved in the exposure of animals to mycotoxins. Figure adapted from slide courtesy of John L. Richard, Romer Labs, Union, Missouri.

Figure 6.8. In this situation, one can see all of the aspects of an animal’s life that may affect the outcome of an intoxication. A diagnosis may be enhanced by investigating fungi in the feed. In some cases, the mycotoxin-producing fungus may no longer be viable, although the product of its growth and metabolism remains in the specific commodities incorporated in the feed mix. Therefore, a fungus responsible for producing a specific mycotoxin may be absent. Also, it is possible to isolate a toxigenic fungus and find no respective mycotoxin in a sample as not all strains of a species produce toxins. Environmental conditions, e.g., the appropriate substrate, may not have been met for toxin production.

Accurate diagnosis involves several criteria but one of the most important is obtaining a representative sample of the feed involved in the toxicosis. Thus, a mycotoxicosis should be considered as a possibility early in the manifestation of the respective disease and a feed sample should be preserved. It may be necessary to obtain a sample from the trough or bunk. Far too often, a mycotoxicosis is considered only after other potential causes for the disease have been eliminated; then it is too late to obtain an adequate sample that could be linked to the toxicosis.

### Diagnostic Criteria

Major criteria for making a thorough mycotoxicosis diagnosis include the following:

#### All Aspects of Disease Manifestation

1. Observed changes in animal condition and behavior, e.g., coat quality, salivation, eye movements, gait.
2. Measured changes in animal condition and behavior, e.g., temperature, respiration rate.
3. Nonintervention, with measurement of urine using chemistry and microbiology and of feces using chemistry.
4. Blood chemistry, including immune status and microbiology.

#### Postmortem Examination

1. Gross visible observations, including position at death.
2. Histopathological observations of tissues selected during postmortem examination.
3. Chemistry of tissues and fluids collected during postmortem examination, e.g., urine, blood, ru-

men or stomach content.

**Examination of Feed**

(See also management and detection section in Chapter 7.)

By carefully observing the animal during the intoxication and following the established criteria, one can get a good idea of which mycotoxin(s) could have produced the effects seen. Once an accurate diagnosis has been made, it is important to report the case in the literature, so that others can learn from the experience. The article should include observations plus

the analytical procedure used in the analysis of the feed sample involved in the intoxication to make the diagnosis of the mycotoxicosis. When the observed and measured changes in the animal fit the description of the mycotoxicosis from the scientific literature, and the amount and type of mycotoxin found in the feed involved in the intoxication are commensurate with the mycotoxicosis, then the best effort has been made to achieve an accurate diagnosis.

Further information on diagnosis of mycotoxicoses can be found in the publication edited by Richard and Thurston (1986).

# 7 Management and Detection of Mycotoxins

## Summary

Avoiding mycotoxin occurrence in the food chain involves understanding elements of strategies to manage mycotoxins (Figure 7.1). This understanding relies on research results from academia, industry, private companies, and government. This information can be applied to institute a quality assurance program for mycotoxins in feed and food manufacturing facilities, to attempt to achieve quality food free of mycotoxins for human and animal consumption. To effectively manage mycotoxins, detection and quantification are important.

It is difficult to estimate accurately and precisely the mycotoxin concentration in a large bulk lot because of the large variability associated with the overall mycotoxin test procedure. A mycotoxin test procedure is a complicated process and generally consists of three steps: (1) a sample is taken from the lot, (2) the sample is ground in a mill to decrease particle size and a subsample is removed from the comminuted

sample, and (3) the mycotoxin is extracted from the comminuted subsample and quantified. Even when using accepted test procedures, there is variability associated with each of the above steps of the mycotoxin test procedure. Because of this variability, the true mycotoxin concentration in the lot cannot be determined with 100% certainty by measuring the mycotoxin concentration in the sample taken from the lot. There is variability for each step of the mycotoxin test procedure and the variability increases with mycotoxin concentration. Results are presented to show that sampling usually is the largest source of variability associated with the mycotoxin test procedure. Sampling variability is large because a small percentage of kernels are contaminated and the level of contamination on a single seed can be very large. The variability associated with a mycotoxin test procedure can be decreased by increasing sample size, the degree of sample comminution, subsample size, and the number of aliquots quantified.

The Food Analysis Performance Assessment Scheme (FAPAS®) organized by the Central Science Laboratory, an executive agency of the United Kingdom Ministry of Agriculture, Fisheries and Food (MAFF) (1990), has checked the proficiency of laboratories analyzing toxins in foods since September 1990. The scheme was started for U.K. laboratories but was expanded worldwide after requests by analysts in other countries that lacked a home-based scheme. During the first ten years of operation, approximately 4,000 homogeneity-checked test materials were issued for analysis of aflatoxins, ochratoxin A, and patulin. Seventy-nine percent of requested data were returned by participants and more than 9,000 z-score proficiency assessments were made, of which 89% were satisfactory.

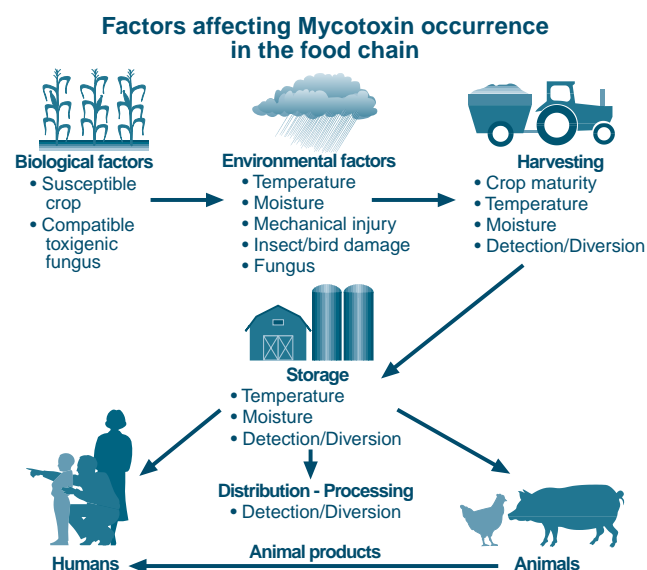


Figure 7.1. Factors affecting mycotoxin occurrence in the food and feed chain (Pestka and Casale 1989).

## Introduction

To reiterate, mycotoxins are essentially unavoidable in commodities. However, a number of practices can be employed to avoid their entry into the food

chain. An understanding of mycotoxins has and will continue to come through integrating research efforts by academia, industry, private companies, and government. In many cases, the studies are collaborative, although significant results also have been accomplished independently by these entities. The outcome is a comprehensive, interdisciplinary effort to identify and implement mycotoxin management strategies that help decrease the exposure of animals and humans to these significant health hazards.

In research, regulatory, and quality assurance activities, it is important to measure accurately and precisely the concentration of a mycotoxin in a commodity so that correct decisions can be made. However, the difficulty in making precise estimates of mycotoxin concentrations in a large bulk of material, i.e., a lot, has been amply demonstrated by Whitaker et al. (1974a, 1974b, 1976, 1979b, 1992, 1994, 1995, 1998), Velasco et al. (1975), Waibel (1977), Knutti and Schlatter (1978), Brown (1974), Jewers (1982), Schatzki (1995), Schatzki and de Koe (1999), Campbell et al. (1986), Park et al. (1991c), Coker (1991), and the Food and Agriculture Organization (1993). Testing generally consists of three steps: (1) a sample is taken from the lot; (2) the sample is comminuted to decrease particle size, then a subsample, or analytical sample, is removed for analysis; and (3) mycotoxin is extracted and quantified. Accuracy depends in part on use of accepted, unbiased test procedures.

General recommendations for sampling products for chemical analysis have been published by Kratochvil and Taylor (1981). Dickens and Whitaker (1982), Campbell et al. (1986), Park and Pohland (1989), Whitaker and Park (1994), and Darnhofer (2000) reviewed accepted procedures for taking and preparing samples of various agricultural commodities for mycotoxin analysis. Nesheim (1979), Schuller et al. (1976), and Park and Pohland (1986) published reviews of accepted procedures to analyze products for aflatoxin. The precision associated with a mycotoxin test procedure depends on the sampling, sample preparation, and analytical frequency used to estimate the mycotoxin concentration of a bulk lot. Even when using accepted procedures, random variation is associated with each step of the testing procedure. This variability means that the actual mycotoxin concentration in a commodity cannot be determined with 100% certainty by measuring the mycotoxin concentration in a sample taken from the lot.

It is important to understand what are considered acceptable methods of sampling, sample preparation, and analysis and the different sources of variability

associated with testing a commodity for mycotoxin. It is important to decrease the variability of test results and design cost-effective inspection programs to detect contaminated commodities as precisely as resources will permit. Most reported research has investigated errors associated with testing commodities for aflatoxin.

## Management of Mycotoxins

The knowledge base accumulated by mycotoxin research that applies to management strategies is voluminous (Sinha and Bhatnagar 1998). An extremely lengthy literature list is available, only a portion of which is referenced in this publication. An understanding of fungal ecology will help considerably in the management of mycotoxin problems. Mycogeography or biogeography of different fungi will indicate their distribution in nature and allow prediction of their occurrence. This, coupled with toxigenicity data, could become a powerful tool in mycotoxin detection and management (Backhouse et al. 2001). Much of the information, however, supports the basic elements of the principles of the HACCP (Hazard Analysis Critical Control Points) program for the management of mycotoxins as described by Lopez-Garcia (2001). These elements are as follows:

1. **Occurrence.** This includes survey, analytical and diagnostic data on commodities that are potentially contaminated with mycotoxins. Often included are the conditions necessary for the given mycotoxin(s) to be present, e.g., weather, other environmental conditions that enhance the likelihood of toxin occurrence.
2. **Prevention.** As information is gathered on occurrence, other data are accrued regarding the means to prevent the occurrence or to at least decrease contamination levels below those of concern.
3. **Detection.** Through the multidisciplinary approach, technological advances have increased chances of detecting mycotoxins in various matrices. To avoid contamination of feeds and foods, we must be able to detect mycotoxins wherever they occur.
4. **Detoxification.** Various integrated strategies for detoxification exist for either eliminating or decreasing the concentration of a mycotoxin from a given matrix or rendering it nontoxic or unavailable for absorption.



## Elements of Mycotoxin Management for Food and Feed Manufacturers

For any manufacturer utilizing a commodity or resource potentially contaminated with mycotoxins, a mycotoxin quality assurance (QA) module should be integrated into the overall QA program. Such a program for mycotoxins utilizes information from the basic elements listed above necessary to develop a good QA program. The obvious reasons to include these QA principles for mycotoxins in the overall program are to (1) maintain product integrity/quality, (2) minimize potential litigation, and (3) comply with regulations.

All employees must be supportive of the company's QA program, so that no weak links decrease its effectiveness. A QA program for mycotoxins includes ten major principles as outlined in the *Total Quality Assurance Program for Mycotoxins*<sup>TM</sup> (Romer Labs 2000a):

1. **Prevention.** Avoiding acceptance of contaminated commodities into the facility. Accomplished through crop reports, surveys, forecasting programs for mycotoxins, preharvest surveys, developing and maximizing buying point guidelines, well-written purchase agreements, and preshipment sampling.
2. **Sampling.** Adequate sampling to obtain representation of entire lot to be tested. Understand the uneven distribution of mycotoxins in commodities. Know how to sample the vessel in which commodity is delivered to facility. Understand that mycotoxins can be formed preharvest as well as postharvest in storage and that significant total amounts may be present in a commodity at low concentrations. Take an adequately sized sample.
3. **Sample preparation.** Grinding and splitting the sample properly before testing. Understand that the portion to be tested must be representative of the sample taken from the vessel. Requires grinding entire sample before subsampling. Never riffle whole grain before taking subsample.
4. **Extraction.** Adequately dissolving the toxin from the sample into the extraction solvent. Use proper extraction protocol for the mycotoxin(s) to be tested. Make sure all particles of the analytical sample are in constant contact with the solvent during extraction.
5. **Evaluation of testing requirements.** Assessing specific needs for testing. Each company's needs vary. Assess the kinds of tests (specificity, sensitivity, ease of method, quantitative or screening, cost), the mycotoxins that need to be assessed, number of samples per day, and location where testing is to be done. In some cases, decisions need to be made whether to do in-house testing or use a contract laboratory.
6. **Testing.** Performing the test procedures adequately to minimize analytical error. Selection of the methods of analysis available at present for in-house testing includes chromatographic methods, i.e., thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography (GC); however, ELISA or fluorometric procedures sometimes are employed. (TLC and ELISA are often employed as a semi-quantitative procedure.) Selection may vary, depending on availability of specific test kits for the desired mycotoxin(s) to be assessed and other evaluation criteria. Individuals must be trained to perform the tests.
7. **Result validation.** Ensuring that results are correct. Accomplished through certification of the technician by using internal check samples to routinely check the method and the technician. Subscribe to a qualified external check sample program and periodically submit positive samples to an outside laboratory that uses a reference method to confirm results.
8. **Documentation.** Accurately documenting all aspects of testing. Keep a detailed log of sampling, sample preparation, testing and results, validation methods. Have a written standard operating procedures manual covering all aspects of testing. Document all unusual circumstances. Document chain of custody of tested materials. These issues become essential if litigation should be initiated.
9. **Supplier involvement.** Transferring some of testing responsibility to suppliers. Actually, this is part of a preventative measure to keep mycotoxin problems away from your facility. Ask suppliers to test to ensure that the product purchased from them is mycotoxin free. Is their mycotoxin QA program similar to yours?
10. **Removal.** Dilute, divert, or destroy mycotoxin-contaminated lots. If operators or a facility find that they have accepted a lot subsequently found, by testing, to be contaminated, decide to stop the

mycotoxin from entering the food chain. Depending on the mycotoxin involved, dilution may be an alternative, to decrease the concentration below the level of concern. Also, the lot may be diverted to some alternative process, e.g., pressing for oil, making animal feed, or the mycotoxin may be rendered nontoxic or unavailable for absorption by certain procedures. If all other means are not acceptable, the contaminated lot would have to be either buried or burned, performed according to regulatory requirements.

A good QA program is integrated, depends on proper implementation of each principle, and has complete company support.

## Detection of Mycotoxins

As stated previously, proper sampling is an important facet for decreasing the variability in the overall testing scheme for mycotoxins. The following information in this chapter is presented to elucidate the importance of these procedures. Supplemental information may be obtained from the following references (Coker 1998; Romer Labs 2001b; Wilson et al. 1998).

### Sample Selection

Because sampling a commodity is extremely important, every individual item in the lot should have an equal chance of being chosen (random sampling). Biases will be introduced by the sample selection methods if equipment and procedures prohibit or decrease the chances of any item in the lot from being chosen (Gy 1982).

If the lot has been blended thoroughly from the various material handling operations, then contaminated particles are likely distributed uniformly throughout. With a well-blended lot, it is not as important where in the lot the sample is drawn, so long as it is selected in a random manner. However, if a commodity has become contaminated by moisture leaks, for example, mycotoxin-contaminated particles may be located in isolated pockets (Shotwell et al. 1975). If the sample is drawn from a single location, contaminated particles may be missed or too many may be collected. Because contaminated particles may not be distributed uniformly throughout the lot, the sample should be an accumulation of small portions taken from many different locations (Bauwin 1982; Hur-

burgh and Bern 1983). If it is unknown whether items are uniformly dispersed throughout the lot, one should act conservatively and accumulate many small increments from different locations, i.e., a bulk sample. If the bulk sample is larger than desired, it should be blended and subdivided until the desired sample size is achieved. The smallest sampling unit used to estimate the lot concentration is often called the test sample. It is generally more difficult to obtain a representative, i.e., unbiased, test sample from a lot at rest, i.e., a static lot, than from a moving stream of the product.

### Static Lots

Commodities contained in storage bins, piles, rail cars, or sacks are examples of static lots. Selecting a truly random sample from a static lot can be difficult because the container may not allow access to all items and/or the items may be physically segregated in the lot due to differences in size and density.

Taking a bulk sample from a container usually requires probing devices to select product from the lot. The probing devices are specially designed for the type of container. The probe (1) should be long enough to reach the limits of the container, (2) should not restrict any item in the population from being selected, and (3) should not alter the items in the lot. Usually, a probing pattern is developed to collect product from different locations in the lot. For example, the U.S. Department of Agriculture's (USDA) Agricultural Marketing Service (AMS) (1991) and the USDA's Grain Inspection Packers and Stockyard Administration (GIPSA) (1983) have designed probing patterns for wagons, trucks, rail cars, etc., when sampling peanuts or grain. Usually, the number of probing points in the pattern increases with lot size. For example, when sampling peanuts, one probe per 1,000 kg lot size is recommended.

When sampling a static lot in separate containers such as sacks, the bulk sample is a composite of many small portions 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 10% of the sacks for large ones (U.S. Department of Agriculture 1991).

If the lot is in a container with limited access, the sample should be drawn when the commodity is either being removed from or being placed into the container. If the cumulative bulk sample is larger than required, it should be thoroughly blended and decreased with a device such as a riffle divider.

### Dynamic Lots

True random sampling can be more nearly achieved when selecting a bulk sample from a moving stream when the lot is transferred, for example, by a conveyor belt. When sampling a moving stream, small increments of product should be taken at predetermined time intervals along the entire length of the stream, and composited to obtain a bulk sample. The FAO (1993) suggests taking at least 100 small increments from a lot.

Automated equipment, e.g., cross-cut samplers, is commercially available with timers that automatically pass a diverter or sampling cup through the moving stream at predetermined, uniform intervals. When automated equipment is not available, the sampling can be done manually. Whether using automatic or manual methods, small increments of product should be collected and combined at frequent, uniform intervals throughout the period that the commodity flows past the sampling point.

Cross-cut samplers should be installed in the following manner: (1) the plane of the opening of the diverter cup should be perpendicular to flow, (2) the diverter cup should pass through the entire cross-sectional area of the stream, and (3) the cup opening should be wide enough to accept all items of interest in the lot. As a general rule, the diverter cup opening width should be two to three times the largest dimensions of the items in the lot.

The size of the bulk sample taken from a lot by a cross-cut sampler is directly proportional to the width of the diverter cup opening and the size of the lot and inversely proportional to the length of time between the cup's movement and velocity through the stream. The interaction among the four variables needs to be fully understood in terms of the amount of sample accumulated and frequency of taking product.

### Sample Preparation

Once a test sample(s) has been taken from a lot, it is comminuted in a mill. It is very important to comminute the entire test sample. Test sample size determines the precision associated with the mycotoxin test procedure.

Following grinding, the comminuted test sample should be blended and a subsample removed for mycotoxin extraction and quantification. Depending on the type of commodity being tested, mills may be commercially available to comminute test samples. Some automatically provide a random subsample of comminuted product for extraction. The Dickens-Satterwhite mill provides a subsample that is approximately

5% of the initial test sample mass (Dickens and Satterwhite 1969). The Romer Series II mill provides a subsample during the grinding process (U.S. Department of Agriculture/GIPSA 1983). However, the Romer mill has the added advantage of adjusting subsample size through an adjustable gate.

For a given commodity, different mills will provide different degrees of grind or particle size. The smaller the particle size, the more homogeneous the comminuted test sample material. Thus, the mycotoxin concentration of the subsample is more likely to be the same as its concentration in the test sample. If a mill does not produce a small enough particle size, the comminuted sample can be slurried in a blender with a suitable liquid such as water or heptane (Campbell et al. 1986). For example, the USDA/AMS currently slurries a 1,100 g subsample of comminuted peanuts with water in a blender. A small portion of the slurry is removed for aflatoxin extraction. The use of a water slurry also decreases solvents needed for extraction and waste disposal problems (Whitaker et al. 1980).

### Analytical Procedures

Besides factors such as accuracy and reproducibility, analytical procedures are characterized by three very practical criteria: (1) the speed with which the analysis can be performed, (2) the level of technical skill required to perform the assay, and (3) whether the assay provides a qualitative or quantitative result. Clearly, the most desirable methods incorporate all three: they are rapid, easy to use, and quantitative. In reality, most methods are a compromise and it is left to users to determine the relative importance of each criterion for their application. This decision is the basis for selection of an analytical method that, in turn, will determine the technical expertise required to run the assays and the overall cost. Regardless of the method chosen, mycotoxin analysis involves extraction, cleanup, and determination.

### Extraction

Most analytical methods require that mycotoxins be extracted from the solid food into a liquid phase. This is done to permit further isolation of the toxins in sufficient concentration to permit their detection. The assumption inherent in an extraction procedure is that the mycotoxin will be distributed evenly among the liquid phase and excluded from the solid phase of the mixture. The extent to which this assumption is valid will be reflected in the efficiency of mycotoxin recovery. Foods are typically extracted with organic

solvents or mixtures of solvents and water. The composition of the extraction solvent is determined empirically from physical and chemical characteristics of the mycotoxin, the commodity, safety considerations, and the analytical procedure. Some test procedures for screening for aflatoxins in corn may use mixtures of methanol/water or acetonitrile/water to extract the toxins. The extraction solvent is often a compromise between the solvent strength required to efficiently extract toxins from grains and the compatibility of solvents with the analytical test system employed in the test procedure. Chlorinated solvents, e.g., methylene chloride, very efficiently extract aflatoxins from corn but their immiscibility with water precludes their use in aqueous-based analytical tests such as ELISAs. Miscibility is not a factor, however, if aflatoxins will be isolated using solid-phase extraction columns. Safety considerations and costs for waste solvent disposal may also affect the selection of extraction solvent.

The physical method for mixing extraction solvent with the sample will influence the ability to efficiently extract mycotoxins from the food. The two most commonly used techniques employ either blenders or shakers to achieve sufficient contact between the extraction liquid and the solid food. Most procedures involve either a blending step for several minutes or a shaking step for 30 minutes to 2 hours. Protocols with blending are more rapid than protocols with shaking when a small number of samples are being analyzed. In blending, care must be taken to ensure that the entire sample is continuously washed with the extraction solvent, i.e., a portion of the sample does not remain in areas of the blending vessel that are not washed by the blending action. However, if large numbers of samples are analyzed, the shaking protocol may be preferable. Apparatus designed for shaking are capable of holding several samples concurrently, allowing samples to be treated in parallel rather than singly. Once the solid sample has been shaken or blended with the extraction solvent, the liquid is separated from the solids either by filtration or centrifugation. The extract is then either cleaned up further to isolate the toxins or applied directly to the determinative step in the procedure.

Methods that do not use extraction also are being developed for mycotoxins. These include presumptive tests for fungal contamination rather than direct determination of toxin content. Fungal infection of corn kernels has been assessed using Fourier transform infrared (FTIR) photoacoustic spectroscopy (Gordon et al. 1997; Greene et al. 1992). Identification of unique spectral characteristics of aflatoxin-contami-

nated corn may permit automated infrared (IR) detection and segregation of grains. This technique has not been adequately evaluated. Sorting of contaminated pistachios has been accomplished using image analysis (Pearson 1997). A mechanical vision system also was recently tested for *Fusarium*-infected wheat (Ruan et al. 1998).

### Cleanup

Once the mycotoxin has been extracted from the solid matrix, the liquid extract is cleaned up to remove impurities before the determinative, or quantitation, step. The cleanup involves isolating the toxin from the extract and is a requirement for some, but not all, analytical methods. In addition to removing sample impurities, the cleanup may further concentrate the mycotoxin prior to the determinative step. Many screening methods, e.g., ELISA, require no cleanup other than dilution of the extract and/or filtration before analysis. The same is true for some laboratory procedures, particularly if achieving the utmost in sensitivity is not an issue.

Most of the common confirmatory procedures and several screening procedures require isolation of the mycotoxin to achieve required selectivity or sensitivity. Generally, cleanup of extracts is accomplished using solid-phase extraction (SPE) columns. SPE columns are usually a porous silica, the surface of which has been modified to provide selective absorption of either the analyte or impurities. In some cases, the analyte is retained on the columns while impurities pass through and are washed off. The analyte is then selectively removed by changing the composition of the rinse solution. In other cases, the SPE columns are designed to trap impurities and permit the analyte to pass through. The advantage of the latter type of column is that only the extraction solvent is needed to purify the sample, eliminating the need for additional solvent for elution. The packing materials of traditional SPE columns have been incorporated into new formats, including disks and 96-well plates, to permit more rapid isolation.

One form of SPE column is the immunoaffinity column. In this format, antibodies attached to an inert support material are used to specifically bind the analyte while sample impurities pass through. The analyte is then removed from the column with a solvent that denatures the antibody. Several commercial affinity columns are available for aflatoxins, ochratoxins, fumonisins, zearalenone, and deoxynivalenol. One advantage of affinity columns is the very specific nature of the interaction between the analyte and the antibody. Although this may be offset some-

what by nonspecific interactions between matrix components and solid-phase support material, in general, affinity columns very efficiently remove sample impurities. Antibodies provide specificity for the cleanup; therefore, factors that influence antibody activity will influence the ability of the column to bind mycotoxins and therefore the ability to recover the toxin from foods. Care must be taken to ensure sufficient antibody is present on the column to adsorb mycotoxins of high concentration. To obtain accurate and reproducible results, factors such as the solvent strength applied to the column, the flow rate, and the volume of extract must fall within manufacturers' guidelines.

### Detection

The final step in an analytical protocol involves determining whether the toxin is present using at least one detection technology. Methods are broadly classified into two groups: those for which the presence of the toxin is indicated but the amount present is less rigorously defined and those for which the amount of toxin can be quantitated. Within this framework, there are rapid (screening) methods, reference methods, and research methods.

### Rapid Methods

Manufacturers of agricultural diagnostics have recognized the need for rapid-screening methods for mycotoxins in foods. Many commercial products are now available for this purpose. The rapid methods for detection and quantification are broadly classified into two groups: those that rely on antibodies to detect mycotoxins (immunological assays) and those that do not. The immunological methods differ according to how the antibody is used in the assay. A special category of SPE uses antibodies attached to a solid-phase support to isolate mycotoxins from the food matrix. The eluted toxins are derivatized, then detected with sensitive fluorometers. Mycotoxins for which immunoaffinity columns are available include aflatoxins, ochratoxins, fumonisins, zearalenone, and DON. Information on mycotoxin immunoaffinity columns was recently reviewed by Scott and Trucksess (1997). In addition to immunoaffinity columns, manufacturers have found ways to rapidly isolate mycotoxins using modified forms of traditional SPE columns, e.g., those that retain impurities and permit the analyte to pass. As with the immunoaffinity columns, the eluted mycotoxins can be derivatized if necessary and quantified with a fluorometer. In many cases, the same fluorometer can be used to perform both types of assay.

The second category of immunological assays is ELISA. Sensitive ELISA kits for a variety of mycotoxins — including aflatoxins, ochratoxins, fumonisins, zearalenone, and DON — are commercially available. Several recent reviews have described the application of ELISAs to mycotoxin analysis (Chu 1996; Dietrich et al. 1995; Pestka 1994). Most commercial ELISAs for mycotoxins rely on a competitive, heterogeneous ELISA format. In this format, the toxin from the sample competes with a labeled toxin (such as a toxin-enzyme conjugate) for a limited number of antibody-binding sites. The greater the amount of toxin present in the sample, the lower the binding of the labeled toxin and the lower the signal generated by the assay. In such assays, the presence of toxin is therefore measured by the absence of a response, i.e., color. This is the Achilles heel of the ELISA tests because any factor that diminishes the binding between the labeled toxin and the antibody can be mistaken for the presence of toxin. Such factors may include structurally related mycotoxins but may also include matrix constituents that are completely unrelated to mycotoxins but simply interfere with conjugate attachment to the antibody by absorbing the conjugate or antibody, by denaturing the antibody, or by inhibiting the enzyme. For these reasons, ELISA kits should be used only with the foods for which they have been extensively tested and demonstrated to work. Also, sufficient controls must be employed for each test, to ensure the validity of the quantitation. A mechanism has been established by the Association of Official Analytical Chemists (AOAC) Research Institute (2001) to evaluate and certify mycotoxin-screening assays (<http://www.aoac.org/testkits/TKDATA5.HTM>). The USDA/GIPSA (2001) also approves kits for the rapid analysis of mycotoxins in grains.

The so-called "black-light" screening method for aflatoxins in corn is based on bright greenish-yellow fluorescence produced by a corn metabolite of kojic acid (Shotwell and Hesseltine 1981). Because this technique is nonspecific for aflatoxins and can render false positives or negatives, it is best to use one of the immunological screening methods, e.g., semiquantitative ELISA-based cup or card tests.

Other practical field methods for aflatoxin screening are based on minicolumns that contain adsorbent 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 ultraviolet light. The meth-

od is semiquantitative and can detect aflatoxins in the low  $\mu\text{g}/\text{kg}$  range, making it adequate for screening. The minicolumn is applicable only to detection of fluorescent mycotoxins. A recent modification has been the use of immunoaffinity columns to concentrate antibodies in the detection band. An alternative approach designated as selectively adsorbed mycotoxins (SAM) 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). A minicolumn procedure capable of detecting the non-fluorescent mycotoxin DON after reaction with aluminum chloride has been described (Ramakrishna et al. 1989).

Mycotoxins can be detected using combinations of chromatographic and mass spectrometric methods, often with minimal sample cleanup (Huopalahti et al. 1997; Musser 1996; Plattner 1995; Scott et al. 1993).

#### Reference Methods

Reference methods have several purposes: one is to confirm samples that have been determined to contain mycotoxins, based on screening tests. The second is to more accurately quantitate the amount of toxin present. Reference methods for mycotoxins generally involve a chromatographic technique such as HPLC, GC, or TLC to further separate mycotoxins from extract impurities. Although instrumental methods such as GC coupled to mass spectrometry (GC-MS) sometimes do not require sample cleanup (other than extraction), in most cases, mycotoxins are isolated using SPE columns. A thorough review of chromatographic methods was published recently (Sydenham and Shephard 1997).

#### High-Performance Liquid Chromatography.

HPLC is the most frequently and widely used method of mycotoxin analysis (Trucksess 1998). HPLC methods that are quite sensitive and have reasonably low levels of detection have been developed for most of the major mycotoxins; thus, these are good quantitative methods. HPLC separates a mixture of compounds, usually present in an extract of a sample by relative affinity of the compounds for a stationary column and a mobile solvent. Eluted compounds from the column pass through a detector of some sort (usually ultraviolet [UV] or fluorescence depending on the physical and chemical attributes of the analyte of interest), and the detector helps quantitate the specific compounds in the original sample injected onto the column. Also, in the case of aflatoxins, photoactiva-

tion may be used to enhance fluorescence before passing through the detector. For many mycotoxins, the time for analysis following injection onto the column is less than 15 minutes but the extract must be substantially cleaned up before injection. The equipment is considerably more expensive than that required for TLC.

**Gas Chromatography.** Gas chromatography often is used in more technical laboratories for some of the mycotoxins and for analysis of trichothecenes such as Type A that do not render themselves readily amenable to HPLC analysis. Components are separated using the relative affinity of the compounds for a stationary column and a mobile, inert gas. Analytes separated on the column and eluted with the inert gas are detected by chemical or physical means. This is a highly sensitive method where analysis time varies according to compound (usually 30 to 45 minutes). Various detection systems may be utilized as coupled to GC. Again, the equipment is expensive and extract cleanup is necessary.

**Thin-Layer Chromatography.** Although TLC is a reference method, it is often used as a mycotoxin-screening assay. Although it is a very powerful tool to determine the presence of one or more mycotoxins in a sample, TLC does not permit critical quantitation that may be required unless densitometry can be used (Trucksess 2001). Typically, TLC involves the spotting of extracts, individually, near one end of a glass plate on which a thin layer of silica gel or similar matrix has been placed. Suitable standards are also spotted on the plate for comparison after the plate has been developed. During development, the edge of the plate nearest the location of the spotted extracts and standards is placed in a specified solvent preparation covering the bottom of a tank that allows the plate to stand nearly vertical. The solvent is adsorbed by the silica or similar matrix and travels up the plate through the spotted extracts and standards. As this occurs, the various compounds in an extract spot are separated, depending on their adsorption to the matrix and their solubility. Because these qualities vary, the compounds are deposited at different heights on the plate. The plate can be removed from the tank when the solvent front nears the top of the plate, dried, and the spots can be visualized.

Densitometric quantitation conducted on a TLC plate is more accurate when the compounds are colored or fluorescent and the analyst does not have to spray or dip the plate to visualize the spots. TLC often can be used with little or no cleanup prior to spot-

ting. In some cases, two-dimensional TLC can be utilized to find mycotoxins in extremely dirty samples. Often, unknown mycotoxins have been found on TLC that would not have been evident using other, more-quantitative methods. Confirming the identity of a specific mycotoxin can sometimes be conducted directly on a thin-layer chromatogram.

**Comments.** Given the vast number of mycotoxins, the variety of foods within which they can be found, and the large number of methods that have been developed for analysis, it is beyond the scope of this report to extensively review these methods. Among the chemical organizations with active programs to evaluate and approve analytical methods for mycotoxins are the AOAC International, the International Union for Pure and Applied Chemistry (IUPAC), the American Association of Cereal Chemists (AACC), and the American Oil Chemists Society (AOCS). In particular, the 17th edition of the *Methods of Analysis of the AOAC International* (Horowitz 2000) provides detailed descriptions of well-established methods for mycotoxin analysis. Excellent reviews of methods of analysis include those by Richard et al. (1993), Gilbert (1993), Sydenham and Shephard (1997), and Trucksess (1998). Additional resources include several books (DeVries et al. 2002; Jackson et al. 1996; Miller and Trenholm 1994; Trucksess and Pohland 2000).

### Research Methods

Studies of the biosynthesis and mode of action of mycotoxins, the discovery of new mycotoxins, the development of new technologies, the need to decrease analytical costs, and the need for environmentally friendly methods are factors that have helped to drive the development of analytical methods. Methods with limited application, or methods that are not yet widely used due to their novelty, are research methods. Immunochemical assays, e.g., ELISA, continue to be developed and expanded into formats that make them easier and quicker to use (Chu 1996; Schneider et al. 1995b). Multimycotoxin screening methods continue to be developed (Abouzieid and Pestka 1994; Pestka 1991; Schneider et al. 1995a), including formats that combine liquid chromatography with immunoassay (Park and Chu 1993).

Biosensors have been devised for several of the major groups of mycotoxins. One format uses the phenomenon of surface plasmon resonance to detect the change in mass that occurs when mycotoxin-specific antibodies attach to a mycotoxin that has been covalently bonded to the surface of the sensor chip (Van

der Gaag et al. 1996). Sensor chips can be used for many analyses, have been successfully used in the pharmaceutical industry, and because the instrumentation is commercially available, this format should find widespread application to future mycotoxin analysis. A second format using fiber optic probes can be adapted for continuous monitoring of mycotoxin levels. This sensor uses the evanescent wave of light that can form around the surface of an optical fiber. Antibodies attached to the surface of the fiber trap fluorescent mycotoxins (e.g., aflatoxins) or fluorescent analogs of mycotoxins (e.g., derivatized fumonisins) with the evanescent zone, permitting their detection. Benchtop devices have been designed for fumonisins and aflatoxins (Maragos and Thompson 1999; Thompson and Maragos 1996, 1997). Portable sensors based on the same technology have been developed by the U.S. Navy to detect chemical warfare agents (Anderson et al. 1996). A third format also uses fiber optics but, rather than the evanescent wave effect, is based on use of fiber optics to direct light at the excitation wavelength onto a semipermeable cellulose acetate membrane to which aflatoxin antibodies have been attached. A very sensitive immunosensor for aflatoxins has been developed based on this technology using commercially available antibodies (Carter et al. 1997). A fourth format uses a mycotoxin-protein conjugate attached to small particles suspended in a small-diameter flow cell (Strachan et al. 1997). Anti-mycotoxin antibody is added and toxin from the sample competes with the toxin conjugate for the limited amount of antibody. A second antibody, labeled with a fluorophore, is added to permit detection of the binding reaction. Optics are arranged around the flow cell to allow detection of the antibody fluorescence. The signal, as with a competitive immunoassay, is indirectly proportional to the amount of toxin present. The assay uses a commercially available instrument to repeatedly fill the flow cell with beads and measure the fluorescence.

Fluorescence intensity has been commonly used as a means of measuring mycotoxins. A related, but distinctly different, technology is fluorescence polarization. Fluorescence polarization instruments measure the extent of fluorophore rotation in solution rather than fluorescence intensity. Fluorescence polarization immunoassays were first described by Dandliker et al. (1973) and there are numerous applications in the clinical area. Recently the technique has been extended to mycotoxin analysis as well (Maragos et al. 2001; Maragos and Plattner 2002).

Analytical instrumentation continues to advance and, with it, applications to mycotoxin analysis. Liq-

uid chromatography coupled with mass spectrometry is a powerful tool for mycotoxin detection, particularly for those mycotoxins for which there is little ultraviolet/visible (UV/VIS) absorbance or native fluorescence (Huopalahti et al. 1997; Musser 1996; Plattner 1995). Capillary electrophoresis (CE) with laser-induced fluorescence detection has been used to analyze fumonisins, aflatoxins, and ochratoxin A at sensitivities comparable to those achieved by more-traditional chromatographic techniques (Corneli and Maragos 1998; Holcomb and Thompson 1995; Maragos 1995; Maragos and Greer 1997). The advantage of CE methods is the potential decrease of solvent usage during the determinative step of the analysis, due to the relatively small volumes of sample injected (nanoliters) and the small volume of waste generated. Because the buffers used in the separation are aqueous, use of solvents beyond the extraction and cleanup steps can be virtually eliminated.

### Validation of Methods

Laboratories that perform mycotoxin testing must have assurances that the methods used are both accurate and precise. New methods are validated by comparing results obtained with those from an existing reference method, generally, a chromatographic method such as HPLC or GC. The new method is compared to existing methods with regards to the recovery of added mycotoxins from the food and the performance of the method with samples of food containing known levels of natural contamination.

Once an assay has been validated in one or more laboratories, it may undergo the process for establishing it as a new reference method. Generally, this involves validation of the assay for precision and accuracy in a larger number of laboratories as collaborative studies under the auspices of chemical societies such as the AOAC International, the AACC, the AOCS, or the IUPAC. Validation is also often performed by regulatory agencies such as the USDA/GIPSA and in Europe by the European Committee for Standardization (CEN) and by the Standards, Measurements, and Testing (SMT) Program of the European Commission. A CEN report CP 13505 gives the performance characteristics for the most important mycotoxins. Collaborative studies indicate the method's performance across a large number of laboratories, using technical personnel of varying skills and resources. Information from the collaborative studies is essential to establish that the method is robust enough for use in multiple analytical laboratories.

### Check Samples of Reference Materials

Laboratories that perform routine mycotoxin testing must validate results internally, even when an established reference method is used. This is best achieved by participation in a proficiency testing program or a check sample program using certified reference material. Check sample programs provide laboratories with samples containing rigorously defined levels of mycotoxins. The materials may be naturally or artificially contaminated. In either case, the sample has undergone substantial analyses in multiple laboratories to establish the mycotoxin concentration. Individual laboratories can gauge the performance of the assay by comparing their results to the established or certified value. A laboratory may analyze the samples "blind" and the results are compared by the check sample provider.

### Reference Materials for Mycotoxins in Foods and Animal Feedstuffs

Reference materials (RMs) — particularly, certified ones — play a role of increasing importance in analytical quality assurance (AQA). In terminology from the International Organisation of Standardisation (ISO), the following definition of a certified reference material (CRM) is used: "A certified material is a material, one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body."

To date, especially the Standards, Measurements and Testing Program of the European Commission and its predecessor, the Bureau Communautaire de Référence (BCR) Program have been active toward the development of CRMs for mycotoxins. The activities to develop mycotoxin CRMs started in the early 1980s within the BCR Program.

In simple jargon, CRMs for mycotoxins are "stable, homogeneous products with certified value(s) of the mycotoxin(s) of interest." Among the matrices and mycotoxins selected at the onset of the BCR-Mycotoxin Program was peanut butter containing aflatoxins  $B_1$ ,  $B_2$ ,  $G_1$ , and  $G_2$ ; peanut meal containing aflatoxin  $B_1$ ; single and compounded animal feed containing aflatoxin  $B_1$ ; milk powder containing aflatoxin  $M_1$ ; corn and wheat flour containing DON; and wheat flour containing ochratoxin A. All these CRMs are available worldwide, with several more planned or under development (corn containing zearalenone, grains containing various trichothecenes, and corn products containing fumonisins) (see Table 7.1). The chosen matrix-mycotoxin combinations represent those that are economically and legally important (in



**Table 7.1. Overview of the different certified reference materials (CRMs) and reference materials (RMs) for mycotoxin analysis as well as their corresponding certified values or current status (after Boenke [1997], updated March 1998)**

CRM No. or RM No.	Matrix	Mycotoxin	Certified value (mass fraction or mass conc.)	Uncertainty (mass fraction or mass conc.)
385	Peanut butter	Aflatoxin B <sub>1</sub>	7.0 µg/kg <sup>d</sup>	± 0.8 µg/kg
		Aflatoxin B <sub>2</sub>	1.1 µg/kg <sup>d</sup>	± 0.2 µg/kg
		Aflatoxin G <sub>1</sub>	1.7 µg/kg <sup>d</sup>	± 0.3 µg/kg
		Aflatoxin G <sub>2</sub>	0.3 µg/kg <sup>d</sup>	± 0.2 µg/kg
		Total aflatoxins	10.1 µg/kg <sup>b</sup>	± 1.5 µg/kg <sup>c</sup>
401	Peanut butter	Aflatoxin B <sub>1</sub>	< 0.2 µg/kg <sup>d</sup>	—
		Aflatoxin B <sub>2</sub>	< 0.2 µg/kg <sup>d</sup>	—
		Aflatoxin G <sub>1</sub>	< 0.3 µg/kg <sup>d</sup>	—
		Aflatoxin G <sub>2</sub>	< 0.2 µg/kg <sup>d</sup>	—
		Total aflatoxins	< 0.9 µg/kg <sup>d</sup>	—
282	Full-cream milk powder	Aflatoxin M <sub>1</sub>	< 0.05 µg/kg <sup>a</sup>	—
283	Full-cream milk powder	Aflatoxin M <sub>1</sub>	0.09 µg/kg <sup>d</sup>	+ 0.04 µg/kg
			– 0.02 µg/kg	
285	Full-cream milk powder	Aflatoxin M <sub>1</sub>	0.76 µg/kg <sup>d</sup>	± 0.05 µg/kg
377	Corn flour	Deoxynivalenol	< 0.05 mg/kg <sup>d</sup>	—
378	Corn flour	Deoxynivalenol	0.43 mg/kg <sup>d</sup>	± 0.04 mg/kg
379	Wheat flour	Deoxynivalenol	0.67 mg/kg <sup>d</sup>	± 0.02 mg/kg
396	Wheat flour	Deoxynivalenol	< 0.05 mg/kg <sup>d</sup>	—
262	Defatted peanut meal	Aflatoxin B <sub>1</sub>	< 3 µg/kg <sup>d</sup>	—
263	Defatted peanut meal	Aflatoxin B <sub>1</sub>	43.3 µg/kg <sup>d</sup>	± 2.8 µg/kg
264	Defatted peanut meal	Aflatoxin B <sub>1</sub>	206 µg/kg <sup>d</sup>	± 13 µg/kg
375	Compound feed	Aflatoxin B <sub>1</sub>	< 1 µg/kg <sup>d</sup>	—
376	Compound feed	Aflatoxin B <sub>1</sub>	9.3 µg/kg <sup>d</sup>	± 0.5 µg/kg
471	Wheat flour	Ochratoxin A	< 0.6 µg/kg <sup>e</sup>	—
472	Wheat flour	Ochratoxin A	8.2 µg/kg <sup>d</sup>	± 1.0 µg/kg <sup>f</sup>
Not yet	Pig kidney	Ochratoxin A	First intercomparison study completed, second planned	
423	Chloroform	Aflatoxin M <sub>1</sub>	Certification study underway	
Not yet	Corn and/or corn products	Fumonisin	Two intercomparison studies completed	
Not yet	Cereal type materials	Trichothecenes (nivalenol, HT-2, T-2)	First intercomparison study completed, feasibility study underway	

<sup>a</sup>The probable content is in the range 0.01–0.02 g/kg; —, not given due to the certification of a less than value.

<sup>b</sup>Calculated by linear addition of the mean of means obtained for the four aflatoxins.

<sup>c</sup>Calculated by quadratic addition of the variances obtained for the four individual aflatoxins and additional application of a safety factor.

<sup>d</sup>Includes allowance for incomplete recovery during certification measurements.

<sup>e</sup>This is a finite value, not corrected for recovery.

<sup>f</sup>This uncertainty is taken as the half-width of the 95% confidence interval of the mean together with applied recovery correction and allowance is included for the uncertainty of the purity of the ochratoxin A calibrant.

the European Union (EU) and in other parts of the world) as well as characteristic for their chemical, physical, and toxicological behavior.

The development of CRMs for mycotoxins in the SMT Program follows an approach whereby a feasibility study, interlaboratory trials, and a certification exercise are undertaken to meet the following requirements:

1. The CRM must be analytically relevant, i.e., the toxin should be present in its natural state and at "normal" concentrations.
2. It must be homogeneous within the variability of methods applied in practice. The toxin(s) must be homogeneously distributed between and within units of a batch.
3. The toxin(s) and matrix must be stable over a long period. Typically, a stability study of one to three years at various conditions, e.g., temperature, is required.
4. Packing of CRMs must be practical with respect to filling, storage, and distribution and in such a way that units cannot be opened without breaking the seal, to allow safe transport.
5. Evidence must exist to justify the certified value and its mathematical uncertainty.

Mycotoxin CRMs developed within the SMT-Program may serve several purposes (Maier et al. 1997). First, they can be analyzed regularly as part of analytical quality control (QC) programs in connection with a control chart. "In-house" RMs can be validated by CRMs and used for in-house QC programs. Surplus materials from proficiency testing programs

also can be purchased to serve as in-house QC materials. Accreditation bodies would expect to see CRMs employed to demonstrate traceability of measurements. Second, CRMs can be applied to validate methods. The objective is to see whether a method (as applied by a particular laboratory or analyst) yields a detectable bias. Third, matrix CRMs can be used to establish a calibration curve, if a series of CRMs are available with equivalent matrix and their matrix type corresponds to the one of a sample to be analyzed (Schnug et al. 1992).

The state of affairs relative to development of mycotoxin CRMs by the SMT-Program can be found in the literature (Boenke 1997) and is summarized in Table 7.1. All mycotoxin CRMs, developed under the SMT Program, and a relevant catalogue are available worldwide from the Institute for Reference Materials and Measurements, Joint Research Centre, European Commission, Belgium (2001).

#### Variation Among Mycotoxin Test Results

Assuming that unbiased test procedures are used to estimate a mycotoxin, random variation still exists among replicate mycotoxin tests on the same bulk lot. For example, 10 replicated aflatoxin test results from each of 12 contaminated shelled peanut lots are shown in Table 7.2 (Whitaker et al. 1972). Each test was made by comminuting a 5.45 kg sample in a subsampling mill developed by USDA's Agricultural Research Service (ARS) (Dickens and Satterwhite 1969; Dickens et al. 1979), extracting aflatoxins from a 280 g subsample with the AOAC Method II (Association of Official Analytical Chemists 1990), and quantifying the aflatoxins densitometrically using TLC (Associa-

**Table 7.2.** Replicated test results for ten 5.45-kg samples from each of twelve contaminated lots of shelled peanuts (Whitaker et al. 1972)

Lot number	Observed aflatoxin concentration (ppb)										Mean (ppb)	Standard deviation	Coefficient of variation (%)	
1	0	0	0	0	0	0	0	0	6	10	14	3.0	5.2	172.9
2	0	0	0	0	2	4	8	14	14	28	43	9.9	14.7	148.0
3	0	0	0	0	0	0	0	16	16	40	69	12.5	23.7	189.6
4	0	0	0	0	0	3	8	26	26	52	70	15.9	25.4	160.0
5	0	0	0	0	3	13	19	41	41	43	69	18.8	24.3	129.0
6	0	0	3	12	12	12	12	25	25	63	103	24.2	33.1	136.7
7	0	0	3	4	4	5	15	60	60	106	165	36.2	57.0	157.5
8	0	0	32	32	34	37	55	67	67	77	134	46.8	39.5	84.5
9	0	3	5	19	32	49	87	91	91	127	168	58.1	57.9	99.7
10	4	7	40	41	55	60	75	95	95	99	230	70.6	64.6	91.5
11	0	4	6	17	36	80	133	148	148	192	216	83.2	82.9	99.6
12	18	50	53	72	82	108	112	127	127	182	191	99.5	56.3	56.6

tion of Official Analytical Chemists 1990). The aflatoxin test results from each lot are ranked from low to high, to demonstrate several important characteristics about replicated aflatoxin test results from a contaminated lot. Other studies have demonstrated that aflatoxin test results in other commodities behave in a manner similar to those for peanuts (Schatzki 1995; Velasco et al. 1975; Whitaker et al. 1979b).

First, the wide range among test results from the same lot reflects the large variability associated with estimating the true mycotoxin content of a bulk lot. In Table 7.2, the variability is described by both the standard deviation and the coefficient of variation (CV). The maximum test result can be as much as five times the lot concentration (the average of the 10 test results is the best estimate of the lot concentration). Second, the amount of variation among the 10 test results seems to be a function of the lot concentration. As the lot concentration increases, the standard deviation among test results increases but the CV (standard deviation relative to the lot mean) decreases. Third, the distribution of the 10 test results for each lot in Table 7.2 is not always symmetrical about the lot concentration (Whitaker et al. 1972). The distributions are positively skewed, meaning that more than half of the sample test results is below the lot concentration. However, the distribution becomes more symmetrical as the lot concentration increases. This skewness can be observed by counting the number of test results above and below each lot concentration (see Table 7.2). As a consequence of this skewed distribution, if a single sample is tested from a contaminated lot, there is greater than a 50% chance that the sample result will be lower than the true lot concentration. The skewness is greater for small sample sizes; the distribution becomes more symmetrical as sample size increases (Remington and Schrok 1970).

The variability shown in Table 7.2 is the sum of the variability associated with each step of the mycotoxin testing procedure. As shown in Figure 7.2, the total variability (VT) associated with a mycotoxin test procedure is equal to the sum of the sampling (VS), sample preparation (VSP), and analytical steps (VA) of the test procedure.

$$VT = VS + VSP + VA \quad (1)$$

#### Sampling Variability

Studies by Whitaker et al. (1974a, 1974b, 1976, 1979b, 1992, 1994), Brown (1974), Coker et al. (1995), Park et al. (1991b, 1991c), and Schatzki (1995) have shown that the sampling step is usually the largest

source of variation in a mycotoxin test procedure. These studies investigated the variability associated with inspecting peanuts, cottonseed, corn, and pistachios for aflatoxin and corn for fumonisin. For aflatoxin, the sampling variability is large because (1) aflatoxin is found only in a small percentage of the kernels in the lot and (2) the concentration in a single kernel may be extremely high (Whitaker and Wisser 1969). Studies on peanut kernels (Whitaker et al. 1972) indicate that the percent contaminated kernels in a contaminated lot at 20 ng/g is 0.095%, which is less than one contaminated kernel per 1,000 kernels. The same studies indicate that the percent contaminated particles in a lot is a function of the lot aflatoxin concentration; the percentage increases with lot concentration. However, these few contaminated particles can have extremely high levels of aflatoxin. Cucullu et al. (1966, 1977) reported aflatoxin concentrations in excess of 1,000,000 ng/g for individual peanut kernels and 5,000,000 ng/g for individual cottonseed. Shotwell et al. (1975) reported finding over 400,000 ng/g of aflatoxin in individual corn kernels. A 5 kg sample of peanut kernels with a single kernel containing  $10^6$  ng of aflatoxin will have a sample concentration of 200 ng/g.

Due to extremes in both the percent contaminated particles and the range in aflatoxin concentrations among individual particles in a contaminated lot, variation among replicated samples tends to be large. For example, the coefficient of variation among 5.4 kg

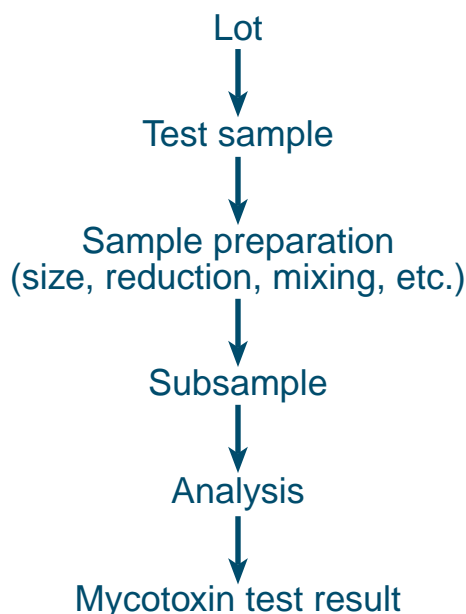


Figure 7.2. Diagram of general steps involved in sampling, sample preparation, and analysis of mycotoxins in agricultural commodities.

samples taken from a raw shelled peanut lot at 20 ng/g is about 110% (Whitaker et al. 1974a). The sampling variability associated with testing several commodities for aflatoxin, fumonisin, and DON has been estimated by Whitaker and associates (1974a, 1976, 1979a, 1992, 1994), U.S. Department of Agriculture/GIPSA (1983), and Hart and Schabenberger (1998). Equations have been developed to predict the sampling variability as a function of the lot concentration and any size sample for the commodities listed above. These variability equations are specific for the type of product, type of mycotoxin, and the average kernel size (count per gram) of the product used in the study.

Studies by Hart and Schabenberger (1998) and the U.S. Department of Agriculture/GIPSA (1983) associated with testing wheat and barley for DON indicated that the sampling step for this mycotoxin is not as large a source of variability as with aflatoxin and fumonisin. It is not clear why DON is different from aflatoxin. However, DON concentrations are about 1,000 times greater than normally reported for aflatoxin. This suggests that a greater percentage of kernels in the lot are contaminated with DON than is the case for aflatoxin, which results in lower sampling variability.

#### Sample Preparation Variability

Once a sample has been taken from the lot, it must be prepared for aflatoxin extraction. Because it is not practical to solvent extract aflatoxin from a large test sample, the mycotoxin is usually extracted from a much smaller portion (subsample) of the test sample. If the commodity is a granular product, e.g., shelled corn, it is essential that the entire test sample be comminuted in a suitable mill before the subsample is removed (Campbell et al. 1986; Dickens and Whitaker 1982; Food and Agriculture Organization 1993; Park and Pohland 1989; Whitaker and Park 1994). Removing a subsample from the test sample before comminution would eliminate the benefits associated with the larger size sample of granular product. After the test sample has been comminuted, a subsample is removed for mycotoxin extraction. It is assumed that the distribution of contaminated particles in the comminuted sample is similar to the distribution of contaminated kernels found in the lot. Thus, subsamples taken from the same comminuted test sample can vary. However, subsampling variability should not be as large as sampling variability, due to the larger number of comminuted particles in the subsample.

Sample preparation variability has been measured for several commodities, several mycotoxins, and sev-

eral types of mills (Dorner and Cole 1993; Velasco et al. 1975; Whitaker et al. 1974a, 1979b, 1994, 1998). These studies have shown that sample preparation variability can be decreased with an increase in subsample size and degree of comminution (more comminuted particles per unit mass).

Equations have been developed to predict sample preparation variability as a function of the sample concentration and any size subsample. These equations are specific for the type of product, mycotoxin, and mill used in the study. For example, at a sample concentration of 20 ng/g, the sample preparation variability, as measured by the standard deviation, associated with a 250 g subsample using the Dickens-Satterwhite mill and a vertical cutter mixer (VCM) mill to grind peanuts is 7.7 and 3.2, respectively. The CV is 38.5% and 16%, respectively. The VCM grinds the sample more finely than the Dickens-Satterwhite mill. The sample preparation variability for the VCM is much lower because it provides a smaller particle size than the Dickens-Satterwhite mill.

#### Analytical Variability

Once the subsample is removed from the comminuted sample, the mycotoxin is usually solvent extracted by official methods (Nesheim 1979; Park and Pohland 1986; Schuller et al. 1976). These methods usually involve steps such as solvent extraction, certification, filtration, drying, dilution, and quantification. As a result, replicated analyses on the same subsample extract can vary considerably. Numerous studies have been conducted to determine the variability of analytical methods for measuring mycotoxins in different commodities (Dorner and Cole 1988; Hagler and Whitaker 1991; Park et al. 1991a, c; Velasco et al. 1975; Whitaker et al. 1974a, 1976, 1979b, 1994, 1998). Within a given laboratory, liquid chromatography (LC) generally has the lowest variability and TLC the highest variability. Variability of the newer ELISA methods generally falls between LC and TLC. TLC studies by Whitaker and Dickens (1989) indicate that the quantification step is the major source of variability when measuring peanuts for aflatoxin. If extraction and cleanup contribute only a small portion of the total analytical variability, then immunoassay and HPLC analytical methods should have lower variability than methods that use TLC quantification techniques.

Whitaker et al. (1996b), using data collected by the FDA (Horwitz et al. 1993), sorted 1,019 analytical precision estimates into two precision-type measurements (among-lab and within-lab), three analytical methods (TLC, LC, and ELISA), and 11 commodities.

Sufficient data existed to study the analytical variability associated with 36 sorted combinations (of a possible 66). As with the sampling and sample preparation variability, analytical variability was also a function of the aflatoxin concentration. When results are pooled across the 11 agricultural commodities, LC had the lowest analytical variability, while ELISA and TLC had similar but higher variability. For a given analytical method, among-lab variability was approximately double the within-lab variability. For example, the average within-lab coefficients of variation (averaged across all commodities studied) associated with measuring aflatoxin in a comminuted subsample at 20 ng/g by the LC, ELISA, and TLC analytical methods are 16%, 30%, and 29%, respectively.

### Decreasing Variability of Test Results

The large variability associated with mycotoxin test procedures makes it difficult to estimate with a high degree of confidence the true concentration of a lot. The only way to achieve a more-precise estimate of the true lot concentration is to decrease the total variability of the test procedure by decreasing the variability associated with each step. Sampling variability can be decreased by increasing sample size (or number of sampling units). Sample preparation variability can be decreased either by increasing the size of the subsample and/or the degree of comminution, i.e., increasing the number of particles per unit mass in the subsample. Analytical variability can be decreased by either increasing the number of aliquots quantified by the analytical method and/or using more-precise quantification methods, e.g., HPLC instead of TLC or ELISA. If the variability associated with at least one of these steps can be decreased, then total variability associated with the test procedure can be decreased.

Whitaker et al. (1974a) showed the expected variation (as measured by the total variance [VT]) associated with a test procedure to estimate aflatoxin in a shelled peanut lot at 20 ng/g aflatoxin. VT was estimated at

$$VT = 575.1 + 13.5 + 20.9 = 609.5 \text{ (2)}$$

using a 1,100 g subsample of a 5.45 kg sample comminuted in the Dickens-Satterwhite mill and analyzed by using the BF method with TLC quantification.

From the VT of 609.5, the standard deviation (SD) and coefficient of variation (CV) associated with the

testing procedure described above are 24.7 and 123%, respectively. The sampling, sample preparation, and analytical variances account for 94.4% (575.1/609.5), 2.2% (13.5/609.5), and 3.4% (20.9/609.5) of the total variation, respectively. It seems that the best use of resources to decrease the total variability would be to increase sample size.

The range of aflatoxin test results associated with any size sample and subsample and number of analyses can be estimated from the SD, which can be determined by taking the square root of the total variance in Eq. (2). Approximately 95% of all mycotoxin test results will range about the lot concentration  $\pm 1.96$  SD. The expressions are valid only for a normal distribution where test results are symmetrical about the mean. While aflatoxin tests are not usually normally distributed, mycotoxin test results will approach a symmetrical distribution as sample size becomes large (Whitaker et al. 1972, 1996a). The effect of increasing sample size on the range of results when testing a contaminated cottonseed lot that has 100 ppb aflatoxin is shown in Table 7.3. The range does not decrease at a constant rate as sample size increases. For example, doubling sample size has a greater effect on decreasing the range at small sample sizes than at large sample ones. This characteristic suggests that increasing sample size beyond a certain point may not be the best use of resources. Instead, increasing subsample size or number of analyses may more effectively decrease the range of test results once sample size has become significantly large.

Methods other than increasing sample size exist to decrease the variability associated with testing a com-

**Table 7.3. Estimated range of aflatoxin test results for 95% confidence limits when testing a contaminated lot of cotton seed with 100 ppb using different sample sizes**

Sample size	Standard deviation <sup>a</sup>	Low <sup>b</sup> (ppb)	High <sup>c</sup> (ppb)	Range (high-low) (ppb)
1	87	0	271	271
2	62	0	222	222
4	45	13	187	174
8	32	37	163	126
16	24	53	147	95
32	19	64	136	72

<sup>a</sup>Standard deviation reflects sample sizes shown in the table plus a 100-g subsample comminuted in a Dickens mill with a 1-mm screen and immunoassay analytical method.

<sup>b</sup>Low = 100 - 1.96 (standard deviation). If low was negative, a value of 0 was recorded.

<sup>c</sup>High = 100 + 1.96 (standard deviation).

modity for aflatoxin. Different costs are associated with each method, so careful study is required to determine the testing procedure that will provide the least variability for a given cost. The optimum balance in sample size, degree of comminution, subsample size, and number and type of analysis will vary with the costs involved with each step of the mycotoxin test procedure. In general, costs of properly designed mycotoxin-testing procedures will increase as the total variation is decreased.

### Designing Aflatoxin Testing Programs

Bulk shipments of a food or feed product often are tested for mycotoxins. If the estimated lot concentration exceeds a defined tolerance, the lot is diverted from human or animal use. The tolerance can be a regulatory guideline or a company tolerance established for quality control. The large variability among test results causes two types of mistakes in any mycotoxin inspection program. First, good lots, i.e., those with a concentration less than or equal to the guideline, will test as bad and be rejected by the inspection program. This type of mistake is called a "false positive" or "seller's risk," since these lots will be rejected at an unnecessary cost to the seller. Second, bad lots, i.e., those with a concentration greater than the guideline, will test as good and be accepted by the inspection program. This type of mistake is called a "false negative" or "buyer's risk," because contaminated lots have a potential for being processed into a consumer-ready product. To maintain an effective quality control program, these two risks must be evaluated and decreased. Then, the costs and benefits (benefits refers to removal of mycotoxin-contaminated lots) associated with a mycotoxin inspection program can be evaluated.

The magnitude of the seller's and buyer's risk is uniquely defined for a particular mycotoxin-testing program with designated values of sample size, type of mill, subsample size, type of analytical method, number of analyses, and accept/reject level (tolerance). Decreasing the variability of the test procedure, e.g., increasing the sample size, decreases both the seller's and buyer's risk. The same effect can be obtained by increasing either the degree of sample comminution, the subsample size, or the number of analyses. Changing the accept/reject level also affects the magnitude of the seller's and buyer's risks. Decreasing the accept/reject level relative to the regulatory guideline decreases the buyer's risk but increases the seller's. Increasing the accept/reject level relative to the regulatory guideline decreases the sell-

er's risk but increases the buyer's. Only one of the two risks can be decreased by changing the accept/reject level of the inspection program relative to the regulatory guideline because decreasing one risk will automatically increase the other.

Methods have been developed to predict the seller's and buyer's risks, the total number of lots accepted and rejected, the amount of mycotoxin in the accepted and rejected lots, and the costs associated with a mycotoxin inspection programs for several commodities (Coker et al. 1995; Food and Agriculture Organization 1993; Whitaker 1977; Whitaker et al. 1974a, 1979b). These methods have been used by the USDA/AMS and the peanut industry to design aflatoxin-testing programs for shelled peanuts (Whitaker and Dickens 1979) and the FAO (1993) to design the aflatoxin-testing plan for corn and peanuts. Regulatory agencies also have used the methods to evaluate existing aflatoxin inspection programs for peanuts (Whitaker et al. 1995).

Regarding testing, the European Committee for Standardization (1999) has developed methods criteria to apply testing for mycotoxins so that standards are in place for trading purposes. These numerical criteria (CEN report CP 13505) are performance based (de Koe 1995) and are published by Codex in the Procedural Manual adopted by the European evaluations, a working group that established criteria for rather low levels of selected mycotoxins that were below rather low regulatory limits existing already in most of the EU member states for targeted foodstuffs. Most of the methods already existing in the literature at that time failed to meet the performance criteria. Therefore, there was a call for validation of analytical methods to determine the content of aflatoxins, ochratoxin A, and patulin in foods of vegetable origin and aflatoxin M<sub>1</sub> in liquid milk. This working group has established validated methodology at lower limits and for a wider range of matrices than previously was the case. Now the working group is called CEN/TC 275/WG 5-Biotoxins and their work has recently been published in a series of EUR reports after collaborative trial studies were reviewed by AOAC International and CEN (Dragaci et al. 2001; Entwisle et al. 2000, 2001; MacDonald et al. 2000; Stroka et al. 2000).

### Proficiency of Toxin Analysis: The Experiences of the United Kingdom Food Analysis Performance Assessment Scheme

In 1990, MAFF initiated the FAPAS® to test the

**Table 7.4. Typical matrices and analytes for the toxin series of the Food Analysis Performance Assessment Scheme (FAPAS®) (Thompson and Wood 1993, 1999)**

Series title	Typical matrix	Concentration ranges (ppb)
Aflatoxin analysis	Maize, peanut butter, spices, dried fruit/fig paste	4–134
Patulin analysis	Apple juice	19–94
Ochratoxin A	Wheat flour, coffee, dried fruit	5

proficiency of analytical laboratories in the U.K. (Patey 1996). Three of the series of test materials were introduced for toxin analysis, and the number of laboratories participating in these series has risen annually to more than 400 in 1999/2000.

The requirements for organizations such as MAFF that wish to develop and operate proficiency-testing schemes are outlined in the International Harmonised Protocol for Proficiency Testing of (Chemical) Analytical Laboratories (Thompson and Wood 1993) together with ISO Guide 43 (International Organisation of Standardisation 1984). The system that FAPAS® uses is outlined in its own Protocol (Central Science Laboratory 1997), which follows the International Protocol and the ISO Guide.

#### Participation

Before FAPAS® started, MAFF anticipated that it would only be of interest to U.K.-based laboratories. Once it was established and laboratories in other countries came to know of it, however, many applied to participate, to the extent that half the laboratories involved were not located in the United Kingdom. During the first seven years of operation, three toxin series of test materials were introduced for analysis. The aflatoxin series started in 1990, with test materials distributed every four months, a time period recommended in the International Protocol (Thompson and Wood 1993), with over 100 laboratories participating in each round. The patulin series started in 1996, offering two distributions each year. The ochratoxin A series began in 1997, also offering two distributions per annum. Typical test material matrices, analytes, and concentration ranges for each of the toxin series are given in Table 7.4. More recently, a *Fusarium* toxins series was introduced in early 2000, with over thirty laboratories registered for participation in the first round for analysis of fumonisins B<sub>1</sub>, B<sub>2</sub>, and deoxynivalenol in a corn/wheat test material.

#### Homogeneity Analysis

A condition for successful proficiency testing is that

each participant receives an identical test material. The International Protocol (Thompson and Wood 1993, 1999) requires scheme organizers to test each material for homogeneity by a prescribed procedure. The test material can only be issued for assessment of a particular analyte if either of two statistical tests is passed: the F-test or the  $s_g/\sigma$  test, where  $s_g$  is the square root of the sampling variance and  $\sigma$  is the target value for standard deviation (International Organisation of Standardisation 1984). The latter test is passed if the analytical precision (repeatability) of the method used in the homogeneity test, defined as  $s_g/\sigma$ , is less than 0.4 (Thompson and Lowthian 1996). If neither of these tests is passed, homogeneity is not demonstrated and the test material cannot be issued. Occasionally, test materials analytes fail the F-test but pass the  $s_g/\sigma$  test, and these materials can therefore be issued for analysis.

#### Performance Markings

The International Protocol (Thompson and Wood 1993, 1999) recommends that a laboratory's performance be measured by z-score, which is defined as

$$z = (x - \hat{X})/\sigma$$

where  $x$  = the participant's reported result,  
 $\hat{X}$  = the assigned value, and  
 $\sigma$  = the target value for standard deviation.

The assigned value was the best estimate of the "true" concentration of the analyte, calculated from participants' results using a robust statistical procedure (Analytical Methods Committee 1989). Target values for standard deviation ( $\sigma$ ) were obtained from collaborative trial data (where available), governmental regulations, or the appropriate form of the Horwitz equation (Thompson 2000).

It is important to understand the statistical limitations of this external means of quality assessment when gauging the competence of a laboratory. The results of a typical chemical analysis will be normally distributed. That is to say, the majority of results

**Table 7.5. Food Analysis Performance Assessment Scheme (FAPAS®) z-score performance data (Thompson and Wood 1993, 1999)**

Analytical area	Z-score assessments	Satisfactory z-scores	Percentage satisfactory
Aflatoxin	8,702	7,793	90
Patulin	277	239	86
Ochratoxin A	378	312	83
Total/average	9,357	8,344	89

will be centered on a mean value but, inevitably, some results will lie at the extremes of the distribution. The statistics of a normal distribution mean that about 95% of data points will lie between a z-score of  $-2$  and  $+2$ . Performance in a FAPAS® proficiency test, therefore, is considered 'satisfactory' if a participant's z-score lies within this range. It follows that if a participant's z-score lies outside  $\tilde{a}|z|\tilde{a} > 2$ , there is about a 1 in 20 chance that their result is in fact an acceptable result from the extreme of the distribution. If a participant's z-score lies outside  $\tilde{a}|z|\tilde{a} > 3$ , the chance that their result is actually acceptable is only about 1 in 300.

#### Rate of Return of Data and Overall Performance

At the end of a FAPAS® round, which is 8 weeks after a test material is distributed, proficiency reports for individual participants are prepared and z-score markings are shown in both tabular and ordered histogram form. Not every analyst that receives the test material, however, returns data within the required timescale. From the issue of some 4,000 test materials between 1990 and 2000, the overall return rate for results from laboratories analyzing toxins was 79%. The total number of z-score proficiency assessments made for toxins from 1990 to 2000 is shown in Table 7.5. More than 9,000 z-scores were issued, of which 89% were in the satisfactory range. This is a very high

success rate, especially when compared with the figure of approximately 80% satisfactory for other FAPAS® series.

#### Performance Trend

In the rounds of testing for toxins, participants are not given a specified method to follow and choose their own analytical method. Thus, participants use a wide range of methods, so performance comparison between different groups of methods is possible. The final test reports for FAPAS® rounds contain a section that summarizes the methods of analysis used. Thus, it is possible to identify the methods used by satisfactory, questionable, and unsatisfactory performers alike, so that decisions may be taken by laboratories to change aspects of the methods they use. This is one way that performance can improve by continued participation in proficiency testing.

Some unsatisfactory performance markings for laboratories are due to simple errors such as confusing units requested (milli- versus micro-) and misreporting the result by three orders of magnitude. However, by studying the methods section of reports, use of inappropriate methodology may be spotted. Method sections also list equipment manufacturers and sources of standards used by participants. Scanning the section will show the popularity of individual items, e.g., which method is in greatest use.



## 8 Risk Assessment and Regulations for Mycotoxins

### Summary

Mycotoxins are considered unavoidable contaminants in foods in that agronomic technology has not yet advanced to the point where preharvest infection of susceptible crops can be completely eliminated. Mycotoxins may exhibit various adverse toxicological manifestations in humans and animals. They can enter food directly or indirectly during the production, transportation, processing, or storage of food. Small amounts of these contaminants may be legally permitted in foods and feeds because they are not entirely eliminated by good agricultural practices (GAP) and good manufacturing practices (GMP), provided the amounts involved are not injurious to human and animal health.

Many countries have established regulatory limits or food laws that restrict the concentrations of mycotoxins that are permitted in certain foods and feeds; at least 77 countries involved in international trade have now established regulatory limits for some of the mycotoxins. Factors that should play a role in deciding appropriate regulatory limits for a mycotoxin in a particular country include the availability of analytical methods, toxicological and analytical survey data for use in preparing risk assessments, and knowledge concerning the occurrence and distribution of the mycotoxin in various commodities. It is also important that each country recognizes the need to maintain an adequate supply of food at reasonable cost to consumers in their country, and to be aware of legislation in other countries with which trade contacts exist.

### Introduction

The control of mycotoxins in foods and feeds is a constantly evolving process. The occurrence of mycotoxins is influenced by certain environmental factors; hence, the extent of contamination of a particular commodity with a particular toxin is unpredictable and will vary with geographic location, agricultural practices, and the susceptibility of commodities to fungal invasion during preharvest, storage, and/or process-

ing periods.

Mycotoxins may exhibit various adverse toxicological manifestations in humans and animals. In many cases, the effects of mycotoxins on human health, at the levels at which humans are sometimes exposed, are not known. In highly developed countries, acute toxic effects of mycotoxins are rarely observed because advances in processing technology and quality control programs, such as GAP and GMP prevent heavily contaminated food from entering the food supply. From a public health perspective, there is concern regarding the health effects of long-term exposure to low levels of mycotoxins in foods.

Because the occurrence of these toxins in foods and feeds is not entirely avoidable, small amounts of these contaminants may be legally permitted, provided the amounts involved are not considered injurious to human and animal health. The legal basis for regulating toxin or poisonous substances in foods in the United States is the Federal Food, Drug and Cosmetic Act, which is enforced by the FDA. In one section of this Act, a food is considered adulterated if it bears or contains any poisonous or deleterious substance that may render it harmful to health. By enforcing this statute, the FDA can prohibit the entry of, and remove from interstate commerce, any food or feed that is so adulterated. One strategy used by the United States to minimize mycotoxins in the food supply is to establish regulatory limits for certain mycotoxins in various commodities. Once these limits are established, the food industry is monitored routinely to determine if it is adhering to established regulatory limits.

The worldwide occurrence of mycotoxins in foods and feeds has been recognized by the FAO, and the World Health Organization (WHO) for many years. Many countries worldwide have established food laws that impose limits on the concentrations of specific mycotoxins in foods. Analytical data obtained from food monitoring programs are coupled with available toxicological data and are used for making science-based risk assessments that can serve as a basis for establishing a particular regulatory level. The objective of this chapter is to give an overview of the importance of the use of science-based risk assessments,

coupled with the establishment of regulatory limits as an ideal approach to minimize the levels of mycotoxin contamination in the food supply and thereby protect the health of consumers.

## Risk Assessment

Many mycotoxins have potent acute and chronic biological activities as determined by animal studies, in vitro bioassays, and human epidemiological studies. These substances are present in the food supply on an intermittent or chronic basis.

Regulatory agencies regulate unavoidable contaminants of foods, i.e., those that cannot be avoided by good manufacturing practices, which usually is the case with mycotoxins. In the United States, the FDA regulates mycotoxins under Section 402 or 406 of the Food, Drug and Cosmetic Act (FDCA). The FDA balances the benefits of food availability against the risk that a contaminant in that food might pose to the public health (Bolger et al. 1996). Quantitative risk assessment for carcinogenic contaminants and safety assessment for noncarcinogenic contaminants are tools used by the FDA to estimate the upper limit of risk to public health posed by the contaminant.

Safety/risk assessment requires determining the severity and scope of adverse effects of a hazard (toxicity assessment) and also the degree of exposure of an individual or population (exposure assessment). The FDA's current approach to toxicity assessment can be traced to a paper by Lehman and Fitzhugh (1954) that introduced the use of 10-fold safety factors, later known as uncertainty factors. Such factors are used to extrapolate data on the effects of specific chemicals on laboratory animals to humans, to derive an acceptable or tolerable daily intake (ADI/TDI); in particular, the no-observed-adverse-effect level (NOAEL) is divided by the safety/uncertainty factors (multiples of 10) (Bolger 1997).

One process of cancer risk estimation, generally used for foodborne substances, employs a simple, direct method. A point on the dose-response curve (tumor incidence versus dose) for a chemical is chosen below which the data no longer appear to be reliable (generally in the range of a 1% to 10% tumor incidence). A straight line is drawn from the upper confidence limit on risk at that point to the origin. This provides the slope of the line used to provide upper bound estimates of cancer risk at low doses where risk  $\geq$  slope  $\times$  dose. For this procedure, the target tissue dose for the active carcinogenic agent is desirable; without this information, the whole dose administered

to an animal in a bioassay is used. In the absence of information to the contrary, dose scaling across species is based on body weight to the 3/4 power (arrived at by an interagency committee) or dose is expressed as concentration in the diet. The FDA recognizes that food consumption and body weight of animals influence survival, tumor incidence in some tissues, and toxicity. Carcinogenic potency in animals tends to overestimate carcinogenic potency in humans for about 20 chemicals where data are available for making comparisons (Gaylor et al. 1997).

### Safety Assessment for Deoxynivalenol

As discussed in Chapter 3, DON is a natural metabolite produced by various members of the genus *Fusarium*. These fungi grow on various grains and are ubiquitous.

The FDA evaluated a number of hazards of exposure to DON (Carrington 1991, 1993), which will be described in the following section.

#### Acute Toxicity

Swine appear to be the species that is most sensitive to vomitoxin, with vomiting, diarrhea, muscular weakness, tremors and coma observed after 24 hours following dietary doses as low as 2 mg/kg. In acute studies in mice, LD<sub>50</sub> values were calculated to be 78 mg/kg bw by gavage and 49 mg/kg bw intraperitoneally. In these animals, lesions were observed in the GI tract, kidney, heart and lymphoid tissue. In rats fed 10 mg/kg bw vomitoxin, no signs of toxicity, e.g., feed refusal, emesis, unusual behavior, were observed. Chickens seem to be less sensitive than swine. In laying hens, concentrations of up to 5 mg/kg vomitoxin in the diet decreased egg weights and shell thickness. Dairy cattle are apparently able to tolerate relatively high levels of vomitoxin; nonlactating cows can consume 1 mg vomitoxin/kg bw/day without any serious side effects.

#### Teratogenicity and Reproductive Toxicity

In swine-feeding studies using 3.5 mg/kg bw, treated gilts had a lower feed consumption and growth rate than did controls; increasing levels of vomitoxin yielded a linear trend toward lower fetal weight and length. Mice embryos are fairly sensitive to vomitoxin. Doses of 2.5, 5, 10, and 15 mg/kg bw were associated with a specific lethal effect on the mouse embryo with or without maternal vaginal bleeding but not with any apparent maternal toxicity. Multiple teratogenic effects occurred at doses of 2.5 and 5 mg/kg bw, while skeletal malformations were present in the 1, 2.5, and

5 mg/kg bw groups in a dose-related manner. Resorptions at the 10 and 15 mg/kg bw dose were 100% and 80%. The mean number of live fetuses/litter at these doses showed no statistically significant decrease, although resorptions were increased. In rats, dietary concentrations of vomitoxin of up to 20 ppm caused neither maternal effects nor convincing evidence of fetal effects. In rabbits, increased resorptions were observed at doses of 1.8 or 2 mg/kg bw and fetal weight was decreased at maternal doses of 1 and 1.6 mg/kg bw. All doses caused maternal decreases in body weight and feed intake.

#### Effects on the Immune System

Chronic exposure to DON has been shown to impart decreased humoral immunity, cell-mediated immunity, and host resistance. An increase in serum IgA can be demonstrated in mice fed levels as low as 2 ppm DON in some experiments, with the greatest effect shown at 25 ppm. Vomitoxin induced elevations in the percentages of IgA+ cells, total T cells, and CD4+ cells in mice, suggesting that it induced regulation of IgA production originating in the Peyer's patches. Increases in serum IgA and the pIgA to mIgA ratio have been associated with IgA nephropathy in mice.

The following discussion shows how toxicology data can be used to estimate a tolerable daily intake. In actual practice, the FDA has employed other data in addition to these mice teratology data. The teratogenicity data in mice (Khera et al. 1982, 1984) were selected as being most appropriate for modeling a quantitative relationship between DON and fetal resorption in mice; this model was used to estimate a maximum likelihood estimate of a dose that leads to a 5% increase in fetal resorption. This dose may also be called a benchmark dose and is equivalent to a NOAEL. A dose that results in a 5% increase in fetal resorption of 1.15 mg/kg/day was obtained (Carrington 1991, 1993).

Evidence indicates that minor renal dysfunction associated with dietary exposure to vomitoxin has a dose-response function similar to that for reproductive hazard. However, insufficient evidence exists to estimate the magnitude, severity, or dose-response relationship of the hazard that may result.

By applying a factor of 10 to extrapolate across species and a factor of 10 to account for individuals who may be more sensitive, a TDI of 12 µg/kg/day can be estimated. For a 60 kg adult, this corresponds to an exposure of 0.7 mg/kg/day. This endpoint is based on an effect that may occur from subchronic exposure (weeks to months). Therefore, a short-term exposure

to this concentration from a single crop may be as hazardous as a long-term exposure.

#### Quantitative Risk Assessment for a Carcinogenic Mycotoxin, Aflatoxin

An excellent example of using animal studies, human epidemiology studies, and *in vitro* metabolism data to perform a quantitative risk assessment for the carcinogenic mycotoxin class of aflatoxins was done by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1997 (United Nations 1997) and in 2001 (United Nations 2001).

This subcommittee of internationally recognized experts was charged with reviewing the experimental evidence concerning aflatoxin carcinogenicity, evaluating potencies, linking potencies to intake estimates, and discussing the impact of hypothetical aflatoxin standards on sample populations and their overall risks.

A number of factors influence the risk of primary liver cancer, most notably, carriage of hepatitis B virus as determined by the presence in serum of the hepatitis B surface antigen. Aflatoxin potency seems to be significantly enhanced in individuals with simultaneous hepatitis B infection. This interaction makes it difficult to interpret the epidemiological studies, e.g., from Shanghai, Thailand, China, Gambia, Taiwan, in the context of aflatoxin as an independent risk factor.

In addition, identification of hepatitis C virus is an important recent advance in understanding etiology of liver cancer. A limited number of studies with inconclusive results have investigated interactions between hepatitis C infection, aflatoxin, and liver cancer. An estimated 50 to 100% of liver cancer cases are associated with persistent infection with hepatitis B and/or C.

A review of aflatoxin carcinogenic potencies estimated from laboratory animal studies and human epidemiological research yielded potency estimates ranging from 0.01 (cancers/year per 100,000 people per ng aflatoxin/kg bw per day) for humans without hepatitis B virus infection to 1.0 in the Fischer rat (one of the most-sensitive animal species). Observations concerning the interaction of hepatitis B and aflatoxin suggested two separate aflatoxin potencies in populations where chronic hepatitis infections were common versus those where such infections are rare.

To evaluate population risk from intake of aflatoxin, potencies were estimated from the positive epidemiological studies for both hepatitis B-negative and

hepatitis B-positive individuals. Potency values of 0.3 cancers/year per 100,000 population per ng aflatoxin B<sub>1</sub>/kg bw per day (with an uncertainty range of 0.05 to 0.5) in hepatitis B-positive individuals and of 0.01 cancers/year per 100,000 population per ng aflatoxin B<sub>1</sub>/kg bw per day (with an uncertainty range of 0.002 to 0.03) in hepatitis B-negative individuals were calculated.

These estimates were subject to potential biases for several reasons: (1) only studies showing a positive association between aflatoxin and liver cancer were used, as opposed to considering all studies, leading to overestimation of aflatoxin potency; (2) relating current levels of intake to current levels of liver cancer (presumably with a long induction period) ignored historical levels (likely to have been higher); (3) the earliest studies systematically underestimated hepatitis B prevalence in cases of liver cancer by a factor of up to 30%, due to limitations in the methodology used to detect hepatitis B, which also led to overestimation of the relative potency of any other factor, including aflatoxins; and (4) histological confirmation of liver cancer cases was limited in most epidemiological studies, so nonprimary liver cancer cases could have been included, underestimating or overestimating aflatoxin potency.

Thus, estimated potencies may be considered overestimates of aflatoxin potency, leading to the hypothesis that humans may be less sensitive to aflatoxin than species tested in laboratory experiments. Thus, the fraction of the incidence of liver cancer in populations attributable to aflatoxin intake was derived by combining aflatoxin potency estimates (risk per unit dose) and estimates of aflatoxin intake (dose per person). Many of the data on aflatoxin contamination levels around the world were derived from nonrandom samples that appeared to be biased upward, since monitoring studies often focus on commodity lots that are thought to be contaminated. Hence, these contamination levels must be used with caution when inferring patterns of importance in setting aflatoxin standards.

Several important conclusions relevant to the impact of aflatoxin exposure on public health and conservation of important food crops have been reached. First, aflatoxin potency in hepatitis B-positive individuals is substantially higher than in hepatitis B-negative individuals. Thus, decreasing aflatoxin intake in populations with many hepatitis B-positive individuals will have a greater impact on decreasing liver cancer rates than decreasing intake in populations with few such individuals. Second, aflatoxin M<sub>1</sub>, the hydroxylated metabolite of B<sub>1</sub>, may be considered

to have a potency approximately one order of magnitude less than B<sub>1</sub>. Third, vaccination against hepatitis B will decrease the prevalence of carriers; the JEC-FA analysis suggests that this would decrease the potency of aflatoxin in vaccinated populations and consequently decrease liver cancer risks (United Nations 2001). Fourth, analysis of the application of hypothetical aflatoxin standards (10 µg/kg or 20 µg/kg in food) to model populations indicates that (1) populations with a low prevalence of hepatitis B-positive individuals and/or with a low mean aflatoxin intake (less than 1 µg/kg bw/day) are unlikely to exhibit detectable differences in population risks for a standard in the range of the hypothetical cases, i.e., western European and U.S. populations and (2) populations with a high prevalence of hepatitis B-positive individuals and high mean aflatoxin intake would benefit from decreased aflatoxin intake.

### Biomarkers for Selected Mycotoxins

The exposure risk in humans to selected mycotoxins can be more precisely determined if there are biomarkers that can be used. These biomarkers can be selective for certain mycotoxins and epidemiological studies can assess the validity of their use. If the biomarkers prove to be valid, the exposure of individuals or populations of individuals can be determined with a high degree of accuracy.

Presently, only sufficient work has been done with a few mycotoxins to determine the possibility of using biomarkers to assess exposure. The mycotoxins having biomarkers of interest for further study are aflatoxins, fumonisins, and ochratoxin.

Several biomarkers have been developed for the aflatoxins, especially aflatoxin B<sub>1</sub>, that can potentially be used in risk assessments for human exposure. These included the use of aflatoxin-albumin adducts in the serum, aflatoxin N7-guanine adducts in the urine, aflatoxin M<sub>1</sub> (a metabolite of aflatoxin B<sub>1</sub>) in the urine, and mutational patterns in the P53 tumor suppressor gene (Robens and Richard 1992; United Nations 2001). The report by the Joint FAO/WHO Expert Committee on Food Additives (United Nations 2001) suggested, however, that sensitive markers for exposure to hepatitis B and/or C viruses likely were more importantly related to liver cancer assessment in humans. Regardless, in determining the exposure to aflatoxins, the biomarkers listed above could be used in the exposure assessments.

One of the earliest known indications of the exposure of humans and other animals species to fumonisin B<sub>1</sub> is an increase in the sphinganine/sphingosine

(Sa/So) ratio in urine or serum. This has been proposed as a biomarker for exposure to fumonisins. (See chapter on fumonisins in this report.) The basis for this change is that fumonisins disrupt lipid metabolism, especially sphingolipids, through the inhibition of ceramide synthase leading to the increase in concentration of the sphingoid bases. In human volunteers, there was an increase in the urinary Sa/So ratio during a one month period of consumption of corn containing fumonisin B<sub>1</sub> ranging from 0.08 to 41.1 mg/kg (Qiu and Liu 2001).<sup>1</sup> The study results suggested that males are more sensitive to fumonisin B<sub>1</sub> disruption of sphingolipid metabolism than females. Notably, fumonisins are not genotoxic and no DNA adducts are formed (United Nations 2001). Therefore, no biomarkers are available based on DNA modification.

While obtaining evidence for the involvement of ochratoxin A in human disease, especially cancer, remains somewhat problematic, there have been confirmations of human exposure based on the presence of ochratoxin A in blood samples of both apparently healthy individuals and of patients with nephropathy in various countries (Fink-Gremmels et al. 1995a; Frolich et al. 1991; Hald 1991). Its presence in human milk was also found (Micco et al. 1991). Interestingly, although ochratoxin A has been found in human blood and breast milk, the major routes of exposure remain unidentified and data from food sources only partially explain the high exposure rates found in humans. Little information is available regarding use of absolute biomarkers for exposure especially when considering the routes of ingestion versus that of, perhaps, inhalation. Studies in rats show that ochratoxin binds to albumin in the serum, is transported throughout the circulatory system, and is released and reabsorbed in various tissues especially renal tubular epithelium (Tsuda et al. 1999). Thus, the major biomarker for exposure to ochratoxin A is identification of this compound in milk, serum, or perhaps urine of humans.

## Regulatory Control Programs for Mycotoxins in the United States

In the United States, the primary mission of the FDA is to protect the public health. The FDA is responsible for enforcing several statutes; one of the most important is the FDCA. This statute and its amendments provide FDA authority to regulate poisonous or deleterious substances, such as mycotoxins, in human food and animal feed. The FDCA also prohibits introduction of adulterated food products into

interstate commerce. Under the FDCA, the producer is responsible for ensuring that food products are safe, wholesome, and truthfully labeled.

Under Section 402(a)(1) of the FDCA, "A food shall be deemed to be adulterated if it bears or contains any poisonous or deleterious substance that may render it injurious to health; but in case the substance is not an added substance such food shall not be considered adulterated under this clause if the quantity of such substance in such food does not ordinarily render it injurious to health." Mycotoxins — specifically, aflatoxins — are considered added poisonous and deleterious substances because their presence in human food and animal feeds can be, in part, avoided by good manufacturing or agronomic practices. Therefore, strategies used by the FDA to minimize mycotoxin contamination in the U.S. food supply include (1) establishing standards or guidelines, e.g., action levels or advisory levels; (2) monitoring foods to determine compliance with established standards and guidelines; (3) taking appropriate enforcement action, e.g., seizure of adulterated domestic foods, detention of imports; (4) working with other federal agencies such as USDA, the states, and foreign countries; and (5) guiding and encouraging industry to address contamination problems by developing and implementing HACCP programs.

One approach that the FDA uses to control mycotoxin contamination in human food and animal feed is establishing action levels. For example, the FDA established an action level of 20 ppb (ng/g) total aflatoxin in most human foods. However, as a result of a decision by the U.S. Court of Appeals in a case involving aflatoxin contamination, the FDA, in its 1988 notice, clarified the status of action levels (U.S. Food and Drug Administration 1988). Specifically, action levels are not binding on the courts, the public (including food processors), or the agency. Action levels are used as a guide by field staff to determine when enforcement actions should be taken. Situations may occur where enforcement action should be considered at levels below an action level or a decision not to consider enforcement action where an action level is exceeded.

Another approach used by the FDA to minimize human and animal exposure to mycotoxins is to issue advisory levels directed at state and federal officials as well as trade associations. FDA advisory levels are not enforceable levels. An advisory level for a particular contaminant in human food or animal feed is a guideline below which, based on available toxicological information, a public health hazard is not anticipated. While enforcement is not the fundamental

purpose of an advisory level, the FDA reserves the right to take appropriate enforcement actions when circumstances warrant such actions.

Further, the FDA can establish tolerances or regulatory limits for added poisonous or deleterious substances in human foods through formal rulemaking and notice-and-comment rulemaking processes, respectively. However, the FDA has not established any tolerances or regulatory limits for the control of mycotoxin contamination in human foods and animal feeds.

FDA standards, e.g., action levels, for control of mycotoxin contamination in human food and animal feeds are based, in part, on practical considerations relating to the capabilities of sampling procedures, analytical methodology, agronomic practices, and availability of technological procedures to minimize human and animal exposure to these contaminants. In addition, FDA mycotoxin standards weigh heavily on the unavoidability of the toxins and therefore do not represent permissible levels of contamination where it is avoidable. Furthermore, blending human food or animals feeds containing a contaminant at levels that would be considered adulterated with unadulterated food or feeds is not permitted; the result of blending would be considered adulterated, regardless of contamination level.

### Aflatoxins

Mycotoxins, particularly aflatoxins, have been regulated under Section 402(a)(1) of the FDCA since 1965. In addition to aflatoxins, major mycotoxins that occur naturally on foods and feeds include DON, fumonisins, ochratoxins, zearalenone, and patulin. To establish the need for regulatory control programs for these and other toxins, background exposure data and toxicological data must be obtained and carefully evaluated. More studies have been reported on aflatoxins than any of the other mycotoxins that have been identified since 1960. The motivating force was a series of findings that aflatoxins were potent liver toxins, carcinogens in susceptible animal species, and contaminants in a variety of human foods. Aflatoxins ( $B_1$ ,  $B_2$ ,  $G_1$ ,  $G_2$ ,  $M_1$ ) are the only mycotoxins for which action levels have been established; all others are advisory levels. The current action levels for aflatoxins in foods and feeds are shown in Table 8.1. These levels represent the best guidance available on levels that FDA considers to be of regulatory interest and will thus enhance the safety of the food supply when implemented and adhered to by the food and feed industries. However, the FDA expects, based on

surveillance data, that aflatoxin levels typically are well below action levels due to good agronomic and manufacturing practices. These levels are reviewed periodically as more knowledge regarding analytical, toxicological, and technological procedures become available.

Experience and knowledge obtained through creating an effective control program for aflatoxins has been used to address regulatory considerations involving other mycotoxins in the United States.

### Deoxynivalenol

FDA advisory levels were first issued in 1982 for DON in wheat and wheat products. This action was taken because spring wheat crops in some parts of the United States were heavily infected with head blight or pink scab; unusually high levels of DON were also reported. DON is a trichothecene mycotoxin produced by several molds of the genus *Fusarium*, especially *F. graminearum*, a common contaminant of several grains. When the advisory levels were issued, there was great concern about the safety of this wheat, which might be used for human food as well as animal feed. Toxicological data on DON were extremely limited; therefore, it was difficult for FDA to estimate its potential public health hazard. The agency believed it was important to provide guidance to assist state and local government to deal with the problem; hence, advisory levels for DON in wheat and wheat products were developed. Products that contained the toxin at or below these levels were not believed to present a public health hazard. The FDA has reviewed scientific data that became available after 1982, including reports of DON-associated acute GI illness in humans in China in 1984–1985 and in India in 1987. DON's precise role in those outbreaks is

**Table 8.1. U.S. Food and Drug Administration action levels for total aflatoxins in food and feed<sup>a</sup>**

Commodity	Concentration (ng/g)
All products, except milk, designated for humans	20
Corn for immature animals and dairy cattle	20
Corn and peanut products for breeding beef cattle, swine, and mature poultry	100
Corn and peanut products for finishing swine	200
Corn and peanut products for finishing beef cattle	300
Cottonseed meal (as a feed ingredient)	300
All other feedstuffs	20
Milk	0.5 <sup>b</sup>

<sup>a</sup>U.S. Food and Drug Administration (FDA) Compliance Policy Guides, Sections 527.400, 555.400, and 683.100 (1996).

<sup>b</sup>Aflatoxin  $M_1$ .

not certain but the data suggest an association between human exposure to DON-contaminated wheat and occurrence of the illnesses.

In 1993, based on new scientific information, updated advisory levels were announced (Chesemore 1993). The advisory levels (Table 8.2) amended those for DON found in milled wheat products intended for human consumption, e.g., flour, bran, and in wheat and other grains used for animal feed. An advisory level was not set for raw wheat intended for milling because normal manufacturing practices and additional technology available to millers can substantially decrease DON levels in the finished wheat product. These advisory levels were sent to state agriculture directors; state feed control officials; and food, feed, and grain trade associations.

### Fumonisin

The FDA has issued a guidance document to the food industry concerning fumonisins; however, no action level has been established to date. The fumonisins ( $B_1$  and  $B_2$  are the major ones) are natural byproducts of the fungi *Fusarium verticillioides* (syn., *moniliforme*) and *F. proliferatum*, common contaminants of corn. The finding of a high incidence of fumonisins in corn-based products for human consumption, epidemiological investigations indicating a possible association of *F. verticillioides* and its metabolic products with esophageal cancer, the association of *F. verticillioides* and fumonisin  $B_1$  with ELEM, and animal studies demonstrating the carcinogenicity of fumonisin  $B_1$  in rats, have heightened concerns about the public health impact of these contaminants. Surveys of corn, corn products, and other foods and feeds have been conducted by the FDA, with a number of chronic toxicological studies underway. Results suggest that fumonisins do not accumulate in edible tissues of food animals and no detectable levels have been found in milk from cows dosed with high levels of fumonisins (Miller et al. 1996; Prelusky et al. 1996b; Richard et al. 1996b). This information, along with additional data from ongoing toxicological studies, will be used to assess human health risks and determine the need for regulatory programs.

Based on the existing health hazard data, the FDA believes that exposure to fumonisins should be decreased to the extent practicable. This can be accomplished through issuance of guidance levels or implementation by industry of an appropriate voluntary monitoring and control program. The FDA has issued a guidance document to the food industry that contained proposed maximum fumonisin levels that

**Table 8.2. U.S. Food and Drug Administration advisory levels for deoxynivalenol in wheat/derived products, other grains<sup>a</sup>**

Product	Concentration ( $\mu\text{g/g}$ )
All finished wheat products, e.g. flour, bran and germ, that may be consumed by humans	1
All grains and grain by-products destined for ruminating beef and feedlot cattle older than 4 months and for chickens; these ingredients should not exceed 50% of the diet of cattle or chickens	10
All grains and grain by-products destined for swine; these ingredients should not exceed 20% of the diet	5
All grains and grain by-products for all other animals; these ingredients should not exceed 40% of the diet	5

<sup>a</sup>Letter to state agricultural directors, state feed control officials, and food, feed and grain trade organizations from R. G. Chesemore, Associate Commissioner for Regulatory Affairs, FDA, dated September 16, 1993.

FDA considers adequate to protect human and animal health and that are achievable in human foods and animal feeds with the use of good agricultural and manufacturing practices (U.S. Food and Drug Administration 2000a). These levels will be in effect until a final document is published. The recommended levels for total fumonisins ( $FB_1$ ,  $FB_2$ , and  $FB_3$ ) in human food products and animal feeds are shown in Table 8.3.

### Patulin

Patulin is most frequently found as a natural contaminant of apples and apple products. It has been isolated almost exclusively from apples and apple products contaminated with *Penicillium expansum*. The FDA believes that patulin levels in apple products, particularly apple juice (major source of patulin in the human diet) can be controlled by processors principally by removing spoiled and visibly damaged apples from the product stream used for the production of apple juice. The FDA recently published a document titled "Draft Guidance Document of FDA Components and Industry on Apple Juice, Apple Juice Concentrates, and Apple Juice Products — Adulteration with Patulin" (U.S. Food and Drug Administration 2000b). This document and its accompanying Draft Compliance Policy Guide supports and establishes an action level of 50  $\mu\text{g/kg}$  for patulin in apple juice, apple juice concentrates, and apple juice products.

## Other Mycotoxins

Other mycotoxins of concern to the FDA are ochratoxin A, zearalenone, penicillic acid, sterigmatocystin, citrinin, cyclopiazonic acid, and the *Alternaria* toxins. No regulatory standards have been initiated for these toxins; results from exposure data and/or risk assessments have indicated that regulatory standards are not warranted at this time. The FDA continuously follows the development of newer data regarding these mycotoxins, thereby constantly evaluating the need to set regulatory standards.

## Worldwide Regulations for Mycotoxins

### Introduction

Worldwide food legislation safeguards the health of consumers and the economic interests of producers and traders. Food laws often impose limits on the concentrations of specific contaminants, e.g., mycotoxins, in foods. Overviews of regulations for mycotoxins and their rationales have been published (Food and Agriculture Organization 1997; Gilbert 1991; Moy 1998; Stoloff et al. 1991; van Egmond 1989a, b; 1991). This section will focus on factors that may influence the establishment of regulations for mycotoxins and summarizes current regulations that exist worldwide for mycotoxins.

The need for legislation imposing limits on myc-

otoxin concentration in foods and feeds is generally recognized by the industrialized world. Virtually all countries with fully developed market economies have regulations. In contrast, many developing countries where subsistence farming is significant have not established regulations for mycotoxins, as shown in Figure 8.1. How the limits are chosen and for which commodities depends on several factors, such as (1) availability of toxicological data, (2) availability of data on mycotoxin occurrence in various commodities, (3) homogeneity of the concentration in a lot, (4) availability of analytical methods, (5) legislation in other countries with which trade contacts exist, and (6) the need for sufficient food supply.

### Toxicological Data

Measures are primarily taken on the basis of known toxic effects. For the mycotoxins currently considered most significant (aflatoxins, ochratoxin A, patulin, some trichothecenes including deoxynivalenol, fumonisins, zearalenone), the Joint Expert Committee on Food Additives (JECFA) of the World Health Organization and the Food and Agriculture Organization, has recently evaluated the hazard. JECFA is a scientific advisory body. It provides a mechanism for assessing the toxicity of additives, veterinary drug residues, and contaminants. Safety evaluation of contaminants incorporates various steps in a formal health risk assessment approach. The qualitative indication that a contaminant can cause adverse effects on health (hazard identification) is

**Table 8.3. Proposed guidance levels for fumonisin B<sub>1</sub> in human foods and animal feed (U.S. Food and Drug Administration 2000a)**

	Total fumonisins (FB <sub>1</sub> + FB <sub>2</sub> + FB <sub>3</sub> )
Human food product	
Degermed dry milled corn products	2 µg/g
Whole/partially degermed dry milled corn product	4 µg/g
Dry milled corn bran	4 µg/g
Cleaned corn intended for masa production	4 µg/g
Cleaned corn intended for popcorn	3 µg/g
Corn and corn byproducts for animals	
Equids and rabbits	5 µg/g < 20% of diet
Swine and catfish	20 µg/g < 50% of diet
Breeding ruminants, poultry, mink, dairy cattle, and laying hens	30 µg/g < 50% of diet
Ruminants > 3 months before slaughter and mink for pelt production	60 µg/g < 50% of diet
Poultry raised for slaughter	100 µg/g < 50% of diet
All other species or classes of livestock and pet animals	10 µg/g < 50% of diet



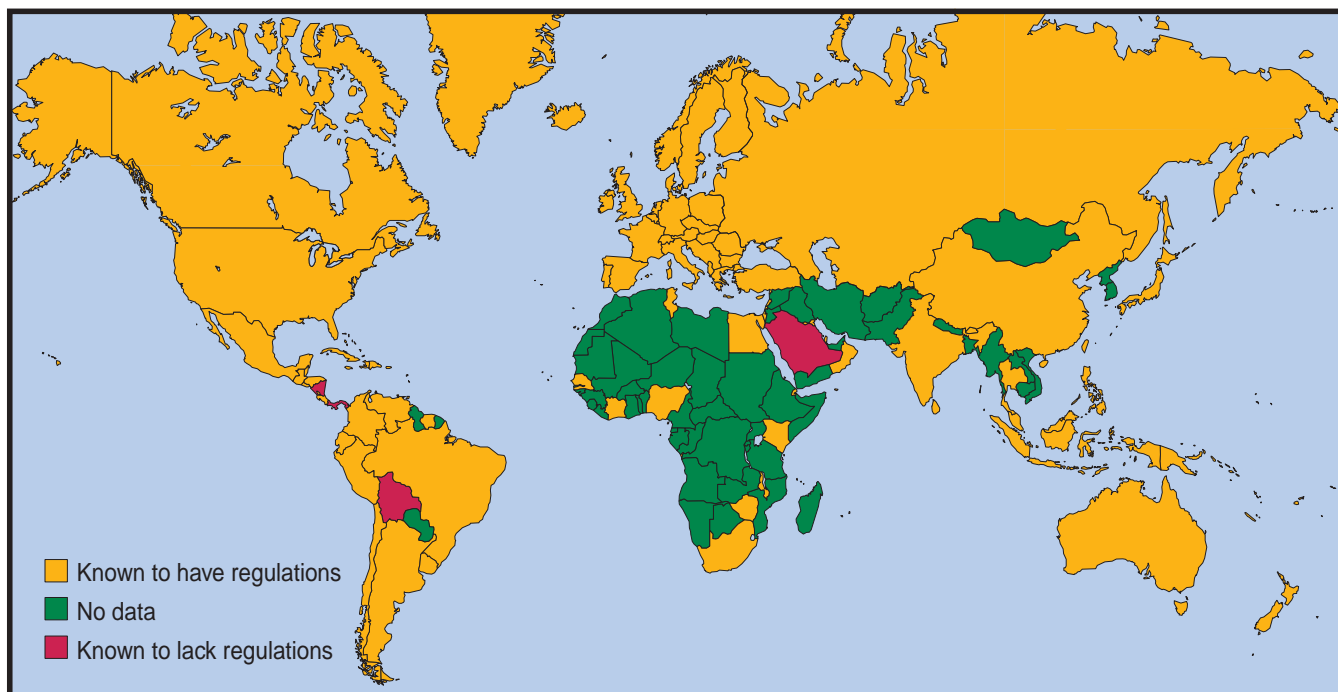


Figure 8.1. Counties known to regulate mycotoxins in food and feed (yellow), those where it is unknown whether regulations exist (green), and nations known to have no specific regulations (red) (Food and Agriculture Organization 1997).

usually included in the information presented to JECFA for evaluation. Similarly, qualitative and quantitative evaluation of the nature of the adverse effects (hazard characterization) is embodied in the data sets that are present.

Toxicological evaluation carried out by JECFA normally results in the estimation of a Provisional Maximum Tolerable Weekly Intake (PMTWI) or a Provisional Maximum Tolerable Daily Intake (PMTDI). The use of the term “provisional” expresses the tentative nature of the evaluation, in view of the paucity of reliable data on the consequences of human exposure at levels approaching those with which JECFA is concerned. In principle, the evaluation is based on the determination of a NOAEL in toxicological studies, and the application of an uncertainty factor. The uncertainty factor means that the lowest NOAEL in animal studies is divided by 100, 10 for extrapolation from animals to humans, and 10 for variation between individuals, to arrive at a tolerable intake level. In cases where the data are inadequate, JECFA uses a higher safety factor.

This hazard assessment approach does not apply for toxins where carcinogenicity is the basis for concern as is the case with the aflatoxins. Assuming that a no-effect concentration limit cannot be established for genotoxic compounds such as the aflatoxins, any

small dose will have a proportionally small effect. Imposing the absence of any amount of aflatoxins would then be appropriate, if these were not natural contaminants that can never completely be eliminated without outlawing the contaminated food or feed. In these cases, JECFA does not allocate a PMTWI or PMTDI. Instead it recommends that the level of the contaminant in food should be decreased so as to be As Low As Reasonably Achievable (ALARA). The ALARA level, which may be viewed as the irreducible level for a contaminant, is defined as the concentration of a substance that cannot be eliminated from a food without involving the discard of that food altogether or without severely compromising the availability of major food supplies. This covers the case of the JECFA evaluation of aflatoxins made in 1987 and 1997.

In February 2001, a special JECFA session was devoted to mycotoxins (World Health Organization 2001). The mycotoxins evaluated or re-evaluated at this 56th JECFA meeting included the fumonisins, ochratoxin A, deoxynivalenol, T-2 and HT-2 toxins, and aflatoxin M<sub>1</sub>. Other significant mycotoxins such as patulin, the aflatoxins, and zearalenone were evaluated at earlier occasions, in 1995, 1997, and 1999, respectively. In the further development of tolerable daily intake (TDI) levels to maximum tolerated levels in food and feed for national or international (Co-

dex Alimentarius) purposes, factors other than hazard assessment play a role; these will be discussed below.

### Occurrence of Mycotoxins in Commodities

In addition to hazard assessment, another factor is necessary to make risk assessment possible: exposure assessment. Data on mycotoxin occurrence in various commodities are needed to enable exposure assessment. They also allow the effects of regulations to be estimated on the availability of the foods and feeds concerned.

Risk assessment is the product of hazard assessment and exposure assessment. Among the factors it involves are (Kuiper-Goodman 1989) (1) hazard assessment based on animal toxicity and possibly epidemiological data, (2) exposure assessment based on data concerning actual concentrations in commodities, (3) extrapolation of hazard assessment from high-exposure animal data to low-exposure human data, and (4) comparison of the product of hazard assessment and exposure assessment with accepted risk.

### Distribution of Mycotoxins over Commodities

The homogeneity of mycotoxin concentration in products is a key factor in carrying out proper sampling. Distribution can be very nonhomogeneous, as with aflatoxins in peanuts (see also Chapter 7). While the number of contaminated peanut kernels in a lot is usually very low, the contamination level within a kernel can be very high. Similar results are known for corn. If insufficient care is taken to achieve a representative sample, the mycotoxin concentration in an inspected lot may be easily estimated incorrectly. Consumption of peanuts may lead to an accidental high single dose of aflatoxins, instead of chronic intake at a relatively low level. A similar situation may occur with pistachios and figs and other commodities.

### Methods of Analysis

Legislation calls for control, so reliable, accurate analytical methods must be available. Tolerance levels that do not have a reasonable expectation of being measured may waste resources and condemn products that are perfectly fit for consumption (Smith et al. 1994b). Besides accuracy and reliability, simplicity is required, as it will influence the amount of data that will be generated and the practicality of the

ultimate measures taken. Data reliability can be improved by using methods that fulfill performance criteria (as demonstrated in collaborative studies). Application of AQA procedures is recommended, including the use of reference materials. Certified reference materials should be used when a high degree of comparison and accuracy is required (see Chapter 7). Improvements in AQA and use of reference materials are likely to emerge for mycotoxin control in foods and feeds.

### Trade Contacts

Preferably, regulations should be harmonized with those in other countries with which trade contacts exist. Unnecessarily strict regulative actions make it difficult for importing countries to obtain supplies of essential commodities such as food grains and animal feedstuffs. Exporting countries may have difficulty finding markets for their products. For example, stringent regulations for aflatoxin B<sub>1</sub> in animal feedstuffs in the European Union (Commission of the European Communities 1991) made it difficult for some developing countries to export feed ingredients to their European trading partners.

### Food Supply

Regulatory philosophy should not jeopardize availability of basic commodities at reasonable prices. Especially in the developing countries, where food supplies are already limited, drastic legal measures may cause food shortages and excessive prices.

Weighing the factors in the decision-making process of establishing mycotoxin tolerances is not trivial. Common sense is the major factor for reaching a decision. Public health officials are confronted with a complex problem: mycotoxins, particularly aflatoxins, should be excluded from food as much as possible. Since the substances are present in foods as natural contaminants, however, human exposure cannot be completely prevented, so exposure of the population to some level of mycotoxins has to be tolerated. Despite the dilemma, mycotoxin regulations have been established in the past decades in many countries.

### Current Regulations

Schuller and colleagues (1983) produced the first overview of worldwide mycotoxin legislation that was updated by van Egmond (1987). Another update has been published as FAO Food and Nutrition Paper 64

(Food and Agriculture Organization 1997). Table 8.4 summarizes the maximum tolerated levels for mycotoxins in foodstuffs, dairy products, and animal feedstuffs, as they existed on October 1, 1996. (This table is a condensed version of the full list published in FAO Food and Nutrition Paper 64.) It shows that tolerated levels for mycotoxins vary widely between countries, making harmonization of regulations highly desirable.

Since 1996, the situation on mycotoxin regulations in foodstuffs and feedstuffs around the world has changed significantly. At the time of publishing this CAST report, an international inquiry was ongoing, conducted by H. P. van Egmond (National Institute of Public Health and the Environment, The Netherlands) on behalf of the Food and Agriculture Organization, to produce an updated document on the mycotoxin regulations. This document is scheduled for publication as an FAO Food and Nutrition Paper in 2003, and it will cover the mycotoxin regulations as they existed worldwide in 2002 in more than 100 countries. The document also will describe the results of regional attempts to harmonize existing regulations. A group of countries in which new (harmonized) regulations for mycotoxins in foodstuffs have recently come into force is the European Union. As a spin-off of the ongoing inquiry, the new EU regulations for mycotoxins in food<sup>1,2</sup> have been summarized in the Table 8.4A (Addendum). The new EU regulations overrule the corresponding existing national regulations in the EU countries, as presented in Table 8.4 under the various country headings. These countries retain the right, however, to keep national regulations for those mycotoxin/matrix combinations not covered in the harmonized EU regulations.

Table 8.4 includes data from 90 countries, which are 36% more than the earlier van Egmond reference (1987). Most new information comes from South

America. Seventy-seven countries regulate mycotoxins, an increase of 38% over the 1987 figure. Thirteen countries are known to have no specific regulations, while no data are available for about 50 countries, most of them in Africa (see Figure 8.1). Most mycotoxin regulations concern aflatoxins and in fact all countries with mycotoxin regulations at least have tolerances for aflatoxins in foods and/or animal feedstuffs. Less frequently, specific regulations exist for other mycotoxins, i.e., patulin, ochratoxin A, DON, diacetoxyscirpenol, zearalenone, T-2 toxin, che-tomin, stachybotryotoxin, phomopsis, fumonisins. Much work is needed to arrive at consensus tolerances for some of these toxins that may significantly impact human and animal health, e.g., fumonisins, ochratoxin A, some trichothecenes, zearalenone.

At the time of publication of this report, many new limits and regulations for mycotoxins have come into force or are in development; therefore, the information contained herein is rapidly changing. The number of countries known to have specific mycotoxin regulations has increased, whereas more mycotoxins are regulated in more commodities and tolerance limits tend to decrease. At the same time, regulations have been harmonized or are in some stage of harmonization. They have become more diverse and detailed with newer requirements regarding official analytical methodology and sampling procedures. Therefore, FAO has decided to produce an update of Food and Nutrition paper No. 64 that is scheduled for publication in 2003.

### Summary

To reiterate, 77 countries regulated mycotoxins in 1996, 13 had no regulations, and no data were available for 50. Compared to 1987, maximum tolerated levels for aflatoxins had not changed dramatically and continued to differ greatly between countries, requiring harmonization to remove the extreme variability of tolerated levels. A new update was in preparation in 2002. This update, scheduled for publication by the FAO in 2003, will show increased and more up-to-date information on mycotoxin regulations in foodstuffs and feedstuffs. It also will show the first results of regional harmonization of regulations for foodstuffs, e.g., in the European Union.

<sup>1</sup>Commission Regulation (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Communities* L77 (2001):1–13.

<sup>2</sup>Commission Regulation (EC) No 472/2002 of 12 March 2002 amending Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Communities* L75 (2002):18–20.

**Table 8.4. Maximum tolerated levels of mycotoxins in foodstuffs, dairy products, and animal feedstuffs (from Food and Agriculture Organization, 1997)**

Country and commodity category	Commodity	(Sum of) Mycotoxin(s) <sup>a</sup>	Level (ng/g)	Analytical method type <sup>b</sup>	
Antigua and Barbuda: situation 1991: No regulations					
Argentina: situation 1991; see also Mercosur					
Food	Baby food	B <sub>1</sub>	0	—	
	Groundnut, maize and by-products	B <sub>1</sub> B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	5 20	— —	
Dairy	Liquid milk, powdered milk	M <sub>1</sub>	0.05	—	
	Milk products	M <sub>1</sub>	0.5	—	
Feed	Soya meal	B <sub>1</sub>	30	—	
Australia: adopted by all states and territories					
Food	All foods	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub> Phomopsis	5 5	— —	
	Peanut butter, nuts and the nut proportion of products	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	15	—	
Austria:					
Food	All foods	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	1 5	TLC	
	Milling and shelled products and derived products	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	2 5	TLC	
	Children foods (in prepared food)	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub> M <sub>1</sub>	0.02	TLC	
	Wheat, rye	Ochratoxin A		5	—
		Deoxynivalenol		500	—
		Zearalenone		60	—
		Durum wheat	Ochratoxin A Deoxynivalenol Zearalenone	5 750 60	— — —
	Dairy	Fruit juice	Patulin	50	—
		Milk (products)	M <sub>1</sub>	0.05	TLC
			Whey powder, whey paste	M <sub>1</sub>	0.4
		Whey, liquid whey products	M <sub>1</sub>	0.025	TLC
			Cheese	M <sub>1</sub>	0.25
		Butter	M <sub>1</sub>	0.02	TLC
			Pasteurized fresh milk for infants/children; children foods	M <sub>1</sub>	0.01
		Powdered milk (products), condensed milk, milk concentrates	M <sub>1</sub>	0.4	TLC
	Feed	See European Union	—	—	HPLC
Bahamas: situation 1991; no national regulations; FDA regulations are used					
Food	All foods, all grains	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	—	
Bahrain: no regulations					
Barbados: situation 1991					
Food	All foods	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	—	
Dairy	Milk	M <sub>1</sub>	0.05	—	
Feed	All feedstuffs	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	50	—	

—continued

Table 8.4. (continued)

Country and commodity category	Commodity	(Sum of) Mycotoxin(s) <sup>a</sup>	Level (ng/g)	Analytical method type <sup>b</sup>
Belgium:				
Food	Peanuts	B <sub>1</sub>	5	TLC
Dairy	Milk	M <sub>1</sub>	0.05	—
Feed	See European Union			
Belize:				
Food	Maize, groundnut	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	—
Bolivia: situation 1991: no regulations				
Bosnia and Herzegovina: situation 1981				
Food	Wheat, maize, rice, cereals	B <sub>1</sub> G <sub>1</sub>	1	—
	Beans	B <sub>1</sub> G <sub>1</sub>	5	—
Feed	Feedstuffs	—	—	—
Brazil: situation 1987; proposals; see also Mercosur				
Food	All foodstuffs	B <sub>1</sub>	15	—
		B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	30	—
	Imported foodstuffs	B <sub>1</sub>	5	—
		B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	10	—
	Industrially prepared foodstuffs for children from 0–2 years and for school meals	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	3	—
	Rice, barley, beans, maize	Ochratoxin A	50	—
	Maize	Zearalenone	200	—
	Maize, groundnut	B <sub>1</sub> G <sub>1</sub>	30	—
Dairy	Milk (products)	M <sub>1</sub>	0.5	—
	Imported milk (products)	M <sub>1</sub>	0.1	—
Feed	Peanut meal (export)	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	50	—
Bulgaria: situation 1992				
Food	Peanut (product)s, kernel (product)s, cocoa beans, cocoa butter, cocoa powder	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	5	—
Dairy	Grain (products), cereal (product)s	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	2.5	—
	Liquid milk products	M <sub>1</sub>	0.5	—
	Powdered milk	M <sub>1</sub>	0.1	—
	Powdered milk for dietetics and infant feeding	M <sub>1</sub>	0	—
	Cheese and similar products	M <sub>1</sub>	0.5	—
Canada:				
Food	Nut (product)s	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	15	HPLC
	Uncleaned soft wheat	Deoxynivalenol	2,000	GC
Feed	Animal feeding stuffs	All aflatoxins	20	EZQC HPLC
	Diets of cattle/poultry	Deoxynivalenol	5,000	GC
		HT-2 toxin	100	MS
	Diets for swine/young calves/ lactating dairy animals	Deoxynivalenol	1,000	GC
		HT-2 toxin	25	MS
	Feedstuffs for reproducing animals	All mycotoxins	0	—

Table 8.4. (continued)

Country and commodity category	Commodity	(Sum of) Mycotoxin(s) <sup>a</sup>	Level (ng/g)	Analytical method type <sup>b</sup>
Chile: situation 1991				
Feed	Feedstuffs	B <sub>1</sub>	20	—
	Feedstuffs	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	50	—
		B <sub>1</sub>	5	—
		B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	—
China (People's Republic of China):				
Food	Rice, edible oils	B <sub>1</sub>	10	—
	Maize, peanut (product)s, maize, peanut oil	B <sub>1</sub>	20	—
	Wheat, barley, oats, beans, sorghum, other grains, fermented foodstuffs	B <sub>1</sub>	5	—
Dairy	Cow milk, milk products (calcd. on the basis of milk)	B <sub>1</sub>	0.5	—
Feed	Compound feed for chickens	B <sub>1</sub>	10	—
	Compound feed for laying hens	B <sub>1</sub>	20	—
	Compound feed and mixed feed for fattening pigs	B <sub>1</sub>	20	—
	Maize, peanut cake, peanut residue	B <sub>1</sub>	50	—
Colombia: situation 1991				
Food	Foods	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	—
	Cereals (sorghum/millet)	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	30	—
	Oil seeds	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	10	—
Feed	Cattle feed	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	50	—
	Sesame seeds	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	—
	Poultry feedstuffs	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	—
Costa Rica: situation 1991				
Food	Maize	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	35	—
Feed	Maize	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	50	—
Côte d'Ivoire: situation 1987; proposal, types of aflatoxins not precisely stated				
Feed	Straight feedstuffs	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	100	—
	Complete feedstuffs	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	10	—
	Complete feedstuffs for pigs/poultry (except young animals/ducks)	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	38	—
	Complete feedstuffs for cattle/sheep/goats	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	75	—
	Complete feedstuffs for dairy cattle	B <sub>1</sub> B <sub>2</sub> G <sub>2</sub> G <sub>2</sub>	50	—
Cuba: situation 1991				
Food	Foods	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	5	—
	Cereals, groundnuts	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	5	—
Feed	Feedstuffs, raw materials for feedstuffs	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	5	—
	Feedstuffs, raw materials for feedstuffs	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	5	ELISA pre-screening kit

— continued

Table 8.4. (continued)

Country and commodity category	Commodity	(Sum of) Mycotoxin(s) <sup>a</sup>	Level (ng/g)	Analytical method type <sup>b</sup>
Cyprus: situation 1992				
Food	Cereals, pulses, dried fruit, sesame and foods produced exclusively from these, caraway seed, poppy seed, seeds used in bakery products and confectionery	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	5	—
Dairy	Milk, dairy products	All mycotoxins	0.5	—
Czech Republic:				
Food	All foods	B <sub>1</sub>	5	HPLC
		B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	10	RIA
	Infant/children foods	B <sub>1</sub>	1	HPLC
		B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	2	RIA
	All foods	Patulin	50	—
		Ochratoxin A	20	—
	Children foods	Patulin	30	—
		Ochratoxin A	5	—
	Infant foods	Patulin	20	—
		Ochratoxin A	1	—
Dairy	All foods	M <sub>1</sub>	5	HPLC
	Milk	M <sub>1</sub>	0.5	HPLC
	Infant/children foods	M <sub>1</sub>	1	HPLC
	Infant foods on milk basis	M <sub>1</sub>	0.1	HPLC
		B <sub>1</sub>	0.1	—
		B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	0.2	—
Denmark:				
Food	Peanut (product)s	B <sub>1</sub>	2	HPLC
		B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	4	—
	Brazil nuts	B <sub>1</sub>	2	HPLC
		B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	4	—
	Dried figs	B <sub>1</sub>	2	HPLC
		B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	4	—
	Pig kidneys	Ochratoxin A	25	TLC
	Pig kidneys	Ochratoxin A	10	TLC
Feed	Cereal (product)s	Ochratoxin A	5	—
	See European Union			
Dominican Republic: situation 1991				
Food	Maize (products), groundnut, soya, tomato (products)	B <sub>1</sub> G <sub>1</sub>	0	—
	Imported maize	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	—
Ecuador: situation 1991; no regulations				
Egypt:				
Food	Peanut (product)s, oil seed (products)s, cereal (product)s	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	10	—
		B <sub>1</sub>	5	—
	Maize	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	—
		B <sub>1</sub>	10	—
	Starch and its derivatives	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	0	—
		B <sub>1</sub>	0	—
Dairy	Milk, dairy products	G <sub>1</sub> G <sub>2</sub> M <sub>1</sub> M <sub>2</sub>	0	—
		M <sub>1</sub>	0	—
Feed	Animal and poultry fodders	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	—
		B <sub>1</sub>	10	—

Table 8.4. (continued)

Country and commodity category	Commodity	(Sum of) Mycotoxin(s) <sup>a</sup>	Level (ng/g)	Analytical method type <sup>b</sup>	
European Union: All European Union tolerances refer to a commodity content of 12%; United Kingdom has extra regulation for feedstuff ingredients; Sweden has own regulations until end 1997.					
Feed	Straight feedstuffs	B <sub>1</sub>	50	TLC HPLC	
	Straight feedstuffs: Groundnut (products), copra (products), palmnut (products), cottonseed (products), babassu (products), maize (products)	B <sub>1</sub>	20	TLC HPLC	
	Complete feedstuffs	B <sub>1</sub>	10	TLC HPLC	
	Complete feedstuffs for pigs and poultry (except young animals)	B <sub>1</sub>	20	TLC HPLC	
	Complete feedstuffs for cattle/sheep/goats except calves/lambs/kids	B <sub>1</sub>	50	TLC HPLC	
	Complete feedstuffs for calves, lambs	B <sub>1</sub>	10	TLC HPLC	
	Complementary feedstuffs	B <sub>1</sub>	5	TLC HPLC	
	Complementary feedstuffs for pigs and poultry (except young animals)	B <sub>1</sub>	30	TLC HPLC	
	Complementary feedstuffs for cattle/sheep/goats except dairy animals/calves/lambs/kids	B <sub>1</sub>	50	TLC HPLC	
	Raw materials: groundnut (products), copra (products), palmnut (products), cottonseed (products), babassu (products), maize (products)	B <sub>1</sub>	200	TLC HPLC	
Finland: Food	All foods	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	5	TLC HPLC GC-MS	
	Feed See European Union	Patulin	50	TLC	
France: Food	All foods	B <sub>1</sub>	10	—	
	Peanuts, pistache nuts, almonds, oil seeds, children foods	B <sub>1</sub>	1	—	
	Wheat meal	B <sub>1</sub>	3	—	
	Wheat bran	B <sub>1</sub>	10	—	
	Vegetable oils, cereals, wheat meal (complete)	B <sub>1</sub>	5	—	
	Apple juice (products)	Patulin	50	—	
	Cereals, vegetable oils	Zearalenone	200	—	
	Cereals	Ochratoxin A	5	—	
	Dairy	Milk, milk powder (calcd. on reconstituted product)	M <sub>1</sub>	0.05	—
		Milk, milk powder (calcd. on reconstituted product) for infants under 3 years	M <sub>1</sub>	0.03	—
Feed	See European Union				

— continued



Table 8.4. (continued)

Country and commodity category	Commodity	(Sum of) Mycotoxin(s) <sup>a</sup>	Level (ng/g)	Analytical method type <sup>b</sup>
Germany:				
Food	All foods	B <sub>1</sub>	2	TLC
		B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	4	
	Enzyme (preparation)s intended for the production of foodstuffs	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	0.05	TLC
Dairy	Foods for infants and young children	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	0.05	TLC-ELISA
	Milk	M <sub>1</sub>	0.05	TLC-ELISA
Feed	Foods for infants and young children	M <sub>1</sub>	0.01	TLC-ELISA
	See European Union			
Greece:				
Food	Peanuts, hazelnuts, walnuts, cashewnuts, pistaches, almonds, pumpkin seeds, sunflower seeds, pine seeds, apricot seeds	B <sub>1</sub> B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	5 10	TLC HPLC-IA
	Maize, dried figs, dried apricots, dried prunes, dates, raisins	B <sub>1</sub> B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	5 10	— —
	Raw coffee beans, apple juice, apple products	Ochratoxin A Patulin	20 50	— —
Feed	See European Union			
Guatemala: situation 1991				
Food	Maize, kidney beans, rice, sorghum	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	—
	Groundnuts, groundnut butter	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	—
Feed	Concentrate	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	—
Honduras: situation 1991				
Food	All foods	B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	1	—
	Maize (grounded or whole grain)	B <sub>1</sub>	1	—
	Baby food	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	0.01	—
		M <sub>1</sub>	0.02	—
Dairy	Milk (products)	M <sub>1</sub>	0.05	—
	Cheeses	M <sub>1</sub>	0.25	—
Hong Kong:				
Food	Foods	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub> M <sub>1</sub> M <sub>2</sub> aflatoxin	15	TLC
	Peanut (product)s	P <sub>1</sub> aflatoxicol B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub> M <sub>1</sub> M <sub>2</sub> aflatoxin	20	HPLC TLC
		P <sub>1</sub> aflatoxicol		HPLC
Hungary:				
Food	All foods	B <sub>1</sub>	5	—
	Groundnut kernels	B <sub>1</sub>	30	—
	Preserved foods	All mycotoxins	0	—
	Groundnuts	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	5	—
India: situation 1987				
Food	All foods	B <sub>1</sub>	30	TLC
Feed	Peanut meal (export)	B <sub>1</sub>	120	—

Table 8.4. (continued)

Country and commodity category	Commodity	(Sum of) Mycotoxin(s) <sup>a</sup>	Level (ng/g)	Analytical method type <sup>b</sup>
Indonesia:				
Food	Peanuts, maize, herbs, seeds	—	—	—
Feed	Copra in cow/pig/duck/sheep feed	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	1,000	—
	Groundnut/sesame seed/rape seed meal	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	200	—
	Cassava in chicken feed	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	120	—
	Capok seed/coconut meal in chicken feed, coconut meal in cow/pig/ duck/sheep feed	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	100	—
	Sunflower seed meal in chicken feed	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	90	—
	Soya bean/capok seed/fish/meat/ bone meal, rice/maize bran, leucaena (?), maize, wheat pollar (?) and sorghum in cow/pig/ duck/sheep feed, maize/meat/ bone/cotton seed meal in chicken feed	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	50	—
	Soya bean/leucaena (?)/fish/meat/ bone meal, rice/maize bran, wheat pollar(?), sorghum, copra in chicken feed	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	—
Iran: no regulations				
Iraq: no regulations				
Ireland:				
Food	All foods	B <sub>1</sub>	5	—
Feed	See European Union	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	30	—
Israel:				
Food	Nut (product)s, peanut (product)s, maize	B <sub>1</sub>	5	—
	Flour (products), fig (products)	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	15	—
	Apple juice	Patulin	50	—
	Cereal (product)s, pulse (product)s	Ochratoxin A	50	—
Dairy	Milk, milk powder (calcd. on the basis of milk)	M <sub>1</sub>	0.05	—
Feed	According to European Union			
	Grain for feed	B <sub>1</sub>	20	—
		Ochratoxin A	300	—
		T-2 toxin	100	—
		Diacetoxyscirpenol	1,000	—
Italy:				
Food	All foods	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	10	HPLC
	Dried figs	B <sub>1</sub>	5	
	Spices	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	10	HPLC
		B <sub>1</sub>	5	
		B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	40	HPLC
Feed	See European Union	B <sub>1</sub>	20	
Jamaica: situation 1991				
Food	Food, grains	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	—

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Table 8.4. (continued)

Country and commodity category	Commodity	(Sum of) Mycotoxin(s) <sup>a</sup>	Level (ng/g)	Analytical method type <sup>b</sup>
Japan:				
Food	All foods	B <sub>1</sub>	10	TLC
Feed	Peanut meal (import)	B <sub>1</sub>	1,000	TLC
Jordan: situation 1981				
Food	Almonds, cereals, maize, peanuts, pistachio nuts, pine nuts, rice	B <sub>1</sub> B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	15 30	TLC
Feed	All feedstuffs	B <sub>1</sub> B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	15 30	— —
Kenya: situation 1981				
Food	Peanut (products), vegetable oils	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	TLC
Kuwait: no regulations				
Luxembourg:				
Food	Peanut (products)	B <sub>1</sub>	5	TLC
Feed	See European Union			
Macedonia: situation 1981				
Food	Wheat, maize, rice, cereals	B <sub>1</sub> G <sub>1</sub>	1	—
	Beans	B <sub>1</sub> G <sub>1</sub>	5	—
Feed	Feedstuffs	—	—	—
Malawi: situation 1987				
Food	Peanuts (export)	B <sub>1</sub>	5	—
Malaysia: situation 1987				
Food	All foods	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	35	—
Mauritius: situation 1987				
Food	All foods	B <sub>1</sub> B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub> M <sub>1</sub> M <sub>2</sub>	5 10	— —
	Groundnuts	B <sub>1</sub> B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	5 15	— —
Mercosur: (Argentina, Uruguay, Brazil, and Paraguay); Proposals for common regulations, probably effective in a few years, will overrule national regulations.				
Food	Maize kernels (whole, pieces, ground, peeled), maize flour/meal, peanuts (in shell, raw, roasted), peanut cream, peanut butter	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	—
Dairy	Fluid milk	M <sub>1</sub>	0.5	—
	Milk powder	M <sub>1</sub>	5	—
Mexico:				
Food	Flours	All aflatoxins	20	—
Feed	Cereals for bovine and porcine fattening	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	200	—
	Feedstuffs			
	Feedstuffs for dairy cattle/poultry	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	0	—
Morocco: currently no regulations; Codex Alimentarius is followed.				

Table 8.4. (continued)

Country and commodity category	Commodity	(Sum of) Mycotoxin(s) <sup>a</sup>	Level (ng/g)	Analytical method type <sup>b</sup>
Netherlands, The:				
Food	All foods and food ingredients except groundnuts used for the preparation of peanut oil	B <sub>1</sub>	5	TLC
	Cereal (product)s, pulse (product)s, legume (product)s	All mycotoxins	0	—
Dairy	Milk (products), milk powder (calcd. on reconstituted product)	M <sub>1</sub>	0.05	TLC HPLC
	Cheese	M <sub>1</sub>	0.2	TLC HPLC
	Butter	M <sub>1</sub>	0.02	TLC HPLC
Feed	Infant foods on milk basis See European Union	M <sub>1</sub>	0.05	—
New Zealand: situation 1987				
Food	All foods	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	5	—
	Peanut butter, shelled nuts, nut portion of products containing nuts	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	15	—
Nicaragua: situation 1991: no regulations				
Nigeria: situation 1987				
Food	All foods	B <sub>1</sub>	20	TLC
	Infant foods	B <sub>1</sub>	0	TLC
Dairy	Fluid milk	M <sub>1</sub>	1	—
Feed	Feedstuffs	B <sub>1</sub>	50	TLC
Norway: situation 1987				
Food	All foodstuffs	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	5	—
	Brazil nuts, buck wheat	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	5	—
Feed	Apple juice	Patulin	50	—
	Mixed feedstuffs depending on type of animal	B <sub>1</sub>	10–50	—
Oman: situation 1987				
Feed	Complete feedstuffs	B <sub>1</sub>	10	—
	Complete feedstuffs for poultry	B <sub>1</sub>	20	—
Panama: situation 1991: no regulations				
Peru: situation 1991; no national regulations, Codex Alimentarius proposals used				
Food	All foodstuffs	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	10	—
Feed	All feedstuffs	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	10	—
	Complementary products for milk, animal products, feedstuffs	B <sub>1</sub>	10	—
	Cereals for porcine growing			
	Feedstuffs	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	100	—
Philippines:				
Food	Nut (products)	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	TLC
Feed	Poultry feedstuffs	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	—
	Livestock feedstuffs	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	50	—

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Table 8.4. (continued)

Country and commodity category	Commodity	(Sum of) Mycotoxin(s) <sup>a</sup>	Level (ng/g)	Analytical method type <sup>b</sup>
Poland:				
Food	All foods	B <sub>1</sub>	0	—
Feed	Feedstuffs, feedstuff ingredients, complete feedstuffs for cattle/ sheep/goats	B <sub>1</sub>	50	—
	Complete feedstuffs for pigs/poultry/ dairy cows	B <sub>1</sub>	20	—
Portugal:				
Food	All foodstuffs	B <sub>1</sub>	20	TLC
	Peanuts	B <sub>1</sub>	25	TLC
	Infant foods	B <sub>1</sub>	5	TLC
Feed	See European Union			
Qatar: no regulations				
Romania: situation 1987				
Food	All foods	B <sub>1</sub>	0	TLC
	All foods	Patulin	30	—
		Ochratoxin A	5	—
		Zearalenone	30	—
Dairy	Milk, dairy products	M <sub>1</sub>	0	—
Feed	All feedstuffs	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	50	TLC
	All feedstuffs	Patulin	30	—
		Ochratoxin A	5	—
		Deoxynivalenol	5	—
		Stachybotriotoxin	0	—
		Chetomin	0	—
Russia:				
Food	Animal fats	B <sub>1</sub>	0	—
		M <sub>1</sub>	0.5	—
	Bottled/canned/potted fruits and berries	Patulin	50	—
	Bottled/potted/canned vegetables	B <sub>1</sub>	5	—
		Patulin	50	—
	Casein	B <sub>1</sub>	0	—
		M <sub>1</sub>	5	—
	Cereals (wheat of hard and strong types), flour, wheat bran	B <sub>1</sub>	5	—
		Zearalenone	1,000	—
		T-2 toxin	100	—
		Deoxynivalenol	1,000	—
	Fruits, berries and vegetables (bottled/canned/potted juices and puree), cacao, cacao powder, chocolate, coffee, eggs, dehydrated egg, meat and poultry (fresh/chilled/frozen/tinned/potted/ bottled), sausage and culinary products from meat and poultry, subproducts of farming animals and poultry, sweets	B <sub>1</sub>	5	—
	Leguminous, protein isolators and concentrators, vegetable oil	B <sub>1</sub>	5	—
		Zearalenone	1,000	—
	Nuts (kernel)	B <sub>1</sub>	0	—
		Zearalenone	1,000	—

Table 8.4. (continued)

Country and commodity category	Commodity	(Sum of) Mycotoxin(s) <sup>a</sup>	Level (ng/g)	Analytical method type <sup>b</sup>
Dairy	Milk, sour dairy products, concentrated milk, cheese and cottage cheese products, cow butter	B <sub>1</sub>	0	—
		M <sub>1</sub>	0.5	—
Salvador, El: situation 1991				
Food	Foods	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	—
Feed	All feedstuffs	B <sub>1</sub>	10	—
	Supplementary feeds for porcine/poultry/dairy cattle; single composite feedstuffs; bovine/caprine/ovine feedstuffs	B <sub>1</sub>	20	—
Saudi Arabia: no regulations				
Senegal: situation 1987				
Feed	Peanut products (straight feedstuffs)	B <sub>1</sub>	50	TLC
	Peanut products (straight ingredients)	B <sub>1</sub>	300	TLC
Serbia: situation 1981				
Food	Wheat, maize, rice, cereals	B <sub>1</sub> G <sub>1</sub>	1	—
	Beans	B <sub>1</sub> G <sub>1</sub>	5	—
Feed	Feedstuffs	—	—	—
Singapore: situation 1987				
Food	All foods	B <sub>1</sub>	0	TLC
		B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	0	HPLC
South Africa:				
Food	All foods	B <sub>1</sub>	5	ELISA-LC
		B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	10	
Spain:				
Food	All foods	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	10	—
Feed	See European Union	B <sub>1</sub>	5	—
Sri Lanka:				
Food	Foods	All aflatoxins	30	—
	Foods intended for children up to 3 years of age	All aflatoxins	1	—
Dairy	Milk (products)	All aflatoxins	1	—
Suriname: situation 1991				
Food	Maize	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	30	—
Feed	Groundnut (products), legumes	B <sub>1</sub>	5	—
	Feedstuffs	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	30	—

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Table 8.4. (continued)

Country and commodity category	Commodity	(Sum of) Mycotoxin(s) <sup>a</sup>	Level (ng/g)	Analytical method type <sup>b</sup>	
Sweden: replacement of Swedish feedstuff regulations with EU regulations to be reconsidered near 31-12-97.					
Food	All foods	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	5	TLC LC-mini	
Dairy	Berries, fruits, juices	Patulin	50	HPLC	
	Liquid milk products	M <sub>1</sub>	0.05	HPLC	
Feed	Feedstuff ingredients	B <sub>1</sub>	50	—	
	Feedstuff ingredients for dairy cattle	B <sub>1</sub>	10	—	
	Cereal grains and forages as feedstuff ingredients for dairy cattle	B <sub>1</sub>	1	—	
	Mixed feedstuffs (excluding forages) for dairy cattle	B <sub>1</sub>	3	—	
	Complete feedstuffs	B <sub>1</sub>	10	—	
	Complete feedstuffs for cattle/ sheep/goats except dairy cattle/ lambs/kids	B <sub>1</sub>	50	—	
	Complete feedstuffs for pigs and poultry except young animals	B <sub>1</sub>	20	—	
	Complete feedstuffs (including forages) for dairy cattle	B <sub>1</sub>	1.5	—	
	Complete feedstuffs for poultry	Ochratoxin A	200	—	
	Complete feedstuffs for pigs	Ochratoxin A	100	—	
	Switzerland:				
Food	All foods (except maize/cereals/ herbs)	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	1 5	— —	
	Maize, cereals (granular or ground)	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	2 5	TLC —	
	Herbs	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	5 5	— —	
	Baby/infant food	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	0.01	—	
	Cereal (product)s	Ochratoxin A	2	—	
	Maize (products)	Fumonisin B <sub>1</sub> +B <sub>2</sub>	1,000	—	
	Fruit juice	Patulin	50	HPLC	
	Dairy	Milk (product)s	M <sub>1</sub>	0.05	—
		Whey (products)	M <sub>1</sub>	0.025	TLC HPLC
		Cheese	M <sub>1</sub>	0.25	TLC HPLC
	Feed	Butter, baby/infant food	M <sub>1</sub>	0.02	TLC HPLC
		Prohibit feeding cattle with peanut bruise			—
	Taiwan, Province of China: situation 1991				
	Food	Cereals	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>1</sub>	50	—
Feed	Feed, oilseed meals for feed under 4% of mixed feed	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	1,000	—	
Thailand: situation 1987					
Food	All foods	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	TLC HPLC	
Trinidad and Tobago: situation 1991; no national regulations, Codex Alimentarius proposals used					
Food	Foods	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	10	—	
Feed	Feedstuffs	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	10	—	
	Complementary products	B <sub>1</sub>	10	—	
Food	Ice cream	All mycotoxins	0	—	

Table 8.4. (continued)

Country and commodity category	Commodity	(Sum of) Mycotoxin(s) <sup>a</sup>	Level (ng/g)	Analytical method type <sup>b</sup>
UAE (United Arab Emirates): no regulations				
UK (United Kingdom):				
Food	Nut (product)s, dried fig (product)s	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	4	—
Feed	See European Union Groundnut, copra, palm-kernel, cotton seed, babassu, maize and derived products (raw materials)	B <sub>1</sub>	20	—
Uruguay: see also Merosur				
Food	Foods and spices	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	TLC HPLC
	Texturized soy protein products: flour, starch, concentrate, isolate	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	30	TLC HPLC
	Peanuts, dried fruit (product)s	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	10	TLC HPLC
	Cocoa beans	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	10	TLC HPLC
	Infant foods, produced industrially	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	3	TLC HPLC
	Rice, barley, beans, coffee, maize	Ochratoxin A	50	TLC HPLC
	Maize, barley	Zearalenone	200	TLC HPLC
	Fruit juice	Patulin	50	TLC HPLC
Dairy	Milk (products)	M <sub>1</sub>	0.5	TLC HPLC
USA (United States of America):				
Food	All foods	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	TLC HPLC
	Finished wheat products	Deoxynivalenol	1,000	TLC HPLC
Dairy	Whole milk, low fat milk, skim milk	M <sub>1</sub>	0.5	TLC HPLC
Feed	Feedstuff (ingredient)s	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	TLC HPLC
	Cottonseed meal intended for beef cattle/swine/poultry feedstuffs (regardless of age or breeding status)	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	300	TLC HPLC
	Maize and peanut products intended for breeding beef cattle/swine or mature poultry	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	100	TLC HPLC
	Maize and peanut products intended for finishing swine of 100 pounds or greater	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	200	TLC HPLC
	Maize and peanut products intended for finishing beefcattle	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	300	TLC HPLC
	Grains and grain by-products destined for ruminating beef and feedlot cattle older than 4 months and for chickens (not exceeding 50% of the cattle or chicken total diet)	Deoxynivalenol	10,000	TLC HPLC
	Grains and grain by-products (not exceeding 40% of the diet)	Deoxynivalenol	5,000	TLC HPLC

— continued



Table 8.4. (continued)

Country and commodity category	Commodity	(Sum of) Mycotoxin(s) <sup>a</sup>	Level (ng/g)	Analytical method type <sup>b</sup>
	Grains and grain by-products destined for swine (not exceeding 20% of their diet)	Deoxynivalenol	5,000	TLC HPLC
Venezuela: situation 1991				
Food	Rice flour	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	5	—
Feed	Feedstuffs	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	20	—
Zimbabwe:				
Food	Foods	B <sub>1</sub>	5	TLC
		G <sub>1</sub>	4	HPLC
	Groundnuts, maize, sorghum	B <sub>1</sub>	5	TLC
		G <sub>1</sub>	4	HPLC
Dairy	Feedstuffs	B <sub>1</sub> B <sub>2</sub> G <sub>1</sub> G <sub>2</sub>	—	TLC HPLC
	Poultry feed	B <sub>1</sub> G <sub>1</sub>	10	TLC HPLC

<sup>a</sup>B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>, and M<sub>1</sub> are aflatoxins.

<sup>b</sup>Analytical method types include TLC = thin layer chromatography, HPLC = high performance layer chromatography, GC = gas chromatography, MS = mass spectroscopy, ELISA = enzyme-linked immunosorbent assay, RIA = radioimmunoassay, LC = liquid chromatography.

Table 8.4A. Addendum. Summary of harmonized maximum admissible levels for mycotoxins in foodstuffs in the European Union (situation May 2002)<sup>a</sup>

Commodity	Mycotoxin(s)	Level (ng/g)
Milk	Aflatoxin M <sub>1</sub>	0.05
Peanuts, nuts and dried fruits (direct human consumption)	Aflatoxin B <sub>1</sub>	2
	Aflatoxins B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub>	4
Peanuts (to undergo physical processing before human consumption)	Aflatoxin B <sub>1</sub>	8
	Aflatoxins B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub>	15
Cereals (for direct human consumption or to undergo physical processing before human consumption)	Aflatoxin B <sub>1</sub>	2
	Aflatoxins B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub>	4
Spices ( <i>Capsicum</i> spp., <i>Piper</i> spp., <i>Myristica fragrans</i> , <i>Zinziber officinale</i> , <i>Curcuma longa</i> )	Aflatoxin B <sub>1</sub>	5
	Aflatoxins B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub>	10
Raw cereal grains (including raw rice and buckwheat)	Ochratoxin A	5
All products derived from cereals (including processed cereal products and cereal grains intended for direct human consumption)	Ochratoxin A	3
Dried vine fruits (currants, raisins, and sultanas)	Ochratoxin A	10

<sup>a</sup> Commission Regulation (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Communities* L77 (2001):1–13.

Commission Regulation (EC) No 472/2002 of 12 March 2002 amending Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Communities* L75 (2002):18–20.

# 9 Decontamination and Detoxification Strategies for Mycotoxins

## Summary

Numerous strategies are evolving for the management and field-practical control of mycotoxins. Some clearly are more practical and effective than others. Novel genetic and molecular approaches to prevent or minimize the occurrence of aflatoxins and other mycotoxins in grains and modification of toxicity and carcinogenicity in animals through diverse chemical and dietary interventions show great promise. More research is required to establish the efficacy, specificity, and safety of these procedures.

## Introduction

Even though recommended agricultural practices to decrease mycotoxin elaboration during crop growth, harvesting, and storage have been implemented, the potential for significant crop contamination still exists (Anderson 1983; Goldblatt 1971; Goldblatt and Dollear 1969; U.S. Department of Agriculture 1968a, 1968b). The most effective way to avoid these toxins involves removal and/or destruction of the contaminated source and the contaminants it contains. Although this would guarantee a mycotoxin-free commodity, it is not economically feasible. The impracticality of this option is compounded when one considers the unavailability of alternative or equivalent foods and feeds. Many foods destined for human consumption are among those most impacted by mycotoxin-producing fungi. Contaminated feed is problematic as well; potential deleterious effects on animal health and possibilities of tainted animal product are some of the issues to consider when mycotoxins have been detected in feed.

Because mycotoxin contamination often is unavoidable, developing and implementing efficient detoxification methods become very important. Guidelines for evaluating mycotoxin detoxification and decontamination procedures have been established. The process should (1) inactivate, destroy, or remove the mycotoxin; (2) not result in the deposition of toxic

substances, metabolites, or byproducts in the food or feed; (3) retain nutrient value and feed acceptability of the product or commodity; (4) not result in significant alterations of the product's technological properties; and, if possible, (5) destroy fungal spores (Park et al. 1988). In addition to these criteria, the process(es) should be readily available, easily utilized, and inexpensive. These latter attributes facilitate administration of a comprehensive decontamination program for affected feed and foodstuffs and are more applicable in regions where less-stringent protocols exist.

Detoxification efforts for mycotoxins have focused primarily on the aflatoxins (Phillips 1999; Phillips et al. 1994); many strategies to decontaminate aflatoxin-contaminated crops and products have been reported and reviewed (Anderson 1983; Cole 1989; Council for Agricultural Science and Technology 1979, 1989; Goldblatt and Dollear 1977, 1979; Marth and Doyle 1979; Palmgren and Hayes 1987; Phillips 1999; Phillips et al. 1994, 1995). Although much more limited in scope, methods to detoxify mycotoxins other than the aflatoxins also have been reported. As the impact of these hazardous toxins is being recognized, successfully removing them from the food supply represents an emerging area of interest and research. Various approaches to the detoxification, inactivation, degradation, or decrease of aflatoxin and other mycotoxins and their applications are outlined in the following sections.

## Physical Methods of Separation

### Mechanical Separation

Toxin levels decrease as clean product is physically separated from contaminated product in corn and wheat (for example); ultimately, this method is not very practical due to incomplete removal of mycotoxin-tainted grains and the converse removal of clean grain (Natarajan et al. 1975; Phillips et al. 1994). Significant decreases in aflatoxin levels from electron-

ic- and hand-sorted peanuts have been reported and is commonly utilized (Dickens and Whitaker 1975; Natarajan et al. 1975). Even though complete removal of all residual contamination cannot be expected with a variety of mechanical methods of separation, aflatoxin concentrations may be markedly decreased following rigorous treatment strategies. Interestingly, in cheese manufacturing, aflatoxin  $M_1$  occurs predominantly with the casein, which results in a higher concentration in the curd than the whey. Consequently, aflatoxin levels in cheese can be significantly enriched, i.e., by a factor of 2.5 to 3.3 in soft cheeses versus 3.9 to 5.8 in hard cheeses (van Egmond 1994; Yousef and Marth 1989). Aflatoxin  $M_1$  apparently is not significantly deactivated during the ripening of cheese.

### Density Segregation

Density segregation of contaminated grain and oilseeds involves sorting and delineating good versus contaminated kernels by flotation; importantly, this method can notably decrease aflatoxin concentrations (Cole 1989; Huff 1980; Huff and Hagler 1982). Peanuts that are contaminated with aflatoxin respond positively to density segregation by floating in tap water. This procedure may be compatible with current wet milling practices and alkaline processing of corn. It should be noted, however, that the appearance and weight of a particular kernel do not always indicate the presence or absence of mycotoxin. The removal of damaged grain by density segregation also has been shown to achieve decreases in deoxynivalenol and zearalenone in corn and wheat (Jackson and Bullerman 1999). Kelfkens et al. from TNO in the Netherlands decreased effective DON in wheat by the developed Aerodynamic Separation Technology called ASTER (de Koe 2001). Combinations of methods involving hand picking and density segregation of contaminated or moldy grains resulted in a decrease of 70 to 90% of aflatoxin and fumonisins (Vasanthi and Bhat 1998). Also, a combination of rinsing and density segregation of floating material was shown to be effective as a practical method for diminishing the toxicity to pigs from moldy corn containing deoxynivalenol and zearalenone (Rotter et al. 1995a). An overall decrease of total fumonisins (as  $FB_1$  and  $FB_2$  by HPLC analysis) of 60% was obtained by screening and gravity separating corn being discharged from a storage silo (Malone et al. 1998a).

## Physical Methods of Detoxification

### Thermal Inactivation

Because aflatoxins are heat stable, they are not completely destroyed by heat treatments, e.g., boiling water, autoclaving (Christensen et al. 1977). Partial destruction of aflatoxin may be accomplished by oil roasting or dry roasting peanuts and oilseed meals (Marth and Doyle 1979) or roasting corn (Conway et al. 1978). In a study by Lee (1989), roasting conditions and initial aflatoxin concentration in raw peanuts determined the degree of mycotoxin reduction, with decreases ranging from 45 to 83%. Other studies using roasting demonstrated that aflatoxin concentrations could be decreased in nuts and oilseed meals and in corn (Conway et al. 1978). Aflatoxin destruction, however, is not complete (nor uniform) and is affected by the commodity's temperature, heating interval, and moisture content (Mann et al. 1967). In contrast to aflatoxins, thermal processing is usually ineffective for decreasing the content of fumonisins and zearalenone in foods (Jackson and Bullerman 1999).

Aflatoxin levels in dough are not significantly altered by baking temperatures (Reiss 1978), and aflatoxin in contaminated wheat shows only partial destruction, exhibiting a similar resistance to heat and other processes involved in the bread making process.

Aflatoxin  $M_1$  apparently was stable in raw milk and was resistant to pasteurization and processing (Stoloff 1975, 1980). A wide range of decrease in aflatoxin  $M_1$  concentration during the preparation of freeze-dried milk has been reported (Purchase et al. 1972). These steps include pasteurization, sterilization, evaporation, roller drying, and spray drying.

### Irradiation

Exposing peanut oil to shortwave and longwave UV light has been reported to decrease aflatoxin levels (Shantha and Sreenivasa 1977). Other studies have shown that exposure to gamma irradiation (2.5 rad) does not degrade aflatoxin in contaminated peanut meal, while UV light produced no observable change in fluorescence or toxicity (Feuell 1977). Sunlight (14-hour exposure) destroyed between 77 and 90% of the aflatoxin  $B_1$  added to groundnut flakes, although only half the toxin was destroyed in the naturally contam-

inated product (Shantha 1987). Exposure of aflatoxins to UV light has been reported to activate these chemicals to mutagens (Stark et al. 1990). Applying UV light for 20 minutes at 25°C decreased the concentration of aflatoxin M<sub>1</sub> in contaminated milk by 89.1% in the presence of 0.05% peroxide, compared to 60.7% without peroxide (Yousef and Marth 1989). There is concern by some that this treatment could cause peroxidation leading to more toxic products. Microwave irradiation also has been suggested as a method for the detoxification of certain mycotoxins in model systems and in foodstuffs. Farag and coworkers (1996) report that aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub> respond to microwave treatment in both model and food systems; the rate of mycotoxin destruction was positively correlated with the power setting and exposure time.

### Solvent Extraction

Aflatoxins can be extracted efficiently from contaminated grains using carefully selected solvent mixtures, including binary and tertiary systems; importantly, this toxin reduction method has a minimal effect on protein content and nutritional value of the contaminated commodity (Goldblatt and Dollear 1979; Rayner et al. 1977). Examples include 95% ethanol, 90% aqueous acetone, 80% isopropanol, hexane-ethanol, hexane-methanol, hexane-acetone-water, and hexane-ethanol-water combinations. Although effective, such treatment is considered cost prohibitive and impractical for most applications (Shantha 1987).

## Biological Methods of Inactivation

### Microorganisms

Strategies that shift the focus from product decontamination (post-harvest) to prevention of aflatoxin production (preharvest) using biological controls have been reported. Nontoxigenic strains of *Aspergillus flavus* and *A. parasiticus* may compete with (and exclude) toxigenic (wild-type) strains and significantly decrease aflatoxin contamination in peanuts and cottonseed (Cole and Cotty 1990). Microorganisms, e.g., yeasts, molds, and bacteria, have been screened for their ability to modify or inactivate aflatoxin. *Flavobacterium aurantiacum* (NRRL B-184) was shown to significantly remove aflatoxin from a liquid medi-

um without producing toxic byproducts or metabolites (Ciegler et al. 1966). These same investigators also determined that certain acid-producing molds could catalyze hydration of aflatoxin B<sub>1</sub> to B<sub>2a</sub> (a less-toxic product). Applications of microbial detoxification of aflatoxins have been reviewed (Ciegler et al. 1978; Marth and Doyle 1979). Hao and coworkers (1987) reported that *F. aurantiacum* removed aflatoxin B<sub>1</sub> from peanut milk. This bacterium grew in both defatted and partially defatted peanut milk and was not inhibited by aflatoxin. Aflatoxins in contaminated grains are degraded by fermentation (Dam et al. 1977) but ensiling contaminated high-moisture corn was not as effective. A variety of microorganisms have been shown to interfere with aflatoxin production, and this interference is thought to occur by competition for nutrients and space or through the production of substances that interfere with toxigenesis (Bhatnagar et al. 1994).

In the case of the trichothecenes, it is known that the 12,13-epoxide ring is responsible for their toxic activity, and removal of this epoxide group entails a significant loss of toxicity. Several authors described this de-epoxidation reaction of ruminal or intestinal flora (He et al. 1992; Kollarczik et al. 1994; Yoshizawa et al. 1983), but Binder et al. (2000) were the first to isolate a pure bacterial strain (a new strain of *Eubacterium*) that is able to bio-transform the epoxide group of trichothecenes. Active, DON-transforming mixed cultures could be isolated from bovine rumen content, using anaerobic techniques described by Hungate (1969) and media according to Caldwell and Bryant (1966), which were modified for screening purposes. Through variation of medium components (energy source, minerals, antibiotics) and subsequent subcultivation in dilution series and highly active enriched cultures, an isolate finally was obtained. Molecular biological analyses as well as physiological characteristics indicated a new species of the genus *Eubacterium*, referred to as BBSH 797. For the use of BBSH 797 as a feed additive, the fermentation and stabilization processes were optimized with respect to fast growth of the microbe and high biotransformation activity of the resulting product. For enhancement of stability during storage and within the GI tract, a three-step encapsulation process was implemented.

For optimizing the cell count of the final product, *in vitro* experiments using pieces of pig intestine were carried out: within 24 h, 31% of 50 ppm DON were transformed at a bacterial concentration of  $3.55 \times 10^4$  CFU/g, while after 48 h, the total amount of DON was

de-epoxidized. Cell counts of  $3.55 \times 10^5$  CFU/g resulted in 83% transformation, while  $3.55 \times 10^6$  CFU/g metabolized 100% within 24 h. Through observing the mechanism of microbial detoxification, two important reactions were detected (Fuchs et al. 1999): besides the transformation of the epoxide into a double bond, hydrolyzation of ester groups into corresponding hydroxyl groups occurred in some cases. While a direct de-epoxidation occurred in the case of nivalenol, fusarenon X was first transformed into nivalenol and then de-epoxidated. The same could be observed with 3- and 15-acetyl-DON, which also were deacetylated prior to de-epoxylation. A partial deacetylation of A-trichothecenes was found prior to or together with de-epoxidation.

The efficiency of the final formulation (i.e., stabilized bacteria) was tested in feeding trials, which were conducted under the surveillance of the University for Veterinary Medicine, Vienna, Austria. Highly significant results ( $P < 0.001$ ) were obtained in piglet feeding trials with a contamination of 2.5 ppm DON. The trial started on the 25th live day of the animals that weighed 6.7 kg. After 45 days, the animals that were fed contaminated feed gained up to 16.4 kg at a feed conversion rate (FCR) of 2.0, while those groups that had received uncontaminated feed plus different concentrations of BBSH 797 weighed between 22.3 and 23.6 kg and a FCR of 1.6.

It could be shown *in vitro* that the bacterial isolate BBSH 797 is able to biotransform trichothecenes into less toxic de-epoxy metabolites. Field trial results also suggest that GGSB 797 can alleviate performance-decreasing effects of DON in growing piglets and broilers under field conditions and may prove beneficial in the preventive management of such mycotoxin contaminations.

Schatzmayer and coworkers (2000) used crude enzyme preparations and pure carboxypepsidase A for degradation of ochratoxin to nontoxic products.

### Modification of Biological Effects of Aflatoxins (Chemoprotection)

Dietary factors that influence the toxicity of aflatoxin B<sub>1</sub> have been widely reported and continue to be an area of active research. These factors can be classified into two categories: nutrient and nonnutrient food components. Of the former, attention has been directed to the presence of methyl donors such as choline and methionine (often in conjunction with folate) in the diet as ameliorating factors in induction

of hepatic preneoplastic foci. Protein deficiency has been shown to affect the incidence of these foci. Dietary fat content (as well as effects of saturated versus unsaturated fats), vitamins (e.g., vitamin A, folic acid, carotene), and trace minerals such as selenium appear to modulate the hepatocarcinogenicity of aflatoxin B<sub>1</sub>. Examples of dietary components that can be classified as nonnutrients include butylated hydro-toluene, butylated hydroxyanisole, ellagic acid (a plant phenolic), indole 3-carbinol (a component of cruciferous vegetables), various garlic extracts, and capsaicins. For a thorough discussion, the reader is directed to the following reviews (Cullen and Newberne 1994; Eaton et al. 1994; Galvano et al. 2001; Kensler et al. 1994; Rogers 1994).

## Chemical Methods of Detoxification

### Structural Degradation Following Chemical Treatment

Numerous chemicals have been tested for their ability to degrade or detoxify aflatoxin; these include acids, bases, aldehydes, bisulfite, oxidizing agents and various gases (Anderson 1983; Feuill 1966; Goldblatt and Dollear 1977, 1979; Hagler 1991; Mann et al. 1970; Park et al. 1988; Phillips et al. 1994; Samara-jeeva et al. 1991; Trager and Stoloff 1967). Although many proposed treatments may successfully destroy aflatoxin, they may be impractical or potentially harmful due to generation of toxic byproducts and/or significant alteration of product quality. Numerous chemical strategies (which may be practical as well as effective) for the aflatoxins include ammoniation, ozonation, and reaction with food-grade additives such as sodium bisulfite. This is not the case for the fumonisins, however, which have been shown to be very resistant to degradation and detoxification by a variety of methods including milling, fermentation, ammoniation, and ozonation. In recent studies (Lemke et al. 2001b), fumonisin B<sub>1</sub> was significantly deaminated in aqueous solution under conditions of acidic pH and low temperature by the addition of NaNO<sub>2</sub>. Diazotization of fumonisins makes use of common, relatively inexpensive components (sodium nitrite [NaNO<sub>2</sub>] and hydrochloric acid [HCl]) whose concentrations can be optimized in larger-scale processes to maximize HONO production.

### Ammoniation

Degradation using ammonia is purportedly a feasible method to detoxify aflatoxin-contaminated products. Ammoniation involves use of gaseous ammonia or ammonium hydroxide and, when performed under appropriate conditions, has been shown in some cases to decrease aflatoxin levels by more than 99% (Brekke et al. 1977, 1979; Dollear et al. 1968; Gardner et al. 1971; Masri et al. 1969; Park et al. 1984; Phillips et al. 1994).

Ammoniation purportedly results in the conversion of aflatoxin B<sub>1</sub> to less toxic products, including aflatoxin D<sub>1</sub> and a derivative with molecular weight 206. Because the initial step is reversible and the lactone ring may reform, it is "extremely important" to allow the reaction to proceed to completion.

Two different ammoniation procedures are currently utilized: a high-pressure and high-temperature process (HP/HT) and an atmospheric pressure and ambient temperature procedure (AP/AT). The HP/HT process frequently is used to decontaminate whole cottonseed and cottonseed meal and AP/AT is used mainly for whole cottonseed. Arizona and California have approved ammoniation to decontaminate cottonseed products; Texas, North Carolina, Georgia, and Alabama permit ammoniation of aflatoxin-contaminated corn. Internationally, this procedure is an accepted decontamination practice in Mexico, Sudan, South Africa, Senegal, and Brazil. Aflatoxin detoxification using ammonia and other chemical treatment methods has been extensively reviewed (Anderson 1983; Goldblatt and Dollear 1979; Palmgren and Hayes 1987; Park et al. 1988).

### Treatment with Bisulfite and Other Food Additives

Promising results have been achieved in aflatoxin detoxification using accepted food additives; sodium bisulfite has received particular attention. Sodium bisulfite reacts with aflatoxins B<sub>1</sub>, G<sub>1</sub>, M<sub>1</sub>, and aflatoxicol to form water-soluble products (Doyle and Marth 1978a, 1978b; Hagler et al. 1982; Moerck et al. 1980). Information on the chemistry of the reaction can be found in studies by Yagen et al. (1989) and Phillips et al. (1994).

### Ozonization

Another effective method of mycotoxin degradation is based on reaction with ozone (O<sub>3</sub>) gas, a powerful oxidant with a preference for double bonds. Several studies indicate that ozone gas (generated by corona discharge) degrades aflatoxins in corn and cottonseed meals (Dollear et al. 1968; Dwarakanath et al. 1968)

and in aqueous solution (Maeba et al. 1988). This procedure has been shown to degrade other mycotoxins such as DON (Young 1986; Young et al. 1986) and moniliformin (Zhang and Li 1994). Studies by Maeba and coworkers (1988) report that ozone gas chemically degrades and detoxifies aflatoxins B<sub>1</sub>, G<sub>1</sub>, B<sub>2</sub>, and G<sub>2</sub> (present as pure standards) *in vitro*.

A novel (electrochemical) method of ozone production couples anodic decomposition of water (hydrolysis) at the water/porous anode interface and utilizes a proton exchange membrane in an electrolysis cell to produce up to 20% by weight (242.0 mg/liter (L) air) O<sub>3</sub> gas (Rogers et al. 1992), as compared to a maximum of 6% by weight O<sub>3</sub> in dried, oxygen-fed systems (Foller and Tobias 1982). This concentrated ozone has been used to degrade and detoxify numerous mycotoxins *in vitro*, including the four commonly occurring aflatoxins, cyclopiazonic acid, ochratoxin A, patulin, secalonin acid D, and zearalenone (McKenzie et al. 1997). Fumonisin B<sub>1</sub> was degraded to a keto-substituted analog of FB<sub>1</sub> (3k-FB<sub>1</sub>), although detoxification was not demonstrated by two separate bioassays. In another study, electrolytically generated ozone gas significantly degraded high concentrations of aflatoxins present in naturally contaminated, toxic corn. Importantly, ozone treatment of this contaminated corn significantly protected young turkeys from the deleterious effects of aflatoxins (McKenzie et al. 1998).

Ozone is a fairly stable gas, but in an aqueous environment, its half-life drops to approximately 20 minutes. Ozone decomposes to form oxygen and, therefore, can be classified as a nonpersistent chemical; however, it must be generated at the location of its intended use. Ozonization may help remediate bulk quantities of corn at a minimal cost with minimal destruction of important nutrients. These findings indicate a potentially practical approach to the remediation of unprocessed corn contaminated with aflatoxin (McKenzie et al. 1998).

## Decreased Bioavailability of Mycotoxins

### Clay and Zeolitic Minerals

The strategy of decreasing foodborne exposure to mycotoxins by the inclusion of various binding agents or sorbents in the diet has been given considerable attention. Many of these binding agents are purported to prevent the deleterious effects of diverse mycotoxins in a variety of animals (primarily poultry and swine) — apparently by diminishing mycotoxin uptake and distribution to the blood and target organs.

As early as 1979, adsorbent clay minerals were reported to bind aflatoxin B<sub>1</sub> in liquids (Masimango et al. 1979). Also, bleaching clays that had been used to process canola oil were found to lessen the effects of T-2 toxin (Carson and Smith 1983; Smith 1984).

In the first enterosorbent study with aflatoxins, hydrated sodium calcium aluminosilicate (HSCAS) (NovaSil™), a calcium montmorillonite clay that is sold as an anticaking additive for animal feeds, was reported to adsorb aflatoxin B<sub>1</sub> with high affinity and high capacity in aqueous solutions and to notably protect broiler and Leghorn chicks from the toxic effects of 7.5 ppm of aflatoxin in the diet (Phillips et al. 1987, 1988). Since these early studies, HSCAS and other similar montmorillonite and bentonite clays have been reported to diminish the effects of aflatoxins in a variety of young animals including rodents, chicks, turkey poults, ducklings, lambs, pigs, mink, and trout (Abdel-Wahhab et al. 1998; Bonna et al. 1991; Cerdchai et al. 1990; Colvin et al. 1989; Ellis et al. 2000; Harvey et al. 1991b, 1991c, 1993, 1994b; Jayaprakash et al. 1992; Kubena et al. 1990a, 1990b, 1991, 1993; Ledoux et al. 1999; Lindemann et al. 1993; Marquez and Hernandez 1995; Nahm 1995; Phillips 1999; Phillips et al. 1990, 1991, 1994, 1995; Smith et al. 1994a; Voss et al. 1993b). HSCAS clay also has been shown to decrease the bioavailability of radiolabeled aflatoxins and decrease aflatoxin residues in poultry (Davidson et al. 1987; Jayaprakash et al. 1992), rats (Mayura et al. 1998; Sarr et al. 1995), and pigs (Beaver et al. 1990). Levels of aflatoxin M<sub>1</sub> in milk from lactating dairy cattle and goats were also diminished in the presence of HSCAS in the diet (Ellis et al. 1990; Harvey et al. 1991c; Smith et al. 1994a).

Chung and Baker (1990) reported that HSCAS clay did not impair phytate or inorganic phosphorous utilization. The addition of HSCAS to basal diets at concentrations of 0.5 or 1.0% did not impair utilization of riboflavin, vitamin A, or manganese; however, there was a slight decrease in zinc utilization in the presence of HSCAS clay (Chung et al. 1990). This decrease was small but statistically significant at the 1.0% level. In another study in chicks that were fed nutrient-deficient diets and clay minerals, HSCAS and sodium bentonite clays did not adversely affect growth performance or tibial mineral concentrations (Southern et al. 1994).

Based on equilibrium adsorption isotherms and animal studies (Grant and Phillips 1998; Grant et al. 1998; Phillips 1999; Phillips et al. 1995; Ramos and Hernandez 1996; Sarr 1992), HSCAS clay has been shown to have a preference for the aflatoxins. Recent-

ly, ligands having at least one functional group in common with aflatoxin B<sub>1</sub> were reacted with NovaSil™ (HSCAS clay) in an attempt to elucidate the specificity and mechanism of adsorption (Grant and Phillips 1998). The dicarbonyl system of aflatoxin was found to be essential for tight binding by HSCAS clay. Through these studies and computer modeling, evidence suggests that aflatoxins may react at multiple sites on NovaSil™ clay particles, including the inter-layer region, edges, and basal surfaces (Grant and Phillips 1998).

In studies with other mycotoxins, the inclusion of HSCAS in the diet did not significantly alter the hyperestrogenic effects of zearalenone (Bursian et al. 1992). Effects of HSCAS clay also were investigated in pigs exposed to diets containing corn that was naturally contaminated with DON. Adding HSCAS at either 0.5 or 1.0% did not influence the average daily gain of the pigs exposed to DON. Diluting contaminated corn with uncontaminated corn was the only effective method of decreasing the effect of DON (Patterson and Young 1993). The possibility of supplementing livestock diets with HSCAS clay to protect from fescue toxicity has also been investigated (Chestnut et al. 1992). *In vitro* experiments have suggested a high affinity of HSCAS for ergotamine in aqueous solution (Chestnut et al. 1992; Huebner et al. 1999). However, HSCAS clay (at levels of 2.0% by weight) did not protect sheep from fescue toxicosis and apparently impaired the absorption of magnesium, manganese, and zinc. Although lower levels of HSCAS may have less of an effect on these minerals, further work is warranted to determine the dosimetry of this effect in livestock.

Mayura et al. (1998) compared the effects of HSCAS and a commonly used zeolite. Pregnant rats were used as a sensitive model, because the developing rat embryo is susceptible to nutritional deficit and vulnerable to toxic insult from aflatoxin. Results suggested that HSCAS (but not zeolite) prevented the maternal and developmental toxicity of aflatoxin. Moreover, nodules of regenerating hepatocytes in the liver were observed only in animals treated with zeolite plus aflatoxin, suggesting that this aluminosilicate may be inactivating a chemoprotective factor or nutrient in the GI tract or feed source that modulates aflatoxin toxicity/carcinogenicity. Clearly, clay and zeolitic minerals, which comprise a broad family of diverse aluminosilicates, are not created equal; all mycotoxin binding agents should be tested for efficacy and safety in sensitive animals before inclusion in animal diets.

### Other Mycotoxin Adsorbents

Granulated activated carbon (GAC) has been studied for its ability to bind aflatoxins, both *in vivo* and *in vitro* (Piva et al. 1995). Galvano and coworkers (1996) investigated numerous GACs to determine their affinities for aflatoxin B<sub>1</sub> in aqueous solution. The results varied according to the type of GAC used, suggesting that those with a high methylene blue index and a low surface acidity could adsorb greater than 99% of the aflatoxin B<sub>1</sub> present. Certain GACs decrease conversion of aflatoxin B<sub>1</sub> to aflatoxin M<sub>1</sub> in Friesian cows by 40.6 to 73.6% when included in the diet at concentrations of 2.0%.

Cholestyramine (an insoluble quaternary ammonium anion exchange resin) has been used to lower the levels of cholesterol. In studies in mice, cholestyramine at a level of 2.5 g/kg diet was reported to decrease the toxic effects of 6 ppm zearalenone (Underhill et al. 1995). From *in vitro* binding studies with zearalenone, it was suggested that the addition of cholestyramine or crospovidone to feedstuffs would efficiently prevent zearalenone-induced toxicosis; although *in vivo* studies are needed first to determine the stability of the complex in the GI tract of animals (Ramos et al. 1996). In other work, acidic montmorillonite clay was chemically modified by ex-

change with various ammonium- and pyridinium-based organic cations. These organoclays were then shown to adsorb zearalenone with an efficacy related to the exchanged cation hydrophobicity, as gauged by estimated critical micelle concentration values and length of the alkyl chain (Lemke et al. 1998). It is important to note that these same organoclays failed to protect prepubertal mice from the estrogenicity of 35 mg zearalenone/kg of diet (Lemke et al. 2001a). In fact, diets containing 0.5% organoclay plus zearalenone resulted in decreased body weight gain and increased uterine weight versus zearalenone alone. These findings suggest that alkylamine groups may assist the transport or uptake of zearalenone in the animal and result in enhanced toxicity. Clearly all mycotoxin binding agents should be tested *in vivo* before inclusion in the diet (Lemke et al. 2001a). The reader is referred to Huwig et al. (2001) for a recent review of mycotoxin binders.

In other studies, ultrafine activated carbon was bonded onto granular quartz to produce a composite carbon adsorbent (CCA) with a high carbonaceous surface area, good bed porosity, and increased bulk density. CCA was shown to effectively decrease patulin concentrations in naturally contaminated apple juice by filtration (Huebner et al. 2000).



# 10 Potential Economic Costs of Mycotoxins in the United States<sup>3</sup>

## Summary

The combined effects of the feed and food losses due to mycotoxins decrease the supply and raise the price of various farm commodities. Mycotoxins also lead to expenditures on research, regulatory enforcement, mitigation, lawsuits, testing, and quality control.

The authors provide a tentative estimate of the potential costs of mycotoxin contamination in the United States economy (U.S. Food and Drug Administration 2000c). It is not possible to estimate precisely the economic costs of mycotoxins. Therefore, the uncertainty was built into the cost model, which used Monte Carlo computer simulations to estimate the distributions of commodity outputs, prices, and contamination levels. Potential annual costs are estimated to be the sum of the value of food losses, feed losses, and mitigation efforts. The potential annual cost of mycotoxin contamination of crops is estimated to range from \$418 million to \$1.66 billion, with the mean estimated cost about \$932 million. In addition, mitigation costs and livestock losses could add another \$466 million and \$6 million, respectively, to the mean simulated costs.

## The Potential Costs

The potential harm to humans and animals by certain mycotoxins makes highly contaminated crops unacceptable for food or feed. Therefore, potential costs are estimated as the value of potentially lost food and feed that may be attributed to the effects of mycotoxins. Also, the cost of potential livestock losses if livestock are exposed to high levels of mycotoxins in their feed was estimated.

Cost estimates for the United States were derived with a series of single-sector models of the most widely consumed food and feed commodities that have been contaminated by aflatoxins, fumonisins, and DON. It

was assumed that simple supply and demand models accurately characterized the markets for these commodities. Crop price and quantity data from recent U.S. Department of Agriculture sources were used. Contamination was estimated from analytical sample data obtained from several sources including the U.S. FDA compliance program. Because the data came from different testing and sampling sources, and, more importantly, because the number of samples was small relative to the size of the industries being investigated, the sample results were particularly uncertain.

Because exact estimates of the economic costs of mycotoxins could not be produced, the uncertainty was built into the model and Monte Carlo simulations were used to estimate the distributions of commodity outputs, prices, and contamination levels. The simulations embodied the uncertainty about

- the extent and level of contamination,
- the variability of contamination,
- the variability of the price and quantity of the affected commodities,
- the costs of efforts to mitigate the contamination, and
- the loss in livestock value from contaminated feed.

## Extent and Level of Contamination

It was assumed that the fraction of contaminated crops in the sample data provided the best evidence on the percentage of contaminated crops in the entire U.S. commodity supply. The rates of contamination of samples nevertheless do not demonstrate that the entire domestic wheat or corn crops have this rate of sample contamination. The sample data sets were small relative to the size of the industry and may not have been geographically diverse enough to be representative of the range of climatic and growing conditions. Some of the FDA data generated by compliance sampling rather than by surveillance sampling presented another problem. Compliance samples are taken when there is reason to suspect that contamination is a problem, whereas surveillance samples are

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taken as a random survey to estimate average levels of contamination. Because so little data were available, compliance and surveillance sample results were combined. Although combining the results of the two types of samples is unconventional, the compliance results did not necessarily show more contamination than the surveillance results. The similarity may reflect the difficulty in identifying crops that are likely to be contaminated. When surveillance samples were not available to compare with compliance samples, however, the compliance samples were not used. Reliance on mixed and incomplete sample data on contamination accounts for the largest uncertainty embodied in the estimates.

### Variability of Contamination

Mycotoxin contamination varies greatly from year to year. This large variability in annual contamination cannot be eliminated by the most optimal growing and handling regime, because the best practices for one weather condition are different than the best practices for another weather condition, and weather changes unpredictably or randomly. This randomness means that past levels of contamination do not predict future levels, even when growing practices remain about the same. Moreover, currently used practices are not ideal. The ideal practice is costly to achieve at any time and ideal practices do not remain the same as new technologies and practices are introduced.

### Variability of the Price and Quantity of Affected Commodities

Commodity prices and quantities vary seasonally, annually, by region, and by quality for reasons unrelated to mycotoxins. Even if the fraction of crops contaminated were constant (and it is not), the value of the lost product would nevertheless be variable because of variable prices and outputs. Obtaining statistically representative sample data might decrease the uncertainty from this source, but such a solution is quite expensive because of the cost of testing the thousands of sample units that would be needed to create a representative sample.

### Costs of Efforts to Mitigate the Contamination

To estimate the costs from crop loss mitigation

strategies, it was assumed that growers and elevator owners would only incur the expense to decrease the contamination when the additional cost incurred was less than or equal to the expected costs prevented. Mitigation costs (Shane 1994) could include the cost of

- developing fungus-resistant grains;
- altered farming practices, such as more intensive irrigation;
- applying additional insecticides, fungicides, and fertilizer;
- improved handling after the harvest, such as additional storage facilities;
- improved storage and transportation conditions to achieve water resistant, aerated, or heated grain storage;
- more efficient drying to achieve desired moisture levels;
- additional quality control procedures to monitor moisture and toxins; and
- amelioration of contaminated grains through physical and chemical treatments.

The total amount spent for these efforts is not known. If these costs were known, those attributable to mycotoxins would still have to be separated from those costs that would have been incurred due to other (nonmycotoxin) related farm risks. For example, a peanut grower who would irrigate more intensively under drought conditions might do so with or without the threat of the aflatoxins to the harvest. The additional costs from irrigation could not be considered a cost of the aflatoxins alone and should not be included in the estimate of mitigation costs.

### Loss in Livestock Value from Contaminated Feed

There were no dose-response data or models to estimate how exposure to various amounts of mycotoxins affects livestock health, growth, or survival. The intake of the various mycotoxins is also uncertain. The livestock losses, potential or actual, remain unknown. As shown below, these losses were estimated indirectly.

The economic impact from the health effects of mycotoxins on humans was not quantified. There is little potency, exposure, or epidemiological data from which to derive such an estimate for the United States. Dose-response experiments or large-scale epidemiology studies need to be carried out to establish the carcinogenic potency and other human health

Table 10.1. Sources of analytical sample data

Commodity	Mycotoxin	Food or feed	Data source	Years	Total number of samples
Shelled corn	Aflatoxin	Food	FDA Compliance Program	1990 to 1996	3,087
Milled corn	Aflatoxin	Food	FDA Compliance Program	1989 to 1996	1,918
Shelled corn	Aflatoxin	Food	FDA and Romer Laboratories	1984 to 1989	1,789
Shelled corn	Aflatoxin	Feed	FDA Compliance Program	1989 to 1996	2,002
Shelled corn	Aflatoxin	Feed	FDA and Romer Laboratories	1984 to 1989	1,317
Peanuts	Aflatoxin	Food	Peanut Administrative Committee	1967 to 1994	NA <sup>a</sup>
Wheat products	DON	Food	FDA Compliance Program	1995 to 1997	478
Wheat	DON	Food	Romer Laboratories	1996–1997	194
Wheat	DON	Feed	FDA Compliance Program	1995–1996	55
Dry-milled corn	Fumonisin	Food	North American Millers Association	1998	672
Corn	Fumonisin	Food	FDA Compliance Program	1990 to 1998	1,208

<sup>a</sup>The dollar impact is based on the average expected annual claims made by peanut growers, and is not based on sample data.

outcomes for many of the mycotoxins. Until the harmful health effects are well established, the magnitude of their economic impact will remain unknown. It is noted, however, that there could be a trade-off between long-term human health costs and short run food and feed losses. If crops are tested and found to be contaminated, they will be diverted at some cost. If these same crops are contaminated and not diverted, then there are potential human health costs.

To include the uncertainty associated with the losses caused by mycotoxins, the costs of mycotoxins were estimated with simulations that used probability distributions for the main variables, including rates of contamination, output, prices, and the fraction of costs attributable to mitigation efforts. The probability distributions were based on the available sample or time series data.

The simulations were used to estimate the distributions of four main potential costs of contamination:

1. the value of the food crop losses,
2. the value of the feed crop losses,
3. the costs incurred to mitigate the contamination, and
4. the value of the livestock losses.

In the next section, the assumptions underlying the simulations are described.

## Assumptions Used in the Simulations

It was assumed that crop contamination occurred randomly and could be characterized with Monte Carlo techniques (Vose 1998). To model crop contamination, all available analytical sample data were examined to assess the extent of the contamination

over time and across regions. The sample data as described in Table 10.1 came from several sources and represented different testing and sampling conditions. In the simulations, available data were used to create probability distributions representing the possible levels of contamination. From the probability distributions, the simulations generated estimates of the commodity quantities that would be at action or advisory levels, supposing that the samples were representative.

Although it was assumed that these distributions of losses derived from sample results could apply if all crops were tested for the relevant mycotoxins, there was no attempt to estimate how much of each crop is currently tested. The potential losses estimated, then, cannot be directly linked to actual losses. That link could be made only if more was known about current testing.

The expected economic value of the food crop losses was simulated by multiplying the rejected quantity by the average annual market price (minus the salvage value) of the contaminated crop. The estimating formula was

$$\text{Value of food loss} = (\text{price per unit} - \text{salvage value per unit}) (\text{number of rejected units}).$$

For feed, it was assumed that the salvage value was zero, so the estimating formula was

$$\text{Value of feed loss} = \text{price per unit} \times \text{number of rejected units}^4.$$

Because the values of the rejected quantities were

<sup>4</sup>It was assumed there are no industrial uses for contaminated feed or if there are, the value of these alternative uses is relatively small. It was recognized that some contaminated feed might be diverted for use in methanol production or for comparable uses.

Table 10.2. Potential total economic costs of mycotoxins (in million dollars)

	5th percentile	Median	Mean	95th percentile
Crop costs	418	882	932	1,656
Mitigation costs	209	441	466	828
Livestock costs	2	6	6	12
Total	629	1,329	1,404	2,496

uncertain, they were estimated with values drawn from probability distributions. Each simulation had 1,000 iterations. The calculation was performed for each of the three major mycotoxins, and for all of the output likely to be affected.

Mitigation costs were estimated as a uniform distribution ranging from zero to the mean estimated crop losses. In other words, mitigation costs were derived from the value of crop losses. This indirect procedure was used because specific production costs could not be assigned to mitigation. It is possible that no additional costs are attributable to mitigation efforts directed at mycotoxins, but mitigation expenditures could be as high as the expected cost of mycotoxins, which should be about the same as the mean crop loss. Although the minimum and maximum mitigation costs could be identified, nothing could be said about their relative likelihood. Rather than attach a mitigation cost to each mycotoxin and crop separately, mitigation costs were estimated as a fraction of the total crop losses associated with mycotoxins. The uniform distribution generates mean mitigation costs equal to one-half of mean crop costs.

Costs of potential livestock losses were estimated

separately from the costs of crop losses. For livestock, it was assumed that the loss of value would be proportional to the exposure to mycotoxins above the action or advisory levels. For example, if 2% of feed were contaminated, the assumption would mean that either 2% of livestock would lose 100% of value, 100% of livestock would lose 2% of value, or some combination of the two types of losses would occur. If, however, all contaminated feed were diverted and destroyed, there would be no exposure and no losses. The livestock losses, then, occur only if feed that is contaminated above the action levels is fed to animals instead of being diverted or destroyed. It was assumed that if feed contained mycotoxins at concentrations below action or advisory levels, there would be no adverse effects on livestock.

The same set of assumptions was used to simulate the effects of each of the three mycotoxins associated with substantial economic costs. The simulations used distributions for the inputs and generated distributions for the outputs. In this chapter, the mean values will be reported only for the inputs to the simulations. Tables 10.2 and 10.3 contain the distributions of simulated outputs.

Table 10.3. Potential crop and livestock costs for each mycotoxin (in million dollars)

	5th percentile	Median	Mean	95th percentile
<b>Aflatoxins</b>				
Food crops	8	34	47	127
Feed crops	44	176	225	571
Livestock	1	4	4	9
<b>Fumonisin</b>				
Food crops	5	10	11	18
Feed crops	< 1	< 1	< 1	< 1
Livestock	< 1	< 1	< 1	< 1
<b>Deoxynivalenol</b>				
Food crops	249	564	637	1,195
Feed crops	1	12	18	55
Livestock	1	2	2	5
Total	311	804	946	1,982

## Aflatoxin

1. Potential losses come from contaminated corn and peanuts.
2. The food crop action level is 20 ppb.
3. The feed crop action level is 300 ppb<sup>5</sup>.
4. The mitigation efforts include testing and other methods of quality control for all products and blanching for peanuts.
5. The potential hazards to human health include acute GI problems and primary liver cancer.

Although it can be found in virtually all crops at low concentrations, aflatoxin contamination at detectable levels is most common in peanuts and corn, because these commodities are grown in climates that are favorable to fungal growth (Phillips et al. 1994). Aflatoxin M<sub>1</sub> may be found in milk when the feed for dairy cattle, especially corn, is highly contaminated. Because there was insufficient sample data to estimate the fraction of milk that would contain levels of aflatoxin, no attempt was made to estimate the value of these losses. The small number of compliance samples may indicate that the loss from this source is small.

Contamination of the various commodities by aflatoxins varies by year, by region, and by the mitigation efforts of producers and distributors. Periods of drought induce aflatoxin growth, which in turn leads to higher crop losses. Good storage practices can mitigate the effect of initial contamination, but poor storage practices even during nondrought years can increase the contamination. The variability of weather and storage conditions makes predicting and estimating aggregate cost difficult. The relative prices of insecticides and fungicides affect the incidence of aflatoxin contamination, because higher relative prices for these inputs decrease their use and thereby increase the risk of contamination. Finally, as yield per harvested acre has grown in the United States, more of the total crop production can be stored in elevators for longer periods, which in turn increases the risk of aflatoxin contamination (Shane 1994).

The estimated loss in peanuts used for food occurs when the contamination levels in the peanut samples exceed 20 ppb. The food loss for peanuts has previ-

ously been estimated to be close to \$5 million (Whitaker 1999).

To estimate the potential economic impact of aflatoxin contamination of corn, the percentage of the analytical sample data that exceeded the action levels established as guidelines by the FDA was determined first. Both the feed and food corn losses were simulated. The action level for food corn is 20 ppb; the action level for feed is 300 ppb. Because much of the food corn that was contaminated at levels above 20 ppb could be diverted to use as animal feed, the value of the corn has a measurable but imprecise salvage value. The salvage value was assumed to be 25 to 40% below its value as food. It was assumed that feed exceeding 300 ppb would be destroyed or at least would lose its market value.

The sample data indicated that about 6.6% of corn samples exceeded the aflatoxin action level for food. From recent price and output data, it was estimated that the mean food crop price was \$3.50/bushel (bu) and that the mean food output was about 900 million bu. About 3.6% of feed corn samples exceeded the aflatoxin action level for feed. The mean feed crop price was estimated to be \$2.68/bu, and mean feed output was about 4,660 million bu.

Cattle, swine, and poultry that have been fed contaminated feed suffer multiple adverse effects. To estimate the economic cost of these diverse effects, it was assumed that the percentage value of the livestock loss equaled the percentage of feed that was contaminated.

The mean simulated potential value of crops lost because of aflatoxin contamination was \$47 million per year in food crops (corn and peanuts) and \$225 million per year in feed corn. The potential livestock loss and the potential feed loss are alternative, mutually exclusive outcomes. Therefore, the livestock loss is reported separately from the feed costs. The mean simulated livestock cost was about \$4 million per year.

## Fumonisin

1. The potential loss is estimated for contaminated dry-milled and masa corn only.
2. The food crop lowest limit is 2 ppm<sup>6</sup>.
3. The U.S. feed crop limits used in this model were 5 ppm for horses, 10 ppm for swine, and 50 ppm

<sup>5</sup>This is not strictly true. There are different action levels for different animals because not all animals are equally sensitive. Beef cattle are less sensitive than poultry. However, these differences were not accounted for because the authors believe the economic impact of the differences is small.

<sup>6</sup>The FDA guidance level for industry varies from 2 to 4 ppm depending on product (U. S. Food and Drug Administration 2001). The authors chose 2 ppm on which to base their analysis as a best guess for a safe level.

for cattle (nonroughage portion of diet) and poultry<sup>7</sup>.

Although fumonisins have recently been detected in sorghum and millet, they are most prevalent in corn. To estimate the economic costs, it was assumed that corn with contamination levels in excess of 2 ppm would be diverted to feed. The economic loss would be the lost revenue to corn producers as measured by the difference in price between the food price and the authors' estimate of the feed price. To estimate the quantity of corn lost, sample corn meal data supplied by the North American Millers Association was used as a measure of the percentage of samples that exceeded 2 ppm. Corn meal was selected for the sample data because meal is believed to be found in all food and feed corn mixtures and therefore is representative of all corn products. Also, the sample is representative geographically for the United States. It was assumed that the distribution of percentages of corn meal samples contaminated with fumonisin represented an unbiased estimate of the percentage contamination in all corn products. From determination of the percentage of the contaminated samples, the percentage that exceeded the recommended safe limits for food was determined. Feed losses were estimated in a similar manner, using the higher action levels associated with fumonisin contamination in feed.

The sample data indicated that about 2.1% of dry-milled corn samples exceeded 2 ppm. From recent price and output data, the mean food corn price was estimated to be \$3.50/bu and the mean output of dry-milled corn to be about 152 million bu. The sample data for masa corn showed that less than 0.1% of samples exceeded 2 ppm. The mean masa corn price was estimated to be \$3.68/bu, and mean output to be about 85 million bu<sup>8</sup>.

In the feed sample data, about 0.1% of dry-milled corn samples exceeded the assumed fumonisin feed limits. The mean feed corn price was estimated to be \$2.68/bu, and mean feed output was about 450 million bu.

As discussed before, the mitigation costs, even if known with certainty for all farm commodities, would still have to be disaggregated from those costs that would be incurred due to nonfumonisin-related farm risks. Because fumonisin is such a recently discovered health risk, the additional mitigation costs from

fumonisin were the costs to test food and feeds and the costs of additional measures undertaken solely to decrease fumonisins.

To estimate the value of the livestock and other farm animals harmed by fumonisin consumption, the mean estimated lost value was assumed to be the percentage of feed that exceeded the recommended safe limits of fumonisin feed intake.

The simulated fumonisin costs were small, with mean food crop costs of about \$11 million per year (dry milled corn and masa corn) and other costs negligible. The livestock and feed crop losses were estimated in separate simulations, but both costs were negligible in the simulated outcomes.

## Deoxynivalenol

1. The potential loss comes from contaminated wheat and corn.
2. The U.S. food crop advisory limit is 1 ppm.
3. The U.S. feed crop advisory limits are 10 ppm for cattle, 10 ppm for poultry, 5 ppm for swine, and 5 ppm for all other animals.
4. The mitigation costs include the costs of dry storage, physical separation during milling, and testing.
5. The most likely human health hazard is acute GI illness.

Deoxynivalenol occurs in several grains, especially wheat, corn, and barley (Trucksess et al. 1996; Troxell 1996). No data, however, are available on the contamination of barley with DON, so wheat and corn only were used in the DON simulations.

To estimate the economic cost of DON in wheat and corn, sample results were used to derive distributions of the percentages that exceeded the recommended safe limits for food. As with all of the mycotoxins, it was assumed that the distributions based on sample results represented unbiased estimates of the percentages of the entire wheat and corn crops that would likely be contaminated. Feed losses were estimated in a similar manner, using the higher advisory levels associated with DON contamination in feed.

The sample data indicated that about 6.9% of corn samples exceeded the advisory level for food. From recent price and output data, the mean food crop price was estimated to be \$3.50/bu and mean food output to be about 900 million bu. For wheat, sample data indicated that 12.4% of samples exceeded the action level for food. The mean wheat price was estimated to be \$3.65/bu, and mean wheat food output to be

<sup>7</sup>Because there were no recommended limits at the time of writing, these were used as de facto limits. See U.S. Food and Drug Administration (2001) for current guidance levels.

<sup>8</sup>These figures were provided by W. J. Duensing, Director of Technical Services, Lauhoff Grain Company.

about 900 million bu.

Wheat represented the only substantial potential feed loss associated with DON. In the sample data, 1.8% of wheat exceeded the action level for feed. The mean feed crop price was assumed to be \$3.65/bu, and the mean feed output to be about 275 million bu.

The mitigation costs, even if known for all farm commodities, would still have to be separated from those costs that would be incurred due to non-DON related farm risks. The additional mitigation costs from DON probably include the costs of separating the contaminated wheat and corn, diverting the contaminated products to alternative uses, and storing wheat and corn under drier conditions.

To estimate the potential value of the livestock and other farm animals harmed by DON consumption, it was assumed that the lost value would be the percentage of feed that exceeded the recommended limits of feed intake. The FDA has established an advisory for feed contaminated with DON based on recent assessments of the toxicity, exposure and epidemiological evidence. The FDA advises that feed should not contain more than 10 ppm DON for ruminating beef and feed lot cattle older than four months. For chickens, feed containing more than 10 ppb should not exceed 50% of the diet. For swine, feed containing more than 5 ppb should not exceed 20% of the diet. For all other animals, feed containing more than 5 ppb should not exceed 40% of the diet.

The simulations generated mean simulated annual costs for DON of \$637 million in crop losses (mainly wheat and corn) and \$18 million in feed losses. The mean simulated annual cost of livestock losses was about \$2 million.

## Results and Conclusions

The results of the simulations, as shown in Table 10.2, indicate that the potential economic costs of crop

losses from mycotoxins are large. The 5th to 95th percentile range for annual food and feed costs was \$418 million to \$1.66 billion. The mean simulated potential cost was about \$932 million. Mitigation costs were not estimated directly. If it is assumed that mitigation costs range from zero to the mean estimated value of crop losses, the mean expected mitigation costs were about \$466 million. Mean simulated livestock costs were about \$6 million per year. Table 10.3 shows the costs of each mycotoxin separately.

Because the evidence on human health outcomes is too slight to permit an analysis, measurements of human health costs from aflatoxins, fumonisins, and DON were not attempted. If these mycotoxins do impose human health costs, then those costs (if measured) should be added to the estimates presented here.

By limiting the estimates to the potential costs associated with three major mycotoxin contaminants, and by not including human health effects, an attempt was made to avoid imparting an upward bias to the results. Because many dozens of mycotoxins have been discovered, it is possible that more will appear that can cause losses. Determination of the relationship between actual and potential costs also was not attempted. The potential costs assume that all shipments are tested and, if above the action or advisory level, diverted or destroyed. Because most shipments are not tested, the actual crop losses are much smaller than what has been simulated.

These first simulated estimates of the economic effects of mycotoxins should be regarded as a first pass at a difficult problem of estimation. They demonstrate that, under certain assumptions, mycotoxins could potentially impose large costs on the economy. Better data are needed, however, before the plausibility of these assumptions can be judged. The most pressing data needs are more surveillance samples and studies of the effects of mycotoxins on people and animals.

## 11 Mechanisms of Mycotoxicity

Mechanisms of mycotoxicity were recently reviewed (de Koe et al. 2000; Eaton and Gallager 1994; Norred and Riley 2001; Riley 1998). Many potential pitfalls are encountered when interpreting the results of *in vitro* studies and extrapolating them to *in vivo* studies or to diseases associated with mycotoxin consumption. The factors that tend to confound *in vitro* mechanistic studies include (1) failure to differentiate secondary effects from the primary biochemical lesion (Figure 11.1), (2) failure to relate the effective intracellular concentration *in vitro* to the tissue concentration of toxin that induces disease *in vivo*, (3) an *in vitro* model that is either deficient in the biochemical target or unresponsive due to other inadequacies of the model system, and (4) failure to adequately model the complexity of the *in vivo* exposure with regard to potential interactions with other toxins, drugs, environmental, and/or nutritional factors.

The study of mechanism of action of toxic compounds is founded in the belief that cells are molecular machines. To understand how mycotoxins interfere with cellular machinery, it is necessary to understand how toxic compounds alter the behavior of the molecules of life. Examples of how mycotoxins interact directly with the molecules of life or prevent their biosynthesis are common in the literature (Table 11.1).

Investigating mechanism of action is an important area of mycotoxin research for several reasons: (1) to reveal the initial biochemical lesion leading to the onset and progression of diseases, (2) to differentiate between biological effects that only occur at high dosages and those more likely to occur at environmentally relevant ones, (3) to predict downstream biochemical effects that will develop as a consequence of the initial biochemical lesion, and (4) to predict potential chronic toxicity and potential for interaction with other mycotoxins or bioactive agents, e.g., toxins, drugs. Because biochemical targets can be similar in all eucaryotic cells, e.g., AAL-toxin (Riley et al. 1996), understanding the mechanism of action in animal cells may provide insight into strategies to decrease the phytotoxicity of mycotoxins that are viru-

lence factors. This information could be used to engineer biochemical target molecules so that they do not interact with the toxin or so that bioactive metabolites are rapidly metabolized or eliminated.

Mechanism of action studies can provide insight into why fungi produce chemicals that appear to functionally mimic bioactive animal and plant molecules. Understanding the mechanism of action can engender testable hypotheses concerning the role of mycotoxins in pathogenesis (Cutler and Han 1996; Riley et al. 1996).

Unfortunately, mycotoxins as a group cannot be classified according to their mechanism of action. This is not surprising when one considers the diversity of chemical structures that are encompassed by the group of secondary fungal metabolites called mycotoxins. To grasp the potential diversity of mycotoxin mechanisms of action, it is necessary to understand the potential number of mycotoxins in nature.

New mycotoxins are being discovered at a high rate and, while few human diseases are definitively caused by mycotoxins (Wild and Hall 1996), there is considerable evidence to support mycotoxins being agents of animal disease. There is little doubt that mycotoxins kill farm animals; however, perhaps of greater importance is the fact that consumption of mold-contaminated feeds by farm animals has been associated with clinical cases involving reproductive effects, feed refusal, emesis, decline in productivity, lameness, bruising, decreased resistance to infectious agents, and other nonlethal effects (Hamilton 1982). Thus, mycotoxins are potentially contributing factors in chronic disease and may confound therapeutic and/or other interventions.

Given the possibly large number of mycotoxins and the diversity of the mechanisms of action, the potential for extremely complex toxin interactions is also great. Mycotoxins with similar modes of action would be expected to have at least additive effects. Conversely, some interactions could have subtractive effects. For example, cyclopiazonic acid prevents the lipid peroxidation induced by patulin (Riley and Showker 1991) and fungal serine palmitoyltrans-



**Table 11.1. Probable primary biochemical lesions and the early cellular events in the cascade of cellular events leading to toxic cell injury or cellular deregulation of selected mycotoxins<sup>a</sup>**

Mycotoxin	Initial Lesion → Cascade of Events	References
Aflatoxin	Metabolic activation → DNA modification → cell deregulation → cell death/transformation (metabolic activation → disruption of macromolecular synthesis → cell deregulation → cell death [apoptotic])	Eaton and Gallager 1994; Ueno et al. 1995
Adenophostins	ER IP <sub>3</sub> receptor → Ca <sup>2+</sup> release → cell deregulation → ?	Takahashi et al. 1994
Anthraquinones	Mitochondrial uncoupler → loss of respiratory control → cell death (apoptotic?)	Kawai et al. 1990
Beauvericin	K <sup>+</sup> ionophore → K <sup>+</sup> loss → cell deregulation → cell death/apoptosis (inhibition of cholesterol acyltransferase → disruption of cholesterol metabolism → ?)	Ojcus et al. 1991; Tomada et al. 1992
Citrinin	Loss of selective membrane permeability → cell deregulation → cell death (apoptotic?) (disruption of macromolecular synthesis → ?)	Ansari et al. 1991
Cyclopiazonic	ER and SR C <sup>2+</sup> -ATPase → disruption of Ca <sup>2+</sup> homeostasis → cell deregulation → cell death	Riley et al. 1995
Cytochalasins	Cytoskeleton → disruption of endocytosis → cell deregulation → cell death	Carlier et al. 1986
Deoxynivalenol	Inhibition of protein synthesis → disruption of cytokine regulation → altered cell proliferation → cell death/apoptosis?	Rotter et al. 1996
Fumonisin	Sphinganine N-acyltransferase → disrupted lipid metabolism → cell deregulation → cell death/apoptosis (disrupted delta-6-desaturase activity → disrupted fatty acid and arachidonic acid metabolism → cell death)	Merrill et al. 1996; Riley et al. 1996; Gelderblom et al. 1996
Glilotoxin	Calcium homeostasis → zinc homeostasis → endonuclease activation → apoptosis (radical mediated damage → oxidative stress → cell death) (inhibition of protein synthesis → apoptosis ?)	Waring 1990
Griseofulvin	Cytoskeleton → deregulation of cytoskeletal control → cell death	Cadrin et al. 1991
Luteoskyrin	Radical mediated damage → oxidative stress → lipid peroxidation → cell death (apoptotic?)	Masuda et al. 1991
Moniliformin	Pyruvate and α-ketoglutarate decarboxylation → loss of respiratory control → cell death	Thiel 1978
Ochratoxin	Disruption of phenylalanine metabolism → reduced PEPCK → reduced gluconeogenesis → cell death (metabolic activation → inhibition of protein/DNA synthesis → apoptosis ?) (altered membrane permeability → disrupt calcium homeostasis → cell deregulation → cell death)	Creppy 1995; Fink-Gremmels et al. 1995a, b; Marquardt and Frolich 1992
Patulin	Nonprotein sulfhydryl depletion → altered ion permeability and/or altered intercellular communication → oxidative stress → cell death (inhibition of macromolecular biosynthesis → cell death)	Barhoumi and Burghardt 1996; Burghardt et al. 1992; Riley and Showker 1991
Sphingofungin and ISP1	Serine palmitoyltransferase → decrease sphingolipids → cell deregulation → cell death/apoptosis	Zweerink et al. 1992; Miyake et al. 1995
Sporidesmin	Radical mediated damage → oxidative stress → thiol depletion → cell death (disrupt calcium homeostasis → zinc homeostasis → endonuclease activation → apoptosis)	Waring et al. 1990
Swainsonine	Manosidase II → disrupted glycoprotein processing → cell deregulation → cell death	Hino et al. 1985
Tremorgens	GABA receptors → altered Cl <sup>-</sup> permeability → disrupted neuromuscular control → ?	Gant et al. 1987

Table 11.1. (continued)

Mycotoxin	Initial Lesion → Cascade of Events	References
T-2 toxin	Inhibition of protein synthesis → ? → cell death (apoptotic?) transient Ca <sup>2+</sup> elevation → endonuclease activation → apoptosis (altered membrane structure → disruption of membrane function → cell deregulation → cell death)	Ueno 1991; Bunner and Morris 1988; Welsh et al. 1994
Wortmannin	Inhibition of phosphatidylinositol 3-kinase → responsiveness to insulin/growth factors/apoptosis (inhibition of myosin light chain kinase → inhibition of IP3 signaling pathway →?) (Inhibition of phospholipase A2 → ?)	Cross et al. 1995; Yao and Cooper 1995; McLachlan 1993
Zearalenone	Cytosolic estrogen receptor → estrogenic response → disruption of hormonal control → ?	Cutler and Han 1996

<sup>a</sup>Additional references in Riley and Norred (1996). Primary cellular biochemical lesion = the molecular interaction between the parent toxin and target biomolecules, which occur within the cell and from which the cascade of events (secondary effects) leading to cellular deregulation and/or toxic cell injury are initiated. It should be recognized that there is often more than one mechanism of action with different dose dependencies and time courses and that these mechanisms may run parallel paths both leading to toxic cell injury (see Figure 11.1). Where there is more than one proposed mechanism of action the alternative mechanism is presented in parentheses. Question marks indicate uncertainty, or events not documented in vivo, or repeated in more than one study or model system. From Riley (1998).

ferase inhibitors such as sphingofungins prevent the fumonisin-induced accumulation of free sphingoid bases (Riley et al. 1996).

Mycotoxins occur naturally in feeds and foods in low concentrations, with several mycotoxins present. For example, *Fusarium proliferatum* on corn might produce several fumonisins (e.g., B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>4</sub>, etc.), fusaric acid, beauvericin, fusarins, moniliformin, and fusaproliferin (Nelson et al. 1993; Ritieni et al. 1995). Co-occurrence of other *Fusarium* spp. and *Aspergillus* spp. can co-contaminate corn with fumonisins, T-2 toxin, DON, nivalenol, aflatoxin B<sub>1</sub>, cyclopiazonic acid, zearalenone, and nitrosamines (Chamberlain et al. 1993; Chu and Li 1994; Rheeder et al. 1994; Wang et al. 1995; Widiastuti et al. 1988). Thus, with a diverse diet, humans will be exposed to multiple mycotoxins, intermittently and at low concentrations, over a long period of time. Acute toxicity studies cannot reveal the risks associated with this type of exposure; chronic toxicity studies of all mycotoxins and their combinations are logistically impossible. Understanding the mechanism of action in simple in vitro systems can provide a rational basis for predicting possible toxin, drug, and/or nutritional interactions. Coupling this approach with the known co-occurrence of mycotoxins will make discovery of those interactions that pose a health risk from consumption of contaminated foods or feeds more likely. For example, fumonisins and aflatoxins are hepatotoxic, co-occur on corn, and have quite different mechanisms of action (Table 11.1). Similarly, DON, fumonisins, and ochratoxins are nephrotoxic, are common contaminants of

human foods, and have quite dissimilar mechanisms of action. Because the mechanisms are different, is there a possibility for synergy or additive risk from multiple mycotoxin exposure? Are there potential adverse interactions with infectious agents, drugs, pesticides, stress, or nutritional deficiencies that might be aggravated by exposure to specific mycotoxins or their combinations (Figure 11.1)?

Many potential mycotoxin interactions can be stud-

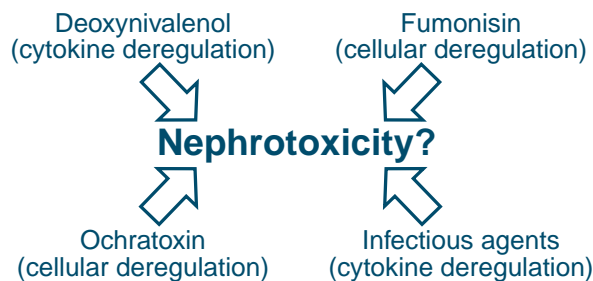


Figure 11.1. A theoretical example of mycotoxins and an infectious agent acting in concert to initiate or exacerbate renal dysfunction. Fumonisins act as a nephrotoxin through disruption of the sphingolipid metabolism (Riley et al. 1994) and alterations in cytokine signaling pathways (Dugyala et al. 1998); DON acts as a nephrotoxin through induction of an increased cytokine response (Azcona-Olivera et al. 1995a, b); ochratoxin A acts as a nephrotoxin (Creppy 1995; Marquardt and Frolich 1992); and Shiga toxin produced by *Escherichia coli* O157:H7, internalized by a fumonisin-sensitive sphingolipid receptor (Sandvig et al. 1996), initiates renal dysfunction by disruption of endothelial cell function acting through a cytokine-mediated pathway (Tesh et al. 1994).

ied. Unfortunately, resources to conduct these studies are limited. Understanding the mechanism of action can help select those interactions relevant to human, animal, and plant safety. Because many mycotoxins remain to be discovered, the actual combined health risk from mycotoxin exposure is unknown. Therefore, McLachlan (1993) proposed that chemicals be classified by their function *in vitro* as a guide for toxicological studies. This same concept, which has been used extensively in drug discovery efforts, is also appropriate for studying feeds and foods naturally contaminated with fungi and suspected of being involved in human or animal disease outbreaks. Functional “food toxicology” would screen suspected feeds and foods for mechanisms known to be under-

lying causes of chronic disease. For example, cereal grains, milling fractions, or fungal culture material could be screened for ability to inhibit sphinganine N-acyltransferase (fumonisin-like activity), inhibit IP<sub>3</sub> binding to the IP<sub>3</sub> receptor (adenophostin A-like activity), produce G→T transversion mutations at codon 249 (aflatoxin-like activity), bind to the SERCA-ATPase (cyclopiazonic acid-like activity), or bind to the cytosolic estrogen receptor (zearalenone-like activity). The number of possible biochemical targets is great and unraveling the source of the biological activity would be difficult but not impossible. Screening fungal isolates would pinpoint those biological activities of fungal origin. The possibility of nonfungal sources of the toxic principal would also need to be assessed.

# 12 Research and Policy Needs

## Introduction

Tremendous progress has been made over the last ten years in understanding the factors influencing mycotoxin production and in detecting and diagnosing mycotoxins. However, mycotoxins continue to present a threat to food safety. Changes in agricultural production practices and food processing, along with global changes in environmental and public policy, challenge us to develop and refine strategies and technologies to ensure safe food and a healthy environment. Increasing globalization of trade also adds a new dimension to the importance of mycotoxins not only as toxins, but also as impediments to free trade among countries.

An important effect of mycotoxins is the tremendous increase in mycotoxin-related litigation during the past five to seven years. Although some of the lawsuits are frivolous, many are not, and there has been a marked increase in insurance claims and litigation due to mycotoxins.

In Chapters 5 and 6 of this report, the authors addressed the need for an increased ability to diagnose mycotoxicoses in humans and animals. Because of the lack of funding by a number of agencies for animal studies involving mycotoxins, especially long-range, low-toxin-concentration studies in large domesticated animals, there is a void in information necessary to make positive diagnoses for many of the mycotoxins that occur in animal feed. Furthermore, because of the delayed recognition by the medical community of the importance of mycotoxins, there is an even greater lack of information of human intoxications caused by mycotoxins.

Presently, considerable information is needed on the association of molds and mycotoxins involved with indoor air quality and human health. In the case of stachybotryotoxicosis, there is an assumed toxic entity involved in human disease that is associated with loci contaminated with the organism *Stachybotrys chartarum*. However, the toxic entity, if that is the cause of disease, has not been identified. Even know-

ing the identity of several toxic components of this organism, this information is still lacking.

Because mycotoxins are unavoidable, they have become one of the leading liability perils in our society involving both the feed and food industry. Therefore, management of the mycotoxin-contaminated matrices is important, and adequate testing is the key to any mycotoxin management program.

Finally, mycotoxins now must be considered as possible bioterrorism agents. Concern for such use dictates increased understanding about the biosynthesis of these toxins, and how they can be rapidly detected and diagnosed.

Listed below are areas of research and public policy that need to be addressed to provide a safer food and feed supply in the twenty-first century.

## Critical Needs

### Public Policy

1. Develop uniform standards and regulations for mycotoxin contamination.
2. Support joint international cooperation (FAO/WHO/UNEP) to adopt standardized regulations.
3. Develop a safe food supply for local populations.

### Mycotoxin Detection

1. Develop new technologies for mycotoxin analysis, including multiple-toxin analyses, and improve detection (with specificity) of mycotoxins in prepared foods.
2. Develop biomarkers for human and animal exposure to mycotoxins, including multipanel arrays that can detect exposure to multiple toxins.

### Human and Animal Interactions

1. Assess mycotoxins as virulence factors.

2. Research the effect of mycotoxins as immunosuppressors.
3. Evaluate toxicological interactions of toxins with the host (activation and detoxification of mycotoxins by host metabolism).
4. Examine population variation for sensitivity to mycotoxins
5. Assess interactions among mycotoxins and with drugs, diet, and nutrition.
6. Assess role of fumonisins on humans and their involvement in esophageal cancer.
7. Assess risks of ochratoxin exposure due to its occurrence in a variety of foods and, perhaps, environmental loci.

#### Plant and Fungus Interactions

1. Establish a better understanding of the factors affecting mycotoxin formation in the field and in storage.
2. Improve understanding of the ecology and epidemiology of mycotoxin-producing fungi.
3. Develop sound agronomic-management practices to decrease mycotoxin contamination.
4. Develop host-plant resistance to mycotoxin-pro-

ducing fungi and to mycotoxin occurrence.

5. Develop models to better forecast the potential of mycotoxin contamination.
6. Research the genetic regulation and biosynthesis of mycotoxins by the producing organisms.

#### Indoor Air Quality

1. Determine mycotoxins responsible for indoor air-quality problems.
2. Develop sound sampling protocols for assessing fungal populations.
3. Establish limits for respiratory exposure to mycotoxins.

#### Economics of Mycotoxin Contamination

1. Develop accurate loss estimates for mycotoxin contamination.

#### Bioterrorism

1. Assess potential for use of mycotoxins as bioterrorism agents.
2. Assess mycotoxin-producing fungi as bioterrorism-agent candidates.

# Appendix A: Abbreviations, Acronyms, and Symbols

AACC	American Association of Cereal Chemists	g	gram
ADI/TDI	accepted daily intake/tolerable daily intake	GAC	granulated activated carbon
ALARA	as low as reasonably achievable	GAP	good agricultural practice
AMS	Agricultural Marketing Service	GC	gas chromatography
AOACI	Association of Official Analytic Chemists, International	GC-MS	gas chromatography coupled to mass spectrometry
AOCS	American Oil Chemists Society	GI	gastrointestinal
AP/AT	atmospheric pressure, ambient temperature	GIPSA	Grain Inspection Packers and Stockyard Administration
AQA	analytical quality assurance		
ARS	Agricultural Research Service	GMP	good manufacturing practice
ATA	alimentary toxic aleukia	HACCP	hazard analysis and critical control point
$a_w$	water activity	HBsAg	hepatitis B surface antigen
BCR	Bureau Communautaire de Référence (Community Bureau of Reference) (no longer exists)	HBV	hepatitis B virus
Bt	<i>Bacillus thurengiensis</i>	HCl	hydrochloric acid
bu	bushel	HP/HT	high pressure, high temperature
bw	body weight	HPLC	high-performance liquid chromatography
Ca	calcium	HSCAS	hydrated sodium calcium aluminosilicate
CAST	Council for Agricultural Science and Technology	IARC	International Agency for Research on Cancer
CCA	Composite carbon adsorbent	IgA	immunoglobulin A
CE	capillary electrophoresis	IgG	immunoglobulin G
CEN	European Committee for Standardization	IgM	immunoglobulin M
CNS	central nervous system	IR	infrared
Con A	concanavalin A	ISO	International Organisation for Standardisation
CPA	cyclopiazonic acid	IUPAC	International Union for Pure and Applied Chemistry
CRM	certified reference material	JECFA	Joint FAO/WHO Expert Committee on Food Additives
CV	coefficient of variation	K	potassium
DAS	diacetoxiscirpenol	kg	kilogram
DNA	deoxyribonucleic acid	L	liter
DNase	deoxyribonucleic acid enzyme	LC	liquid chromatography
DON	deoxynivalenol	LPS	lipopolysaccharide
ELEM	equine leukoencephalomalacia	LSD	lysegeic acid diethylamide
ELISA	enzyme-linked immunosorbent assay	MAFF	Ministry of Agriculture, Fisheries and Food (U.K.)
EPA	U.S. Environmental Protection Agency	mg	milligram
EU	European Union	$\mu\text{g}$	microgram
FAO	United Nations Food and Agriculture Organization	MPa	megapascal
FAPAS <sup>®</sup>	Food Analysis Performance Assessment Scheme	mRNA	messenger ribonucleic acid
FCR	food conversion rate	NaNO <sub>2</sub>	sodium nitrite
FDA	U.S. Food and Drug Administration	ng	nanogram
FDCA	U.S. Food, Drug and Cosmetic Act	NOAEL	no-observed-adverse-effect level
FTIR	Fourier transform infrared	O <sup>2-</sup>	superoxide anion
		O <sub>3</sub>	ozone

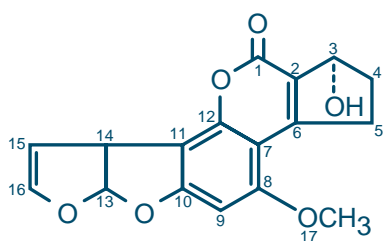
ppb	part per billion		the European Commission)
ppm	part per million	SPE	solid-phase extraction
PMTDI	provisional maximum tolerable daily intake	SRBC	sheep red blood cell
PMTWI	provisional maximum tolerable weekly intake	TDI	tolerable daily intake
QA	quality assurance	TLC	thin-layer chromatography
QC	quality control	USDA	U.S. Department of Agriculture
RNA	ribonucleic acid	UV	ultraviolet light
RNase	ribonucleic acid enzyme	UV/VIS	ultraviolet visible light
RM	reference material	VA	analytical steps
SAM	selectively adsorbed mycotoxins	VCM	vertical cutter mixer
SA/SO	sphinganine/sphingosine	VSP	sample preparation
SD	standard deviation	VT	total variance
SMT	Standards Measurements and Testing (Program of	WHO	World Health Organization

## Appendix B: Glossary

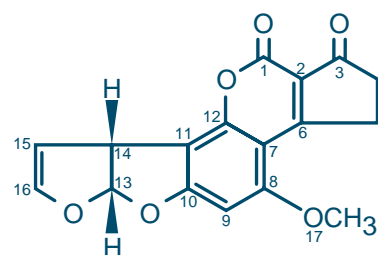
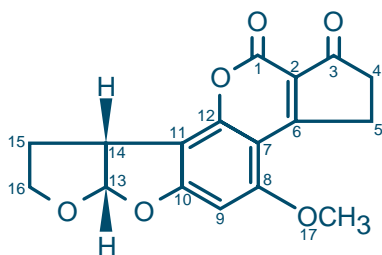
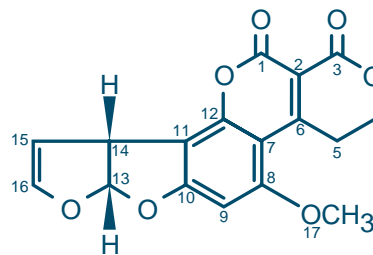
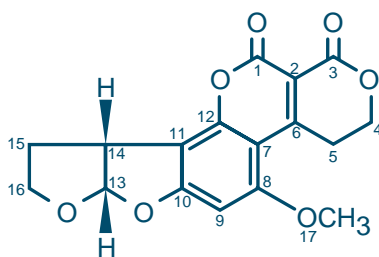
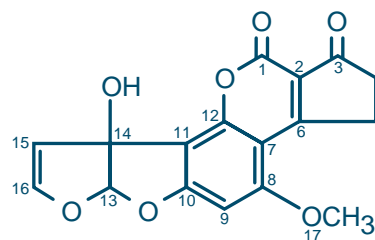
- Aflatoxins.** Polyketides derived from acetate as a starter unit.
- Akakabi-byo.** Red-mold disease of cereal grains caused by trichothecenes. (See also kokuten-byo.)
- Antibiosis.** An association between microorganisms in which one is adversely affected; a type of amensalism.
- Ascospore.** A sexual (meiotic) spore; one of usually eight haploid spores characteristically formed inside a specialized cell in fungi of the Ascomycotina.
- Buyer's risk.** The type of mistake when "bad" commodity lots (i.e., mycotoxin concentration greater than the guidelines) test as good and are accepted by an inspection program; also called a "false negative." (See also seller's risk.)
- Conidium(a) (conidiospore).** An asexual spore produced by many fungi, especially ascomycetes, often borne on specialized erect hyphae.
- Dry milling.** A dry process that separates grain components into various particle sizes, resulting in fractions such as grits, germ, meal, and flour. (See also wet milling.)
- Early splits.** A condition in pistachios where the hull splits before the nut is mature.
- Ergot.** A hardened mass of fungus tissue; the sclerotium of *Claviceps* spp.
- Fescue foot.** The gangrenous loss of extremities in cattle that occurs during colder periods.
- Fescue toxicosis.** Ergot-like poisoning on tall fescue pastures.
- Flag leaf.** The innermost leaf surrounding an immature inflorescence.
- Head blight.** Wheat and barley head scab.
- Honeydew.** A sweet liquid suspension of asexual spores transferred by insects from floret to floret.
- Inoculum.** Fungal propagules that initiate disease in plants or animals.
- Kokuten-byo.** Black-spot disease of cereal grains, caused by trichothecenes. (See also akakabi-byo.)
- Kwashiorkor.** A disease linked to consumption of aflatoxin-containing food. Attributes include hypoalbuminemia, fatty liver, and immunosuppression.
- LD<sub>50</sub>.** The single dose of a substance required to cause 50% mortality in a population.
- Macroconidia.** Long, multicelled spores produced by fungi.
- Microconidia.** One-celled, asexual spores.
- Mycotoxigenesis.** A disease resulting from exposure to a fungal toxin.
- Mycotoxin.** A compound produced by a fungus that is toxic to humans and other animals.
- Nixtamalization.** The process used to make masa flour from corn.
- Pericarp.** The wall of a fruit, derived from the maturing ovary wall.
- Perithecia.** Fruiting bodies containing the sexual spores of fungi.
- Phylloerythin.** A breakdown product of chlorophyll.
- Replacement parasite.** A parasite that replaces the developing plant structures with fungal structures, e.g., ergots or sclerotia.
- Reye's syndrome.** A disease linked to consumption of aflatoxin-containing food. Attributes include acute encephalopathy with fatty degeneration of viscera.
- Saprophyte.** A plant, fungus, or bacterium that obtains its nutrition from dead organic materials.
- Sclerotium.** The resting body of certain fungi, which can survive long periods of adverse conditions to produce either a mycelium or fruiting bodies and serve as important sources of inoculum.
- Secondary metabolite/metabolism.** Compounds not essential for the growth of the producing organism in culture, but may be important in the ecology of the organism in nature.
- Seller's risk.** The type of mistake when "good" commodity lots (i.e., mycotoxin concentration less than or equal to the guidelines) test as "bad" and are rejected by an inspection program; also called a "false positive." (See also buyer's risk.)
- Shoshin kakke.** Acute cardiac beriberi caused by the fungal extract citreoviridin.
- Staggers.** Disease of cattle caused by tremorgenic mycotoxins. Clinical signs include muscle tremor, uncoordinated movements, general weakness in the hind legs, and stiff, stilted movements of the forelegs.
- St. Anthony's fire.** Description of ergotism in the Middle Ages in humans whose early symptoms are swollen limbs with burning sensations.
- Stroma.** Proliferating fungal structure; a solid mass of plectenchyma (a form of "tissue" commonly found among the higher fungi composed of a mass of interwoven hyphae) that may bear perithecia on its surface or embedded within the tissue.
- Tombstones.** Chalky white seeds present in head scab or blight; premature bleaching caused by *Fusarium* infection.
- Water activity (a<sub>w</sub>).** The ratio of the vapor pressure of the product to that of pure water.
- Wet milling.** (See also dry milling.) A wet process that separates grain components into its various constituents such as starch, gluten feed, and gluten meal.

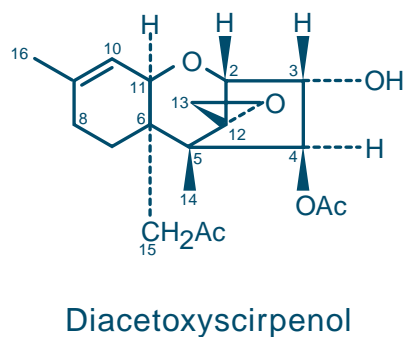
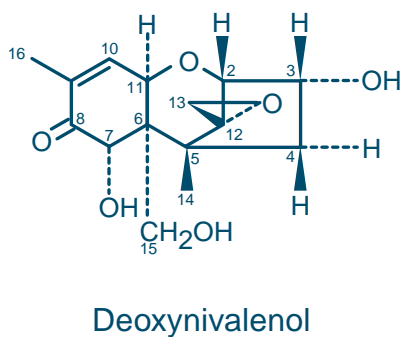
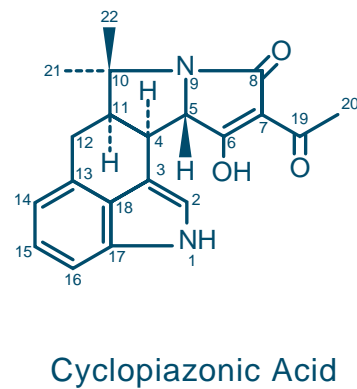
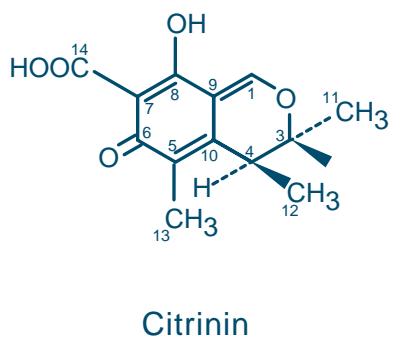
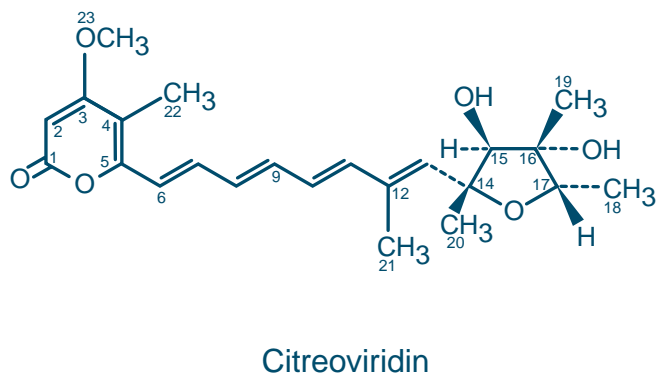
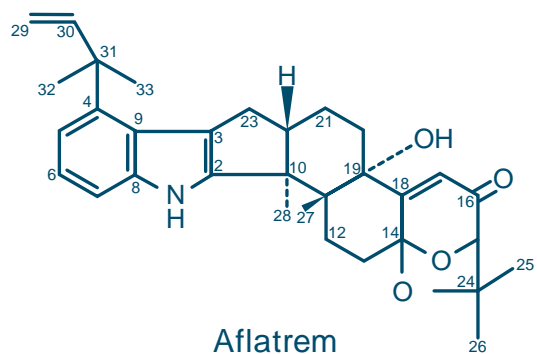


## Appendix C: Chemical Structures of Selected Mycotoxins

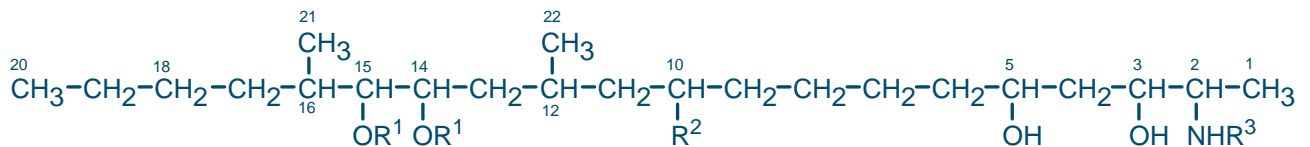
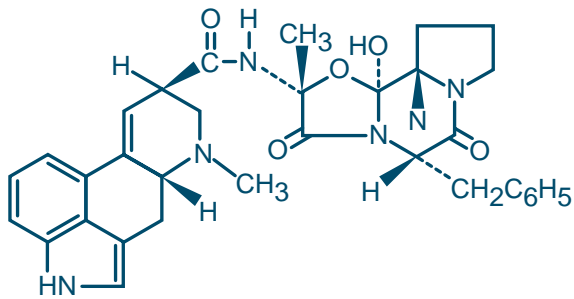


Aflatoxicol

Aflatoxin B<sub>1</sub>Aflatoxin B<sub>2</sub>Aflatoxin G<sub>1</sub>Aflatoxin G<sub>2</sub>Aflatoxin M<sub>1</sub>



Ergotamine



Fumonisin

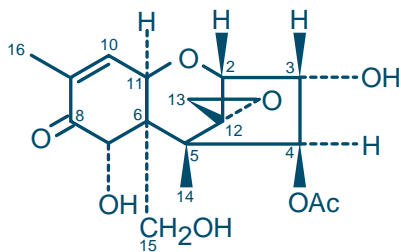
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A<sub>2</sub>: R<sup>1</sup> = COCH<sub>2</sub>CH(CO<sub>2</sub>H)CH<sub>2</sub>CO<sub>2</sub>H; R<sup>2</sup> = H; R<sup>3</sup> = OH; R<sup>4</sup> = COCH<sub>3</sub>

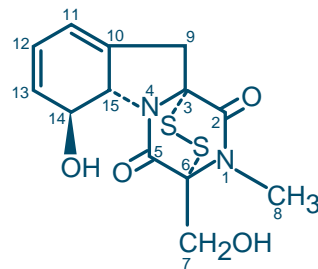
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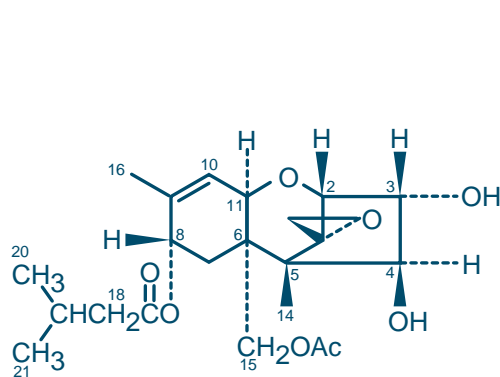
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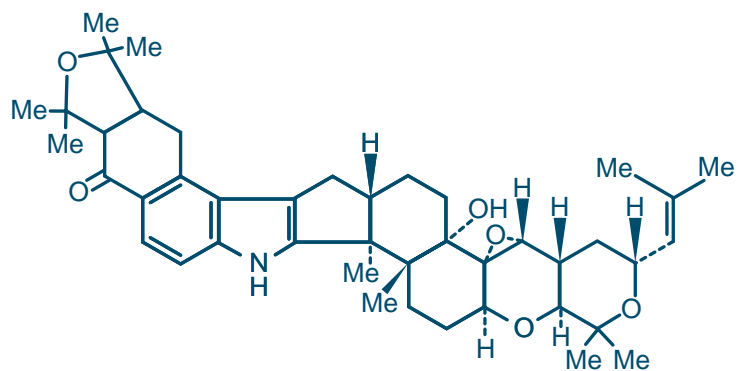
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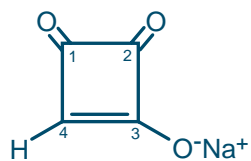
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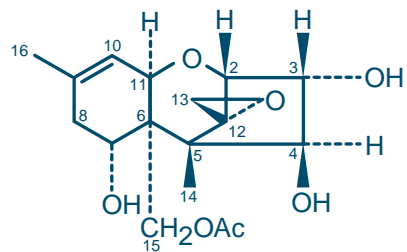
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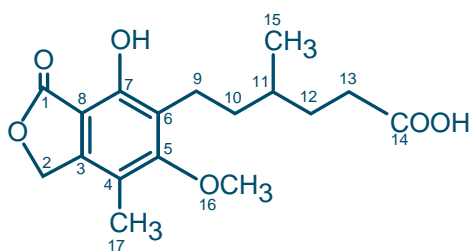
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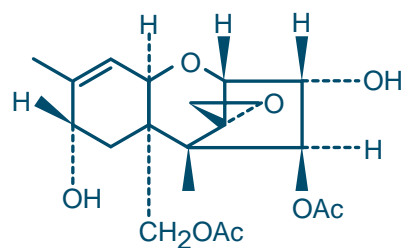
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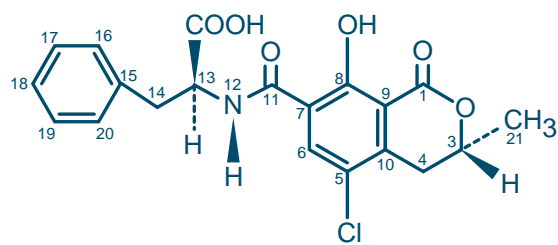
Monoacetoxyscirpenol



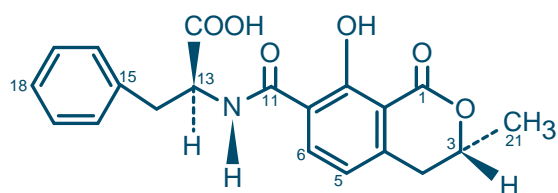
Mycophenolic Acid



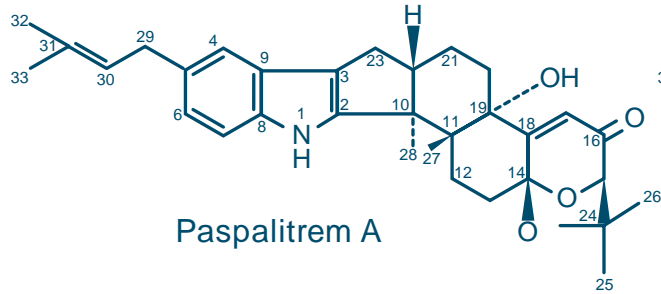
Neosolanol



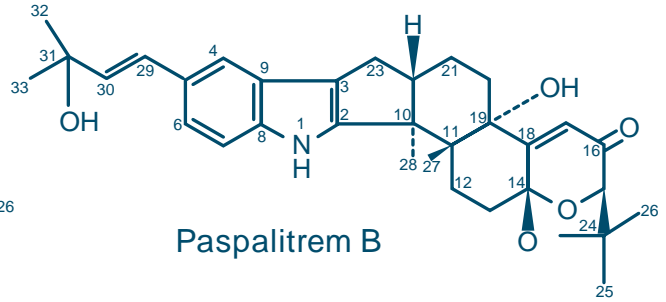
Ochratoxin A



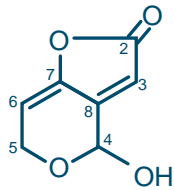
Ochratoxin B



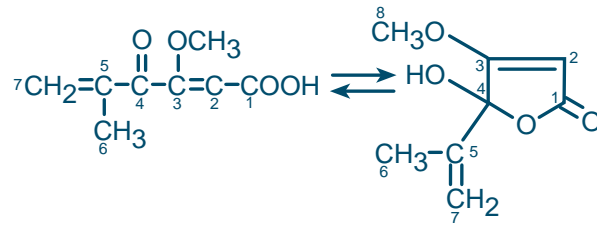
Paspalitrems A



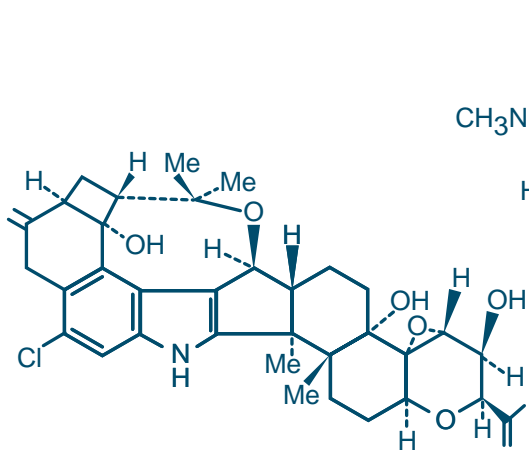
Paspalitrems B



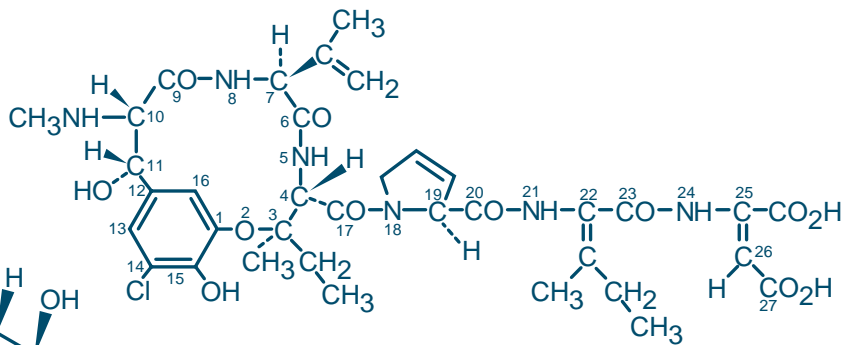
Patulin



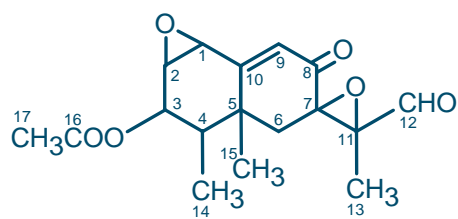
Penicillic Acid



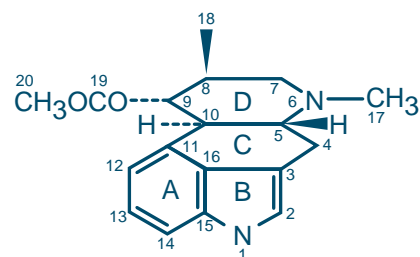
Penitrem A



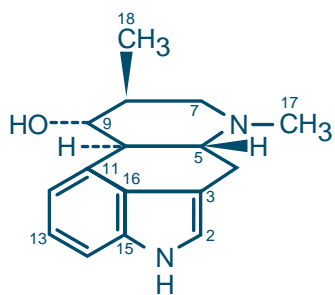
Phomopsis A



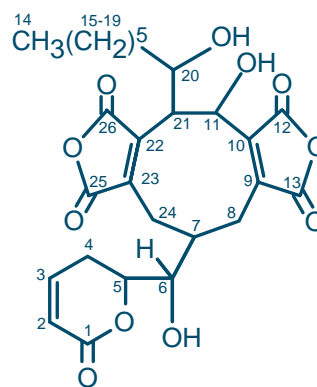
PR-Toxin



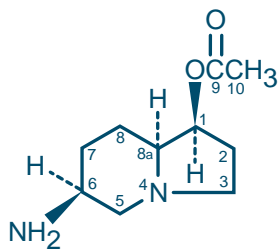
Roquefortine A



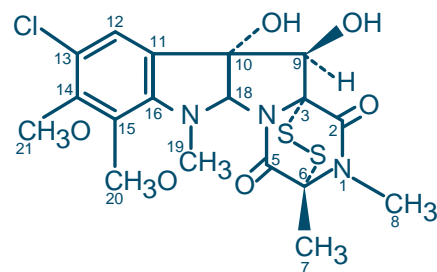
Roquefortine B



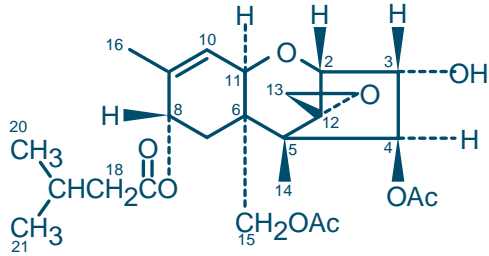
Rubratoxin B



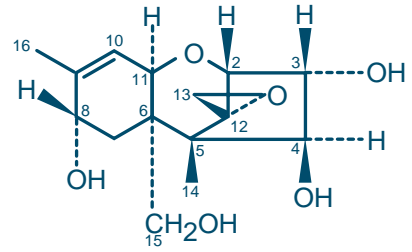
Slaframine



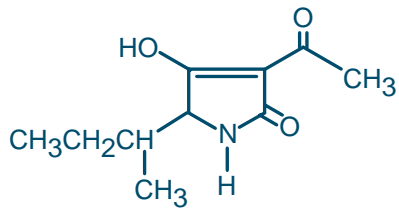
Sporidesmin



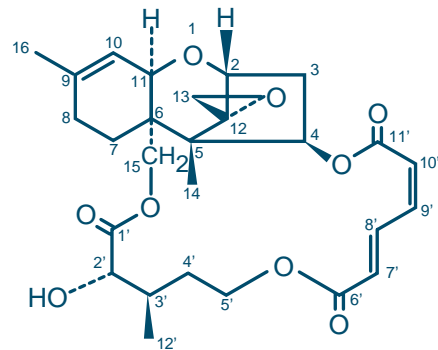
T-2



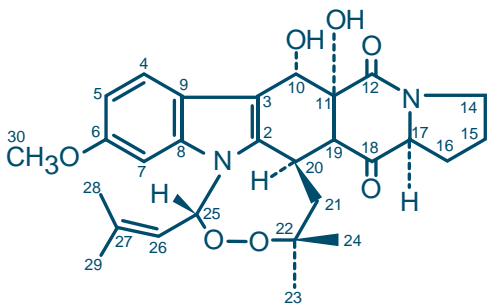
T-2 Tetraol



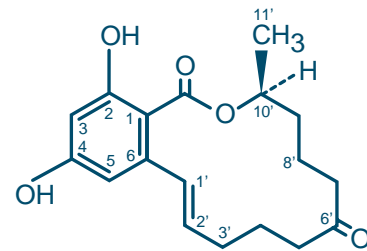
Tenuazonic Acid



Verrucarin A



Verruculogen



Zearaleone

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