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# IMPROVING PUBLIC HEALTH THROUGH MYCOTOXIN CONTROL

EDITED BY JOHN I. PITT, CHRISTOPHER P. WILD, ROBERT A. BAAN, WENTZEL C.A. GELDERBLOM, J. DAVID MILLER, RONALD T. RILEY, AND FELICIA WU

International Agency for Research on Cancer
World Health Organization

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# Table of contents

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# Dedication: Professor Wally Marasas

Walter Friedrich Otto Marasas was born on 25 October 1941 in Boksburg, South Africa. After obtaining his B.Sc. and M.Sc. from the University of Pretoria, he received a Ph.D. in Plant Pathology from the University of Wisconsin, USA, in 1969. Dr Marasas returned to South Africa with the Plant Protection Research Institute and, together with Dr Fanie Kellerman of the Onderstepoort Veterinary Research Institute, investigated equine leukoencephalomalacia, a disease that causes brain lesions in horses. Mouldy maize was a suspected cause, and Wally identified *Fusarium verticillioides* (then known as *F. moniliforme*) as the culprit. This launched a decades-long examination of this fungus and its impact on animal and human health.

Wally moved to the South African Medical Research Council (MRC) in 1975 and remained there until his retirement in 2006, when he was the Unit Director of the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC Unit). Throughout this period, he made major inroads into understanding the health effects of mycotoxins, based on his expertise in fungal taxonomy, specifically of *Fusarium* species and the toxic effects of their mycotoxins. Notably, he investigated the high incidences of oesophageal cancer in the former Transkei region of Eastern Cape Province, at first in relation to the occurrence of *F. verticillioides* and later in relation to fumonisins, which Wally's team characterized from *F. verticillioides* (strain MRC 826) brought back from the Transkei. He published more than 300 scientific articles, and the Institute for Scientific Information in 2002 rated him as one of the most cited researchers worldwide in two categories.

Photograph courtesy of South African Medical Research Council.

Professor Marasas enjoyed wide international recognition and was admitted to membership of the prestigious National Academy of Sciences of the USA. Among his many awards were the Gold Medal from the South African Society for Plant Pathology, the Wellcome Gold Medal from Wellcome Pharmaceuticals, the African Academy of Sciences/CIBA Prize for Agricultural Biosciences, the MRC Silver Medal for Excellence, the MT Steyn Gold Medal from the South African Academy for Science and Arts, and the distinguished service award from Kansas State University. Professor Marasas was only the second South African to be elected a Fellow of the American Phytopathological Society. He held several visiting academic positions, at Pennsylvania State University, USA; the University of Sydney, Australia; and Kansas State University, USA. He was also appointed as honorary professor at the University of the Orange Free State and as extraordinary professor at the University of Pretoria, South Africa.

Aside from his own academic achievements, Wally was a great encourager and a scientific enthusiast who inspired others. He supervised theses by more than 60 postgraduate students and was a warm and generous collaborator with many more colleagues across the world. His friendship combined with his scientific excellence and strong motivation for public health distinguished him as a remarkable colleague.

# Executive summary

Mycotoxins are fungal toxins that contaminate staple foods consumed by many of the poorest and most vulnerable populations in the world. These toxins have the potential to contribute to a diversity of adverse health effects in humans, even at low concentrations. Given the ubiquitous nature of exposure in many countries, an urgent need exists for a coordinated international response to the problem of mycotoxin contamination of food. The topic has been marginalized for far too long. One priority is development of a better understanding of the consequences of consuming large quantities of toxin-contaminated food daily, as is often the case in subsistence farming areas in developing countries. A second priority is development of effective, accessible, and practical intervention measures to prevent or minimize exposure in the populations at the greatest risk.

The knowledge and methodologies exist to control mycotoxins in food. However, these are currently only applied with any rigour in some high-income countries with well-established analytical methods to screen for mycotoxins and with strong regulatory controls. In low-income countries, the situation is often in marked contrast: subsistence or small-scale farmers produce foods for local consumption that may be heavily contaminated with mycotoxins; these foods are untested; and regulatory controls either do not exist or are not enforced.

In low-income countries, the main consequence of mycotoxin awareness may be the commercial sale of the best quality food and local consumption of the worst. Grains and other foods for export must comply with quality requirements and sanitary regulations, which are established by the target markets. Contaminated commodities may also be exported from high-income countries to those where regulations either are not in place or are ineffective or unenforced. Because of a lack of resources for an efficient control system, these contaminated imports can be diverted to the poorest sectors of society. One man's meat is truly another man's poison. As a result, for example, some populations are exposed daily to aflatoxins at levels well above those legally permitted in other countries; these are toxins that the international health community has recognized for two decades as being human carcinogens.

In low-income countries, exposure to mycotoxins at high levels is often closely associated with inequality and poverty. Therefore, affordable and feasible solutions should be a part of the international development agenda. Much can be done; much should be done. Failure to act in a timely manner could pose a grave risk to consumers in low-income countries where basic commodities like wheat, maize, and groundnuts are potentially contaminated with harmful levels of mycotoxins and are consumed in large quantities.

This book from the International Agency for Research on Cancer, the specialized cancer agency of the World Health Organization, aims to sensitize the international community to the mycotoxin problem in a format that is accessible to a wide audience and is useful to decision-makers across a broad spectrum of disciplines, including agriculture, public health, marketing, and economics. The editors hope that this book will be a stimulus to governments, nongovernmental and international organizations, and the private sector to initiate measures designed to minimize mycotoxin exposure in high-risk populations. The book not only provides a scientific description of the occurrence and effects of mycotoxins but also goes further by outlining approaches to reduce mycotoxin exposure aimed at improving public health in low-income countries. This executive summary briefly outlines the book's content and provides some context to its preparation.

## Mycotoxin occurrence

Mycotoxins are secondary fungal metabolites that contaminate many of the most frequently consumed foods and feeds worldwide. Therefore, human and animal exposure to one or more of this broad group of toxins is widespread. The types of fungi – and therefore mycotoxins – found in different regions depend on climatic conditions. The main mycotoxins of significance for human disease are aflatoxins, fumonisins, ochratoxin A, deoxynivalenol, and zearalenone. These toxins are produced by just a few species of fungi from the common genera *Aspergillus, Penicillium*, and *Fusarium*. These fungi may either grow on the crop or invade the crop after harvest and produce toxins during drying and storage.

Wheat is the most important cereal for human consumption, as the staple food for nearly half of the world's population, followed by rice and maize. With some exceptions (e.g. parts of West Africa), consumption of the remaining staple crops, including sorghum and millet, is minor by comparison. Natural contamination of the three major crops by mycotoxins can be a serious food safety problem in some regions, but the crops differ in susceptibility to the various fungi that produce toxins. The incidence of mycotoxins also depends on a wide variety of agronomic and climatic conditions as well as on whether a particular cultivar is grown within the area to which it is adapted.

Of the *Aspergillus* species occurring in warmer climates, *A. flavus* produces aflatoxins in maize, groundnuts, tree nuts, and, less frequently, other commodities, whereas *A. parasiticus* is common in groundnuts. Fumonisins are produced mainly by *Fusarium verticillioides*, a fungus that is ubiquitous in maize. Ochratoxin A is produced mainly by *A. carbonarius,* which is commonly found in grapes, dried vine fruits, wine, and coffee, and by *A. ochraceus* and related species, which produce this toxin in coffee and sometimes in stored grains. *Penicillium verrucosum* also produces ochratoxin A but occurs only in cool temperate climates, where it infects small grains. *F. graminearum* is the major producer of deoxynivalenol and zearalenone in maize, wheat, oats, and barley and produces these toxins whenever it infects these grains before harvest. Indeed, wheat can be highly affected by deoxynivalenol in southern China and parts of South America as well as in cooler, damper climates in Europe and Canada. Maize is at considerable risk for contamination by aflatoxin, fumonisin, deoxynivalenol, and zearalenone, sometimes all four at once. Significant contamination of maize with aflatoxins and fumonisins occurs in the tropical and subtropical growing areas of the world. Rice is not infected before harvest by any of the major toxigenic fungi and seldom, if ever, contains significant mycotoxins immediately after harvest, drying, and hulling. However, rice can be contaminated by aflatoxins if post-harvest storage systems are inadequate.

## Mycotoxin measurement

Investigating the health and economic impacts of mycotoxins requires measuring their occurrence. However, accurately assessing the level of one or more mycotoxins in a crop or food commodity is a major challenge because the mycotoxins are heterogeneously distributed, which makes representative sampling extremely difficult. Therefore, considerable care and planning are needed in sampling and sample preparation to ensure the accuracy of the estimated levels of contamination. In any investigation of mycotoxin levels in relation to health effects or for regulatory purposes, careful attention must be paid to the need for accurate estimates of contamination. Representative sampling can pose particular problems in the context of small-scale farmers or subsistence farming communities, where only

smaller sample sizes can be obtained, and thus sampling approaches must be adapted accordingly.

Once an appropriate sample has been prepared, an analytical procedure must be selected to measure the mycotoxin(s). Many modern methods require highly sophisticated technology and skilled technicians. Although these techniques are applicable in settings of developed economies handling bulk commodities, a need still exists for methods that are applicable to smaller-scale farms, where resources are limited and rapid decisions are needed about the contamination of foods to be consumed locally. Validated thin-layer chromatography and immunoassays are existing technologies capable of meeting this challenge. Indeed, one of the outstanding requirements in the management of mycotoxins is the production of reliable and easily applicable tests for use in low-income countries. Commercial companies and development agencies should consider production of appropriate testing kits, sampling equipment (e.g. grinders), and training models specifically tailored for use in these regions.

## Effects in food-producing animals

Animals suffer a variety of adverse effects from consuming mycotoxin-contaminated feeds, and these in turn may pose problems to those communities that rely on the animals for food. Several indices can be used to associate a disease in animals with a particular mycotoxin through awareness of the outward adverse signs typical of exposure to a given mycotoxin. As well as the direct effect in animals, mycotoxicoses in domestic animals can be a warning sign of a risk of high-level contamination of foods for human consumption.

Aflatoxins can cause acute toxicity in many species, most notably evident in poisoning outbreaks in poultry. In addition, chronic exposure leads, among other effects, to reduced weight gain, decreased egg production, impaired immunity, and altered susceptibility to infectious agents. Fumonisins were first identified through the occurrence of equine leukoencephalomalacia and, later, the occurrence of porcine pulmonary oedema. The main target organ of ochratoxin A is the kidney, and nephropathy is accompanied by renal dysfunction and oedema; this is most common in pigs but has also been reported in horses. Ochratoxicosis in poultry is associated with feed refusal and high mortality. Deoxynivalenol can provoke feed refusal, e.g. in cattle, pigs, and chickens, and pigs are particularly sensitive; high intakes are also associated with emesis. Zearalenone causes a variety of reproductive problems as a consequence of estrogenism.

In relation to cancer, aflatoxins are hepatocarcinogenic in several different species, and fumonisins have been shown to induce kidney tumours in mice and liver tumours in rats. Ochratoxin A is also carcinogenic in rats and mice, inducing kidney carcinoma in rats and both liver and kidney tumours in mice. In contrast, there is little evidence of carcinogenicity of deoxynivalenol. Zearalenone treatment increased incidences of liver cell and pituitary tumours in mice, consistent with a hormonal mode of carcinogenic action; no carcinogenic effect was seen in rats.

## Effects in humans

Despite the fact that the potential adverse effects of mycotoxins on human health have been recognized for centuries, much remains to be defined. For aflatoxins, a strong causal association with liver cancer has been identified, with a particularly elevated risk in people chronically infected with hepatitis B virus (HBV). Acute aflatoxin poisoning, termed aflatoxicosis, is also a known consequence of exposure to high dietary toxin levels. The recently identified association between aflatoxin exposure early in life and impairment of child growth is an important observation that, together with possible immunomodulatory effects, demands greater scrutiny to evaluate its importance in many vulnerable populations worldwide. If the link between aflatoxin exposure and child growth impairment is causal, this will transform the public health impact of minimizing exposure.

For fumonisins, studies indicate a possible role in oesophageal cancer and in neural tube defects, but further investigation is needed as a priority. For deoxynivalenol and other trichothecenes, exposure has been linked to acute poisoning outbreaks in large numbers of subjects. The effects of chronic exposure need to be defined, given the extent of exposure occurring worldwide. For ochratoxin A and zearalenone, the human health effects remain largely undefined and also merit greater attention. However, investigating the effects of mycotoxins on human health is challenging, not least because of the difficulties in measuring exposure, and this is also reflected in the relative paucity of epidemiological studies on the toxins mentioned above. Newly established biomarkers of exposure at the individual level are proving valuable in improving exposure assessment and should be prioritized in terms of development and validation as well as application in studies of etiology.

As with other environmental toxins, individual differences in susceptibility to mycotoxins are likely. Susceptibility may be influenced by genetic, demographic, nutritional, infectious, or immune factors. For example, individuals may differ in the way in which mycotoxins are metabolized or, alternatively, other co-exposures may be critical, as mentioned above for aflatoxin exposure and HBV chronic carriers. The consequence of these interactions is that the same mycotoxin exposure may have different effects in different populations or individuals. The influence of malnutrition on susceptibility to toxicity induced by mycotoxins has been little studied. In developing countries, most people consuming maize as a monocereal staple diet also lack sufficient levels of micronutrients and thus may have enhanced susceptibility to adverse effects of contaminating mycotoxins. Young children may be particularly susceptible, as may be the fetus. In fact, there is a dearth of data on the possible effects of mycotoxins in women and children in populations where mycotoxin exposures are highest. Increased awareness of the risks posed to vulnerable populations by mycotoxins should stimulate more research on the interactions with other risk factors and elicit caution when risk assessments are conducted.

## Risk assessment and management

The well-documented toxicity of mycotoxins in animals and humans has provided the impetus for the conduct of risk assessments on all the major mycotoxins (aflatoxins, fumonisins, ochratoxin A, deoxynivalenol, and zearalenone) by various regulatory agencies. These risk assessments have had a variety of impacts in relation to the global burden of diseases related to mycotoxins as well as regulatory measures on food supply and international trade. Overall, in line with the limited evidence from epidemiology referred to already, many uncertainties remain. The health end-points used to set regulatory levels need to be periodically reviewed, given that mycotoxins may cause a diverse set of effects. For example, with aflatoxins the risk assessments have been dominated by the association with liver cancer, but the recent studies on child growth impairment, if confirmed, may provide an important additional parameter for consideration. These adverse effects may occur at exposure levels below those that induce cancer, in which case the current regulatory levels may provide inadequate protection, even if adhered to.

Many differences are also apparent in risk management strategies and government regulations and technologies to reduce levels of mycotoxins in the food-chain. However, it is evident that regulatory measures have very little impact on remote rural and subsistence communities.

## Economics of mycotoxins

In the past, the biology and economics of mycotoxins have been considered separately, but if the mycotoxin problem is to be addressed, a rounded consideration is needed of the broader consequences of these environmental contaminants. Therefore, the economic impacts of mycotoxins should be thought of in two ways: (i) the direct market costs associated with lost trade or reduced revenues due to contaminated food or feed, and (ii) the human health losses from the adverse effects mentioned above.

Losses related to markets occur within systems in which mycotoxins are being monitored in the food and feed supply. Such monitoring systems can result in a commodity with excessively high mycotoxin levels being rejected for sale or having a lower market value, but human exposure to the toxins is thus reduced. Losses related to health occur when mycotoxins are present in food at levels that can cause illness, and such losses can be measured using disability-adjusted life years (DALYs) or quality-adjusted life years (QALYs). It is estimated that aflatoxin-related liver cancer alone may cause more than 2 million DALYs annually worldwide. The total socioeconomic burden of all mycotoxins and all associated diseases and conditions is likely to be much higher. From a market standpoint, in the USA alone, mycotoxins cost crop growers more than 1 billion US dollars annually. Similar losses are sustained by producers in three Asian countries – Thailand, the Philippines, and Indonesia – from aflatoxin alone.

An important component, which merits more research, is the economic assessment of costs of interventions to reduce mycotoxins compared with benefits, in terms of either improved markets or improved human health. The cost-effectiveness of an intervention is evaluated by comparing the cost of implementing the intervention in a given population, per DALY saved, with the per capita gross domestic product for the country in which the intervention is applied. Aside from direct economic considerations, interventions that are feasible in low-income countries should be prioritized.

## Reducing exposure to mycotoxins

Approaches to minimize mycotoxin levels in food are varied, encompassing good agricultural and manufacturing practices as well as approaches at the household or individual level. In general, interventions at the different stages include pre-harvest, post-harvest, and dietary approaches. What is needed most is the evaluation and adaptation of these approaches into cost-effective, simple, and sustainable intervention methods, predominantly at the population level, suitable for low-income countries.

One possibility to reduce mycotoxin exposure is avoiding contaminated foodstuffs by modifying the diet. However, this is dependent on having the necessary wealth and access to different foods to enable choice. It also presupposes that lack of food is not driving the need to eat contaminated food. Some examples exist where economic development has resulted in a change in diet and lower mycotoxin levels, notably in parts of China where a shift from maize to rice consumption has lowered aflatoxin intake. This option is not an obvious one for other populations to follow but does demonstrate both the detrimental effects of overreliance on a potentially contaminated crop and the positive effects of resources flowing to low-income countries, permitting a greater dietary diversity. Nevertheless, any such changes are likely to be slow in coming.

The possibility has been explored of using dietary interventions that either reduce mycotoxin bioavailability or modulate metabolism in ways that reduce the harmful effects of ingested toxins. For example, some enterosorbent clays and micronutrients have been added to the diet to adsorb aflatoxins in the gastrointestinal tract, thus reducing absorption. An alternative approach, also for aflatoxins, has involved the use of chemopreventive agents (e.g. chlorophyllin) that increase the detoxification of reactive metabolites in the body. Although these approaches can be used to target exposed individuals, their application as a broader, sustainable public health intervention is unclear.

A wide range of agricultural strategies, both pre-harvest and post-harvest, exist that may reduce the quantity of aflatoxin in food. Genetic modification of crops to reduce insect damage or enhance fungal resistance offers a promising opportunity for some toxins. Biocontrol by inoculating fields with non-toxigenic fungi is another approach, already in use in some high-income countries. Post-harvest interventions include the removal of infected and/or insect-damaged food components by hand sorting, preferably combined with correct drying and storage conditions, which avoid fungal proliferation and toxin production. Significant reductions in exposure can be achieved by simply removing and discarding visibly mouldy maize or groundnuts, for example. In addition, under some circumstances the food commodity can be processed to reduce mycotoxin levels; an example is chemical deactivation via nixtamalization. Each of these agricultural and individual approaches is more or less accessible to different populations, depending upon available resources and cultural acceptance of the practice.

## Food security versus food sufficiency

Human exposure to mycotoxins is determined by the level of contamination in a given food commodity and the quantity of that commodity consumed. Thus, in some areas of the world mycotoxin levels are relatively high but exposure is modest because of a varied diet. In other areas, a similar level of contamination may translate into a much higher exposure because the diet is more uniform. This is why communities relying on dietary staples such as wheat, groundnuts, or maize, which are frequently contaminated by mycotoxins, are particularly vulnerable. These communities tend to be in regions where national regulations on mycotoxins either do not exist or are seldom implemented.

While facing mycotoxin contamination and high levels of exposure, these same regions often face challenges of adequate food production and preservation combined with a generally poor nutritional status of the population. Food availability may have a strong seasonality, which threatens the adequacy of nutrition at the household and national level. Importation of food may be constrained by currency problems, among other things. This is the context in which many countries have to face the challenge of mycotoxins as they balance the adequacy of the food supply with the safety of food.

One key problem is the general lack of awareness about the presence and health effects of mycotoxin contamination of food, compared with the immediacy of not having enough to eat. As a consequence, in many cases food security simply translates to having sufficient food for everybody throughout the year, as viewed from both household and government perspectives. This constant concern about food sufficiency is itself, therefore, a barrier to addressing the issue of mycotoxins as a component of food security. At the household and community level, misconceptions about food security result from a lack of information and nutritional

education. At the government level, the information may also be lacking, but the overriding focus is on food supply, whereas the problem of food security is a matter that awaits economic development.

At an international level, the responsibility to safeguard the health of a specific population from mycotoxins is placed on the importing governments because of the way mycotoxin regulations are applied. This leads to heterogeneity as a result of economic factors, international trade agreements, and other factors, such as the protection of certain agricultural commodities by government subsidies. The result of these varying pressures is that mycotoxin regulation varies among countries, from strict implementation based on the latest risk assessments through to a total lack of implementation and control. Therefore, the various influences on regulatory decisions in a specific country are often played out against a background of tension between the health authorities and the political and economic powers controlling international trade and industry.

Despite the political, structural, and economic challenges to mycotoxin control outlined here, the means and the motivation for change already exist. Subsistence and small-scale farmers are familiar with problems of the quality of seed varieties, pests (e.g. insects, rodents), and storage management. The understanding that fungi damage crops exists and solutions are welcomed, even though an understanding that fungi produce mycotoxins and that these can have adverse health effects remains uncommon.

A limited awareness of the danger of mycotoxins in foodstuffs is also apparent among the local traders who, in some regions of the world, may purchase from local growers and sell to customers or larger businesses. In fact, regulations applied by importing developed countries to crop exports from developing countries are often the point where governments and large traders are brought face to face with the mycotoxin problem.

Overall, therefore, it is important, through education, to improve awareness of the problems of mycotoxin contamination of foods and to promote the potential solutions within different sectors of society, including householders, farmers, and traders as well as governments and nongovernmental and international organizations.

## Responses to the mycotoxin problem – seven priorities

This executive summary provides a flavour of how this book addresses the major issues and of the complexity of the mycotoxin problem in low-income countries. However, mycotoxins are one problem embedded within many other challenges to health, resulting from poverty and inequality.
Therefore, mycotoxins can only really be addressed by taking into account the local situation with respect to food sufficiency, economic pressures, competing health problems, and the social environment. In this context, we propose the following seven priority areas for mycotoxin control worldwide.

1. Education leading to improved awareness of mycotoxins across different sectors of society, including householders, farmers, traders, governments, and nongovernmental and international organizations.
2. Better description of the prevalence and level of mycotoxin exposure in the regions of the world most affected, as a basis for prioritizing control and estimating risk.
3. Development of accurate and applicable mycotoxin testing kits, sampling equipment, and training models for use in low-income countries, to permit rapid assessment of mycotoxin contamination of staple foods.
4. Conduct of epidemiological studies to assess the acute and chronic health effects of mycotoxin exposure; priorities include the impact of mycotoxins on child growth and immune status.
5. Identification of individuals and groups who are particularly susceptible to mycotoxins so that the risk assessment can be focused on those at greatest risk for adverse health effects.
6. Development and evaluation of cost-effective, simple, and sustainable intervention methods suited to low-income countries.
7. Detailed economic assessments of interventions, in terms of either improved markets or improved human health.

CHAPTER 1.

# Fungi producing significant mycotoxins


## Summary

Mycotoxins are secondary metabolites of microfungi that are known to cause sickness or death in humans or animals. Although many such toxic metabolites are known, it is generally agreed that only a few are significant in causing disease: aflatoxins, fumonisins, ochratoxin A, deoxynivalenol, zearalenone, and ergot alkaloids. These toxins are produced by just a few species from the common genera *Aspergillus*, *Penicillium*, *Fusarium*, and *Claviceps*. All *Aspergillus* and *Penicillium* species either are commensals, growing in crops without obvious signs of pathogenicity, or invade crops after harvest and produce toxins during drying and storage. In contrast, the important *Fusarium* and *Claviceps* species infect crops before harvest. The most important *Aspergillus* species, occurring in warmer climates, are *A. flavus* and *A. parasiticus*, which produce aflatoxins in maize, groundnuts, tree nuts, and, less frequently, other commodities. The main ochratoxin A producers, *A. ochraceus* and *A. carbonarius*, commonly occur in grapes, dried vine fruits, wine, and coffee. *Penicillium verrucosum* also produces ochratoxin A but occurs only in cool temperate climates, where it infects small grains. *F. verticillioides* is ubiquitous in maize, with an endophytic nature, and produces fumonisins, which are generally more prevalent when crops are under drought stress or suffer excessive insect damage. It has recently been shown that *Aspergillus niger* also produces fumonisins, and several commodities may be affected. *F. graminearum*, which is the major producer of deoxynivalenol and zearalenone, is pathogenic on maize, wheat, and barley and produces these toxins whenever it infects these grains before harvest. Also included is a short section on *Claviceps purpurea*, which produces sclerotia among the seeds in grasses, including wheat, barley, and triticale. The main thrust of the chapter contains information on the identification of these fungi and their morphological characteristics, as well as factors influencing their growth and the various susceptible commodities that are contaminated. Finally, decision trees are included to assist the user in making informed choices about the likely mycotoxins present in the various crops.

## 1. Introduction

Mycotoxins have been defined as "fungal metabolites which when ingested, inhaled or absorbed through the skin cause lowered performance, sickness or death in man or animals, including birds" (Pitt, 1996). This definition is widely accepted, although interpretation of "animals" is still under discussion in some quarters. The definition as it stands may be taken as including lower (invertebrate) animals; as it seems likely that some mycotoxins are indeed aimed at insects, such as the mites that prey on fungi, this interpretation is reasonable. However, most mycotoxicologists consider that mycotoxins are of relevance only when they affect humans and domestic animals, and this much narrower definition is used here.

It has been known for a long time that it is hazardous to eat some species of the larger fungi, i.e. mushrooms and "toadstools", but until comparatively recently the occurrence of common moulds on foods has generally been considered an aesthetic problem, not a health hazard. The realization that metabolites of some common foodborne fungi were responsible for animal disease and death came only in the 1960s, despite a few excellent studies in the first half of the 20th century. It is now well established that mycotoxins have been responsible for major epidemics in humans and animals during recent historical times. The most important epidemics have been ergotism, which has killed hundreds of thousands of people in Europe during the last millennium (Smith and Moss, 1985); alimentary toxic aleukia (ATA), which was responsible for the deaths of at least 100 000 people in the USSR between 1942 and 1948 (Joffe, 1978); stachybotryotoxicosis, which killed tens of thousands of horses in the USSR in the 1930s (Moreau, 1979); and aflatoxicosis, which killed 100 000 young turkeys in the United Kingdom in 1960 and has caused disease and death in many other animals, including humans (Rodricks *et al*., 1977; Lubulwa and Davis, 1994).

By general consent, the name "mycotoxin" is usually restricted to toxic compounds produced by microfungi and excludes toxins formed by the Basidiomycetes, the mushrooms or macrofungi consumed as foods in many parts of the world.

Many thousands of metabolites have been described from microfungi; even the large number dealt with in extensive reviews (Cole and Schweigert, 2003a, 2003b; Cole *et al*., 2003) are only a fraction of those known. It seems likely that many of these metabolites are produced not randomly but in attempts to alter the ecology surrounding the fungus, by inhibiting growth of competitor microorganisms, insects, etc. Only a limited number of compounds, a few hundred, are known to be toxic to humans or domestic animals.

## 2. Mycotoxigenic fungi

### *2.1 Which genera are important?*

If we look at the worldwide occurrence of fungi in foods, and at which might be capable of mycotoxin production, three genera stand out: *Aspergillus*, *Penicillium*, and *Fusarium* (Pitt and Hocking, 2009). *Fusarium* species are destructive pathogens on cereal crops and other commodities, and produce mycotoxins before, or immediately after, harvest. Certain species of *Aspergillus* and *Penicillium* are also plant pathogens or commensals, but these genera are more commonly associated with commodities and foods during drying and storage. *Aspergillus flavus* is an exception: it can be a pathogen, a commensal (growing in a plant without affecting it), or a storage fungus, and it produces mycotoxins under all three conditions.

While not of worldwide significance, a fourth genus is of sufficient importance to be included here. *Claviceps* is pathogenic on a wide variety of cereals and other crops, producing resting structures called ergots, which often contain toxins.

### *2.2 Which mycotoxins are important?*

As noted above, several hundred compounds are known to be toxic to humans or animals. However, many of these cause little concern because they are produced by fungi that are rarely encountered in foods or feeds. Many species of *Penicillium*, for example, are found almost exclusively in soils and rarely, if ever, in foods or feeds. Therefore, many highly toxic compounds produced by *Penicillium* species have not been found in foods or feeds in appreciable quantities. These include verruculogen, produced by *P. simplicissimum* and related species; janthitrems, produced by *P. janthinellum*; rugulosins, produced by *P. rugulosum* and *P. variabile*; and many others.

In a second category are compounds that are demonstrably toxic under some test conditions, e.g. by injection, but are not toxic when taken by a natural route. Compounds of this type may be inactivated by stomach acids or are so insoluble as to be excreted without harm. Sterigmatocystin, produced by the quite common storage fungus *Aspergillus versicolor*, is so insoluble in water, or acid, that its true toxicity to mammals has been difficult to measure, and it has not been known to cause illness.

In a third category, of potentially greater human health significance, are compounds that are produced by fungi known to occur in foods but that

under normal conditions are present in such low concentrations that they present no real hazard, i.e. their effects, if any, are not measurable. In most cases the reason is that although the fungi are readily isolated from some types of foods, they do not normally grow to the extent required to produce hazardous levels of toxin. Many examples exist: cyclopiazonic acid, from *Aspergillus flavus*; citrinin, from *Penicillium citrinin* and several other species; citreoviridin, produced by *P. citreonigrum* and *Eupenicillium ochrosalmoneum*; roquefortine, produced by *P. roqueforti* and related species; penitrem A, from *P. crustosum*; many of the trichothecenes produced by various *Fusarium* species; and tenuazonic acid, from *Alternaria* species. Under favourable growth conditions, however, the fungi in this category are capable of extensive growth and significant toxin production, so these and some other toxins should be kept in mind when fungal spoilage of foods and feeds occurs.

Some toxins are produced by rare species. For example, the species that produces rubratoxins is known from only a few isolates and does not even have a recognized name. Rubratoxin A is known to have caused one disease outbreak, in two people who consumed mouldy home-made rhubarb wine (Richer *et al*., 1997). However, rubratoxin can be ignored when overviewing mycotoxin occurrence worldwide.

A few mycotoxins are considered to be significant in feeds but not foods. These are of known toxicity to birds, in particular, and are mainly water-soluble toxins. The reason appears simple: whereas mammals excrete water-soluble toxins, often with little ill effect, birds excrete only solid waste, so are unable to get rid of these toxins so readily. In this category are cyclopiazonic acid, citrinin, and tenuazonic acid.

Some other mycotoxins are important in limited areas of the world. Sporidesmin is a mycotoxin that causes facial eczema in sheep. It is produced in pasture by the fungus *Pithomyces chartarum* in some areas of New Zealand and Australia, and can cause large economic losses in local areas. The fungus *Phomopsis leptostromiformis* produces the mycotoxin phomopsin in lupin plants and seeds in Western Australia, and phomopsin is of great importance to the cattle raising and lupin seed export industry in that state. However, its global impact is minimal.

Patulin is sometimes included in lists of important mycotoxins, and concentrations in foods are subject to regulatory control in some countries. Patulin is produced by the growth of *Penicillium expansum* in apples and pears. The production of significant levels of patulin is accompanied by visible rotting of the fruit, so patulin is primarily of concern in juices. Nearly all the toxin can be removed if rotting fruit are rejected by visual inspection or rotten parts are removed by hand trimming or by washing them out with high-pressure water jets. Hence, patulin in apple juices and other products is controllable by simple food technological procedures, and its occurrence does not warrant consideration here.

The mycotoxins treated in detail in this book are based on those considered by Miller (1995) to be the most important on a worldwide basis: aflatoxins, ochratoxin A, fumonisins, specific trichothecenes (deoxynivalenol and nivalenol), and zearalenone. These toxins are produced in foods and feeds by species of *Aspergillus*, *Penicillium*, and *Fusarium*. A limited taxonomic treatment of these fungi and the species producing important mycotoxins is given in this chapter. Also included is *Claviceps purpurea*, the species that produces ergot and ergot toxins in small grains.

## 3. Taxonomic overview of the fungal genera producing important mycotoxins

The genera *Aspergillus*, *Fusarium*, and *Penicillium* all reproduce by asexually produced spores, known as conidia, which are formed from specialized cells called phialides, where mitosis takes place and from which conidia are generated rapidly and in great numbers. Some species in each genus also produce a sexual stage defined by the production of asci, specialized cells that result from meiosis, usually in well-defined macroscopic bodies (up to 1 mm in diameter) called cleistothecia (Kirk *et al*., 2001). A few species produce hard resting cells called sclerotia, essentially immature cleistothecia.

In *Aspergillus* and *Penicillium*, phialides are borne in clusters, while conidia are single-celled, more or less spherical, and very small, usually not exceeding 5 µm in diameter. The two genera are closely related and are distinguished by the way in which phialides are grouped. In *Aspergillus*, phialides are always borne in tight clusters around the swollen apices (vesicles) of long stalks (stipes), with or without an intermediate row of supporting cells called metulae (Raper and Fennell, 1965; Pitt and Hocking, 2009; Samson *et al*., 2010); see Figs 1.1–1.5. In *Penicillium*, phialides are usually borne in finger-like clusters on more diminutive stipes, again with or without one or two intermediate rows of supporting cells (metulae and rami) (Pitt, 1979; Pitt and Hocking, 2009); see Fig. 1.6. Colonies of *Penicillium* species grown on identification media in Petri dishes are usually green, the colour of *Penicillium* conidia, and often have other pigments from the mycelium or excreted from the colonies. In *Aspergillus*, colony colours are those of the conidia, which may be black, yellow, brown, white, or green.

Colonies of most *Aspergillus* species show no other colours. *Fusarium* colonies generally consist of loose, fluffy mycelium, coloured white, pink, or purple, and often show similar colours in the colony reverse. In *Fusarium*, phialides are not clustered, and conidia may be of two types: those characteristic of the genus are large and crescent-shaped (although sometimes formed only under natural conditions or on special media), whereas the second type, produced by only some species, are small and usually cylindrical (Marasas *et al*., 1984; Pitt and Hocking, 2009; Samson *et al.* 2010); see Figs 1.7–1.9.

The taxonomy of all three genera is complex. Overall taxonomies for mycotoxigenic fungi occurring in foods are given in *Fungi and Food Spoilage* (Pitt and Hocking, 2009) and *Food and Indoor Fungi* (Samson *et al*., 2010). The most useful introductions to the major species in each genus are found in laboratory guides: *A Laboratory Guide to Common* Penicillium *Species* (Pitt, 2000), *Identification of Common* Aspergillus *Species* (Klich, 2002), and *The* Fusarium *Laboratory Manual* (Leslie and Summerell, 2006).

*Claviceps* differs from the three genera mentioned above because most species cannot be cultivated in the laboratory. Species of *Claviceps* grow on a wide variety of grasses, where they infect only the ovaries, forming hard bodies called sclerotia that replace normal seed heads. Conidia are released in droplets called honeydew, attractive to insects, which then disseminate the fungus throughout the crop.

The taxonomy (classification) of these genera is described in more detail below.

## 4. Genus *Aspergillus*

*Aspergillus* is a large genus, with 100 or more recognized species, most of which grow and sporulate well on common synthetic or semisynthetic media. The most widely used taxonomy is by Raper and Fennell (1965), although some of their concepts are out of date. For more modern taxonomic concepts, see Samson and Pitt (1990) or Klich (2002). A minority of *Aspergillus* species make a sexual stage (known as a teleomorph) in which spores (ascospores) are borne in asci, in turn borne in cleistothecia. Species with teleomorphs are correctly classified in teleomorph genera, of which *Eurotium*, *Neosartorya*, and *Emericella* are the best known. Few of the important mycotoxigenic species produce teleomorphs.

The most significant mycotoxigenic species in *Aspergillus* are *A. flavus* and *A. parasiticus*, which make aflatoxins, and the species that make ochratoxin A: *A. ochraceus* and related species, the black species *A. carbonarius*, and (uncommonly) *A. niger. A. flavus* and *A. parasiticus* are very closely related and are treated together.

### *4.1 Fungi producing aflatoxins:* A. flavus *and* A. parasiticus

Taxonomy. Aflatoxins are now known to be produced by at least 10 *Aspergillus* species. However, most are rare or are rarely found in foods: the principal fungi producing aflatoxins remain *A. flavus* and *A. parasiticus*. Of some importance is a new species found in groundnuts in the southern hemisphere, called *A. minisclerotigenes*. This species looks like a variant of *A. flavus* that produces unusually small sclerotia, but like *A. parasiticus* it produces both B and G aflatoxins. The other species of some importance is *A. nomius*, which also makes B and G aflatoxins and looks like *A. flavus* but produces bullet-shaped sclerotia. *A. nomius* is associated with insects, and not usually with foods, but recently has been shown to be a common cause of aflatoxin production in Brazil nuts (Olsen *et al*., 2008).

Enumeration. Satisfactory enumeration of *A. flavus* and *A. parasiticus* can be achieved on any antibacterial enumeration medium that contains appropriate inhibitors to reduce colony spreading. Dichloran rose bengal chloramphenicol agar (DRBC) or dichloran 18% glycerol agar (DG18) are recommended (Pitt and Hocking, 1997, 2009; Samson *et al*., 2010). Relatively rapidly growing, moderately deep, yellow green colonies exhibiting "mop-like" fruiting structures under the stereomicroscope can be presumptively counted as *A. flavus* plus *A. parasiticus*. Microscopic examination of colonies can provide supporting evidence, but representative colonies must be grown on standard identification media for confirmation.

A more effective medium for enumerating these species is Aspergillus flavus and parasiticus agar (AFPA; Pitt *et al*., 1983), a medium formulated specifically for this purpose. AFPA has two major advantages: enumeration can be carried out after incubation at 30 °C for 2 days, and both species are readily recognized, even by untrained eyes, by intense orange yellow colours in the reverses of the colonies. Under the incubation conditions specified for AFPA (30 °C for 42–48 hours), the presence of the bright orange yellow reverse is diagnostic for these species.

Descriptions. The descriptions of *Aspergillus* species given here are taken from *Fungi and Food Spoilage* (Pitt and Hocking, 2009). The fungi are grown as colonies on Czapek yeast extract agar (CYA) and malt extract agar (MEA) at 25 °C and CYA at 37 °C for 7 days. Fungi are inoculated onto these plates at three

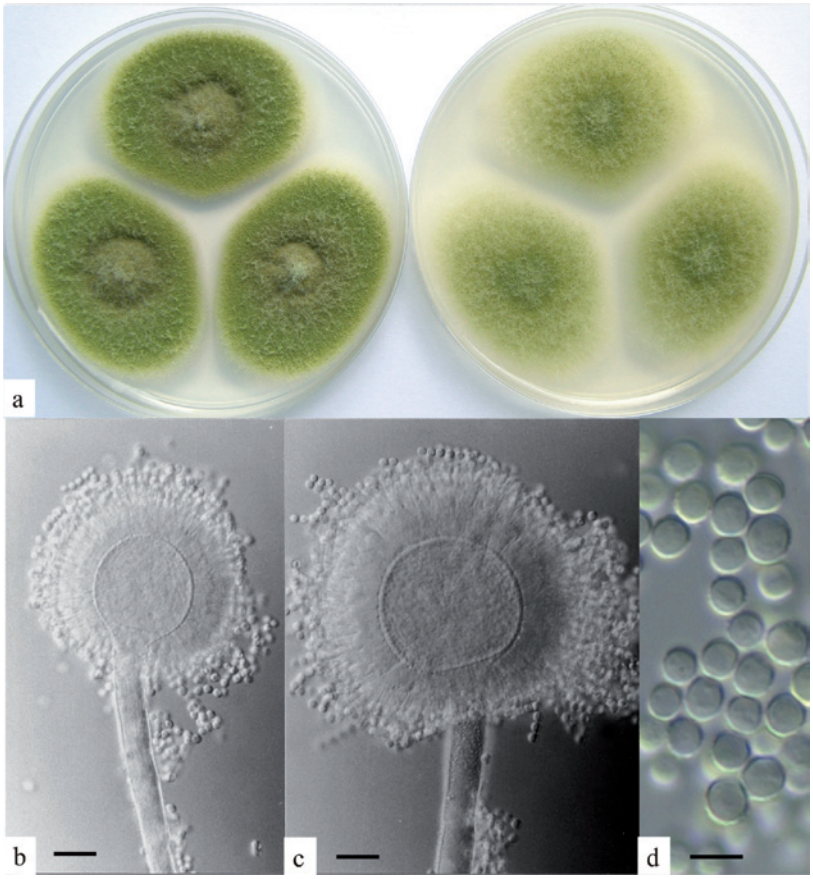
points, equidistant from each other and midway between the rim and centre of the Petri dish. Inoculation is facilitated by first dispersing a needle point of conidia in small vials containing 0.2 mL of 0.2% agar and 0.05% Tween 80 or similar detergent (Pitt and Hocking, 2009), as this reduces colonies from stray spores. Formulations for these and other media are given in the Annex (p. 29).

*Aspergillus flavus* Link. See Fig. 1.1. Colonies on CYA 60–70 mm in diameter; conidial heads usually borne uniformly over the whole colony but sparse or absent in areas of floccose (cotton wool) growth or sclerotial production, characteristically coloured greyish green but sometimes pure yellow, becoming greenish in age; sclerotia produced by about 50% of isolates, at first white, becoming deep reddish brown, density varying from inconspicuous to dominating colony appearance and almost entirely suppressing conidial production. Colonies on MEA 50–70 mm in diameter, similar to those on CYA, although usually less dense. At 37 °C, colonies usually 55–65 mm in diameter, similar to those on CYA at 25 °C.

Sclerotia spherical, usually 400–800 µm in diameter. Teleomorph developing from sclerotia, but only after selected isolates are mated. Structures bearing conidia 400 µm to 1 mm or more long; vesicles (terminal swellings) spherical, 20–45 µm in diameter, fertile over three quarters of the surface, typically bearing both phialides and metulae (cells supporting phialides), but in some isolates a proportion of, or even most, heads bear phialides alone; conidia spherical or nearly, usually 3.5–5.0 µm in diameter, with relatively thin walls, finely roughened or, rarely, smooth.

The teleomorph is *Petromyces flavus* (Horn *et al*., 2011), but in culture is seen only after suitable strains are mated (Horn *et al*., 2009b).

**Fig. 1.1.** *Aspergillus flavus* (a) colonies on CYA (left) and MEA (right), 7 days, 25 °C; (b, c) heads, bars = 20 µm; (d) conidia, bar = 5 µm. Source: Pitt and Hocking (2009), Fig. 8.13, p. 305; reproduced with kind permission from Springer Science+Business Media B.V.



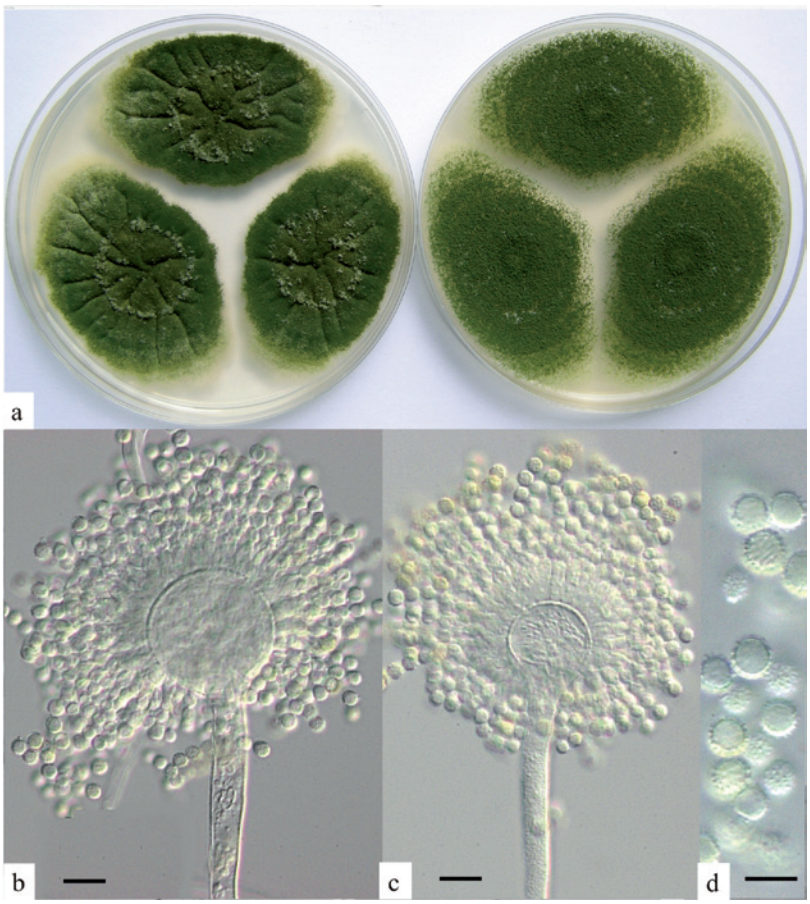

*Aspergillus parasiticus* Speare. See Fig. 1.2. Colonies on CYA 50–70 mm in diameter, conidial heads in a uniform, dense layer, coloured dark yellowish green; sclerotia occasionally produced. Colonies on MEA 50–65 mm in diameter, generally similar to those on CYA. At 37 °C, colonies covering the available area, similar to those on CYA at 25 °C.

Sclerotia occasionally produced, white at first, becoming black, spherical, 400–800 µm in diameter. Teleomorph developing from sclerotia, but only after selected isolates are mated. Structures bearing conidia 250–500 µm long; vesicles spherical, 20–35 µm in diameter, fertile over three quarters of the surface, mostly bearing phialides only, but in some isolates up to 20% of heads bearing metulae as well; conidia spherical, mostly 4.0–6.0 µm in diameter, with distinctly roughened walls.

The teleomorph is *Petromyces flavus* (Horn *et al*., 2011), but in culture is seen only after suitable strains are mated (Horn *et al*., 2009a).

Distinctive features. *Aspergillus flavus* and *A. parasiticus* together are distinguished by their rapid growth at both 25 °C and 37 °C and their bright yellow green (or, less commonly, yellow) conidial colour. The definitive difference between the two species is that *A. flavus* produces conidia that are rather variable in shape and size,

**Fig. 1.2.** *Aspergillus parasiticus* (a) colonies on CYA (left) and MEA (right), 7 days, 25 °C; (b, c) heads, bars = 10 µm; (d) conidia, bar = 5 µm. Source: Pitt and Hocking (2009), Fig. 8.18, p. 321; reproduced with kind permission from Springer Science+Business Media B.V.




have relatively thin walls, and range from smooth to moderately rough, the majority being finely rough. In contrast, conidia of *A. parasiticus* are spherical and have relatively thick, rough walls. In addition, vesicles of *A. flavus* are larger, up to 50 µm in diameter, and usually bear metulae, whereas vesicles of *A. parasiticus* rarely exceed 30 µm in diameter and metulae are uncommon (Klich and Pitt, 1988). The teleomorphs are not seen in pure culture of single isolates.

Differentiating toxigenic from non-toxigenic isolates of *A. flavus* or *A. parasiticus* can also be of value. A variety of media have been proposed to achieve this, one of which is coconut cream agar (Dyer and McCammon, 1994). Recently, the use of a cyclodextrin, incorporated into any standard medium, has been proposed (Jaimez Ordaz *et al.*, 2003). Visualization of aflatoxin production is by examination of the reverse of colonies on Petri dishes under ultraviolet (UV) light.

Factors influencing growth. Growth temperatures for *A. flavus* most often reported are a minimum of 10–12 °C, a maximum of 43–48 °C, and an optimum of about 33 °C (Pitt and Hocking, 2009). The minimum water activity ($a_w$) permitting growth is 0.82 at 25 °C, 0.81 at 30 °C, and 0.80 at 37 °C (Pitt and Miscamble, 1995). A predictive model for *A. flavus* growth in relation to $a_w$ and temperature was derived from those data (Gibson *et al.*, 1994). Growth of *A. flavus* occurred over the pH range 2.1–11.2 (the entire range examined) at 25, 30, and 37 °C, with optimal growth over a broad pH range of 3.4–10 (Wheeler *et al.*, 1991).

The heat resistance of *A. flavus* has been studied under various conditions by several authors. The most reliable figures indicate a $D_{45}$ value (the time required at 45 °C to kill 90% of the population) of more than 160 hours, a $D_{50}$ of 16 hours, a $D_{52}$ of 40–45 minutes, and a $D_{60}$ of 1 minute, at neutral pH and high $a_w$, with z values (the increase in temperature required to reduce the D value by 90%) of 3.3–4.1 C° (ICMSF, 1996).

The addition of phosphine, used to control insects, to grain at 0.80 or 0.86 $a_w$ reduced growth of *A. flavus* while having little effect on the survival of conidia (Hocking and Banks, 1991, 1993).

Available data indicate that the influence of physical factors on the growth of *A. parasiticus* is very similar to that on *A. flavus* (Pitt and Hocking, 2009). However, *A. parasiticus* grows at somewhat lower temperatures, up to 42 °C. The effect of $a_w$ is similar to that found for *A. flavus* (Pitt and Miscamble, 1995).

### *4.1.1 Commodities and foods at risk from aflatoxin contamination*

*Aspergillus flavus* and to a lesser extent *A. parasiticus* have been isolated from a very wide range of food commodities (Pitt and Hocking, 2009). Indeed *A. flavus* may be regarded as truly ubiquitous in foods produced in tropical and subtropical countries. Although evidence of *A. flavus* at low levels in foods cannot be taken as an indicator of the presence of aflatoxins, high levels of infection, i.e. plate counts of greater than $10^5$/g, or infection levels of more than

50% of grains when direct plated, provide reasonable presumptive evidence that aflatoxin may be present. However, quantification of the association between levels of *A. flavus* infection and aflatoxin production is not possible, so these figures can only serve as guidelines.

Although it is possible to induce aflatoxin production in a very wide range of foods or raw materials under experimental conditions, research and experience have shown that only certain commodity types are likely to contain aflatoxin in the absence of obvious signs of fungal growth or other deterioration in appearance.

Based on the results of many surveys, commodities most at risk in international trade are groundnuts, maize, and cottonseed. Lesser, but still substantial, risk is associated with tree nuts of all types, especially Brazil nuts, pistachio nuts, and semi-processed coconuts (i.e. copra). Walnuts, hazelnuts, and cashews are only occasionally affected. Spices from tropical countries are also a frequent source of aflatoxin, but these spices are usually present at only low levels in foods. Oilseeds of all kinds are affected from time to time (Pohland and Wood, 1987), and figs may carry a substantial risk (Le Bars, 1990).

Aflatoxins have been reported from smoked and dried or cured fish in Sierra Leone (Jonsyn and Lahai, 1992) but are not considered to be a problem in salted dried fish produced under South-East Asian conditions (Pitt and Hocking, 1996). Other meat products, including prepared hams (Rojas *et al*., 1991), are not considered to be at risk.

Cereals, legumes, and pulses may also be infected with *A. flavus* (Pitt and Hocking, 2009), but unacceptable levels of aflatoxin occur only under poor storage conditions and are rarely of concern.

*Aspergillus flavus* is capable of causing spoilage of some kinds of fresh fruit and vegetables, including citrus, tomatoes, peppers, litchis, pineapples, and pomegranates (Snowdon, 1990, 1991), but aflatoxin production is unlikely.

### *4.1.2 Formation of aflatoxins in susceptible crops*

Groundnuts. Groundnuts are susceptible to infection by both *A. flavus* and *A. parasiticus* (Hesseltine *et al*., 1973; Diener *et al*., 1987; Pitt and Hocking, 2009). The primary source of these fungi is soil, where high numbers may build up because some groundnuts are not harvested but remain in the ground and act as a nutrient source (Griffin and Garren, 1976a). Uncultivated soils contain very low numbers of *A. flavus*, but soils in groundnut fields usually contain 100–1000 propagules/g (Pitt, 1989). Under drought stress conditions, this number may rise to $10^4$–$10^5$/g (Horn *et al*., 1995). Large numbers of *A. flavus* spores are also airborne over susceptible crops (Holtmeyer and Wallin, 1981).

Direct entry to developing groundnuts through the shell by *A. flavus* in the soil appears to be the main method for nut infection (Diener *et al*., 1987). Infection can also occur through the pegs and flowers (Wells and Kreutzer, 1972; Griffin and Garren, 1976b; Pitt, 1989). *A. flavus* sometimes grows within groundnut plants themselves: growth in plant tissue is not pathogenic but commensal. The seedpod (Lindsey, 1970) or the plant (Pitt, 1989; Pitt *et al*., 1991) show no visible sign of colonization by the fungus.

A variety of factors influence invasion of developing groundnuts by *A. flavus*. Infection before harvest occurs only if substantial numbers of fungal propagules (perhaps $10^3$/g) exist in the soil. Other important factors are drought stress (Sanders *et al*., 1981) and soil temperatures around 30 °C (Blankenship *et al*., 1984; Sanders *et al*., 1984; Cole *et al*., 1985; Cole, 1989; Dorner *et al*., 1989) during the last 30–50 days before harvest (Sanders *et al*., 1985).

Maize. Maize is usually infected only by *A. flavus*, not by *A. parasiticus* (Lillehoj *et al*., 1980; Angle *et al*., 1982; Horn *et al*., 1995). It appears probable that the most important route for entry of *A. flavus* to maize is through insect damage (Lillehoj *et al*., 1982; Bilgrami *et al*., 1992). Invasion down the silks is also possible (Marsh and Payne, 1984; Diener *et al*., 1987). High temperature stress increases infection (Jones *et al*., 1980), the critical time for infection being between 16 and 24 days after inoculation at silking (Jones *et al*., 1980; Payne, 1983).

Cottonseed. *A. flavus* is also a commensal in the cotton plant (Klich *et al*., 1984). Infection occurs through the nectaries, natural openings in the cotton stem that are important in pollination (Klich and Chmielewski, 1985), or through cotyledonary leaf scars (Klich *et al*., 1984). Upward movement occurs in the stem towards the boll, but not downwards from boll to stem (Klich *et al*., 1986). Insect damage is also a potential cause of infection (Lee *et al*., 1987), but insects are often well controlled in cotton crops. As in groundnuts and maize, temperature appears to be a major environmental factor in pre-harvest infection of cottonseed (Marsh *et al*., 1973; Simpson and Batra, 1984). High minimum temperatures, above 24 °C, appear to lead to high aflatoxin formation (Diener *et al*., 1987).

### *4.1.3 Formation of aflatoxins in other crops*

With other crops, *Aspergillus flavus* is not associated with the plant, so entry to nuts or seeds or other food parts is opportunistic and usually occurs

only after the crop matures. Entry of *A. flavus* into pistachio nuts depends on the time of splitting of hulls. Nuts in which hull splitting occurs early are much more susceptible to *A. flavus* invasion on the tree (Doster and Michailides, 1994). It is known that some cultivars are more prone to early splitting than others, and this is especially important where nuts are harvested from the ground, after contact with the soil.

Brazil nuts are harvested from the ground, beneath trees growing naturally in Amazonian forests. Harvesting is intermittent, up to a month apart, providing time for the ever present *A. flavus* and other related species to infect the nuts (Johnsson *et al*., 2008). *A. nomius* appears to be an important source of aflatoxins in Brazil nuts (Olsen *et al*., 2008).

In other tree nuts, formation of aflatoxins occurs sporadically, usually as the result of insect damage or poor storage practices.

Figs are sometimes infected by *A. flavus*. The unique structure of the fruit evolved to enable fertilization by insects, and insects may carry *A. flavus* spores into the seed cavity. Also, figs are harvested from the ground in some countries. Immature figs are not colonized by *A. flavus*, but once they are ripe infection occurs readily and fungal growth continues during drying (Buchanan *et al*., 1975; Le Bars, 1990). The proportion of the crop infected is low, 1% or less (Steiner *et al*., 1988). The problem has been serious for some exporting countries (Sharman *et al*., 1991) but is now well controlled by examination of individual figs under UV light.

### *4.1.4 Formation of aflatoxins in storage*

Crops susceptible to aflatoxin formation are mostly nuts and oilseeds, where soluble solids (sugars) in the dried commodity are low, and oil content high. Sorption isotherms of these commodities are similar (Iglesias and Chirife, 1982). *A. flavus* and *A. parasiticus* cannot grow below about 0.80 $a_w$, equivalent to about 10% moisture content in these commodities. However, storage above 8% moisture content (about 0.7 $a_w$) can lead to fungal spoilage. Fungal growth may result in a moisture increase, creating conditions under which *A. flavus* can grow, so 8% moisture must be considered the safe moisture content for these commodities.

Such a low moisture content can be difficult to maintain in practice. Shipment of nuts in containers across the tropics is a particular hazard as unsuitable stowage, on decks or near engines, can lead to moisture migration sufficient to cause sporadic spoilage or even total loss. Cases of rampant growth of *A. flavus* accompanied by high aflatoxin production have been observed under these conditions.

Storage of nuts in tropical countries is sometimes inadequate, again leading to spoilage or aflatoxin production.

## *4.2 Ochratoxin A*

### *4.2.1 Formation of ochratoxin A*

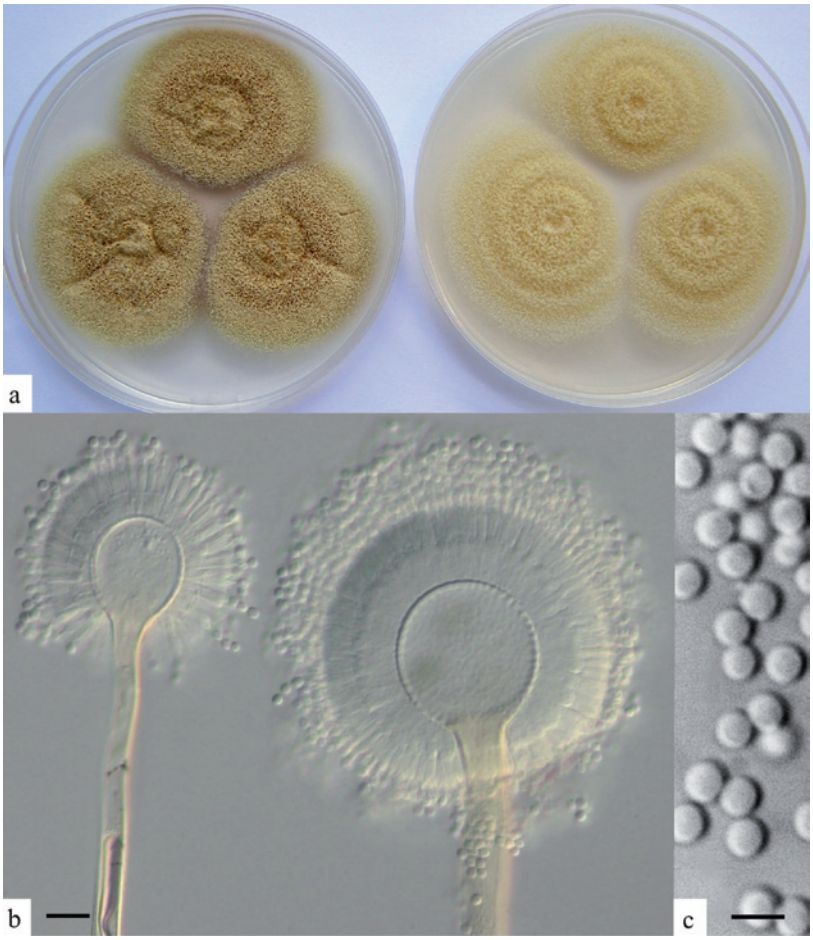
Ochratoxin A (OTA) was originally described as a metabolite of *Aspergillus ochraceus* from laboratory experiments (van der Merwe *et al*., 1965), and subsequently from several related *Aspergillus* species. However, the first report of natural occurrence, and the potential importance, of OTA was from a *Penicillium* species (Scott *et al*., 1970; Krogh *et al*., 1973), reported as *P. viridicatum*, but later corrected to *P. verrucosum* (Pitt, 1987). Larsen *et al*. (2001) reported that *P. nordicum*, closely related to *P. verrucosum*, is also a producer of OTA. Many reports refer to OTA production by other, often unspecified, *Penicillium* species, but these reports are known to be erroneous (Frisvad 1989; Frisvad and Filtenborg, 1989; Pitt and Hocking, 2009).

Recently, *Aspergillus carbonarius* has been identified as a third major source of OTA, together with a low percentage of isolates of the closely related species *A. niger* (Abarca *et al*., 1994; Téren *et al*., 1996). It is now clear that OTA is produced by two closely related *Penicillium* species, *P. verrucosum* and *P. nordicum*, and by a rather remarkable range of *Aspergillus* species. The following sections deal with these species in more detail.

### *4.2.2* Aspergillus ochraceus *and related species*

Taxonomy. Recent work has shown that *Aspergillus ochraceus* is not a common producer of OTA. Most OTA in foods originally attributed to *A. ochraceus* is now known to be due to *A. westerdijkiae* and *A. steynii*, newly described species very similar morphologically to *A. ochraceus* (Frisvad *et al*., 2004). Apart from these species, this group of OTA producers includes two ascosporic fungi, *Neopetromyces muricatus* (asexual state *A. muricatus*) and *Petromyces alliaceus* (asexual state *A. alliaceus*), and two that do not produce a teleomorph, *A. sclerotiorum* and *A. sulphureus*. *N. muricatus* is the correct name for isolates that produce OTA, previously identified as *A. melleus*. Both *N. muricatus* and *P. alliaceus* are uncommon species. *A. sclerotiorum* isolates produce OTA only rarely, and although isolates of *A. sulphureus* are usually OTA producers, this is a rare species. Apart from *A. westerdijkiae* and *A. steynii*, all of these species are very uncommon in foods and are not known to cause food spoilage.

**Fig. 1.3.** *Aspergillus ochraceus* (a) colonies on CYA (left) and MEA (right), 7 days, 25 °C; (b) heads, bar = 20 µm; (c) conidia, bar = 5 µm. Source: Pitt and Hocking (2009), Fig. 8.17, p. 318; reproduced with kind permission from Springer Science+Business Media B.V.




Enumeration. *Aspergillus ochraceus*, *A. westerdijkiae*, and *A. steynii* grow slowly on media of high $a_w$ as they are all xerophilic. Enumeration on a medium of reduced $a_w$, such as DG18, is recommended (Pitt and Hocking, 2009).

Colonies of *A. ochraceus* and closely related species can be presumptively recognized by relatively deep colonies, uniformly coloured pale brown to yellow brown, that under the low-power stereomicroscope exhibit long fruiting stalks bearing radiate *Aspergillus* heads, with spore chains splitting into two or three dense columns in age. Confirmation of identity by growth in pure culture is necessary.

Satisfactory enumeration should usually be possible also on DRBC, a selective medium of higher $a_w$ (King *et al*., 1979). Dilute media, such as potato dextrose agar (PDA) or bacteriological enumeration media, and incubation temperatures above 25 °C, are unsatisfactory.

*Aspergillus ochraceus* Wilhelm. See Fig. 1.3. Colonies on CYA 40–55 mm in diameter; conidial heads closely packed, coloured light to golden yellow; sclerotia sometimes produced, white when young, later pink to purple. Colonies on MEA 40–55 mm in diameter, plane, similar to those on CYA but quite sparsely sporing. At 37 °C, colonies of 25–30 mm in diameter produced.

Structures bearing conidia 1.0–1.5 mm long, with yellowish to pale brown walls, finely to conspicuously roughened; vesicles spherical, 25–50 µm in diameter, bearing tightly packed metulae and phialides over the entire surface; conidia spherical or near, 2.5–3.5 µm in diameter, with smooth to finely roughened walls.

Distinctive features. *Aspergillus ochraceus* and closely related species producing ochratoxin all grow moderately slowly on standard identification media such as CYA and MEA (Pitt and Hocking, 2009). Colonies are coloured pale brown to yellow brown from the conidia. *A. ochraceus* grows strongly at 37 °C, whereas the closely related species *A. westerdijkiae* and *A. steynii* do not grow at that temperature. Apart from that distinction, *A. westerdijkiae* produces spherical, finely roughened conidia, whereas those of *A. steynii* are smooth-walled and ellipsoidal, not spherical.

Factors influencing growth. *Aspergillus ochraceus* and the closely related species described here are mesophilic xerophiles. Growth occurs between 8 °C and 37 °C, with the optimum at 24–31 °C (Pitt and Hocking, 2009). Optimal conditions for growth are 0.95–0.99 $a_w$, while the lower limit for growth is 0.79 $a_w$ on media containing sugars and down to 0.81 $a_w$ on media based on NaCl. *A. ochraceus* grows slowly at pH 2.2 and well between pH 3 and 10 (Pitt and Hocking, 1977).

Commodities and foods at risk. *Aspergillus ochraceus* has been reported from a wide range of food products, more commonly in dried and stored foods than elsewhere. However, it is likely that many of these reports relate to the recently described *A. westerdijkiae* or *A. steynii*. Stored foods from which these species have been isolated include

smoked or salted dried fish and meat, beans, chickpeas, and nuts, especially pecans and pistachios. These species have been reported (usually as *A. ochraceus*) from cereals and cereal products but, rather infrequently, also from cheese, spices, black olives, and cassava. However, these species rarely cause spoilage, and are often found in foods at only low levels, so their presence is not a good indicator of significant mycotoxin production (Pitt and Hocking, 2009).

Several studies have detected *A. ochraceus* in green coffee beans (Levi *et al*., 1974; Cantafora *et al*., 1983; Tsubouchi *et al*., 1984; Micco *et al*., 1989; Studer-Rohr *et al*., 1994). Coffee cherries are usually picked by hand, or sometimes mechanically on large farms, and are usually dried in the sun. The beans may be dried directly and separated from the hull afterwards, or mechanically dehulled and dried, or dehulled by fermentation before drying. Coffee beans are stored after drying (as "green" coffee), then graded and shipped to manufacturers.

Picking cherries and spreading them on drying yards frequently causes damage, allowing ingress of fungi. If cherries are picked from the ground, contamination is likely to be high. Drying is often a slow process, in particular because of the environment in which coffee is grown. Coffee trees will not flower above 19 °C but require high temperatures to mature, so coffee is commonly grown on upland areas in the tropics. In consequence, drying is often conducted under less than ideal conditions, with morning mists or rain common in some growing areas (Teixera *et al*., 2001). Fungal growth frequently occurs.

Although the possibility of significant levels of OTA being present in coffee beans has been known for some time, the fungal cause remained elusive. Only recently has it been established that *Aspergillus ochraceus*, and no doubt its close relatives *A. westerdijkiae* and *A. steynii*, are major sources of OTA in coffee (Taniwaki *et al*., 1999, 2003; Pitt *et al*., 2001; Batista *et al*., 2003). Other known OTA producers, *A. niger* and *A. carbonarius*, have also been isolated from coffee (Frank, 2001; Pitt *et al*., 2001). As detailed mycological studies have not yet been conducted in some major coffee growing areas, the relative importance of *A. ochraceus* and *A. carbonarius* as the main source of OTA in coffee is difficult to assess.

Available evidence indicates that the sources of these fungi are environmental and that entry to cherries is gained during picking and drying (Taniwaki *et al*., 1999). OTA is produced during drying (Taniwaki *et al*., 1999; Bucheli *et al*., 2000; Teixera *et al*., 2001). Coffee picked and dried under good agricultural practice appears to contain OTA only rarely (Taniwaki *et al*., 1999, 2003).

### *4.2.3* Aspergillus carbonarius *and related species*

Taxonomy. *Aspergillus carbonarius* was recognized as a source of OTA relatively recently (Horie, 1995; Téren *et al*., 1996; Wicklow *et al*., 1996). It is now known that most, if not all, isolates of *A. carbonarius* produce OTA when grown in pure culture (Heenan *et al*., 1998; Taniwaki *et al*., 1999), although the extent of production is variable. The closely related species *A. niger* has also been reported reliably as a producer (Ueno *et al*., 1991; Abarca *et al*., 1994; Heenan *et al*., 1998; Taniwaki *et al*., 1999). However, all reports agree that OTA production by *A. niger* is very uncommon; OTA is formed under pure culture conditions by only 1–2% of isolates.

Enumeration. Satisfactory enumeration of *A. niger* and *A. carbonarius* and the closely related (but always non-toxigenic) species *A. japonicus* can be achieved on any antibacterial enumeration medium that contains appropriate inhibitors to reduce colony spreading. DRBC or DG18 is recommended (Pitt and Hocking, 1997; Samson *et al*., 2010). Rapidly growing, very dark brown to black colonies exhibiting "mop-like" fruiting structures under the stereomicroscope can be presumptively counted as *A. niger* plus *A. carbonarius*. Microscopic examination of colonies can provide supporting evidence, but representative colonies must be grown on standard identification media for confirmation.

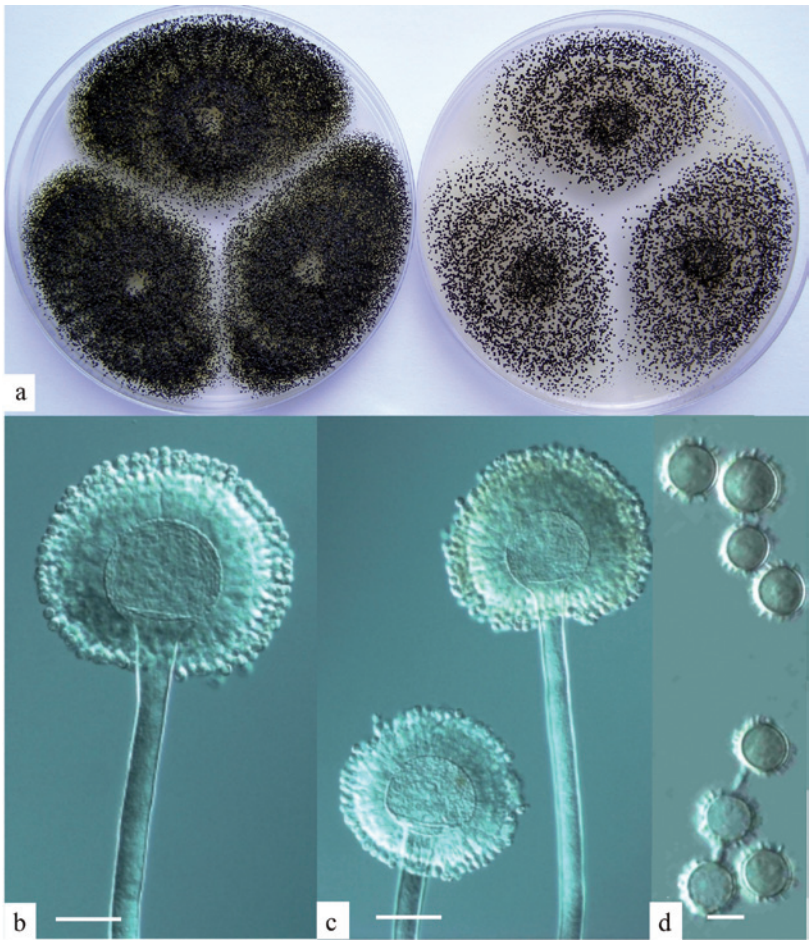
*Aspergillus carbonarius* (Bainier) Thom. See Fig. 1.4. Colonies on CYA 60 mm or more in diameter, usually covering the whole Petri dish; conidia black or nearly black. Colonies on MEA 50–60 mm in diameter, usually smaller than those on CYA, otherwise similar. At 37 °C, colonies 10–20 mm in diameter.

Structures bearing conidia 1.0–3.0 mm long, with heavy, hyaline, smooth walls; vesicles spherical, usually 60–85 µm in diameter, bearing closely packed metulae and phialides over the whole surface; conidia spherical, 7–10 µm in diameter, black, with walls extremely roughened.

*Aspergillus niger* Tiegh. See Fig. 1.5. Colonies on CYA 60 mm or more in diameter, usually covering the whole Petri dish; conidia black. Colonies on MEA varying from 30 mm to 60 mm in diameter, usually smaller than those on CYA and often quite sparse. At 37 °C, colonies 60 mm or more in diameter, covering the available space.

Structures bearing conidia 1.0–3.0 mm long, with heavy, hyaline, smooth walls; vesicles spherical, usually 50–75 µm in diameter, bearing closely packed metulae and phialides over the whole surface; conidia spherical, 4–5 µm in diameter, brown, with walls conspicuously roughened or sometimes striped.

**Fig. 1.4.** *Aspergillus carbonarius* (a) colonies on CYA (left) and MEA (right), 7 days, 25 °C; (b, c) heads, bars = 40 µm; (d) conidia, bar = 5 µm. Source: Pitt and Hocking (2009), Fig. 8.10, p. 300; reproduced with kind permission from Springer Science+Business Media B.V.




Distinctive features. Differentiation of *Aspergillus niger*, *A. carbonarius*, and *A. japonicus* from nearly all other species is not difficult. These species grow rapidly and produce very dark brown to black conidia. *A. niger* and *A. carbonarius* produce metulae, whereas *A. japonicus* does not. *A. niger* produces conidia that are 4–5 µm in diameter, whereas those of *A. carbonarius* are larger, on average 7 µm or more in diameter. If these species are grown on CYA for 7 days at 37 °C, separation on colony diameters can be very useful: *A. niger* grows very quickly (60 mm or more), whereas *A. carbonarius* and *A. japonicus* grow much more slowly (less than 20 mm) (Mitchell *et al*., 2003).

It is important to remember that very few isolates of *A. niger* produce OTA. *A. niger* is an exceptionally common species, and recovery of this species from foods should not be regarded as evidence that OTA is likely to be present.

Factors influencing growth. *Aspergillus carbonarius* can grow down to 10 °C, with an optimum near 30 °C and a maximum near 41 °C. The optimal $a_w$ for growth is 0.96–0.98, with a minimum near 0.85 at 25 °C. *A. niger* grows up to 45 °C, with an optimum of 35–37 °C, and has been reported to germinate down to 0.77 $a_w$ (Pitt and Hocking, 2009).

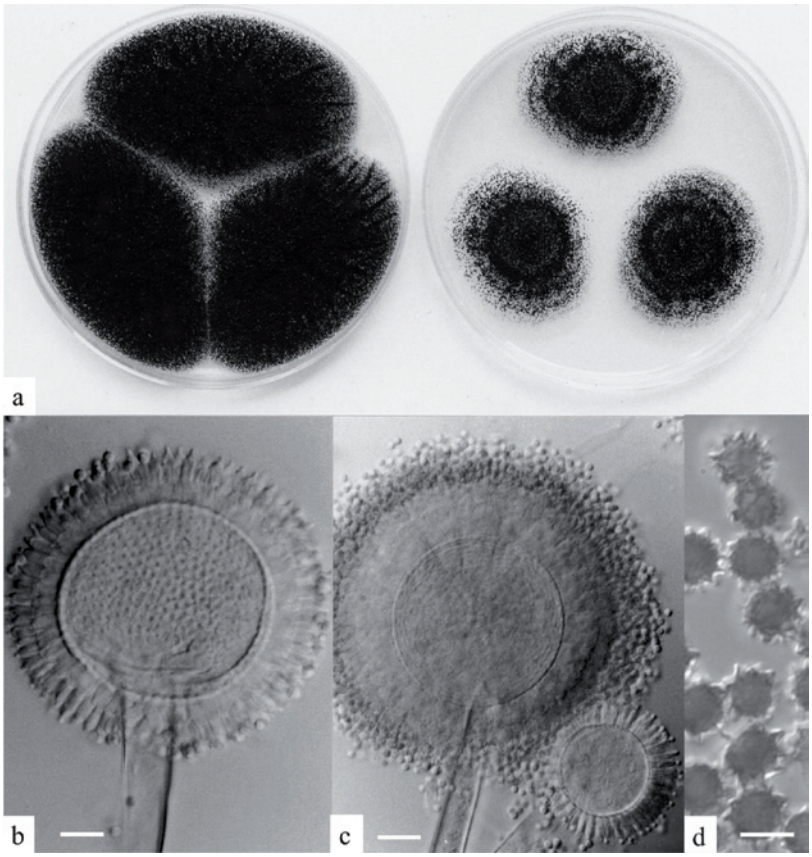
Commodities and foods at risk. Some black *Aspergillus* species, i.e. *A. niger*, *A. carbonarius*, and *A. japonicus*, are common inhabitants of vineyards, as these fungi grow rapidly at relatively high temperatures (above 30 °C) and their pigmentation renders them highly resistant to the deleterious effects of sunlight and UV light. These species appear to have no pathogenicity towards grapes and to be unable to penetrate an intact grape skin. Entry to maturing grapes results from attack by other pathogenic fungi (e.g. *Rhizopus stolonifer*, *Botrytis cinerea*, or powdery mildews), from mechanical damage due to cultivating or harvesting equipment, or, in some cultivars, from the splitting of berry skins that results from rain near harvest time. Once entry to a berry is gained, these fungi thrive in the acid, high-sugar environment.

Where grapes are dried, the black Aspergilli enjoy a considerable ecological advantage, and will continue to grow and produce OTA until the grapes dry below 0.8 $a_w$. As grapes are normally dried in the sun, this usually takes several days, allowing ample time for OTA production to occur (Hocking *et al*., 2003).

None of the three species of black Aspergilli appears to enjoy a particular ecological advantage, at least in Australia, as all three species are commonly recorded from maturing grapes, with proportions of each varying with seasonal factors (Leong *et al*., 2004). For OTA formation, *Aspergillus carbonarius* is the significant species: only a small proportion of *A. niger* isolates are capable of producing OTA, and *A. japonicus* isolates do not produce this toxin.

In grapes intended for wine-making, the time interval is usually short between infection and crushing, when fermentation stops fungal growth

**Fig. 1.5.** *Aspergillus niger* (a) colonies on CYA (left) and MEA (right), 7 days, 25 °C; (b) head, bar = 15 µm; (c) heads, bar = 10 µm; (d) conidia, bar = 5 µm. Source: Pitt and Hocking (2009), Fig. 8.15, p. 314; reproduced with kind permission from Springer Science+Business Media B.V.




and toxin production ceases. So control of OTA in wines relies on good vineyard management, i.e. control of bunch rots and skin splitting, and short time intervals between harvest and crushing.

Occasional contamination of figs with OTA has been reported (Özay and Alperden, 1991). In a study of Turkish figs sampled during different stages of processing, only 3 of 100 samples contained OTA, and in each case levels were between 5 µg/kg and 10 µg/kg.

*Aspergillus niger* and *A. carbonarius* also occur in a wide variety of other fruits (Snowdon, 1990, 1991). Generally, other fruits are handled in ways that minimize fungal infection, or damaged fruit is discarded, not eaten, so OTA formation is not a hazard.

## 5. Genus *Penicillium*

Taxonomy. *Penicillium* is a large genus, with more than 200 recognized species, of which 50 or more are of common occurrence (Pitt, 2000). All common species grow and sporulate well on synthetic or semisynthetic media, and usually can be readily recognized at genus level. Most *Penicillium* species grow slowly, and have green conidia.

Classification within *Penicillium* is based primarily on microscopic morphology: the genus is divided into subgenera based on the number and arrangement of phialides (elements producing conidia) and metulae and rami (elements supporting phialides) on the main stalk cells. The classification of Pitt (1979, 2000) includes four subgenera: *Aspergilloides*, in which phialides are borne directly on the stalk cells without intervening supporting elements; *Furcatum* and *Biverticillium*, in which phialides are supported by metulae; and *Penicillium*, in which both metulae and rami are usually present. The majority of important toxigenic and food spoilage species are found in subgenus *Penicillium*.

Enumeration. Enumeration procedures suitable for all common *Penicillium* species are similar. Any effective antibacterial enumeration medium can be expected to give satisfactory results. However, some *Penicillium* species grow rather weakly on dilute media, such as PDA or dichloran chloramphenicol peptone agar (DCPA), so DRBC is recommended. Penicillia can also be effectively enumerated on DG18 (Pitt and Hocking, 1997; Samson *et al*., 2010).

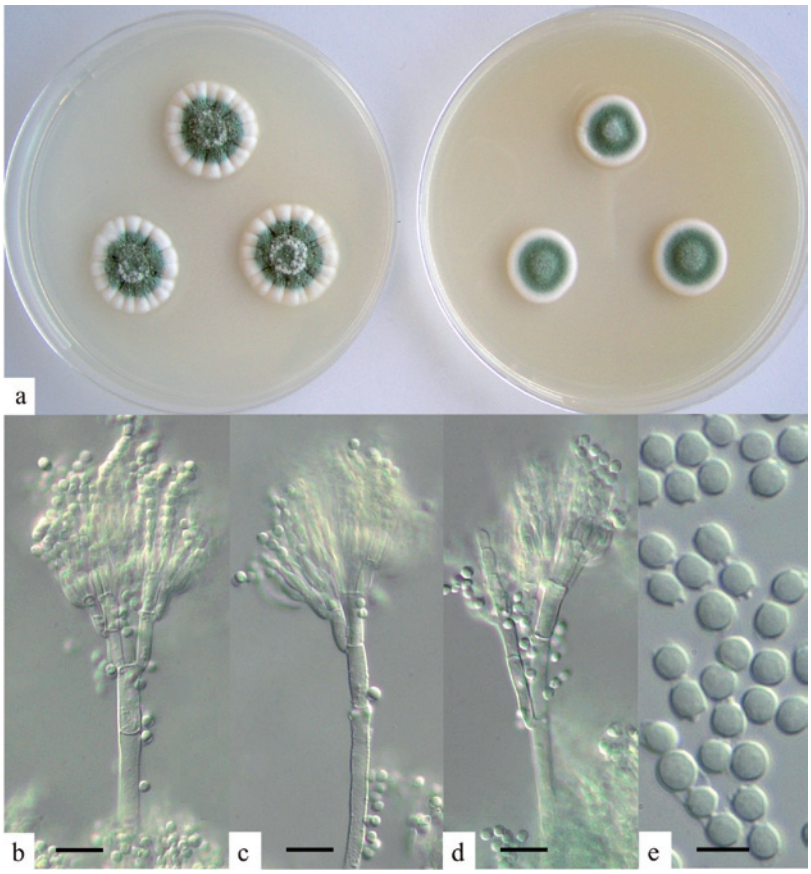
Identification. For a comprehensive taxonomy of *Penicillium*, see Pitt (1979). Keys and descriptions to common species are provided by Pitt (2000) and to foodborne species by Pitt and Hocking (2009) and Samson *et al*. (2010).

Identification of *Penicillium* isolates to species level is not easy, preferably being carried out under carefully standardized conditions of media, incubation time, and temperature. As well as microscopic morphology, gross physiological features, including colony diameters, colours of conidia, and colony pigments, are used to distinguish species.

### *5.1 Ochratoxin A production by* Penicillium verrucosum

Soon after the discovery of OTA from *Aspergillus ochraceus*, the formation of OTA by a *Penicillium* species, *P. viridicatum*, was reported (van Walbeek *et al*., 1969) and natural occurrence confirmed (Krogh *et al*.,

**Fig. 1.6.** *Penicillium verrucosum* (a) colonies on CYA (left) and MEA (right), 7 days, 25 °C; (b, c, d) penicilli, bars = 10 µm; (e) conidia, bar = 5 µm. Source: Pitt and Hocking (2009), Fig. 7.48, p. 260; reproduced with kind permission from Springer Science+Business Media B.V.




1973). The view that *P. viridicatum* was a major source of OTA contamination in foods and feeds in some parts of the world was accepted for more than a decade. The species involved was later correctly identified as *P. verrucosum* (Pitt, 1987), and this was confirmed (Frisvad, 1989; Frisvad and Filtenborg, 1989). *P. viridicatum* does produce mycotoxins, but these have only rarely been implicated in animal health.

*Penicillium verrucosum*, and the closely related *P. nordicum*, are the only *Penicillium* species that produce OTA. *P. verrucosum* commonly occurs in cereals in temperate climates, whereas *P. nordicum* has been isolated, uncommonly, from processed meats.

Enumeration. The media specified above for general enumeration of *Penicillium* species are effective for *P. verrucosum*. On dichloran rose bengal yeast extract sucrose agar (DRYS), a selective medium for the enumeration of *P. verrucosum* and *P. viridicatum*, *P. verrucosum* produces a violet brown reverse colouration (Frisvad, 1983). Isolation and identification of *P. verrucosum* in pure culture is essential for confirmation.

*Penicillium verrucosum* Dierckx. See Fig. 1.6. Colonies on CYA 15–25 mm in diameter; mycelium white; conidial formation light to moderate, grey green to dull green; reverse yellow brown to deep brown. Colonies on MEA 12–15(–20) mm in diameter; mycelium white; conidial production moderate, coloured as on CYA; reverse dull brown or olive. No growth at 37 °C. Structures bearing conidia 200–500 µm long, with walls finely to conspicuously roughened; fruiting structures variable, with two or three supporting cells beneath phialides; conidia usually spherical, 2.5–3.0 µm in diameter, with smooth walls.

Distinctive features. *Penicillium verrucosum* is characterized by slow growth on CYA and especially on MEA, by conidia coloured relatively bright green, by the absence of other conspicuous pigmentation, and by rough walls on the stalk cells (Pitt, 2000). It is similar in general appearance to *P. viridicatum* and *P. solitum*. *P. verrucosum* and *P. viridicatum* produce a distinctive violet brown reverse on DRYS (Frisvad, 1983). It should be noted that recognition of this species requires specialist knowledge, or detailed comparison with known cultures (of this and other related species).

Only one other species of *Penicillium* is known to produce ochratoxin A: *P. nordicum*. This species was segregated from *P. verrucosum* by small physiological differences (it produces a yellow reverse on DRYS; Larsen *et al.*, 2001), but it is ecologically distinct, occurring mainly on meat and cheese. Its significance in terms of human health is unknown.

Factors influencing growth. *P. verrucosum* grows from 0 °C to 31 °C, with the optimum at 20 °C. The minimum $a_w$ for growth is about 0.80 (Pitt and Hocking, 2009). Growth occurs over the pH range 2.1–10.0 at least (Wheeler *et al.*, 1991). The ability of *P. verrucosum* to produce significant levels of OTA at 4 °C and $a_w$ as low as 0.86 is noteworthy (Northolt *et al.*, 1979). The physiology of *P. nordicum* is likely to be very similar.

Commodities and foods at risk. The major food habitat for *P. verrucosum* is cereal crops grown in cool temperate climates, ranging across northern and central Europe and Canada. The occurrence of this species in European cereals has two consequences: OTA is present in many kinds of European cereal products, especially bread and flour-based foods, and in animals that eat cereals as a major dietary component. OTA was detected in Danish pig meats nearly 40 years ago (Krogh *et al*., 1973), and its implications for human and animal health were recognized at the same time. As bread and other cereal products and pig meats are major components of the European diet, the further consequence is that most Europeans who have been tested have shown appreciable concentrations of OTA in their blood (WHO, 2007).

Recent information indicates that *P. verrucosum* is not a commensal on cereal crops, i.e. it does not grow in grain crops before harvest, but its presence is due to post-harvest contamination. The primary sources of infection of the grain appear to be from harvesting, processing, and storage equipment (Magan and Olsen, 2004; Olsen *et al*., 2004).

A maximum growth temperature near 30 °C restricts *Penicillium verrucosum* geographically. It appears to be uncommon, indeed almost unknown, in warm temperate or tropical climates, or in other kinds of foods. *P. verrucosum* is never a source of OTA in foods from warmer climates, such as coffee, wines, or other grape products.

*Penicillium nordicum* has been isolated quite commonly from meats, especially refrigerated products.

## 6. Genus *Fusarium*

*Fusarium* is one of the most important genera of plant pathogenic fungi, with a record of devastating infections in many kinds of economically important plants. *Fusarium* species are responsible for wilts, blights, root rots, and cankers in legumes, coffee, wheat, maize, carnations, pine trees, and grasses. The importance of *Fusarium* species in the current context is that infection may sometimes occur in developing seeds, especially in cereals, and also in maturing fruits and vegetables. An immediate potential for toxin production in foods is apparent.

The very important role of *Fusarium* species as mycotoxin producers appears to have remained largely unsuspected until the 1970s. Research has now strongly associated alimentary toxic aleukia (ATA) with *Fusarium* species. An epidemic of this human mycotoxicosis in the USSR killed at least 100 000 people between 1942 and 1948 (Joffe, 1978). ATA outbreaks are also known to have occurred in the Russian Federation in 1932 and 1913, and there is little doubt that outbreaks occurred in earlier years as well (Joffe, 1978). Matossian (1981, 1989) has argued persuasively that ATA outbreaks occurred in other countries, including England, in the 16th to 18th centuries at least.

Research since 1970 has shown that *Fusarium* species are capable of producing a bewildering array of mycotoxins. Foremost among these are the trichothecenes, of which at least 50 are known; the majority are produced by *Fusarium*. The most notorious trichothecene is T-2 toxin, which was linked to ATA. Of no less importance in modern times are the fumonisins, which are especially toxic to horses, and are suspected to be responsible for chronic human diseases also.

Taxonomy. The signature micromorphological characteristics of *Fusarium* species are uncoloured, multiseptate, large (25 µm to 50 µm or more) curved conidia called macroconidia, which are produced from phialides. Most species produce macroconidia in cushion-like structures called sporodochia. In some species, macroconidia are sparsely formed in Petri dish culture and recognition of these species as belonging to *Fusarium* requires experience.

In addition to macroconidia, some *Fusarium* species can make one or two kinds of smaller, one- or two-celled conidia called microconidia. Microconidia are usually produced in the aerial mycelium in culture. Most often, microconidia are produced in slimy heads, but some species produce them in chains or singly. Microconidia are also produced from phialides, which may have a single spore-bearing opening (monophialides) or multiple openings (polyphialides).

The taxonomy of *Fusarium* has been difficult, with several competing taxonomic schemes, recognizing from 9 to 60 species in the genus. The taxonomy of Nelson *et al*. (1983), which accepted 30 species, has met with widespread approval, and is still widely used in conjunction with the laboratory manual of Leslie and Summerell (2006). Recent molecular studies have suggested that Nelson *et al*. (1983) greatly underestimated species numbers in *Fusarium*. For example, O'Donnell *et al*. (1998) recognized 36 phylogenetic species (species recognizably different by molecular techniques) in a grouping corresponding to *Fusarium* section Liseola in which four species had been recognized by Nelson *et al*. (1983). Nirenberg and O'Donnell (1998) described 10 new species in this section. However, for practical identification of the species important for mycotoxin production, the manual of Leslie and Summerell (2006) is recommended.

**Table 1.1.** Media of value for isolation and enumeration of *Fusarium* species

| Medium | Advantages | Disadvantages | Reference |
|---|---|---|---|
| Pentachloronitrobenzene (PCNB) agar | Often used | Carcinogenic; no spore production by *Fusarium* species | Snyder and Hansen (1940) |
| Dichloran chloramphenicol peptone agar (DCPA) | Sporulation allows recognition of *Fusarium* species | Little pigmentation for differentiating *Fusarium* species | Andrews and Pitt (1986) |
| Czapek–Dox iprodione dichloran agar (CZID) | Pigmentation helpful in distinguishing *Fusarium* species | Sterile colonies do not permit ready identification of *Fusarium* species | Abildgren *et al.* (1987) |
| Dichloran 18% glycerol agar (DG18) | Sporulation allows recognition of *Fusarium* species | Low-$a_w$ medium, not ideal for *Fusarium* growth | Hocking and Pitt (1980) |

A direct consequence of confusion in taxonomy has been confusion over species–mycotoxin associations. *Fusarium* isolates producing a particular toxin have often been given different names. However, Desjardins (2006) has provided a comprehensive clarification of the important mycotoxigenic species and the mycotoxins they each produce. The species judged to be most important from the viewpoint of human health are discussed here.

Enumeration and isolation. Growth of *Fusarium* species is favoured by dilute media of high $a_w$. Enumeration of Fusaria can be effectively carried out on media such as PDA provided chloramphenicol or other broad-spectrum antibiotics are added to suppress bacteria. However, acidified PDA, a frequently used antibacterial medium, is not recommended because it may inhibit sensitive cells. DCPA (Andrews and Pitt, 1986) and Czapek–Dox iprodione dichloran agar (CZID) (Thrane, 1996) are effective enumeration and isolation media for most foodborne *Fusarium* species. In addition, half-strength PDA is used by many laboratories isolating directly from plant tissue, where the number of unwanted fungi is much lower than in soil or plant debris. It should be pointed out that, although pentachloronitrobenzene (PCNB) agar is still widely used, PCNB is a known carcinogen. DCPA is to be preferred as it contains pentachloronitroaniline (dichloran), a molecule with similar properties to PCNB but that is not carcinogenic.

Recognition of *Fusarium* colonies on these media requires careful observation and experience. Presumptive identification to genus level can usually be made from colony appearance: low to floccose colonies, coloured white, pink, or purple, with pale to red or purple reverses, are indicative of *Fusarium*. Confirmation requires microscopic examination, in which the crescent-shaped macroconidia characteristic of the genus should be observed. However, these are not always produced on enumeration media, especially PDA. Differentiation of some species on enumeration media is possible, but also requires experience.

Identification. All contemporary identification schemes based primarily on morphology use two media: a weak medium for stimulation of sporulation and a richer medium for measuring growth rates and for stimulation of diagnostic pigment production. The most commonly used rich medium is PDA. Potato sucrose agar (PSA) was used in the manual of Booth (1971) and is still used in some laboratories instead of, or in addition to, PDA. Nelson *et al.* (1983) among others advocated the use of PDA made from old potatoes, rather than commercial formulations, but many laboratories use commercial PDA with satisfactory results. Oatmeal agar is used in some laboratories. The most commonly used weak media are carnation leaf agar (CLA) and Synthetischer Nährstoffarmer Agar (often now called synthetic nutrient agar [SNA]). SNA has the advantage of being a defined medium, but CLA supports superior sporodochial production in some species. However, use of CLA requires access to a source of gamma-irradiated carnation leaves. Banana leaf agar (autoclave-sterilized banana leaves on half-strength cornmeal agar) is also a good medium for stimulation of sporulation, but is not yet widely used. To maintain uniformity for descriptions of all foodborne fungi, Pitt and Hocking (2009) provided *Fusarium* descriptions on PDA, together with media used in *Aspergillus* and *Penicillium* identification. Some readily prepared media of value in *Fusarium* identification are given in Table 1.1.

Opinions differ regarding the necessity to make a single conidium isolate of *Fusarium* species before identification. Some laboratories make a new single conidium isolate at every transfer of the culture. This ensures a highly reproducible growth rate. Other laboratories prefer not to make single spore cultures because this may decrease the vigour of the culture. Because *Fusarium* species often grow in mixed colonies when isolated from soil or plant material, at least one generation of single conidium isolates is advisable for cultures intended for experimental use, especially genetic studies.

Traditionally, *Fusarium* cultures have been cultivated for 7–10 days under mixed fluorescent/near UV light at room temperature, near 25 °C. Recently, particularly for the group of species producing fumonisin, the importance of also growing the

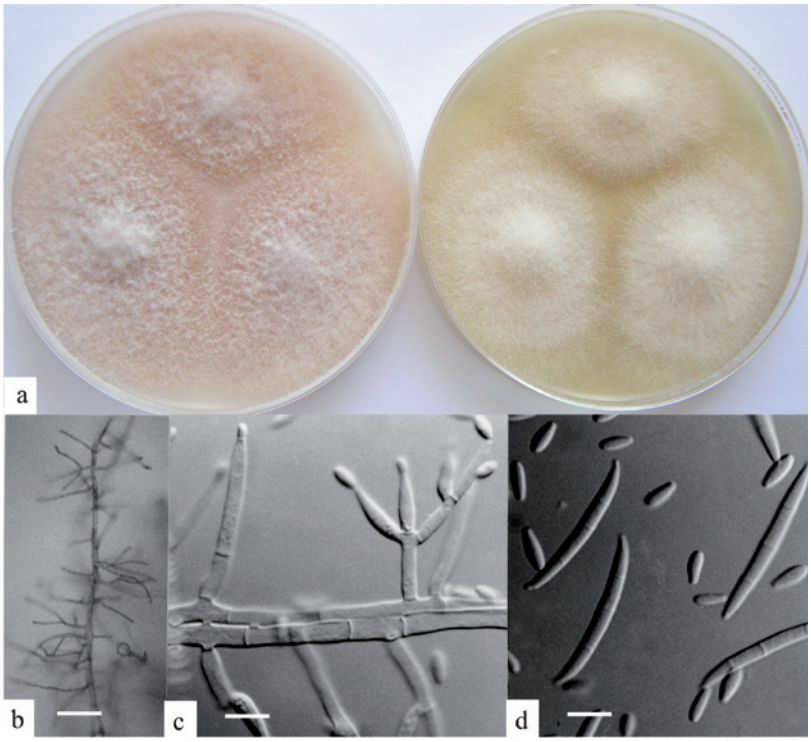
same cultures in darkness to allow the development of some diagnostic features has been emphasized.

The only definitive taxonomy for *Fusarium* is that of Nelson *et al*. (1983). The most useful and up-to-date guide to important species is that provided by Leslie and Summerell (2006). Pitt and Hocking (2009) and Samson *et al*. (2010) provide keys and descriptions for common foodborne species. The descriptions of species given here have been provided by Dr K.A. Seifert (Agriculture and Agri-Food Canada, Ottawa) and are based on the protocols and media described above.

### *6.1 Fumonisin production by* F. verticillioides *and* F. proliferatum

*Fusarium verticillioides* (Sacc.) Nirenberg. See Fig. 1.7. Colonies on rich media (PDA or PSA) at 25 °C grow moderately rapidly, 3.5–5.5 cm in diameter in 4 days. Abundant aerial mycelium is produced, and the reverse usually has rays or large patches of violet or purple. On weak media (CLA or SNA) at 25 °C, sporodochia are sparsely produced or not present in most isolates. When present they are inconspicuous and almost colourless, on the agar surface beneath the often dense aerial mycelium. Macroconidia are usually 3–5 septate, mostly 30–45 µm long, straight or variably curved, with more or less parallel walls and with the widest point near the middle. Microconidia are abundantly produced in the aerial mycelium on highly branched conidiophores. The conidiogenous cells are monophialides that often collapse before the cultures are about 10 days old. Microconidia are produced in long, dry chains visible with the stereomicroscope; these chains, which are often coiled, give the colonies a distinctive texture similar to curly hair. Individual conidia are ellipsoidal, 0–1 septate, 4–19 × 1.5–4.5 µm.

**Fig. 1.7.** *Fusarium verticillioides* (a) colonies on PDA (left) and DCPA (right), 7 days, 25 °C; (b) phialides bearing chains of microconidia, bar = 50 µm; (c) phialides, bar = 10 µm; (d) macroconidia and microconidia, bar = 10 µm. Source: Pitt and Hocking (2009), Fig. 5.36, p. 120; reproduced with kind permission from Springer Science+Business Media B.V




The teleomorph of *F. verticillioides* is *Gibberella moniliformis*.

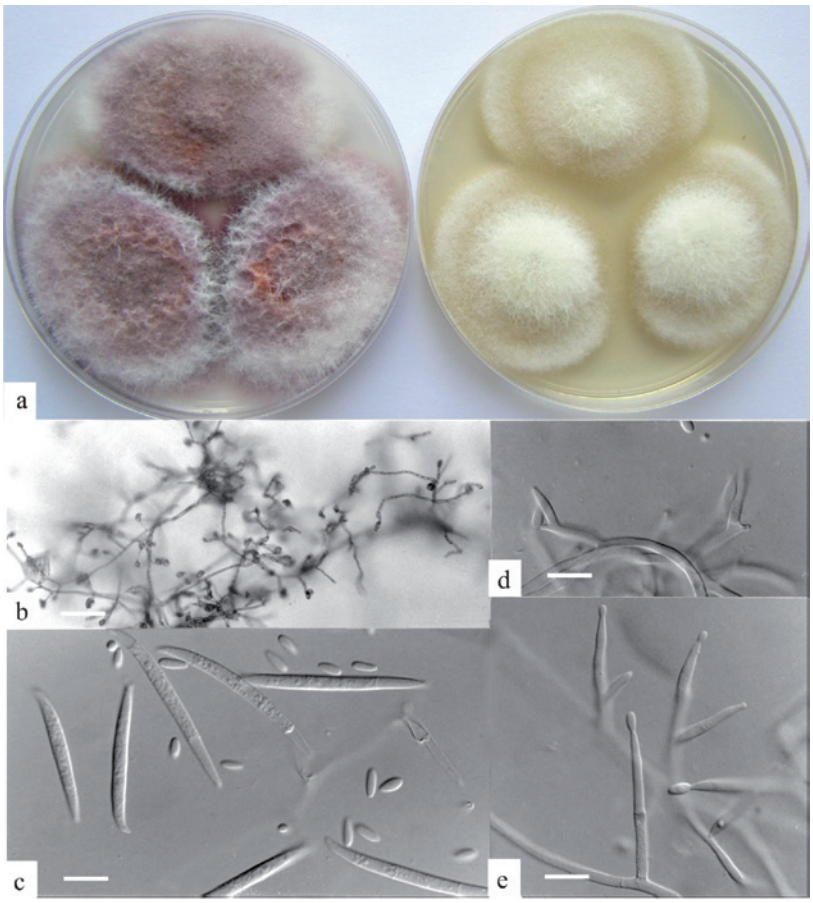
Distinctive features. *Fusarium verticillioides* is recognized by the combination of purplish colours on PDA, and the production of long, curly chains of microconidia from monophialides in the aerial mycelium. Although macroconidia are found in some cultures, many strains do not produce them. Therefore, an experienced eye can be necessary to recognize those strains as belonging to *Fusarium*. The teleomorph, *Gibberella moniliformis*, is produced in culture only when strains of opposite mating type are crossed under appropriate conditions.

Until recently, *Fusarium verticillioides* was known as *F. moniliforme*, a name that is no longer used on the recommendation of an expert committee (Seifert *et al*., 2003). The name *F. moniliforme* is predated by *F. verticillioides* and also cannot reliably be linked to modern species concepts.

*Fusarium thapsinum* (teleomorph: *Gibberella thapsina*) is a closely related species, found primarily on sorghum. It has similar micromorphology, but PDA colonies lack purplish pigmentation and tend to be yellow to yellow brown. Both *F. verticillioides* and *F. thapsinum* produce longer microconidial chains and lack the polyphialides that characterize *F. proliferatum*.

Factors influencing growth. The maximum temperature for growth of *Fusarium verticillioides* has been reported as 32–37 °C, the minimum as 2.5–5 °C, and the optimum near 25 °C. The minimum $a_w$ for growth is about 0.87 (Pitt and Hocking, 2009). Values of these parameters for *F. proliferatum* are essentially identical.

**Fig. 1.8.** *Fusarium proliferatum* (a) colonies on PDA (left) and DCPA (right), 7 days, 25 °C; (b) phialides bearing microconidia in chains and false heads in situ, bar = 50 µm; (c) macroconidia and microconidia, bar = 10 µm; (d) polyphialides, bar = 10 µm; (e) monophialides, bar = 10 µm. Source: Pitt and Hocking (2009), Fig. 5.31, p. 111; reproduced with kind permission from Springer Science+Business Media B.V.




*Fusarium proliferatum* (Matsush.) Nirenberg. See Fig. 1.8. Colonies on rich media (PDA or PSA) at 25 °C grow moderately rapidly, 3.5–5.5 cm in diameter in 4 days. There is abundant white to pink or slightly orange aerial mycelium, and the reverse usually has rays or large patches of violet or purple. On weak media (CLA or SNA) at 25 °C, sporodochia are usually sparsely produced and often are not present. When present they are inconspicuous and almost colourless, on the agar surface beneath the often dense aerial mycelium. Macroconidia are usually 3–5 septate, 30–45 µm long, straight or variably curved, with more or less parallel walls and with the widest point near the middle. Microconidia are abundantly produced in the aerial mycelium on divergently branched conidiophores. The cells producing conidia are predominantly monophialides, but up to 20% may be polyphialides. Microconidia are produced in short, dry chains visible with the stereomicroscope, ovoid to ellipsoidal, 0–1 septate, 7–12.5 × 2–3 µm.

The teleomorph of *F. proliferatum* is *Gibberella intermedia*.

Distinctive features. The production of purplish pigments on PDA, and the occurrence of short, dry chains of microconidia in the aerial mycelium and the sometimes sparse occurrence of polyphialides characterize *F. proliferatum*. Although macroconidia are found in some cultures, many strains do not produce them. Therefore, an experienced eye can be necessary to recognize those strains as belonging to *Fusarium*. The teleomorph, *Gibberella intermedia*, is produced in culture only when strains of opposite mating type are crossed under appropriate conditions.

*Fusarium proliferatum* is distinguished from *F. verticillioides* by the shorter chains of microconidia and the occurrence of polyphialides in the aerial mycelium. Another frequently isolated species that occupies the same ecological niche is *F. subglutinans*, which does not produce fumonisins. It produces slimy heads rather than chains of microconidia from a mixture of monophialides and polyphialides; strains of this species are more likely to produce macroconidia than either *F. verticillioides* or *F. subglutinans*. *F. nygamai* is a similar species that also produces fumonisin in some strains; it can be distinguished from *F. proliferatum* by the production of chlamydospores.

Other species making fumonisins. Several other *Fusarium* species are known to produce fumonisins, including *F. anthophilum*, *F. beomiforme*, *F. dlamini*, *F. globosum*, *F. napiforme*, *F. nygamai*, *F. oxysporum*, *F. polyphialidicum*, and *F. subglutinans* (IARC, 2002). None is of major importance in fumonisin production in foods.

Commodities and foods at risk. *Fusarium verticillioides* and *F. proliferatum*, the major sources of fumonisins, are the most common fungi associated with maize. *F. verticillioides* has been known for many years to occur systemically in leaves, stems, roots, and kernels of maize (Foley, 1962). These fungi can be recovered from virtually all maize kernels worldwide, including those that are healthy (e.g. Hesseltine *et al*., 1981; Pitt *et al*., 1993; Miller,

1994; Miller *et al*., 1995; Ramirez *et al*., 1996; Logrieco *et al*., 2002). *F. verticillioides* has been reported to suppress the growth of other ear fungi (Reid *et al*., 1999), and kernels heat-treated to destroy the fungus germinate but do not thrive (Foley, 1962). Strains of *F. verticillioides* isolated from maize have the potential to produce fumonisins, even for maize from regions where fumonisin accumulations in maize are historically uncommon. These include Africa, Asia, Europe, North America (Canada, the USA, and Mexico), and South America (WHO, 2000).

Fusarium kernel rot in maize due to the growth of *F. verticillioides* and related species causes the formation of fumonisins, whereas Gibberella ear rot or pink ear rot, mainly caused by *F. graminearum*, is associated with deoxynivalenol and zearalenone production (see Section 6.3). These fungi grow under different environmental conditions but with overlap. *F. proliferatum*, which causes kernel rot, concurrently produces fumonisin and moniliformin. Slightly different environmental conditions appear to favour one or the other of the principal species that produce fumonisins. For these reasons, it is sensible to consider the two diseases Fusarium kernel rot and pink ear rot together. In North America and Europe, fumonisins can occur in maize crops in some seasons, but in others, deoxynivalenol can occur, sometimes accompanied by zearalenone. In other regions, such as Africa or South-East Asia, all three toxins can be seen together (Yamashita *et al*., 1995; Doko *et al*., 1996; Ali *et al*., 1998). These differences in mycotoxin occurrence have important toxicological implications.

*Fusarium subglutinans* is also common in maize kernels in North America (Miller, 1994; Munkvold, 2003), but this species apparently causes a higher level of ear rot in some European locations and results in the accumulation of moniliformin (Logrieco *et al*., 2002). From limited data, moniliformin is not commonly found in United States (Gutema *et al*., 2000) or Canadian maize (Farber *et al*., 1988).

After genotype susceptibility, temperature is the primary determining factor for maize diseases caused by *Fusarium* species (Miller, 1994; Munkvold, 2003). *F. graminearum* has a very narrow temperature window for growth in maize. The optimal temperature is between 26 °C and 28 °C. Its growth rate at 24 °C is one quarter that at 26–28 °C, while at 30 °C it is about one half. In contrast, *F. verticillioides* grows well above 26 °C (Miller, 2001; Munkvold, 2003).

In culture, fumonisin $B_1$ is produced under conditions known to favour the production of polyketides and sesquiterpenes. The toxin is optimally produced in media that have moderate $a_w$ and are nitrogen-limited. Fumonisin is produced under relatively high oxygen tensions but apparently has an unusual requirement for low pH (about 2) for optimal production (Miller *et al*., 1995); such conditions arise only when the plant is dead or dying.

Fusarium kernel rot is associated with warm, dry years and insect damage and is caused by *F. subglutinans* (teleomorph: *Gibberella subglutinans*), *F. verticillioides* (teleomorph: *Gibberella moniliformis*), and *F. proliferatum* (Logrieco *et al*., 2002; Munkvold, 2003). In warmer parts of the USA and in the lowland tropics, *F. verticillioides* is one of the most important ear diseases (De Leon and Pandey, 1989).

Studies of the occurrence of fumonisin from natural occurrence and experimental infections clearly demonstrate the importance of drought stress and insect damage at the same time as temperatures are favourable. The fumonisin $B_1$ concentrations found in maize from the two Ontario, Canada, counties with the highest and lowest average concentration after the 1993 harvest were 1.4 and 0.4 times the state average, respectively. The average temperatures in the counties were similar, at 104% and 107% of the 30-year average, respectively. However, rainfall in the county with the highest fumonisin $B_1$ level was only 49% of normal, whereas in the county with the lowest level, it was 95% of normal (Miller *et al*., 1995). After experimental inoculation of 14 maize genotypes in Poland, the average temperature in the year with the highest fumonisin $B_1$ accumulation was 117% of the 30-year average; that in the year with the lowest fumonisin $B_1$ accumulation was 102% of normal. Rainfall in the year with the highest fumonisin $B_1$ accumulation was 6% of normal and in the year with the lowest, 65% of normal (Pascale *et al*., 1997).

A study of fumonisin occurrence in hybrids grown in the USA indicated that fumonisins are produced in higher concentrations in hybrids grown outside their area of adaptation. Fumonisin concentrations were inversely proportional to June rainfall (Shelby *et al*., 1994), again suggesting the important role of drought stress (Munkvold, 2003). Data from samples collected in Africa, Italy, and Croatia also indicate fumonisin accumulation in lines grown outside their area of adaptation, which includes tolerance to moisture stress (Doko *et al*., 1995; Visconti, 1996).

Hybrids with an increased likelihood of kernel splitting show higher levels of Fusarium kernel rot, and kernel splitting is generally worse under drought conditions. Drought stress also results in greater insect herbivory on maize; hence it is not possible to totally separate these variables. Further, in studies of experimental inoculation methods, the severity of Gibberella ear rot is related to wound size (Drepper and Renfro, 1990). It was observed early on that

a strong relationship exists between insect damage and Gibberella ear rot (Lew *et al*., 1991). Transgenic Bt maize genotypes, which contain a gene from the soil bacterium *Bacillus thuringiensis* that results in the accumulation of proteins toxic to key insect pests of maize, had lowered levels of recovery of *F. verticillioides* and fumonisin (Munkvold *et al*., 1997, 1999; Bakan *et al*., 2002; Munkvold, 2003). Under conditions of high disease pressure, the Bt hybrids can make the difference between a crop being fit or unfit for human consumption (Hammond *et al*., 2004; De La Campa *et al*., 2004). In an examination of fumonisin concentration in relation to various climate variables under moderate insect pressure, most of the variation was explained by temperatures above 30 °C (about 40%), followed by insect pressure (about 20%) and hybrid (about 10%; De La Campa *et al*., 2004).

Maize infected by other pathogens that damage ears (such as *F. graminearum*) may be predisposed to *F. verticillioides* damage and fumonisin accumulation. Ears inoculated with *F. graminearum*, *F. verticillioides*, and *F. subglutinans* by wounding produced visible symptoms on a 1–9 scale of 7.3, 4.4, and 4.7, respectively. Despite the fact that *F. graminearum* and *F. subglutinans* do not produce fumonisin, ears inoculated with these fungi contained 42 µg/g and 3 µg/g fumonisin $B_1$, respectively (Schaafsma *et al*., 1993).

Breeding for resistance to Fusarium kernel rot has not been effective. Within areas of adaptation, there are apparent differences in symptom response (Miller, 2001; Munkvold, 2003). In a large trial at the International Maize and Wheat Improvement Center, in Mexico, slight improvements in symptom expression in some tropical maize genotypes were observed after many cycles of selection (De Leon and Pandey, 1989).

Factors that control insects, confer resistance to other ear diseases, and adaptations, including drought and temperature tolerance, are important in reducing the risk of fumonisin accumulations in maize.

Fumonisins are very uncommon in commodities or foods other than maize and maize products (IARC, 2002).

### *6.2 Fumonisin production by* Aspergillus niger *and* Alternaria arborescens

It has been known for 20 years that one particular race of *Alternaria alternata*, described as *Alternaria alternata* f. sp. *lycopersici*, produces fumonisins (Chen *et al*., 1992). This particular race is a host-specific pathogen that causes a stem canker disease on tomato plants. For that reason, fumonisin production by this species has been largely ignored in general discussions of fumonisins in foods and feeds. This taxon has been re-identified as *Alt. arborescens* (Frisvad *et al*., 2007).

Recently, however, the picture has become more complicated. Studies on the genome sequence of *Aspergillus niger* showed, totally unexpectedly, the presence of the genes for fumonisin (Baker, 2006), and this was independently verified by Pel *et al*. (2007). It was soon confirmed that this gene cluster (consisting of at least 15 genes) was active and that at least some strains of *A. niger* can indeed produce fumonisins (Frisvad *et al*., 2007).

The implications are vast. As described in Section 4.2.3, *A. niger* is a very common fungus, of which a few strains produce ochratoxin A. Foods in which OTA is found, produced by *A. niger* and the closely related species *A. carbonarius*, include grapes, dried vine fruits, wines, and coffee. *A. niger* is also common in some fresh fruits, particularly berries, and on onions (Pitt and Hocking, 2009). Therefore, fumonisins produced by *A. niger* can be expected to be widespread if more than a few isolates are producers. Recent information indicates that fumonisin production by *A. niger* is indeed common. In one study of *A. niger* strains from a sample of Californian raisins, 50 of 66 strains (77%) produced fumonisins (Mogensen *et al*., 2010b). In a second study, where isolates were taken from 13 samples of dried vine fruits from several countries, 20 of 30 (67%) produced fumonisins (Varga *et al*., 2010). Of an unstated number of isolates from Thai coffee, 67% were able to produce fumonisins (Noonim *et al*., 2009). *A. niger* isolates do not usually produce fumonisin $B_1$; the major metabolite is fumonisin $B_2$, sometimes with lower amounts of fumonisin $B_4$.

So far, only a few studies have examined food products that are frequently infected by *A. niger* for the presence of fumonisins. Low levels (1–9.7 µg/kg) were found in 7 of 12 coffee samples (Noonim *et al*., 2009). Levels of up to 7.8 mg/kg were found in inoculated dried fruits (Mogensen *et al*., 2010b). More alarming, seven commercial dried fruit samples positive for *A. niger* were all positive for fumonisins $B_1$ to $B_4$: the average total fumonisin level was 7.2 mg/kg, with the range 4.6–35.5 mg/kg (Varga *et al*., 2010). It is not surprising that wines can also contain fumonisins. Of 51 market samples of Italian wines, 9 (18%) contained fumonisin $B_2$, with levels ranging from 0.4 µg/L to 2.4 µg/L (Logrieco *et al*., 2010). Seventy-seven wine samples from 13 countries were examined by Mogensen *et al*. (2010a), and 18 (23%) were found to contain fumonisins, with a range of 1 µg/L to 25 µg/L. These levels are low, but wine has a high consumption rate in some areas.

It has been found that fumonisin production in culture by *A. niger* is enhanced at a slightly reduced $a_w$, about 0.99, by the addition of 5% NaCl to CYA (Mogensen *et*

*al*., 2010c). This additive reduced fumonisin production by *Fusarium* species. Fumonisin was produced optimally by *A. niger* at 25–30 °C, whereas optimal temperatures for production were lower in *Fusarium* species (20–25 °C). Clearly, some evolution has occurred since the genes were transferred to *A. niger* (Mogensen *et al*., 2010c).

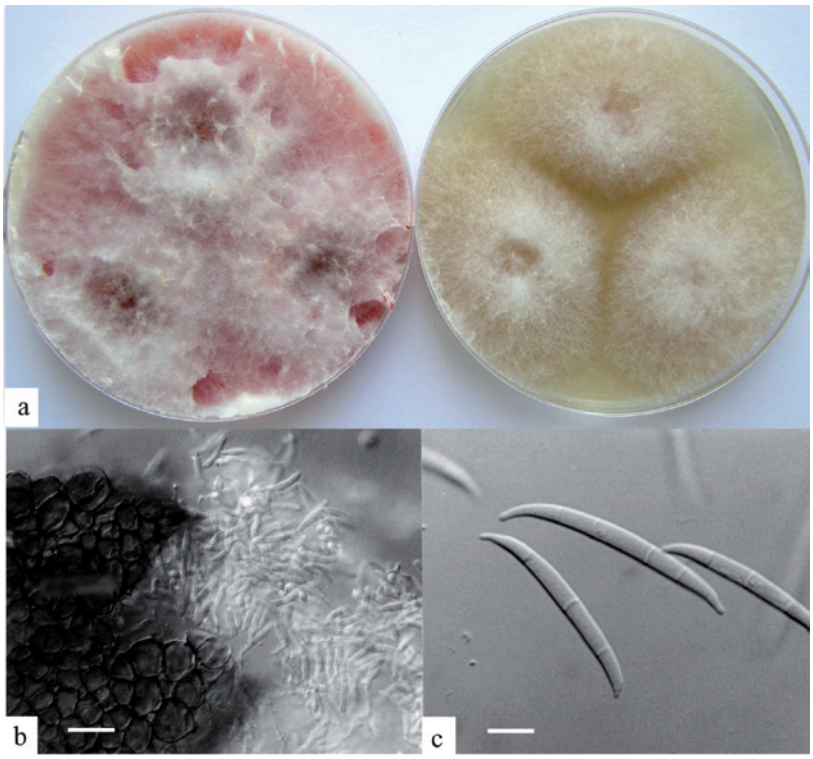
At this time, it is difficult to assess the relative importance of fumonisin production by *A. niger*, but the range of commodities in which fumonisins are found has been extended considerably. However, given the very high consumption of maize in some countries, production by *A. niger* will have lesser importance.

### *6.3 Deoxynivalenol, nivalenol, and zearalenone production by* Fusarium graminearum *and related species*

The most important *Fusarium* species that produce the trichothecenes deoxynivalenol (DON) and (less commonly) nivalenol (NIV) in small grains are *F. graminearum*, *F. culmorum*, and, less frequently, *F. crookwellense*.

The name *Fusarium roseum* as used by Snyder and Hansen (1940) has caused a great deal of confusion in the literature as their very broad species concept included several well-known and important *Fusarium* species, including *F. graminearum*. The literature before the mid-1980s is therefore unreliable, both taxonomically and with respect to mycotoxin production. Since the publications by Nelson *et al*. (1983) and Marasas *et al*. (1984), *F. graminearum* and *F. culmorum* have become well established and species–mycotoxin relationships have become clear. *F. crookwellense* is a widespread species first described in 1982.

**Fig. 1.9.** *Fusarium graminearum* (a) colonies on PDA (left) and DCPA (right), 7 days, 25 °C; (b) *Gibberella zeae* perithecium and ascospores, bar = 25 µm; (c) macroconidia, bar = 10 µm. Source: Pitt and Hocking (2009), Fig. 5.27, p. 103; reproduced with kind permission from Springer Science+Business Media B.V.




*Fusarium graminearum* Schwabe.

See Fig. 1.9. Colonies on rich media (PDA or PSA) at 25 °C grow rapidly, 7.5–9 cm in diameter in 4 days. There is abundant white, reddish or yellowish brown aerial mycelium, and the reverse is usually distinctly red. About 5% of isolates have an orange brown reverse. On weak media (CLA or SNA) at 25 °C, almost colourless sporodochia are produced under a sparse layer of white aerial mycelium. Structures bearing conidia have 1–2 levels of branching, terminating with 1–4 phialides. Macroconidia are usually 4–6 septate and abundantly produced in fresh isolates, mostly 40–60 µm long, more or less straight with parallel walls. No microconidia are produced, although sometimes immature macroconidia are seen and could be confused with microconidia.

Sexual fruiting structures (perithecia) are often produced on CLA or SNA as the medium begins to desiccate, almost black, about 200 µm in diameter, with a warty wall, and exuding a light orange cloud of ascospores. Ascospores are generally fusiform to allantoid (slightly bean-shaped), light brown, and 20–30 µm long. Ascospores are forcibly discharged and can be found on the agar surface away from the perithecia, or on Petri dish lids. The teleomorph of *F. graminearum* is *Gibberella zeae* (Schw.) Petch.

Distinctive features. *Fusarium graminearum* produces straight macroconidia with parallel walls, which in combination with rapid growth and usually red pigments on PDA, are relatively distinctive. The frequent occurrence of perithecia of the teleomorph in culture is also a

reliable characteristic, occurring in about 90% of fresh isolates under appropriate lighting conditions. *F. pseudograminearum* (teleomorph: *Gibberella coronicola*) is an ecologically and phylogenetically distinct species that causes crown rot of wheat. The macroconidia are similar to those of *F. graminearum*, but are reportedly broadest above the middle; the growth rate on PDA is slower. Diagnostic PCR primers for *F. pseudograminearum* were designed by Aoki and O'Donnell (1999) based on β-tubulin sequences. This species also produces DON and zearalenone (ZEA).

*Fusarium culmorum* (W. G. Sm.) Sacc. Colonies on rich media (PDA or PSA) at 25 °C grow rapidly, 7.5–9 cm in diameter in 4 days. There is abundant white to slightly orange, brown or reddish aerial mycelium, and the reverse is usually distinctly red. About 5% of isolates have an orange brown or tan reverse. On weak media (CLA or SNA) at 25 °C, reddish brown or orange sporodochia are produced under a sparse layer of white aerial mycelium. Structures bearing conidia have up to 4 levels of branching, terminating with 1–4 phialides. Macroconidia are usually 3–6 septate and abundantly produced in fresh isolates, mostly 30–45 µm long, with the widest point above the middle and hence somewhat wedge-shaped, often appearing short and fat. No microconidia are produced.

Distinctive features. Broad, wedge-shaped macroconidia with short apical cells and basal cells are distinctive for *F. culmorum*. The related species *F. sambucinum* produces narrower macroconidia than *F. culmorum*. The wedge shape of the conidia distinguishes *F. culmorum* from *F. crookwellense*.

*Fusarium crookwellense* Burgess *et al.* Colonies on rich media (PDA or PSA) at 25 °C grow rapidly, 7.5–9 cm in diameter in 4 days. There is abundant aerial mycelium, white to pink, and the reverse is usually distinctly red. On weak media (CLA or SNA) at 25 °C, sporodochia are usually abundant and distinctly reddish brown. Structures bearing conidia, arising from the sporodochia, have 1–4 levels of branching, terminating with 1–6 phialides. Macroconidia are usually 5 septate, 35–60 × 4.5–6.5 µm, fairly uniform in shape, with a more or less straight ventral wall and curved dorsal wall, with the widest point near the middle, of medium size. Microconidia are not produced, although sometimes immature macroconidia give this impression.

Distinctive features. Distinctive macroconidia, with straight inner walls and curved outer walls, characterize *Fusarium crookwellense*.

Factors affecting growth. The optimal temperature for growth of *Fusarium graminearum* is 25 °C, and the maximum below 37 °C. The minimum $a_w$ for growth is near 0.90 (Pitt and Hocking, 2009). *F. culmorum* is a psychrotroph, growing down to 0 °C but up to only 31 °C (Pitt and Hocking, 2009).

Trichothecenes in small grains: *Fusarium graminearum* and related species. Fusarium head blight is an important plant disease in temperate regions that affects small grains, mainly wheat, but also barley and triticale. Five or six *Fusarium* species are consistently isolated from small grains affected by this disease, and the most pathogenic species, *F. graminearum* and *F. culmorum*, are the most common. These two species are closely related and produce DON or NIV and ZEA, depending on the geographical origin of the isolate (Miller *et al.*, 1991; Waalwijk *et al.*, 2003). *F. graminearum* is common in wheat from North America, South America, and China (Miller, 1994). In cooler parts of Europe, *F. culmorum* has been dominant, but *F. graminearum* appears to be displacing it (Waalwijk *et al.*, 2003).

Concerning the other species involved in Fusarium head blight, *F. avenaceum* is also common in wheat from all regions studied. *F. crookwellense* is relatively common in Australia and South Africa, but is rare in wheat from Canada and the USA. *F. poae*, *F. langsethii*, *F. equiseti*, and *F. sporotrichioides* are also isolated from wheat kernels at low to moderate frequencies, more commonly under cooler conditions (Bottalico and Peronne, 2002). The distribution of head blight species is affected by pathogenicity, with a relative pathogenicity of *F. graminearum* > *F. culmorum* >> *F. crookwellense* > *F. avenaceum*. The regional and annual variation of the pathogenic species is most affected by temperature; species ranked from coldest to warmest areas are *F. culmorum* > *F. crookwellense* > *F. avenaceum* > *F. graminearum* (Miller, 1994; Bottalico and Peronne, 2002).

Isolates of *F. graminearum* and *F. culmorum* produce a fairly large number of other compounds as well as DON and ZEA. Isolates from North and South America produce 15-acetyl deoxynivalenol, the precursor to DON. If isolates from Asia and Europe produce DON or NIV, they also produce the respective 3-acetate, i.e. deoxynivalenol monoacetate or fusarenon-X. Strains of *F. crookwellense* produce NIV regardless of geographical origin (Miller *et al.*, 1991; Bottalico and Peronne, 2002, under the name *F. cerealis*). Some of the minor metabolites are found in small grains along with DON.

Although increased rainfall promotes Fusarium head blight, incidence is most affected by moisture at anthesis as long as the temperature remains in the favourable range for growth (Miller, 1994). Cultivar susceptibility and rainfall at anthesis explain most variability in infection,

but crop rotation also has a large effect. Growing wheat following maize increases disease under favourable weather conditions (Schaafsma *et al*., 2002). Reduced tillage is an equivocal source of variation in the amount of disease observed (Miller *et al*., 1998; Schaafsma *et al*., 2002).

*Fusarium graminearum* is a necrotrophic pathogen, i.e. it invades plants by killing host cells in advance. This was reported by the earliest investigators (see Schroeder and Christensen, 1963). Trichothecenes were recognized to be phytotoxic compounds at the time of their discovery (Brian *et al*., 1961). It was realized much later that large differences exist in the responses of wheat cultivars to Fusarium head blight. Coleoptile tissue of cultivars that were resistant to Fusarium head blight was 10 times as resistant to necrosis in the presence of DON than was that of disease-susceptible cultivars (Wang and Miller, 1988). This difference was shown to be due to the presence of a modified peptidyl transferase involved in protein synthesis (Miller and Ewen, 1997) and to unknown functional changes in the membranes of more resistant types (Snijders and Krechting, 1992; Cossette and Miller, 1995; Miller and Ewen, 1997). Earlier studies had shown that cultivars of wheat in the field appeared to be able to metabolize DON, and this was later shown to be the case in vitro in cultivars resistant to head blight (Miller and Arnison, 1986). Strains that produce high concentrations of DON in the field were more virulent (Snijders, 1994; Mesterhazy *et al*., 1999). This implied that one component of resistance to Fusarium head blight is related to reducing the phytotoxic impact of DON. In addition, DON has been found to appear in wheat kernels in advance of fungal mycelia (Snijders and Perkowski, 1990; Snijders and Krechting, 1992). The wheat cultivar Frontana is substantially more resistant to the membrane-damaging effects of DON than susceptible cultivars (Miller and Ewen, 1997).

## 7. Genus *Claviceps*

The following descriptions of *Claviceps* species are taken from Alderman *et al*. (1999).

The genus *Claviceps*, an Ascomycete with a conidial state, includes several species that are parasitic on grasses, including cultivated cereals throughout the temperate world. *Claviceps* species infect only the flowers of susceptible hosts. Infection involves replacement of the ovary by a specialized structure that develops into a sclerotium, a hard, compact mass of fungal tissue. The sclerotia are usually white, black, or tan and are 1–4 times as large as the seeds they replace. The sclerotia and diseases caused by *Claviceps* species go by the general name ergot. Ergots formed by the most important species, *C. purpurea*, are dark purple to black, and are most prevalent on rye, but also occur to some extent on barley, oats, and wheat, as well as wild and cultivated grasses.

During ergot development, conidia are produced by the fungus and are immersed in plant sap to produce a sugary liquid known as honeydew, which drips from the infected plant as large drops. These are attractive to insects, which act as vectors for dispersing the conidia and spreading infection throughout the crop.

Sclerotia are the resting stage of *Claviceps* species between seasons. Under favourable conditions sclerotia germinate, producing the ascomycete stage of the fungus. Ascospores are formed in closed bodies on stalks arising from the sclerotia, and in many species these provide the initial inoculum for infection of the next season's crop.

The sclerotia of many *Claviceps* species contain toxic alkaloids, poisonous to humans and animals, which have also found major use in pharmaceuticals.

Many *Claviceps* species are restricted to a few grass genera as hosts. However, the species most important from the mycotoxin viewpoint, *C. purpurea*, has a host range of more than 200 grass species. It is distributed worldwide in temperate climates and is responsible for the disease called ergotism in humans and domestic animals.

Identification. Species of *Claviceps* cannot be grown in culture, so must be identified by natural characteristics. *C. purpurea* is a pathogen on grasses, but not on sorghum or maize, whereas *C. africana*, also of importance in terms of mycotoxins, is a cause of a serious disease resulting in male-sterile sorghum seed.

*Claviceps purpurea* (Fr.: Fr.) Tul. The sclerotium of *Claviceps purpurea* comprises a compact mass of fungal tissue encased in a dark pigmented outer rind. This overwintering stage apparently requires 2 months of cold weather (0–10 °C) to induce germination. In warmer regions, sclerotia do not survive well.

In spring, the sclerotia germinate, producing stalked ascocarps, in which small, thread-like ascospores are produced. The ascospores are ejected forcibly from the ascocarp and are carried by air currents to grass flowers.

The period of susceptibility for most grasses is very brief, from flower opening to fertilization, as fertilized ovaries are resistant to infection. Environmental conditions that delay pollination, such as cool temperatures, increase the infective period. Male-sterile lines of grasses are especially susceptible to *Claviceps* infection because they are not pollinated.

Within a week after infection, conidia are produced in abundance, present in the sticky honeydew that drips from the flowers. The honeydew

acts as the main infective stage, being spread by insects, rain splash, or contact with uninfected flower heads.

Within about two weeks after infection, sclerotia begin to appear. Maturity of the sclerotia coincides with maturity of the infected grass seed heads.

## 8. Decision trees

The major commodities susceptible to mycotoxin formation are summarized in Figs 1.10 and 1.11, together with the major fungal species involved.

**Fig. 1.10.** Decision tree for directing risk management decisions or actions based on environmental considerations and probability of fungal contamination in warm climates. Expected toxic effects in susceptible animals are given for each group of mycotoxins.

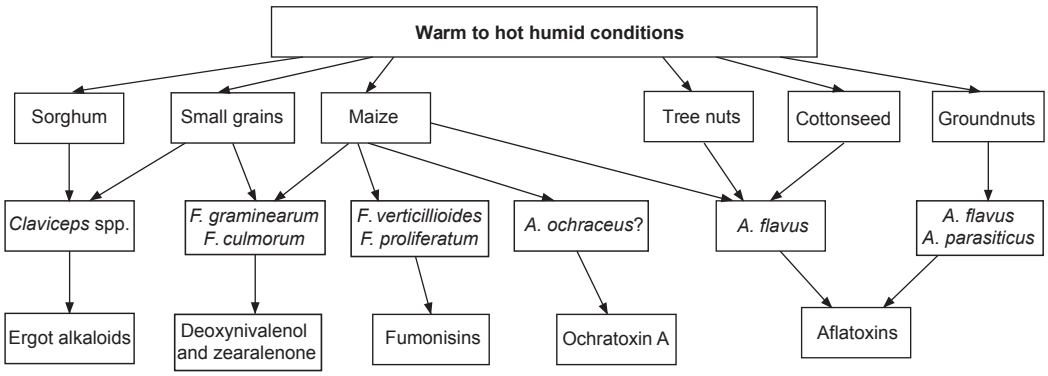
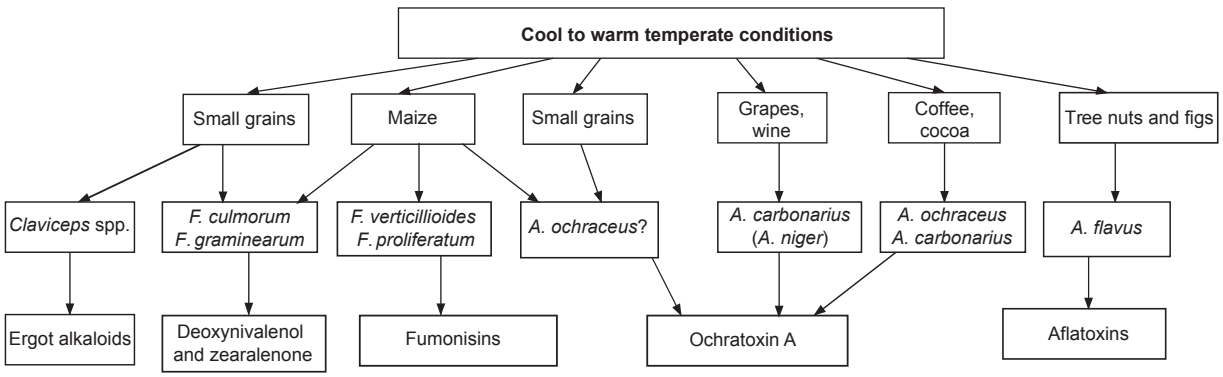



**Fig. 1.11.** Decision tree for directing risk management decisions or actions based on environmental considerations and probability of fungal contamination in cool climates. Expected toxic effects in susceptible animals are given for each group of mycotoxins.



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## ANNEX. MEDIA

The formulations given below are from Pitt and Hocking (2009).

### Aspergillus flavus and parasiticus agar (AFPA)

Peptone, bacteriological: 10 g
Yeast extract: 20 g
Ferric ammonium citrate: 0.5 g
Chloramphenicol: 100 mg
Agar: 15 g
Dichloran (0.2% in ethanol, 1.0 mL): 2 mg
Water, distilled: 1 L

Sterilize by autoclaving at 121 °C for 15 minutes. Final pH is 6.0–6.5.

### Czapek concentrate

$NaNO_3$: 30 g
KCl: 5 g
$MgSO_4.7H_2O$: 5 g
$FeSO_4.7H_2O$: 0.1 g
Water, distilled: 100 mL

Czapek concentrate will keep indefinitely without sterilization. The precipitate of $Fe(OH)_3$ that forms in time can be resuspended by shaking before use.

### Czapek–Dox iprodione dichloran agar (CZID)

Sucrose: 30 g
Yeast extract: 5 g
Chloramphenicol: 100 mg
Dichloran (0.2% in ethanol, 1.0 mL): 2 mg
Czapek concentrate: 10 mL
Trace metal solution: 1 mL
Agar: 15 g
Water, distilled: 1 L
Iprodione suspension: 1 mL

Sterilize by autoclaving at 121°C for 15 minutes. Add iprodione suspension (0.3 g Roval 50WP [Rhône-Poulenc Agrochimie, Lyon, France] in 50 mL sterile water, shaken before addition to medium) after autoclaving. This formulation is an adaptation of the original published formulation (Abildgren *et al.*, 1987), made from basic ingredients rather than using commercial Czapek–Dox broth. Chloramphenicol (100 mg/L) replaces the original combination of chlortetracycline (50 mg) and chloramphenicol (50 mg).

### Czapek yeast extract agar (CYA)

$K_2HPO_4$: 1 g
Czapek concentrate: 10 mL
Trace metal solution: 1 mL
Yeast extract, powdered: 5 g
Sucrose: 30 g
Agar: 15 g
Water, distilled: 1 L

Refined table grade sucrose is satisfactory for use in CYA provided it is free of sulfur dioxide. Sterilize by autoclaving at 121 °C for 15 minutes. Final pH is 6.7.

### Dichloran chloramphenicol peptone agar (DCPA)

Peptone: 15 g
$KH_2PO_4$: 1 g
$MgSO_4.7H_2O$: 0.5 g
Chloramphenicol: 0.1 g
Dichloran (0.2% in ethanol, 1.0 mL): 2 mg
Agar: 15 g
Water, distilled: 1 L

Sterilize by autoclaving at 121 °C for 15 minutes. Final pH is 5.5–6.0.

### Dichloran 18% glycerol agar (DG18)

Glucose: 10 g
Peptone: 5 g
$KH_2PO_4$: 1 g
$MgSO_4.7H_2O$: 0.5 g
Glycerol, A.R.: 220 g
Agar: 15 g
Dichloran (0.2% w/v in ethanol, 1.0 mL): 2 mg
Chloramphenicol: 100 mg
Water, distilled: 1 L

Add minor ingredients and agar to about 800 mL of distilled water. Steam to dissolve agar, then make up to 1 L with distilled water. Add glycerol; note that the final concentration is 18% w/w, not w/v. Sterilize by autoclaving at 121 °C for 15 minutes. Final $a_w$ is 0.955; final pH is 5.5–5.8.

### Dichloran rose bengal chloramphenicol agar (DRBC)

Glucose: 10 g
Peptone, bacteriological: 5 g
$KH_2PO_4$: 1 g
$MgSO_4.7H_2O$: 0.5 g
Agar: 15 g
Rose bengal (5% w/v in water, 0.5 mL): 25 mg
Dichloran (0.2% w/v in ethanol, 1.0 mL): 2 mg
Chloramphenicol: 100 mg
Water, distilled: 1 L

Sterilize by autoclaving at 121 °C for 15 minutes. Final pH is 5.5–5.8. Store prepared medium away from light; photoproducts of rose bengal are highly inhibitory to some fungi, especially yeasts. In the dark, the medium is stable for at least 1 month at 1–4 °C. The stock solutions of rose bengal and dichloran need no sterilization, and are also stable for very long periods.

### Dichloran rose bengal yeast extract sucrose agar (DRYS)

Yeast extract: 20 g
Sucrose: 150 g
Dichloran (0.2% in ethanol, 1.0 mL): 2 mg
Rose bengal (5% w/v in water, 0.5 mL): 25 mg
Chloramphenicol: 50 mg
Agar: 20 g
Water, distilled: to 1 L
Chlortetracycline (l% in water, filter-sterilized, 5.0 mL): 50 mg

Sterilize all ingredients except chlortetracycline by autoclaving at 121 °C for 15 minutes. Add

chlortetracycline after tempering to 50 °C. Chloramphenicol at twice the concentration specified (i.e. 100 mg/L) adequately controls bacteria in most situations, and this avoids the need for a second antibiotic that must be filter-sterilized.

### 25% Glycerol nitrate agar (G25N)

$K_2HPO_4$: 0.75 g
Czapek concentrate: 7.5 mL
Yeast extract: 3.7 g
Glycerol, analytical grade: 250 g
Agar: 12 g
Water, distilled: 750 mL

Glycerol for G25N should be of high quality, with a low (1%) water content. If a lower grade is used, allowance should be made for the additional water. Sterilize by autoclaving at 121 °C for 15 minutes. Final pH is 7.0.

### Malt extract agar (MEA)

Malt extract, powdered: 20 g
Peptone: 1 g
Glucose: 20 g
Agar: 20 g
Water, distilled: 1 L

Commercial malt extract used for home brewing is satisfactory for use in MEA, as is bacteriological peptone. Sterilize by autoclaving at 121 °C for 15 minutes. Do not sterilize for longer as this medium will become soft on prolonged or repeated heating. Final pH is 5.6.

### Potato dextrose agar (PDA)

Potatoes: 250 g
Glucose: 20 g
Agar: 15 g
Water, distilled: to 1 L

PDA prepared from raw ingredients is more satisfactory than commercially prepared media. Wash the potatoes, which should not be of a red skinned variety, and dice or slice, unpeeled, into 500 mL of water. Steam or boil for 30–45 minutes. At the same time, melt the agar in 500 mL of water. Strain the potato through several layers of cheesecloth into the flask containing the melted agar. Squeeze some potato pulp through also. Add the glucose, mix thoroughly, and make up to 1 L with water if necessary. Sterilize by autoclaving at 121 °C for 15 minutes.

CHAPTER 2.

# Chemical and physical characteristics of the principal mycotoxins


## Summary

This chapter provides information about the chemical and physical properties of the mycotoxins considered in this book: aflatoxins; fumonisins; ochratoxin A; trichothecenes, especially deoxynivalenol and nivalenol; zearalenone; and ergot alkaloids. This information about structures reveals the chemical diversity of mycotoxins, which is relevant to the wide range of toxicological effects in animals and humans discussed later in the book.


## 1. Aflatoxins

### *1.1 Formulae and structures*

Aflatoxin $B_1$. Chemical Abstracts (CA) name: (6aR,9aS)-2,3,6a,9a-tetrahydro-4-methoxycyclopenta[*c*]furo-(3′,2′:4,5]-furo[2,3-*h*][*l*]benzopyran-1,11-dione. Chemical Abstracts Service (CAS) registry number: 1162-65-8. Molecular formula: $C_{17}H_{12}O_6$. Molecular weight: 312.3.

Aflatoxin $B_2$. CA name: (6aR,9aS)-2,3,6a,8,9,9a-hexahydro-4-methoxycyclopenta[*c*]furo(3′,2′:4,5]furo[2,3-*h*]-[*l*]benzopyran-1,11-dione. CAS registry number: 7220-81-7. Molecular formula: $C_{17}H_{14}O_6$. Molecular weight: 314.3.

Aflatoxin $G_1$. CA name: (7aR,10aS)-3,4,7a,10a-tetrahydro-5-methoxy-1*H*,12*H*-furo-(3′,2′:4,5]furo[2,3-*h*]pyrano-[3,4-*c*][*l*]benzopyran-1,12-dione. CAS registry number: 1165-39-5. Molecular formula: $C_{17}H_{12}O_7$. Molecular weight: 328.3.

Aflatoxin $G_2$. CA name: (7aR,10aS)-3,4,7a,9,10,10a-hexahydro-5-methoxy-1*H*,12*H*-furo-(3′,2′:4,5]furo[2,3-*h*]pyrano-[3,4-*c*][*l*]benzopyran-1,12-dione.CAS registry number: 7241-98-7. Molecular formula: $C_{17}H_{14}O_7$. Molecular weight: 330.3.

Aflatoxin $M_1$. CA name: (6aR,9aR)-2,3,6a,9a-tetrahydro-9a-hydroxy-4-methoxycyclopenta[*c*]furo-(3′,2′:4,5]-furo[2,3-*h*][*l*]benzopyran-1,11-dione. CAS registry number: 6795-23-9. Molecular formula: $C_{17}H_{12}O_7$. Molecular weight: 328.3.

Structures of aflatoxins are shown in Fig. 2.1.

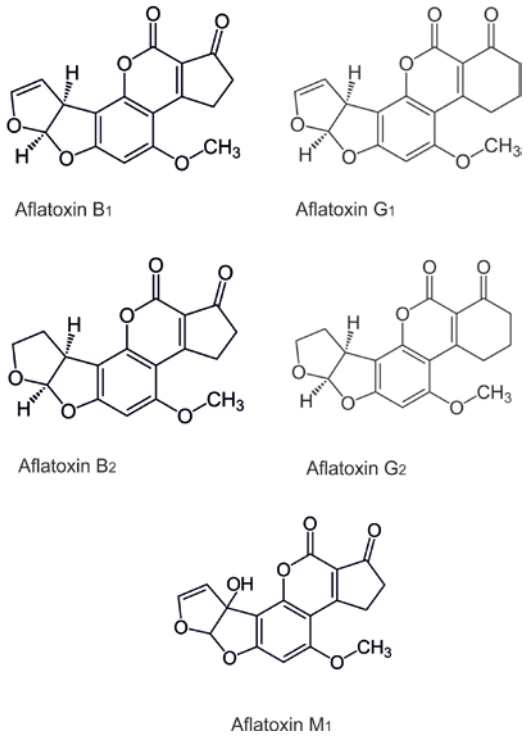
### *1.2 Physical data*

Descriptions. Colourless to pale-yellow crystals. Fluorescence in ultraviolet (UV) light: aflatoxins $B_1$ and $B_2$, blue; aflatoxins $G_1$ and $G_2$, green; aflatoxin $M_1$, blue–violet.

Melting-points. See Table 2.1.

Spectral properties. For UV absorption, see Table 2.1. Fluorescence excitation and emission data are not listed in Table 2.1 because they depend on the type of instrument, the solvent, and the supporting media

**Fig. 2.1.** Structures of aflatoxins




used. For those data, see Wogan (1966), Robertson and Pons (1968), Kiermeier and Kroczek (1974), and Uwaifo *et al.* (1977).

For mass and nuclear magnetic resonance (NMR) spectral data, see Bycroft *et al.* (1970), Stubblefield *et al.* (1970), and Cole and Schweikert (2003).

Specific rotation. $[\alpha]_D$ in chloroform, –558° (aflatoxin $B_1$), –430° (aflatoxin $B_2$), –556° (aflatoxin $G_1$), –473° (aflatoxin $G_2$); $[\alpha]_D$ in dimethylformamide, –280° (aflatoxin $M_1$) (Cole and Schweikert, 2003).

### *1.3 Chemical data*

Solubility. Insoluble in non-polar solvents. Slightly soluble in water (10–20 µg/mL). Freely soluble in moderately polar organic solvents (e.g. chloroform, methanol), especially in dimethyl sulfoxide (Cole and Cox, 1981; O'Neil *et al.*, 2001).

Stability. Unstable to UV light in the presence of oxygen. Unstable to extremes of pH (< 3 or > 10). Unstable in the presence of oxidizing agents (Castegnaro *et al.*, 1980, 1991).

Reactivity. Under alkaline conditions, the lactone ring opens and the aflatoxins are apparently absent. However, the reaction is reversible upon acidification.

Ammoniation at high temperature and high pressure opens the lactone ring and results in decarboxylation. This reaction is not reversible.

## 2. Fumonisins

### *2.1 Formulae and structures*

Fumonisin $B_{1}$. CA name: 1,2,3-propanetricarboxylic acid, 1,1′-[1-(12-amino-4,9,11-trihydroxy-2-methyltridecyl)-2-(1-methylpentyl)-1,2-ethanediyl] ester. CAS registry number: 116355-83-0. Molecular formula: $C_{34}H_{59}NO_{15}$. Molecular weight: 721.

Fumonisin $B_{2}$. CA name: 1,2,3-propanetricarboxylic acid, 1,1′-[1-(12-amino-9,11-dihydroxy-2-methyltridecyl)-2-(1-methylpentyl)-1,2-ethanediyl] ester. CAS registry number: 116355-84-1. Molecular formula: $C_{34}H_{59}NO_{14}$. Molecular weight: 705.

Structures of fumonisins are shown in Fig. 2.2.

### *2.2 Physical data*

Unless otherwise noted, data are from WHO (2000).

Description. White hygroscopic powder.

Melting-point. Not known (compounds have not been crystallized).

Spectral properties. For mass and NMR spectral data, see Bezuidenhout *et al.* (1988), Laurent *et al.* (1989), Plattner *et al.* (1990), Savard and Blackwell (1994), and Cole *et al.* (2003a).

### *2.3 Chemical data*

Solubility. Soluble in methanol, in acetonitrile–water, and in water (at least 20 g/L) (NTP, 2001).

Stability. Stable in acetonitrile–water (1:1) at 25 °C. Unstable in methanol at 25 °C, forming monomethyl and dimethyl esters (Gelderblom *et al.*, 1992; Visconti *et al.*, 1994). Stable in methanol at –18 °C (Visconti *et al.*, 1994). Stable in buffer solutions over the pH range 4.8–9 at 78 °C (Howard *et al.*, 1998).

Octanol–water partition coefficient for fumonisin $B_{1}$. log $P$ = 1.84 (Norred *et al.*, 1997).

## 3. Ochratoxin A

### *3.1 Formula and structure*

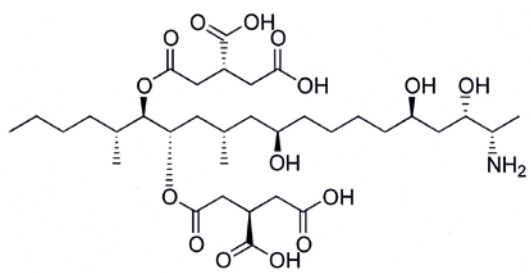
Ochratoxin A. CA name: *N*-[(5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1*H*-2-benzopyran-7-yl)carbonyl]-L-phenylalanine. CAS registry number:

**Table 2.1.** Melting-points and ultraviolet absorption of aflatoxins

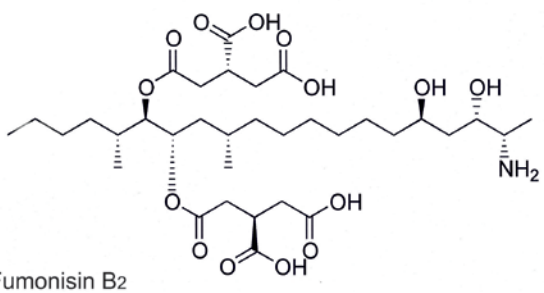
| Aflatoxin | Melting-point (°C) | Ultraviolet absorption | |
|---|---|---|---|
| | | $\lambda_{max}$(nm) | $\varepsilon$ (L·mol$^{-1}$·cm$^{-1}$) × 10$^{-3}$ |
| $B_1$ | 268–269 (decomposition) (crystals from $CHCl_3$) | 223 | 25.6 |
| | | 265 | 13.4 |
| | | 362 | 21.8 |
| $B_2$ | 286–289 (decomposition) (crystals from $CHCl_3$–pentane) | 265 | 11.7 |
| | | 363 | 23.4 |
| $G_1$ | 244–246 (decomposition) (crystals from $CHCl_3$–methanol) | 243 | 11.5 |
| | | 257 | 9.9 |
| | | 264 | 10.0 |
| | | 362 | 16.1 |
| $G_2$ | 237–240 (decomposition) (crystals from ethyl acetate) | 265 | 9.7 |
| | | 363 | 21.0 |
| $M_1$ | 299 (decomposition) (crystals from methanol) | 226 | 23.1 |
| | | 265 | 11.6 |
| | | 357 | 19.0 |

Data from O'Neil *et al.* (2001).

**Fig. 2.2.** Structures of fumonisins




Fumonisin $B_1$




Fumonisin $B_2$

303-47-9. Molecular formula: $C_{20}H_{18}ClNO_6$. Molecular weight: 403.8.

The structure of ochratoxin A (OTA) is shown in Fig. 2.3.

### 3.2 Physical data

Description. White odourless crystalline solid (Pohland *et al.*, 1982). Intensely fluorescent in UV light, emitting green and blue fluorescence in acid and alkaline solutions, respectively, due to two different forms, i.e. closed or open lactone ring, respectively.

Melting-point. 159 °C when recrystallized from benzene–hexane (Natori *et al.*, 1970); 169 °C when recrystallized from xylene (Van der Merwe *et al.*, 1965a, 1965b); 168–173 °C after drying for 1 hour at 60 °C (Pohland *et al.*, 1982).

Specific rotation. $[\alpha]^{20}_D$ –118° (c = 1.1 mmol/L in chloroform) (Van der Merwe *et al.*, 1965a, 1965b); $[\alpha]^{21}_D$ –46.8° (c = 2.65 mmol/L in chloroform) (Pohland *et al.*, 1982).

UV spectrum. At $\lambda_{max}$ of 214, 282, and 332 nm, extinction coefficients of $37.2 \times 10^{-3}$, $0.89 \times 10^{-3}$, and $63.3 \times 10^{-3}$ $L \cdot mol^{-1} \cdot cm^{-1}$, respectively, have been reported (Cole and Cox, 1981).

Other spectral properties. For infrared (IR) spectra, see Van der Merwe *et al.* (1965a, 1965b), Steyn and Holzapfel (1967), and Pohland *et al.* (1982). For NMR spectra, see Pohland *et al.* (1982) and Cole *et al.* (2003b). For mass spectra, see Pohland *et al.* (1982) and Cole *et al.* (2003b).

### 3.3 Chemical data

Solubility. Moderately soluble in polar organic solvents (e.g. chloroform, ethanol, methanol).

Stability. OTA is partially degraded under normal cooking conditions (Müller, 1983). The stability of OTA to heating conditions depends on the water activity of the medium (Subirade, 1996; Van der Stegen *et al.*, 2001).

Reactivity. The lactone ring opens under alkaline conditions, but the reaction is reversible. Solutions of OTA are completely degraded by treatment with an excess of sodium hypochlorite.

## 4. Deoxynivalenol

### 4.1 Formula and structure

Deoxynivalenol. CA name: 12,13-epoxy-3,7,15-trihydroxy-(3α,7α)-trichothec-9-en-8-one. CAS registry number: 51481-10-8. Molecular formula: $C_{15}H_{20}O_6$. Molecular weight: 296.32.

The structure of deoxynivalenol (DON) is shown in Fig. 2.4.

### 4.2 Physical data

Description. White needles.

Melting-point. 151–153 °C.

Specific rotation. $[\alpha]^{20}_D$ +6.35° (c = 0.07 mmol/L in ethanol).

Spectral properties. IR, UV, NMR, and mass spectral data have been reported (Cole and Cox, 1981; Cole *et al.*, 2003c).

### 4.3 Chemical data

Solubility. Soluble in chloroform, ethanol, methanol, and ethyl acetate.

Stability. Autoclaving creamed maize reduced DON content by only 12% (Wolf-Hall *et al.*, 1999). At pH 4.0, DON appeared to be very stable, showing no destruction at 100 °C or 120 °C and only partial destruction at 170 °C after 60 minutes. At pH 7.0, DON was still stable but showed more destruction at 170 °C after 15 minutes. At pH 10.0, DON was partially destroyed at 100 °C after 60 minutes and was totally destroyed at 120 °C after 30 minutes and at 170 °C after 15 minutes (Wolf and Bullerman, 1998).

When DON was gamma-irradiated on maize, breakdown of DON began only after irradiation to 20 kGy, and 80–90% of the DON remained after irradiation to 50 kGy (O'Neill *et al.*, 1993).

No significant decomposition of DON was observed when stored in ethyl acetate for 24 months at 25 °C or 3 months at 40 °C (Widestrand and Pettersson, 2001). DON was relatively stable in buffer solutions over the pH range 1–10 (Lauren and Smith, 2001).

## 5. Nivalenol

### 5.1 Formula and structure

Nivalenol. CA name: 12,13-epoxy-3,4,7,15-tetrahydroxy-(3α,4β,7α)-trichothec-9-en-8-one. CAS registry number: 23282-20-4. Molecular formula: $C_{15}H_{20}O_7$. Molecular weight: 312.32.

The structure of nivalenol (NIV) is shown in Fig. 2.4.

### 5.2 Physical data

Description. White crystals.

Melting-point. 222–223 °C (with decomposition, after drying in the presence of $P_2O_5$ at reduced pressure).

Specific rotation. $[\alpha]^{20}_D$ +21.54° (c = 1.3 mmol/L in ethanol).

Spectral properties. IR, UV, NMR, and mass spectral data have been reported (Cole and Cox, 1981; Brumley *et al.*, 1982; Cole *et al.*, 2003c).

### 5.3 Chemical data

Solubility. Soluble in chloroform, ethanol, methanol, and ethyl acetate; slightly soluble in water; soluble in polar organic solvents (Budavari, 1989).

Stability. No significant decomposition of NIV was observed when stored in ethyl acetate for 24 months at 25 °C or for 3 months at 40 °C. A significant decrease of NIV stored

**Fig. 2.3.** Structure of ochratoxin A

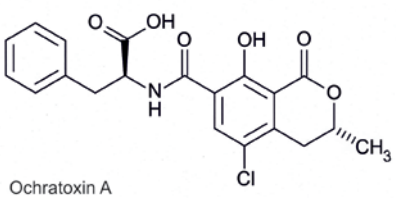



**Fig. 2.4.** Structures of major trichothecenes

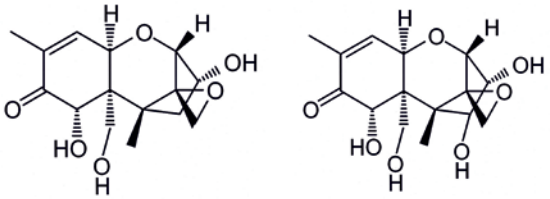



**Fig. 2.5.** Structure of zearalenone

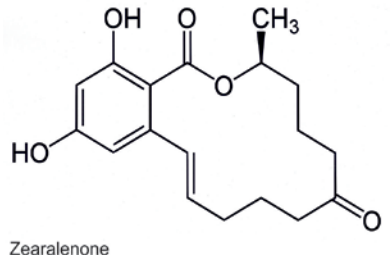
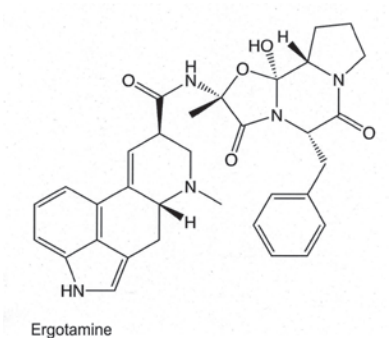



**Fig. 2.6.** Structure of ergotamine




as a thin film was observed after 9 months at 25 °C (Widestrand and Pettersson, 2001). NIV is relatively stable in buffer solutions over the pH range 1–10 (Lauren and Smith, 2001).

## 6. Zearalenone

### *6.1 Formula and structure*

Zearalenone. CA name: 3,4,5,6,9,10-hexahydro-14,16-dihydroxy-3-methyl-1*H*-2-benzoxacyclotetradecin-1,7(8*H*)-dione. CAS registry number: 17924-92-4. Molecular formula: $C_{18}H_{22}O_5$. Molecular weight: 318.4.

The structure of zearalenone (ZEA) is shown in Fig. 2.5.

### *6.2 Physical data*

Description. White crystals.

Melting-point. 164–165 °C.

Specific rotation. $[\alpha]^{25}_D$ –170.5° (c = 1.0 mmol/L in methanol); $[\alpha]^{21}_D$ –189° (c = 3.14 mmol/L in chloroform).

Spectral properties. IR, UV, proton NMR, and mass spectral data have been reported (Cole and Cox, 1981). The molar absorptivities of ZEA in acetonitrile at 236, 274, and 314 nm were established, and a common reference wavelength of 274 nm with molar absorptivity of 12 623 ± 111 $L \cdot mol^{-1} \cdot cm^{-1}$ was recommended for ZEA in acetonitrile (Josephs *et al.*, 2003).

### *6.3 Chemical data*

Solubility. Solubilities at 25 °C in percent by weight are: water, 0.002; *n*-hexane, 0.05; benzene, 1.13; acetonitrile, 8.6; dichloromethane, 17.5; methanol, 18; ethanol, 24; and acetone, 58 (Hidy *et al.*, 1977).

Stability. ZEA was stable when heated at 120 °C; 29% decomposed when heated at 150 °C for 60 minutes and 69% when heated at 200 °C for 60 minutes (Kuiper-Goodman *et al.*, 1987). Stable to hydrolysis in neutral or acid buffer solutions (Müller, 1983). Less than 23% of ZEA was lost when heated in aqueous buffer solution to 125 °C for 60 minutes, but 34–68% was lost after 60 minutes at 150 °C, depending on the pH of the buffer. More than 92% was lost after 60 minutes at 175 °C, and complete loss was observed in < 30 minutes at 225 °C, regardless of pH. ZEA was most stable at pH 7, and the greatest losses occurred above 175 °C (Ryu *et al.*, 2003).

Extrusion cooking of maize grits resulted in significant reductions of ZEA with either mixing screws or non-mixing screws, but use of mixing screws was somewhat more effective (66–83% reduction) overall than non-mixing screws (65–77%). Greater reduction of ZEA content was observed at either 120 °C or 140 °C than at 160 °C (Ryu *et al.*, 1999).

ZEA content was not reduced by heating at 110 °C for 12 days after treatment with a sodium bicarbonate solution (Lauren and Smith, 2001).

## 7. Ergot alkaloids

Ergots, the sclerotia produced by *Claviceps purpurea* and related species, contain a remarkable variety of compounds, which can be divided into three groups: derivatives of lysergic acid, derivatives of isolysergic acid, and clavines. The most important of these is ergotamine.

### *7.1 Formula and structure*

Ergotamine. CA name: 12′-hydroxy-2′-methyl-5′-(phenylmethyl)-ergotaman-3′,6′,18-trione. CAS registry number: 113-15-5. Molecular formula: $C_{33}H_{35}N_5O_5$. Molecular weight: 581.66.

The structure of ergotamine is shown in Fig. 2.6.

### *7.2 Physical data*

Description. White powder.

Melting-point. 180 °C.

Spectral properties. UV, IR, and fluorescence spectral data were reviewed by Hofmann (1964). The electron mass spectrum of ergotamine was described by Vokoun and Řehāček (1975), and the [$^1$H]-NMR spectrum was reported by Pierri *et al*. (1982).

Specific rotation. $[\alpha]^{20}_D$ –160°.

### *7.3 Chemical data*

Solubility. Some data on recrystallization, appearance, and solubility were reviewed by Hofmann (1964).

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CHAPTER 3.

# Sampling and sample preparation methods for determining concentrations of mycotoxins in foods and feeds




## Summary

Sample variation is often the largest error in determining concentrations of mycotoxins in food commodities. The worldwide safety evaluation of mycotoxins requires sampling plans that give acceptably accurate values for the levels of contamination in specific batches or lots of a commodity. Mycotoxin concentrations show a skewed or uneven distribution in foods and feeds, especially in whole kernels (or nuts), so it is extremely difficult to collect a sample that accurately represents the mean batch concentration. Sample variance studies and sampling plans have been published for select mycotoxins such as aflatoxin, fumonisin, and deoxynivalenol, emphasizing the importance of sample selection, sample size, and the number of incremental samples. For meaningful data to be generated from surveillance studies, representative samples should be collected from carefully selected populations (batches or lots) of food that, in turn, should be representative of clearly defined locations (e.g. a country, a region within a country). Although sampling variability is unavoidable, it is essential that the precision of the sampling plan be clearly defined and be considered acceptable by those responsible for interpreting and reporting the surveillance data. The factors influencing variability are detailed here, with reference to both major mycotoxins and major commodities. Sampling of large bag stacks, bulk shipments, and domestic supplies are all discussed. Sampling plans currently accepted in international trade are outlined. Acceptance sampling plans and the variabilities that affect operating characteristic curves of such plans are also detailed. The constraints and issues related to the sampling of harvested crops within subsistence farming areas are also discussed in this chapter, as are the essential rules of sample labelling and storage. The chapter concludes with a short section on sample preparation methods.


## 1. Introduction

Mycotoxin concentrations in foods and feeds are controlled in various ways; the most important are good agricultural practice and good manufacturing practice accompanied by end-product testing, product monitoring by regulators, and safety evaluation of foods on a national or worldwide basis. Each of these approaches requires efficient sampling and sample preparation methods. End-product testing, for

example, requires the collection of representative samples from defined batches, whereas product monitoring by regulators is more likely to focus on the safety of a particular batch (e.g. a 20 t batch of groundnut kernels) or the safety of an individual retail pack (e.g. a jar of groundnut butter). The safety evaluation of mycotoxins requires sampling plans that give acceptably accurate values for the levels of mycotoxin contamination in a variety of foods throughout the world.

When sampling is undertaken, it is essential that the following are clearly defined: the aim of the sampling exercise, the nature of the population being sampled, the sampling method, the efficiency of the sampling method, and the sample preparation method. In addition, the sampling plans may vary according to the kind of mycotoxin being analysed.

## 2. Sampling variability associated with testing for mycotoxins

### *2.1 Aflatoxins*

In sampling of commodities for aflatoxins, sampling variance is known to be a major contributor to the total variance associated with an analysis. Aflatoxin concentrations show a skewed distribution in foods and feeds, especially in whole kernels, so it is extremely difficult to collect a sample that accurately represents the mean batch concentration (Whitaker and Wiser, 1969). This problem has been extensively investigated: Whitaker *et al*. (1995) reported on the sampling, sample preparation, and analytical variances associated with the testing of groundnuts (during farm storage and after shelling), shelled maize, and cottonseed.

Whitaker *et al*. (1976) studied the impact of sample size on the precision of sampling plans used to test batches of cottonseed for aflatoxin. They showed that the collection of a sample of 1 kg from a cottonseed batch contaminated with 100 µg/kg aflatoxin was associated with a standard deviation of 87 µg/kg, i.e. replicate 1 kg samples that fell within 2 standard deviations of the mean had aflatoxin concentrations within the range 0–271 µg/kg (Table 3.1). However, the collection of a 32 kg sample reduced the standard deviation of the sample concentrations to 19 µg/kg and the range for samples that fell within 2 standard deviations to 64–136 µg/kg. In general, the sampling variance was halved each time the sample size was doubled. Similarly, the sample preparation variance was halved by doubling the subsample size and the analytical variance was halved by doubling the number of replicate analyses (Whitaker *et al*., 1976).

For the total variability to be reduced to an acceptable level, a cost-effective balance is needed between the sampling, sample preparation, and analytical variances. For example, increasing the sample size beyond a certain point may be less cost-effective than increasing the subsample size and/or the number of replicate samples. It is important to realize that variability in sampling is much higher than variability in sample preparation or analyses. Adequate sampling is extremely important.

### *2.2 Aflatoxin $M_1$*

A European Commission (EC) Directive (European Commission, 1998a), together with an EC Decision (European Commission, 1991), specifies the sampling method to be used to determine aflatoxin $M_1$ levels in liquid milk. After the batch of milk is mixed, by manual or mechanical means, a minimum sample of 0.5 kg or 0.5 L is collected, composed of at least 5 increments. The batch is accepted if the concentration of aflatoxin $M_1$ in the sample does not exceed the permitted limit.

**Table 3.1.** Effect of sample size on the range of results bracketing the 95% confidence limits in a cottonseed lot containing 100 µg/kg aflatoxins

| Sample size (kg) | Standard deviation (µg/kg) | Concentration of aflatoxins in sample (µg/kg) | | |
|---|---|---|---|---|
| | | Low[a] | High[b] | Range |
| 1 | 87 | 0 | 271 | 271 |
| 2 | 62 | 0 | 222 | 222 |
| 4 | 45 | 13 | 187 | 174 |
| 8 | 32 | 37 | 163 | 126 |
| 16 | 24 | 53 | 147 | 94[c] |
| 32 | 19 | 64 | 136 | 72 |

[a] The low concentration value was calculated as 100 – (1.96 * standard deviation); a value of 0 was recorded if a negative result was obtained.
[b] The high concentration value was calculated as 100 + (1.96 * standard deviation).
[c] CAST (2003) number was 95.
Adapted, by permission of the publisher, from CAST (2003).

The United States Food and Drug Administration/Office of Regulatory Affairs' Compliance Policy Guide (FDA/ORA, 1996) stipulates that samples of milk for the determination of aflatoxin $M_1$ should consist of at least 10 lb (4.5 kg), composed of not less than 10 randomly selected portions.

Although the distribution of aflatoxin $M_1$ in liquid milk may be expected to be reasonably homogeneous, no studies have been reported that evaluated the sampling variance associated with this mycotoxin–commodity combination.

### 2.3 Fumonisins

The sampling variability associated with the testing of shelled maize for fumonisin was analysed by Whitaker *et al*. (1998). A bulk sample of 45 kg was taken from each of 24 batches of shelled maize, which had been harvested from 24 different fields in North Carolina, USA. Each bulk sample was riffle divided into 32 test samples of 1.1 kg, which were comminuted in a Romer mill (Romer Labs, Union, MO, USA). A nested design was used to determine the variability associated with each step (sampling, sample preparation, and analysis) of the fumonisin test procedure. Briefly, 10 of the 24 batches were selected, covering a wide range of fumonisin concentrations. For each selected batch, 10 comminuted test samples were randomly selected, and two 25 g portions were taken from each sample by riffle division. Finally, the concentrations of fumonisins $B_1$, $B_2$, and $B_3$ were determined with AOAC methods (Sydenham *et al*., 1996). At a batch contamination level of 2 mg/kg, the coefficient of variation (CV) associated with sampling, sample preparation (Romer mill and 25 g analytical portion), and analysis was 16.6%, 9.1%, and 9.7%, respectively; these values were independent of the fumonisin type ($B_1$, $B_2$, $B_3$, or total). The CV associated with the total test procedure (sampling, sample preparation, and analysis) was 21%, which was similar to that associated with the testing of shelled maize for aflatoxin with a similar test procedure.

If it is considered desirable to understand sampling variability at locations with quite different climate than the USA, the type of testing detailed above should be carried out using the methods and calculations provided by Whitaker *et al*. (1998).

### 2.4 Ochratoxin A

No sampling plans have been published for the determination of ochratoxin A (OTA) in foods. A preliminary study commissioned by the Food Standards Agency of the United Kingdom evaluated the application of the United States, United Kingdom, and Dutch sampling plans (see Table 3.5) and the EC sampling plan (European Commission, 1998a), which are all plans for the determination of aflatoxins in foods, to the determination of OTA in green coffee and wheat (Food Standards Agency, 2000). The green coffee study was performed on two batches containing low levels of OTA (0.9 µg/kg and 0.4 µg/kg), whereas the wheat study was performed on a single batch containing 5.6 µg/kg OTA. The distribution of OTA in the two coffee batches was relatively uniform, whereas the distribution in the single wheat batch was far more heterogeneous. The sampling simulation exercise indicated that the United Kingdom sampling plan (United Kingdom, 1992) would accurately predict the OTA concentration in batches of coffee but that a plan based on the EC sampling plan was required for the determination of OTA concentration in wheat. However, it was recognized that further studies were required to confirm the results of this preliminary investigation.

### 2.5 Deoxynivalenol

The variability associated with the testing of barley for deoxynivalenol (DON) was studied by Freese *et al*. (2000). Bulk samples of 225 kg were collected from 6 batches of barley, and each was riffle divided into 16 test samples each of 0.1 kg, 0.8 kg, and 7 kg. Each test sample was comminuted in a Romer mill, and 50 g portions were taken from each sample for the determination of DON concentration. An evaluation of the analytical results indicated that the variability associated with the sample preparation and analytical steps was more significant than the sampling variance for all sample sizes, and that variability was not significantly reduced by increasing the sample size. This finding is in contrast to the situation with groundnuts and maize because of the smaller grain size of barley.

In a further experiment by Freese *et al*. (2000), 10 samples of about 2.5 kg were taken from each of 10 truckloads of barley, using sampling methods prescribed by the Grain Inspection, Packers and Stockyards Administration (GIPSA, 1995). Each 2.5 kg sample was comminuted in a Romer mill, and two 50 g portions were taken from each sample. A single determination of DON concentration was performed on the extract from the first portion, whereas duplicate determinations were performed on the second portion, using an enzyme-linked immunosorbent assay (ELISA) procedure. In this instance, an evaluation of the variances associated with the sampling, sample preparation, and analytical steps indicated approximately equal contributions from each step. It was

concluded that sample sizes of 100–200 g were adequate, assuming that these samples were obtained by the riffle division of a large bulk sample. However, it was also concluded that batches may be stratified to different degrees, depending upon the amount of mixing that occurred during handling, and that stratification could have a significant impact on sampling variance. If stratification is suspected, larger sample sizes are desirable.

A similar approach was used in a study of the sampling, sample preparation, and analytical variances associated with the testing of wheat for DON (Whitaker *et al.*, 2000). A 20 kg bulk sample was taken from each of 24 commercial batches, and each sample was riffle divided into 32 test samples of 0.45 kg each. Each 0.45 kg sample was finely comminuted in a Romer mill, which was set to automatically produce a representative, comminuted 25 g portion. DON concentration was determined in each of 768 (24 × 32) 25 g portions using the Romer FluoroQuant fluorometric procedure, and the analytical data were used to determine the total variability (sampling plus sample preparation plus analytical). Next, 20 comminuted samples, with a wide range of DON levels, were selected from the residual 768 comminuted samples, and eight 25 g portions were taken from each of these samples by riffle division. The Romer FluoroQuant method was then used to determine the DON concentration in four replicate extracts prepared from each 25 g portion. The combined sample preparation and analytical variance, and the analytical variance alone, were estimated using SAS procedures (SAS, 1997). The CV associated with the total test procedure varied from 261.8% (for a batch concentration of 0.02 mg/kg DON) to 7.9% (for 14.38 mg/kg DON). For a batch concentration of 5.0 mg/kg DON, the CV associated with the sampling, sample preparation, and analytical steps was 6.3%, 10.0% and 6.3%, respectively. The sampling variance was specific to a 0.45 kg sample, the sample preparation variance to a Romer mill and a 25 g analytical sample, and the analytical variance to the Romer FluoroQuant method. The CV associated with the total test procedure was 13.4%. The low variance associated with the sampling step (relative to that for other mycotoxins and other commodities) is partly due to the high kernel count of wheat (about 30 kernels/g), which is about 10 times that for shelled maize and about 30 times that for shelled groundnuts.

### *2.6 T-2 and HT-2 toxins*

No sampling plans have been published for the determination of T-2 and HT-2 toxins in foods, and details of the sampling variability of these toxins have not been reported. However, the sampling plans outlined above for DON should be applicable.

## 3. Sampling and surveillance

Surveillance studies involve the collection of information on the mycotoxin contamination of selected batches of food or feed within specified regions.

For meaningful data to be generated from surveys or surveillance studies, representative samples should be collected from carefully selected populations of food (e.g. batches or lots, marketplaces, farmers' stores) that, in turn, should be representative of clearly defined locations (e.g. a country, a region within a country). Three examples of different types of materials to be sampled are shown in Fig. 3.1.

The large bag stack of maize (Fig. 3.1a) consists of several thousand bags. However, only a small proportion of this population is accessible for sampling without dismantling the complete stack; the sample collected is representative only of outer bags that are readily accessible. An evaluation of the mycotoxin content of the inner bags can be performed only during the construction or dismantling of the stack. Although the maize crib (Fig. 3.1b) contains far less material than the bag stack, a sample that represents the whole population can be obtained only by dismantling the complete crib. The very large bulk consignment of oilseed meal (Fig. 3.1c) is composed of tens of thousands of tonnes of material, which is discharged, ultimately, into a barge with a capacity of about 500 t. The discharge is a convenient sampling point where the meal may be sampled as it flows into the barge from the weighing tower. In this case, the population being tested is equivalent to the material contained in the barge, and sampling from a flowing stream ensures that the sample is representative of the whole population.

Once a population has been selected, it is equally important that samples are collected using a clearly defined sampling plan designed to produce a reasonably representative sample. Although sampling variability is unavoidable, it is essential that the precision of the sampling plan be clearly defined and be considered acceptable by those responsible for interpreting the surveillance data. If the samples are too small, a wide range of estimated levels of contamination with mycotoxins will be obtained for a given population, and there will be a strong probability that the average value will be significantly lower than the true value. It is equally important that enough samples be collected from each population to ensure that infrequently occurring, highly contaminated samples are included in the surveillance data.

**Fig. 3.1.** Examples of materials to be sampled: (a) large bag stack of maize; (b) crib of maize cobs; (c) very large bulk consignment of oilseed meal. (a, b) Photographs courtesy of Raymond Coker, Founder & Director at Raymond Coker Consulting Limited. (c) Source: Coker (1997);reproduced with permission from the Natural Resources Institute of the University of Greenwich.


## 3.1 Sampling whole kernels

The sampling variance associated with different numbers of grains or nuts in a sample was studied by Whitaker and Wiser (1969). They showed that, for a batch of groundnuts contaminated with 30 µg/kg aflatoxins, the sampling variance increased very significantly if the sample was smaller than 40 000 kernels but changed very gradually for larger samples. The recommended weight of bulk samples thus depends on the grain or kernel size of the commodity. The sample weight should be 30 kg for groundnuts, 10 kg for maize, and 5 kg for rice (European Commission, 1998a). Ideally, a bulk sample is composed of 100 primary samples.

The sampling of groundnut kernels, typically from a 20 t batch, is illustrated in Fig. 3.2. Here, a 20 kg sample is being produced by collecting 200 g primary samples from 100 bags. As mentioned previously, the population being evaluated is composed of only the accessible bags. However, if the stack is reasonably new (and, consequently, spoilage is not likely to have occurred), the accessible population may be assumed to be representative of the inner bags if the stack was assembled in an unbiased manner.

The distribution of aflatoxins in groundnuts and groundnut products is illustrated in Table 3.2. Bags from stacks were sampled systematically by collecting 100 g samples and then determining the aflatoxin level of each sample (Coker, 1998). Of 204 samples of raw, ungraded nuts, 15% contained aflatoxins, and the highest aflatoxin level was about 20 times the mean concentration of the samples. However, in a different set of samples, from graded and roasted nuts, only 1% of the samples were contaminated, but the highest level was about 200 times the mean concentration of the samples.

## 3.2 Sampling processed commodities

The very heterogeneous distribution of aflatoxins in whole groundnuts is illustrated in Table 3.2. After the batch of raw, ungraded nuts was crushed, aflatoxin was more uniformly distributed: every sample contained aflatoxin, and the highest level was 2.5 times the mean concentration of the samples (Coker, 1998).

In general, the distribution of aflatoxins in ground or comminuted commodities has been shown to be relatively homogeneous compared with that in whole kernels. Coker *et al.* (2000) found that the aflatoxin concentration of large (500 t) batches of crushed oilseeds, including copra cake, copra meal pellets, and palm kernel cake, could be determined using quite small samples composed of a small number of primary samples. For example, a sampling plan that had a precision (CV) of 9% involved the collection of just 20 primary samples of 100 g to produce a 2 kg composite sample.

## 3.3 Sampling point

An ideal point for sampling occurs while the batch is being transferred, for example from one holding vessel to another. Examples of this approach to sampling are shown in Fig. 3.3.

## 3.4 Proposed surveillance sampling plans

Some examples of sampling plans proposed for surveillance purposes are given in Table 3.3. In Table 3.3, “sample size” refers to samples produced by riffle division of large (e.g. 40 kg), unground, bulk samples, as described by Freese *et al.* (2000) and Whitaker *et al.* (2000) when evaluating the sampling variance associated with the testing of barley and wheat, respectively, for DON.

**Table 3.2.** Distribution of aflatoxins in groundnuts and groundnut products

| Product (total weight) | No. of samples of 100 g | No. of samples containing aflatoxin (%) | Range of aflatoxin concentrations (µg/kg) | Batch mean aflatoxin concentration (µg/kg) |
|---|---|---|---|---|
| Raw kernels, ungraded (10 t) | 84 | 13 (15%) | < 1–4000 | 200 |
| Roasted kernels, graded (15 t) | 200 | 2 (1%) | 7–600 | 3 |
| Ground kernels (5.6 t)[a] | 204 | 204 (100%) | 90–250 | 100 |

[a] Produced by crushing the 10 t batch of raw, ungraded kernels shown in the first row.
Adapted, by permission of the publisher, from Coker (1998).

**Table 3.3.** Summary of minimum sample sizes suggested for interpretation of surveillance data

| Commodity | Increments ($n$ × $y$ grams) | Minimum sample size (kg) |
|---|---|---|
| AFLATOXIN $M_1$ | | |
| Milk (liquid and dried) (e.g. raw, pasteurized, homogenized, UHT, skimmed, semi-skimmed, evaporated, infant formula)[a] | 5 × 100 | 0.5 |
| Milk products | 5 × 100 | 0.5 |
| FUMONISINS | | |
| Maize | | |
| Whole maize[b] | 50 × 100 | 5.0 |
| Maize on the cob[c] | 50 cobs | 7.5 |
| Maize flour | 10 × 100 | 1.0 |
| Maize meal | 10 × 100 | 1.0 |
| Maize grits | 10 × 100 | 1.0 |
| Bran[d] | 10 × 100 | 1.0 |
| Processed maize foods (e.g. cornflakes, tortilla chips, popcorn, muffin mix, starch)[e] | 10 × 100 | 1.0 |
| OCHRATOXIN A | | |
| Maize | | |
| Whole maize[f] | 50 × 100 | 5.0 |
| Maize on the cob[c] | 50 cobs | 7.5 |
| Maize grits | 10 × 100 | 1.0 |
| Processed maize foods (e.g. cornflakes, tortilla chips, popcorn, muffin mix, starch)[d] | 10 × 100 | 1.0 |
| Wheat | 30 × 100 | 3.0 |
| Barley | 30 × 100 | 3.0 |
| Rice (including dehusked and polished rice) | 30 × 100 | 3.0 |
| Peas and beans (including coffee beans)[f] | 30 × 100 | 3.0 |

**Table 3.3.** Summary of minimum sample sizes suggested for interpretation of surveillance data (continued)

| Commodity | Increments ($n$ × $y$ grams) | Minimum sample size (kg) |
|---|---|---|
| | OCHRATOXIN A | |
| Dried fruit (e.g. raisins, currants, sultanas, figs, dates, apricots) | 30 × 100 | 3.0 |
| Flour, meal, and bran of all origins | 10 × 100 | 1.0 |
| Bread[d] | 10 × 100 | 1.0 |
| Ground and instant coffee | 10 × 100 | 1.0 |
| Cocoa powder | 10 × 100 | 1.0 |
| Beverages (e.g. coffee, wine, grape juice)[a] | 5 × 100 | 0.5 |
| | DEOXYNIVALENOL AND T-2 AND HT-2 TOXINS[g] | |
| Maize | | |
| Whole maize[h] | 20 × 100 | 2.0 |
| Maize on the cob[c] | 20 cobs | 3.0 |
| Maize grits | 10 × 50 | 0.5 |
| Processed maize foods (e.g. cornflakes, tortilla chips, popcorn, muffin mix, starch)[i] | 10 × 50 | 0.5 |
| Wheat[j] | 20 × 50 | 1.0 |
| Barley[k] | 20 × 50 | 1.0 |
| Oats | 20 × 50 | 1.0 |
| Rye | 20 × 50 | 1.0 |
| Flour, meal, and bran of all origins[i] | 10 × 50 | 0.5 |
| Bread | 10 × 50 | 0.5 |

UHT, ultra-high-temperature pasteurized.
[a] European Commission (1998a). The sampling variance for mycotoxins in beverages is assumed to be similar to that for aflatoxin $M_1$ in milk.
[b] Whitaker *et al.* (1998). Sampling variance (CV, %) for fumonisins in maize is similar to that reported for aflatoxins in maize.
[c] Assuming that the core of a cob contributes about 30% of the total weight of a cob and that a cob yields about 100 g of kernels.
[d] Coker *et al.* (2000). Sampling variance in these commodities is assumed to be similar to that for aflatoxin in comminuted feeds. The suggested sampling plan is associated with a sampling precision (CV) of 12.5% for aflatoxin in comminuted feeds.
[e] The minimum sample size for comminuted/processed foods is set at half the sample weight required for estimating fumonisins in whole kernels.
[f] The sampling variability for ochratoxin A is assumed to be similar to that for fumonisins (and aflatoxins; see footnote b).
[g] No data on sampling variance for T-2 or HT-2 toxins; assumed to be similar to sampling variance for deoxynivalenol.
[h] The minimum sample size for maize is set at double the sample weight required for estimating deoxynivalenol in wheat and barley.
[i] The minimum sample size for deoxynivalenol in cereal products is arbitrarily set at half the sample weight required for estimating fumonisins and ochratoxin A in cereal products.
[j] Whitaker *et al.* (2000).
[k] Freese *et al.* (2000).

**Fig. 3.2.** Sampling a 20 t batch of groundnuts: (a) collecting primary samples; (b) a sampling probe. Source: Coker (1997); reproduced with permission from the Natural Resources Institute of the University of Greenwich.

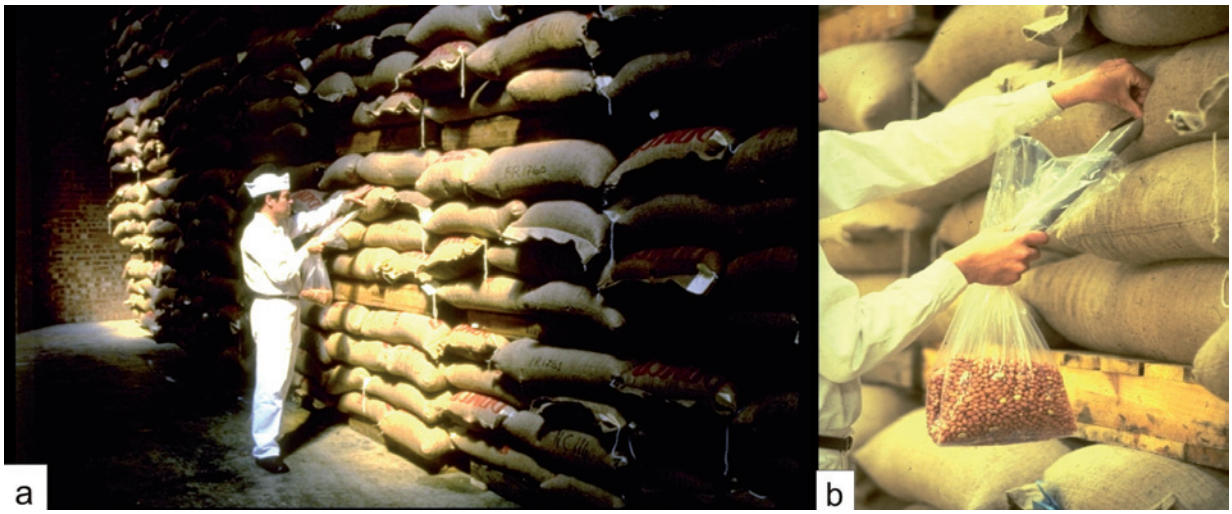
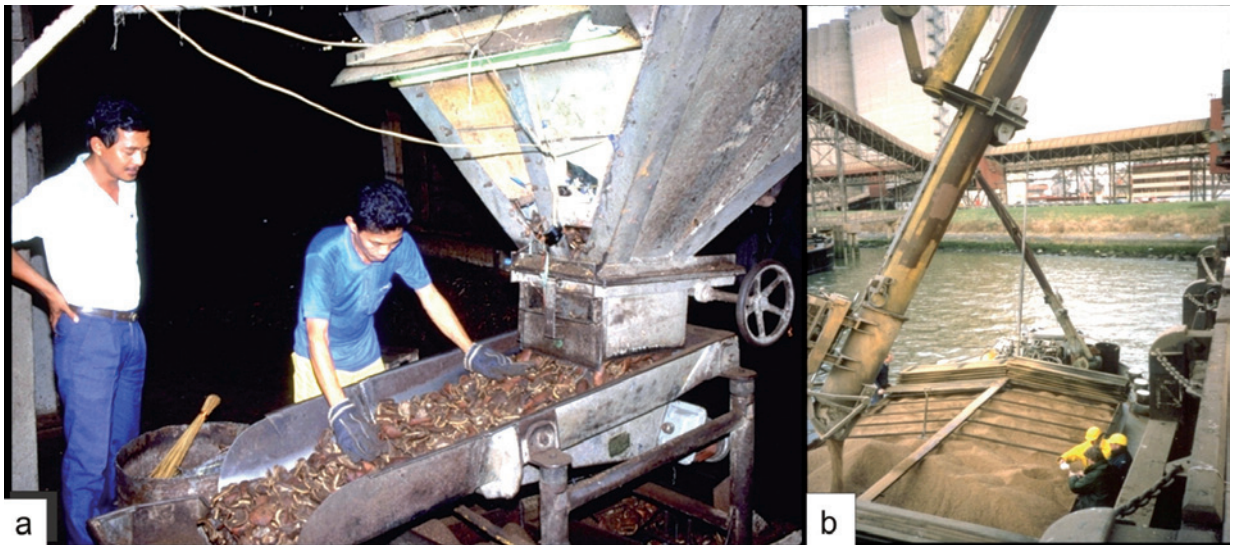



**Fig. 3.3.** Sampling of mobile batches: (a) copra cake emerging from a hopper; (b) sampling a 500 t batch of oilseed meal as it flows into a barge. Photographs courtesy of Raymond Coker, ToxiMet Limited.



The values shown are minimum sample sizes and minimum numbers of incremental samples for effective sampling. Because these values are based on currently available information, these sampling plans may change as more comprehensive data become available.

As batches of foods move along the food-chain, the heterogeneity of their mycotoxin distribution will decrease with increased handling. Therefore, it may be more appropriate to collect smaller samples directly from batches of foods of approximately the size of the samples shown in Table 3.3. The number of incremental samples proposed reflects the distribution (or anticipated distribution) of the mycotoxin in that particular commodity.

It is important that enough batches are sampled to ensure that infrequently occurring, highly contaminated batches are included in the surveillance data. The number of populations required in a given region depends on the variability in mycotoxin concentration among the populations within the region. It is suggested that there should be a 95% probability of capturing at least one population with a mycotoxin concentration that is significantly greater than the acceptable level for that toxin. The number of populations required in a survey will increase as the proportion of highly contaminated populations decreases. Table 3.4 shows the relationship between the proportion of populations with mycotoxin concentrations above the acceptable level and the number of populations required in a survey.

**Table 3.4.** Number of populations required to achieve a 95% probability of capturing at least one population with a mycotoxin concentration that is significantly greater than the acceptable level for that toxin

| True proportion of populations with mycotoxin concentrations above the acceptable level | No. of populations (e.g. batches) required |
|---|---|
| 0.3 | 9 |
| 0.2 | 14 |
| 0.1 | 29 |
| 0.05 | 59 |
| 0.03 | 99 |
| 0.01 | 298 |

## *3.5 Sampling in subsistence farming areas*

Unlike the statistically based sampling plans used for bulk foods and feeds, the sampling of harvested crops, particularly maize, from subsistence farmers is problematic. No elaborate technical sampling plan will work in such situations because there are too many variables to take into consideration. These include limited sample size, varying grades of post-harvest sorting, and different varieties and/or hybrids. It is, nevertheless, imperative that sampling and surveillance studies be conducted among subsistence farmers to determine to what extent they are being exposed to mycotoxin contamination. Such data can then be used to plan detailed intervention studies, which are better suited to the needs of rural communities than are mycotoxin regulations.

Subsistence maize farmers will have the following available: unshelled maize cobs hung on walls or stored in wooden huts or cribs, in metal or plastic containers, or in heaps on homestead floors (Fig. 3.4a–c) and/or shelled maize kept in hessian or nylon bags or in metal or plastic containers (Fig. 3.4d, e).

Sampling shelled maize is relatively easy because a small probe can be used to take samples from bags and open containers, with a sample size (a minimum of 1.5–2.0 kg) that is representative of the overall amount of grain. This technique is also applicable to other small grain crops, including sorghum and millet.

Maize on the cob is more difficult to sample because the larger the individual unit (here, the cob) within the lot, the more skewed the distribution of mycotoxins within that lot (Whitaker *et al.*, 1969; Coker *et al.*, 2000). Sampling whole cobs should be avoided as far as possible. If no alternative is available, given time and other constraining factors in a rural area, the aim should be to collect at least 10 cobs, randomly selected, from the top and as far down as possible in the pile of maize cobs. Once shelled, 10 cobs will result in a sample size of ≤ 1.5 kg, which will need to be milled and then effectively mixed before being ground for analysis. To obtain a composite sample that is representative of a specific region (e.g. a village), it is suggested that subsamples of equal size (100–250 g) be taken from each of the collected samples in a specific region, pooled, thoroughly mixed, and then analysed to give a location value (R.D. Coker, personal communication).

Sampling groundnuts is also difficult. As noted previously, variability in aflatoxin levels among individual groundnut kernels can be very high. It is unlikely that samples from a single farm will be meaningful. Composite samples from an area such as a village will be essential to provide useful data.

Other important issues need to be taken into consideration when sampling in subsistence farming areas; five such issues are discussed here.

First, it is unethical to take large samples from households without some sort of appropriate compensation in the form of commercial maize meal or rice.

Second, very little grain may be available because of poor harvests. To maintain good relationships with rural communities, it is vital that farmers are not left with the impression that their food is being stolen when samples are taken. Before sampling is done, the farmers' consent should be obtained, accompanied by adequate explanations that it is in their best interest to cooperate with the study and that they will receive feedback on its outcome.

Third, rural areas with extreme levels of poverty and/or low crop yields may have locally produced food crops available for only a limited period each year. For example, in southern Africa subsistence maize farmers may only have enough of their own maize to last them for 4–6 months after harvest, before becoming dependent on what little commercial maize meal they can afford until the next cropping season. Sampling should, therefore, be undertaken as soon after harvest as possible to obtain a reasonable number of samples.

Fourth, care should be taken to ensure that subsistence farmers are never told that their crops should not be eaten or that their food is bad for them. A culturally sensitive approach is to explain the occurrence of mycotoxins, the hazards associated with them, and the usefulness of sorting if supplies permit that.

Finally, in most subsistence households in southern Africa, harvested maize cobs are sorted before storage and processing. Maize cobs are sorted by hand into two lots: visibly non-mouldy maize (referred to as good maize) and mouldy maize. The good maize consists of cobs that the people regard as acceptable for direct human consumption. Once these cobs are shelled, any visibly mouldy maize kernels are sometimes removed before food preparation. The sorted mouldy portion of maize cobs is kept separate from the good maize and is used predominantly as animal feed. Sampling protocols need to take this scenario into consideration because only sorted grain intended for human consumption should be sampled for inclusion in surveillance studies.

**Fig. 3.4.** Storage of maize in subsistence farming areas of Guatemala: (a) maize cobs stored outdoors in a highland community; (b) maize cobs stored in a wooden crib in the lowlands; (c) maize cobs for shelling and (background) shelled kernels for storage; (d) metal silo for long-term storage of shelled maize; (e) shelled maize kept in bags for immediate sale. Photographs courtesy of Mario Roberto Fuentes Lopez, Guatemala.


## 4. Sampling and regulation

Most studies have focused on the development of sampling plans for the regulation of aflatoxins, as described here, and little or no work has focused specifically on acceptance sampling plans for other mycotoxins.

### *4.1 Acceptance sampling plans*

Acceptance sampling plans, an essential part of surveillance and regulation of mycotoxins, are designed to assess whether a lot is "acceptable", i.e. has a high probability of containing a level of a particular toxin below some specified limit, after analysis of a specified number of samples of a specified size from that lot.

The efficacy of any plan depends, among other, usually less important, factors, on the variance associated with sampling. The sampling variance associated with the determination of aflatoxins has been studied in a

**Table 3.5.** United States, United Kingdom, and Dutch sampling plans for the determination of aflatoxins in raw shelled groundnuts

| Country | Mc (µg/kg)[a] | χc (µg/kg)[b] | No. of samples | Sample size (kg) | Type of mill | Subsample size (g) | Analytical method |
|---|---|---|---|---|---|---|---|
| USA | 20 | 15 | 3 | 21.8 | Hammer | 1100 | TLC |
| United Kingdom | 10 | 10 | 1 | 10.0 | VCM | 50 | HPLC |
| The Netherlands | 9 | 5 | 4 | 7.5 | VCM | 50 | HPLC |

HPLC, high-performance liquid chromatography; TLC, thin-layer chromatography; VCM, vertical cutter mill.
[a] Mc is the maximum permitted level of total aflatoxins; for the Dutch plan, an Mc of 5 µg/kg aflatoxin $B_1$ is assumed to be equivalent to 9 µg/kg total aflatoxins.
[b] χc is the critical concentration of aflatoxin in the sample (i.e. the maximum level of aflatoxin permitted in the sample if the batch is to be accepted); for the Dutch plan, a χc of 3 µg/kg of aflatoxin $B_1$ is assumed to be equivalent to 5 µg/kg total aflatoxins.
Data summarized from Whitaker *et al.* (1995).



variety of commodities (Coker, 1998; Coker *et al*., 2000; Johansson *et al*., 2000a, 2000b, 2000c; Park *et al*., 2000). For example, Johansson *et al*. (2000b) evaluated the efficacy of three sampling plans for shelled maize, involving sample sizes of 5, 10, and 20 kg and a specified maximum permitted level of contamination (the sample acceptance level, also called the critical concentration) of 20 µg/kg. The efficacy of each sampling plan was determined by simulating the performance of the plan as applied to a collection of batches of maize with aflatoxin concentrations within the range 0–150 µg/kg. The percentage of correct decisions, i.e. acceptance of the lot when the true mean was < 20 µg/kg, increased slightly with increasing sample size, from 97.2% for 5 kg samples to 97.8% for 20 kg samples.

Published sampling plans for the determination of aflatoxins in groundnuts, developed for use in the USA, the United Kingdom, and the Netherlands, were examined by Whitaker *et al*. (1995). The plans are summarized in Table 3.5. The percentage of correct decisions of each sampling plan was determined by evaluating a collection of groundnut lots with a specific distribution of aflatoxin, produced in the USA during 1976–1985. The United States plan, with a sample size of 21.8 kg, gave the greatest percentage of correct decisions (95.6%), followed by the United Kingdom plan, with a sample size of 10.0 kg (91.1%) and the Dutch plan, with a sample size of 7.5 kg (82.4%).

In addition, a sampling plan for the determination of aflatoxins in edible nuts and dried fruit was specified by an EC Directive (European Commission, 1998a). The required sample size and the number of incremental samples depend on both the batch size and the type of commodity. For example, for batches of groundnuts, pistachios, Brazil nuts, or dried figs of > 15 t, a 30 kg sample composed of 100 incremental samples is required. If the commodity is not intended for further processing, the unground 30 kg sample must be mixed and divided into three 10 kg subsamples. The 30 kg sample (or the three 10 kg subsamples) should then be comminuted and mixed before the analytical sample is withdrawn. For unprocessed commodities, the 30 kg sample should contain no more than 15 µg/kg total aflatoxins. For those commodities intended for direct human consumption, the batch is accepted if each of the three 10 kg samples contains no more than 4 µg/kg total aflatoxins (European Commission, 1998b).

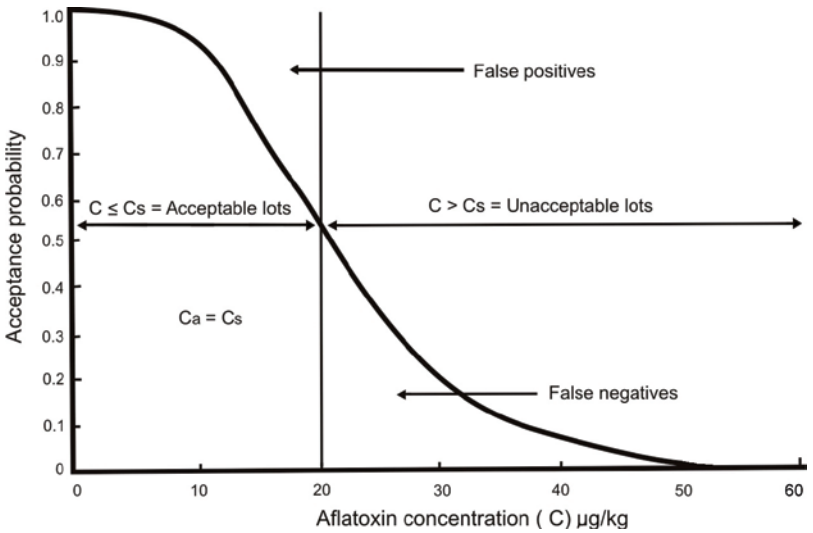
## *4.2 Consumer and producer risks*

The efficacy of acceptance sampling plans may be evaluated by calculating their consumer and producer risks. The consumer risk associated with a specified plan is the probability that the plan will accept an unacceptable batch (i.e. a batch that exceeds the permitted level of toxin), whereas the producer risk is the probability that the plan will reject an acceptable batch (i.e. a batch that does not exceed the permitted level). A well-designed sampling plan will attain an acceptable balance between the two types of risk.

For any given sampling plan, the magnitude of the consumer risk and producer risk can be estimated by constructing an operating characteristic (OC) curve, which is a plot of the probability of the sampling plan accepting a batch as a function of the mean concentration of mycotoxin in the batch. The shape of the OC curve will be determined by the sample size, the size and degree of comminution of the subsample, the type and number of analyses performed, and the critical concentration of the sample. A typical OC curve is shown in Fig. 3.5, where the maximum permitted level of contamination is 20 µg/kg. An ideal sampling plan would be represented

by the vertical line. Any practical OC curve, such as the one shown here, rejects some acceptable batches (designated as false positives, or producer risk) and accepts some unacceptable batches (designated as false negatives, or consumer risk). The consumer risk can be reduced by increasing the sample size (thereby also reducing the producer risk) and/or by decreasing the critical concentration (thereby increasing the producer risk).

**Fig. 3.5.** An operating characteristic curve defining the probability that the concentration of a sample (Cs) will compare to the acceptable concentration (Ca) of 20 µg/kg. Source: Whitaker *et al.* (2010), Fig. 10.2, p. 42; reproduced with kind permission from Springer Science+Business Media B.V.




## 5. Sample labelling and storage

An equally important facet of the whole sampling procedure is the accurate labelling and storage of samples, especially if they have been collected in the field and need to be transported to a laboratory for analysis. The underlying principle is to handle samples in such a way as to prevent spoilage before analysis and thus ensure that analyses reflect the mycotoxin levels in the commodity at the time of sampling. There are no universal guidelines for handling samples because the best practice will depend on the condition of the samples when collected and on the climate in which the work is being performed.

If samples are already dry, it is advisable to store them in polyethylene or glass bottles so that no moisture enters the sample. Storage of moist samples should be avoided. Samples should be dried as quickly as possible, after a subsample has been taken for moisture measurement (if this is to be determined). If drying cannot be carried out immediately, samples should be refrigerated, frozen, or sampled for mycotoxin analysis immediately. The samples should be transported to the laboratory as soon as possible and kept free of insect infestation or moisture damage in transit and in the laboratory. If possible, the samples should be kept in cold storage at < 5 °C until ready for analysis. In the case of visibly moist samples, it is preferable to place these at –20 °C as soon as possible after collection and to maintain the samples at this temperature until ready for analysis. In handling the specimens subsequently, containers should be allowed to reach room temperature before opening. Lengthy storage of samples (> 3 months in cold storage, and > 6 months at –20 °C) before analysis should be avoided.

Care should be taken that samples are labelled with the correct sample number and other relevant information, such as the place and date of collection. A separate record of all collected samples should be kept. A copy of this record should be stored with the samples at all times and must include additional information about the quality of the commodity at the time of collection (e.g. whether the grain was damaged in any way, how long it had been in storage, whether it had been exposed to heat treatment or dusted with any fungicides or insecticides).

## 6. Sample preparation methods

Because representative samples of foods and feeds are typically between 3 kg and 30 kg in size, it is essential that sampling procedures allow the preparation of laboratory samples that are representative of the original lot. Representative laboratory samples require successive comminution and dividing of the total sample, using either static or rotary dividers. Ideally, the comminution and division steps should be performed simultaneously using a subsampling mill. Such mills will convert large samples of edible nuts, oilseeds, or grains into comminuted, representative subsamples in a single operation.

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CHAPTER 4.

# Analysis of mycotoxins




## Summary

The analytical methods for mycotoxin determination used in fully developed countries require sophisticated infrastructure, stable electricity, ready availability of supplies, and qualified and experienced technicians for instrument maintenance. Simple and appropriately validated tools analogous to those used for the management of contaminated bulk commodities at the grain elevator level are needed at the rural level in developing countries. These tools are needed to promote public health and to manage emergency situations in subsistence farming communities with an immediate and severe problem of mycotoxin contamination of food grains, with the goal of working towards feasible reductions in exposure. Two general analytical approaches that require less infrastructure are described here. The first approach is thin-layer chromatography (TLC), which has been used for more than 50 years to analyse mycotoxins. The advantages of TLC include simplicity and proven reliability. Accuracy may be improved by using precision spotters to apply precise amounts of sample to TLC plates and optical readers. The costs of these refinements to TLC are far lower than those of gas or liquid chromatography systems. The disadvantages of TLC include the need for stable supplies of solvents and standards as well as safe conditions for their storage. The second approach described here is based on immunological methods using anti-mycotoxin antibodies. These tests are available as kits, have the necessary standards built in, use little or no organic solvent, and are generally easy to use. The disadvantages of these methods include the need to refrigerate the kits before use and the limited shelf-life. It has been proposed that companies and development agencies could be solicited to develop packages of kits, sampling equipment (e.g. grinders), and training models for deployment in the many areas where mycotoxins are a chronic problem.


## 1. Introduction

Determination of mycotoxin concentrations in staple crops is a challenging exercise because of the problems associated with sampling heterogeneously distributed compounds (see Chapter 3) and the fact that the analytical methods need to have low limits of detection, generally in the mg/kg (ppm) or µg/kg (ppb) range, depending on the individual mycotoxin being analysed.

Analysis at these levels needs to be very specific to avoid analytical interferences and can produce large uncertainties. Given the many advances in analytical science since the discovery of the aflatoxins in the 1960s, it is not surprising that a wide array of analytical methods have been used for mycotoxin testing; most fall into the general categories of either chromatographic methods or immunological methods based on antibody technology (for a tutorial review, see Shephard, 2008). All these analytical methods require solvent extraction of the mycotoxin of interest from the matrix, followed by key analytical steps, which, for chromatographic determination, typically involve extract clean-up and concentration before final determination. Another component of the complexity of mycotoxin analysis is the fact that the varied chemical structures of mycotoxins mean that specific methods are required for individual toxins. This constraint is now being overcome by the use of expensive and sophisticated mass spectrometric methods. Because of this plethora of methods and their individual characteristics, when selecting a method for mycotoxin analysis, one should consider the purpose for which the results are needed, the matrix to be analysed, the detection limit required, and the expertise and infrastructure available.

## 2. Analytical methods used in developed countries

For the survey and control of mycotoxin levels and the implementation of mycotoxin regulations in developed countries, several official analytical methods have been validated by interlaboratory collaborative studies conducted under the auspices of international bodies such as AOAC International and the European Committee for Standardization (CEN). Most official methods are based on high-performance liquid chromatography (HPLC) with various detectors, and the most recent of these methods use immunoaffinity columns (IACs) for sample extract clean-up before the HPLC analysis. More recently, the advances made in coupling mass spectrometry to HPLC have enabled analytical chemists to combine analytical steps with a confirmatory test by measuring the mass spectrum of the HPLC peak. The highly specific nature of mass spectrometry has also been used to avoid extract purification and to develop multitoxin methods, which can be applied to mixtures of mycotoxins in one analytical experiment. However, in addition to these official methods, rapid screening methods have been developed for situations where quick decisions are required, such as at granaries, silos, and factories, and such methods can be adapted for transfer to developing countries. Most of these rapid methods are based on immunological principles and use antibodies raised against specific mycotoxins. They include quantitative enzyme-linked immunosorbent assays (ELISAs), fluorometric methods, lateral flow devices, and a range of tests that give a yes/no result for contamination above or below a set control level. Whereas a full review of these methods lies outside the scope of this chapter, many recent reviews exist of methods for the analysis of mycotoxins in fully developed countries or in export certification laboratories set up in countries to certify bulk commodities for export (Krska *et al.*, 2008; Solfrizzo *et al.*, 2009; Maragos and Busman, 2010; Shephard *et al.*, 2012).

## 3. Analytical methods useful in developing countries

The techniques used in developed countries require sophisticated infrastructure, stable electricity, ready availability of supplies, and qualified and experienced maintenance technicians. The facilities are expensive to build, require highly trained personnel, and generally have a low throughput unless staff numbers are large and spare instruments are available. The difficulties in meeting the challenges associated with mycotoxin testing in Africa, namely a lack of political commitment, infrastructure and trained personnel, sustainable supplies, instrument maintenance and repairs, and laboratory quality control, have been discussed by Waliyar *et al.* (2008). For these reasons, the extent to which such methods can be transferred to developing countries depends on the country's exact stage of development and the importance attached to analytical determinations of mycotoxins. Usually, developing countries rely on less sophisticated methods, such as thin-layer chromatography (TLC) and antibody-based methods. TLC remains a useful tool in developing countries, can be semi-automated with sample spotters and ultraviolet (UV) scanners for detection, and has the advantage of testing several samples simultaneously. Its potential use in rural settings is discussed in Section 4.1.

The wide range of commercial immunological methods has found broad application in laboratories that lack sophisticated instrumentation or in which access to such instrumentation is limited by high demand. Of these, quantitative or semiquantitative ELISAs, which do not require sample extract purification, are widely used and have the advantage of handling many samples in a

single experiment. The purification of sample extracts via IACs has also been commercialized for direct fluorescence measurements using proprietary calibrated fluorometers. In recent years, the technique of fluorescence polarization (FP) immunoassay has been successfully applied to mycotoxin determination. Rather than measuring the total fluorescence, FP measures the orientation of the fluorescence emission, which is related to the rate of molecular rotation. The advantage of FP is that it is performed entirely in the extract solution (Lippolis *et al.*, 2006). Lateral flow devices can provide a yes/no result for contamination above or below a set control level, and they have also been commercialized with optical readers to provide quantitative results. It needs to be recognized that all antibody-based methods are liable to cross-reactivities and matrix effects, but they are extremely useful as a first line of analysis. Where it is possible, problematic samples can be confirmed by HPLC methods.

## 4. Analytical methods useful in rural areas

Methods of analysis suitable for use at the rural level are still a challenge to analytical scientists. Citing Sashidhar (1993), Fernández-Surumay *et al.* (2000) commented (in a rural Latin American context) that methods used in fully developed countries "require highly qualified personnel, as well as sophisticated equipment in advanced laboratories.... [therefore,] simpler methods must be developed that do not require such infrastructure, are easier to manipulate, and at the same time do not compromise the quality of the analysis." A decade earlier, in commenting on mycotoxin analysis in developing countries, Coker (1991) wrote: "It is therefore imperative that the development of efficient, cost-effective sampling and analysis methods is pursued with considerable urgency." Unfortunately, the agenda set by these authors has not yet been addressed.

Appropriate and useful tools analogous to those used for the management of contaminated bulk commodities at the grain elevator level are needed at the rural level in developing countries. These tools are needed to promote public health and to manage emergency situations in rural areas with an immediate and severe problem of mycotoxin contamination of food grains. This is not a question of meeting Codex standards, but rather of working towards feasible reductions in exposure.

Analytical methods applied in rural settings need to relate to a comprehensive risk management strategy designed to address and reduce exposure to relevant mycotoxins. Therefore, the methods must be rapid and easy to perform and should require a minimum of local or transportable infrastructure. Methods should have a wide analytical range because determinations at the rural level mostly require a focus on removing or managing contaminated lots as opposed to determining small differences in contamination that might be relevant for compliance with a regulatory limit. For this purpose, TLC and some immunological methods would be suitable.

### *4.1 Thin-layer chromatography*

Cognizant of the constraints in developing countries, Sashidhar (1993) described a portable mycotoxin analysis kit housed in a large fibreglass (or metal) suitcase-sized package as a suitable method for use at the village market level. The approach used was a simple, reliable, and inexpensive TLC method. The main components of the kit were a portable sample grinder, a robust domestic blender for toxin extraction, a TLC tank, and solvents. Chromatography was carried out using strips of silica-coated plastic sheets for dipsticks and visualization with a handheld UV lamp. The author reported a detection limit of 10 ppb for aflatoxin $B_1$.

TLC methods are useful for the key toxins discussed in this book: aflatoxins and the *Fusarium* mycotoxins fumonisins, deoxynivalenol, and zearalenone (Lin *et al.*, 1998; Schaafsma *et al.*, 1998; Shephard, 1998; Shephard and Sewram, 2004). AOAC International has approved several TLC methods for aflatoxins in groundnuts and maize as well as for ochratoxin A in barley, deoxynivalenol in wheat, and zearalenone in maize (Table 4.1). These procedures are more accurate and reliable if carried out with precision spotters and optical readers (Nawaz *et al.*, 1992); these units are relatively expensive but are far less costly and are easier to maintain than HPLC or gas chromatography (GC) instruments, particularly because of the absence of precision pumps and, in the case of GC, a constant supply of carrier gas.

Like all chemical analyses, TLC requires trained staff; individuals with college-level education need several weeks of intensive training in chemical analysis to perform reliable TLC analyses. Experience has been gained in providing such training in Asia and Africa (FAO, 1990; Boutrif, 1995; Cardwell, 1996). Potential problems with TLC analysis include the acquisition of the essential mycotoxin standards, the preparation of fresh standards in solution, and the stability of the resulting solutions. Pure standards as solids are expensive and are perishable unless stored under very carefully controlled conditions. The preparation of standards in solution requires access to an accurate balance as well as weighing conditions with appropriately conditioned air (ca. 25 °C, 30–40% relative humidity).

**Table 4.1.** AOAC International official TLC methods for some mycotoxins in cereals

| Mycotoxin | AOAC method | Commodity | Remarks |
|---|---|---|---|
| Aflatoxins | 968.22 | Groundnuts and groundnut products | CB method |
| | 970.45 | Groundnuts and groundnut products | BF method |
| | 998.03 | Groundnuts | Alternative BF method |
| | 993.17 | Maize and groundnuts | |
| | 975.37 | — | Aflatoxin $B_1$/aflatoxin $G_1$ confirmation method |
| | 985.17 | — | Aflatoxin $B_1$ confirmation method |
| Ochratoxin A | 973.37 | Barley | |
| Deoxynivalenol | 986.17 | Wheat | |
| Zearalenone | 976.22 | Maize | |

BF, United States Food and Drug Administration Best Foods; CB, United States Food and Drug Administration Contaminants Branch; TLC, thin-layer chromatography.

More typically, standards are purchased as certified solutions or access to a UV spectrophotometer is required for accurate determination of toxin concentration. The stability of standards in solution is limited unless they are kept refrigerated or frozen. Therefore, the challenges of TLC are training, solvent supplies, acquisition of standards, standard preparation, and standard stability.

## *4.2 Antibody-based methods*

As noted previously, many primary testing laboratories in both developed and developing countries use antibody-based methods to assay mycotoxins. The sampling, grinding, blending, and extraction steps are similar to those for TLC, except that solvent use is much reduced and solvents are usually less expensive and less hazardous. Several studies have examined antibody-based tests and have shown that commercially available products from several companies are quite effective (Schaafsma *et al.*, 2009). A comparison of TLC and antibody-based methods showed that the training needs are similar for the two kinds of systems but that for antibody-based methods the equipment and supply costs are lower, and the problems associated with standards are eliminated. Cross-reactivity of related mycotoxins occurs with most ELISA methods and precludes their use as regulatory tools (Tangni *et al.*, 2010). Although antibody-based tests can also suffer from the occurrence of various matrix effects, especially if they are used inappropriately or in matrices for which they were not validated, they could supply the rapid analyses needed in rural settings. The local development of antibodies and immunoassay kits has been proposed to obviate the commercial costs, but care needs to be exercised in adequately validating locally developed kits. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has developed a simple, robust, versatile, and cost-effective ELISA for the determination of aflatoxins in groundnuts (ICRISAT, 2009).

After appropriate validation, the United States Department of Agriculture Grain Inspection, Packers and Stockyards Administration (GIPSA) has approved several antibody-based tests for use as first-action tools at silos. Several companies produce very good dipstick methods based on antibodies with simple-to-use and relatively inexpensive readers. It would seem plausible to build on the portable mycotoxin analysis kit of Sashidhar (1993), replacing the TLC method with antibody-based dipsticks. The sensitivity of these methods can be improved by using nanoparticles to support the capture antibody (Posthuma-Trumpie *et al.*, 2009; Maragos and Busman, 2010).

## 5. Conclusions

The capacity to perform mycotoxin analysis is needed to manage emergency situations and to promote public health. When a rural region has an immediate and severe problem of mycotoxin contamination of food grains, appropriate risk management practices (see Chapter 7) need to be implemented. Suitable portable and robust analytical equipment and methods are needed to identify the most severely affected rural areas and to provide feedback on the effectiveness of the management practices instituted. In addition to mycotoxin analysis for managing emergency situations, general public health can be promoted by regular monitoring of mycotoxin in rural areas, which may also identify priorities for improved agronomy, crop varieties, greater crop diversity, and improved storage. Many commercial ELISAs are available for mycotoxins, each with its strengths and weaknesses, including sensitivity, cross-reactivity, and shelf-life. Evaluating such tests for applicability for field use in rural areas of developing countries would be a useful and important project.

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CHAPTER 5.

# Effects in food-producing animals




## Summary

Unexplained disease outbreaks in farm and domestic animals have suggested the likely presence of mycotoxins in feeds for many years. The manifestations of mycotoxicoses in the field are frequently nondescript and potentially have many contributing factors, which are often difficult to define. Nevertheless, toxigenic moulds were implicated in, and sometimes proven to be the cause of, animal disease in field outbreaks long before the toxins were discovered. The development of methods for the chemical analysis of mycotoxins in feeds and animal tissues has contributed to an improved understanding of the dose–response relationships of farm animal diseases associated with exposure to aflatoxins, fumonisins, ochratoxin A, deoxynivalenol, zearalenone, and ergot alkaloids. In all cases the effect of mycotoxins on animal performance is potentially a major problem for farmers regardless of their scale of operation. Reduced growth, decreased egg and milk production, lower reproductive efficiency, and increased susceptibility to stress are all potentially devastating consequences of mycotoxin exposure. Thus, being aware of the outward signs that might signal the involvement of a mycotoxin in an animal performance problem is the first step to minimizing potential adverse impacts. The target organ affected can provide important clues to involvement of a specific mycotoxin, in which case understanding the toxicokinetics and toxicology will assist in minimizing the cost and maximizing the effectiveness of interventions. The primary objective of this chapter is to provide information that will aid in the field identification of the possible involvement of a mycotoxin in an animal production problem. In conjunction with the information provided in the other chapters, this information will assist farmers in making decisions that will minimize losses due to diseases induced by mycotoxins.


## 1. Introduction

In this chapter, we discuss the effects in farm and domestic animals of the most economically important feedborne mycotoxins. The chapter begins with a summary of field outbreaks, followed by sections that describe the toxicokinetics and metabolism of each mycotoxin or group of mycotoxins. Of the trichothecenes, only deoxynivalenol is covered in depth; however, it should be recognized that the predominant trichothecene encountered in con-

taminated feeds may differ in different geographical locations (Starkey *et al*., 2007; Miller, 2008; Sugita-Konishi and Nakajima, 2010).

Additional information relevant to the toxicology in animals can be found in Chapter 6, which focuses on effects and toxicology in humans. Potential interventions to prevent field outbreaks or minimize their adverse effects are described in Chapter 9. Numerous extensive review articles and monographs provide additional information on effects in farm animals (WHO, 2001, 2011; CAST, 2003; Cousin *et al*., 2005; Roberts *et al*., 2005; Fink-Gremmels and Malekinejad, 2007; Morgavi and Riley, 2007a, 2007b; Voss *et al*., 2007; Fribourg *et al*., 2009; Pestka, 2010a, 2010b; Eaton *et al*., 2010). To aid in the field identification of the main observations characterizing exposure to a particular mycotoxin, Table 5.1 summarizes the expected toxic effects, species sensitivity, and potentially useful biomarkers in farm animals for each of the major groups of mycotoxins.

## 2. Field outbreaks

The interest of the scientific and regulatory communities in mycotoxins began in earnest when aflatoxins were found to be potent carcinogens that occurred in several important food and feed commodities. However, even in the absence of any known causal agent, unexplained disease outbreaks in farm and domestic animals, which often involved mortality or evidence of acute toxicity, served as an indicator of the likely presence of mycotoxins in foodstuffs (Forgacs and Carll, 1962).

Definitively linking a disease outbreak in the field to a specific mycotoxin is very difficult (Hamilton, 1982), even though a great deal of experimental evidence exists to characterize the potential of mycotoxins and mouldy foodstuffs to cause animal disease. A major problem for the veterinarian in the field is that disease expression is seldom pathognomonic, i.e. with a sign or symptom that is so characteristic of a disease as to be diagnostic. Instead, multiple interacting factors often occur that can modify the expression of toxicity. Thus, the manifestations of mycotoxicoses in the field are frequently nondescript and have many potential contributing factors, which are often difficult to define.

An attempt to apply Koch's postulates to demonstrate causality requires a bioassay that reproduces the disease when a pure compound is used. Unfortunately, reproducing the exact conditions that existed in the field is confounded by the potential presence of multiple contributing factors. These include, but are not limited to, environmental stress, multiple toxigenic fungi and mycotoxins, nutrient/vitamin deficiencies, infectious agents, and pre-existing conditions. These co-occurring factors can influence the clinical signs, severity, and progression of the disease (CAST, 2003) in ways that can confound both diagnosis and replication of the observed adverse effects. Also, mycotoxins in feeds are not evenly distributed (see Chapter 3); therefore, reproducing disease at the dosages found in feed samples analysed from field outbreaks can be difficult. It has been suggested that 100–200 kg or more of suspect feed should be saved for confirmatory studies in experimental animals (Osweiler, 2000). In addition, experimental confirmation can require a large number of animals if the incidence of the disease in the suspected field outbreak is low.

Despite these difficulties, toxigenic moulds were implicated as the cause of animal disease in field outbreaks long before the toxins were discovered. This was the case for aflatoxins, fumonisins, deoxynivalenol, zearalenone, and ergot alkaloids, where mould-contaminated feed was associated with disease outbreaks before the toxins were identified. For example, in the 1960s the outbreak of a disease known as turkey "X" disease led to the discovery of aflatoxins. However, before this disease was reported, outbreaks of liver cancer in farm-raised rainbow trout fed diets containing cottonseed (Butler, 1974) and of liver toxicity in pigs and cattle fed maize contaminated with *Aspergillus flavus* were documented in 1935 and 1953, respectively (Raisbeck *et al*., 1991). Equine leukoencephalomalacia (ELEM) was first linked to mouldy maize in 1891 (Haliburton and Buck, 1986). ELEM is now known to be caused by fumonisins. Effects from the consumption of maize on which *Fusarium verticillioides* had been cultured were the first indication that mouldy maize might cause porcine pulmonary oedema (PPE) syndrome (Kriek *et al*., 1981). This was confirmed only after the discovery of fumonisins in 1988 and after the cause of outbreaks of PPE in the USA in 1989–1990 was confirmed by inducing PPE with pure fumonisin $B_1$ (Marasas, 2001).

Feed refusal syndrome in the USA, most likely caused by deoxynivalenol and other trichothecenes, led to an embargo of United States barley by Germany in the 1930s (Hamilton, 1982). The association between consumption of mouldy feed and estrogenism in pigs has been known since 1928 (McNutt *et al*., 1928), and estrogenism has been attributed to consumption of feeds contaminated with zearalenone. The biological activity of ergot (the sclerotia of *Claviceps* spp.) was known in China more than 5000 years ago even though its involvement in animal disease was probably not reported until the Middle Ages (Christensen,

**Table 5.1.** Expected toxic effects, species sensitivity, and potentially useful biomarkers in farm animals for each of the major groups of mycotoxins

| Mycotoxin | Target organs and major effects | Relative sensitivity of species | Biomarkers |
|---|---|---|---|
| Aflatoxins | Reduced performance, jaundice, pale liver, hepatotoxicity with fatty changes, coagulopathy, and increased susceptibility to internal bruising during handling.<br>Liver tumours in trout. | Ducklings > turkeys > chicks > quail.<br>Rabbits > swine > cattle > sheep.<br>Dogs and mink can also be affected.<br>Young animals > mature animals. | Aflatoxin–albumin adducts in serum and DNA adducts in urine.<br>Aflatoxin $M_1$ in milk and urine. |
| Fumonisins | Liver in all species and kidney in many. Brain in horses (ELEM) and lung in pigs (PPE). | Horses and rabbits > pigs and catfish > ruminants and poultry.<br>Breeding animals > animals being raised for slaughter. | Fumonisin $B_1$ in urine and faeces.<br>Elevated sphinganine and sphinganine-1-phosphate in tissues, urine, and serum and elevated sphinganine-1-phosphate in red blood cells. |
| Ochratoxin A | Pale and grossly enlarged kidney. Fatty liver in poultry.<br>Altered performance, including decreased feed consumption, reduced weight gain, and decreased egg production. Increased susceptibility to bruising, decreased tensile strength of the large intestines, reduced pigmentation, decreased immune response, increased susceptibility to infection, and glycogen accumulation in the liver. | Pigs and dogs > poultry > calves.<br>Chicks > turkeys > quail.<br>Mature cattle are considered resistant. | Ochratoxin A or its metabolites in tissues, blood, and urine. |
| Deoxynivalenol | Feed refusal in swine and reduced weight gain and vomiting. Gastrointestinal problems, soft stools, diarrhoea, increased susceptibility to other diseases, and decreased performance. | Pigs > dogs and cats > cattle, sheep, and poultry. | Deoxynivalenol and its glucuronide conjugate in urine. |
| Zearalenone | Estrogenic effects. Swollen red vulva, vaginal prolapse, rectal prolapse, and fertility problems in swine. | Pre-pubertal pigs >> cattle and sheep > poultry. | Zearalenone or metabolites (including glucuronic acid conjugates) in urine or faeces. |
| Ergot alkaloids | In cattle, dry gangrene aggravated by cold weather and heat intolerance in warm weather.<br>In cattle and horses, neurotoxicity, including tremors, convulsions, and agalactia.<br>In swine, reproductive problems and agalactia. | Cattle > horses and sheep. Poultry and swine can also be affected. | Lysergic acid in urine. |

ELEM, equine leukoencephalomalacia; PPE, porcine pulmonary oedema.

1980). Ergot was implicated as the cause of animal production problems in domestic animals (mainly cattle) in the USA in the early 19th century (Hesseltine, 1979), but the alkaloids responsible for ergotism were not identified until the 1930s. The causative agent or agents for haemorrhagic syndrome in the USA in the 1950s caused by mouldy feed (Forgacs and Carll, 1962) have never been identified. However, it has been suggested that multiple mycotoxins could have been involved, including aflatoxins and ochratoxin A (Hamilton, 1982). Ochratoxin A, unlike aflatoxins, fumonisins, deoxynivalenol, zearalenone, and ergot alkaloids, was isolated and characterized (van der Merwe *et al.*, 1965) before it was proven to cause outbreaks of kidney disease in poultry (Hamilton *et al.*, 1977) and pigs (Krogh, 1978a, 1978b).

In the remainder of this section, we provide some examples of the spectrum of documented or strongly suspected field outbreaks for aflatoxins, fumonisins, ochratoxin A, deoxynivalenol, zearalenone, and ergot alkaloids.

### 2.1 Aflatoxins

Turkey "X" disease was responsible for the deaths of > 100 000 turkeys in the United Kingdom in 1960. Mortality was also documented in ducks, chickens, pheasant, calves, and pigs (Butler, 1974). In the USA and elsewhere, field outbreaks causing mortality have been well documented in turkeys, laying hens, pigs, cattle, rainbow trout, and dogs (Butler, 1974; Hamilton, 1982). In the case of poultry, pigs, and farm-raised trout, large numbers of animals were involved (Butler, 1974; Hamilton, 1982). Acute toxicity is easily recognized, but the more subtle effects are probably of greater concern to farmers. For example, decreased rates of weight gain, decreased milk or egg production, increased susceptibility to bruising during processing of poultry and pigs, underpigmentation of meat in poultry, and altered immune function have all been associated with exposure in the field (Hamilton, 1982; Raisbeck *et al*., 1991). Pet food recalls due to aflatoxin contamination resulting in liver toxicity and death in dogs are not uncommon in the USA.

### 2.2 Fumonisins

In the USA, field outbreaks of ELEM caused by mouldy maize have been reported for more than 100 years (Haliburton and Buck, 1986). It was reported that about 5000 horses died of "mouldy corn poisoning" in Illinois in 1934–1935 (Haliburton and Buck, 1986). The disease has also been reported in South America, Hungary, China, Greece, France, Mexico, New Caledonia, Egypt, South Africa, and Germany (Magnol *et al*., 1983; Haliburton and Buck, 1986; Laurent *et al*., 1998; Rosiles *et al*., 1998). After identifying *Fusarium verticillioides* (then known as *F. moniliforme*) as the predominant fungal contaminant of mouldy maize that had caused cases of ELEM in Egypt, Wilson and Maronpot (1971) reproduced ELEM in horses by feeding them maize on which this fungus had been grown. Kriek *et al*. (1981) induced PPE, a disease that has been known in Hungary since 1950 (Fazekas *et al*., 1998), by feeding pigs maize on which *F. verticillioides* had been grown. After isolating and chemically characterizing the fumonisins, Marasas *et al*. (1988) induced ELEM in a horse by using purified fumonisin $B_1$. Serendipitously, in 1989–1990 there were numerous outbreaks of ELEM and PPE syndrome in the USA. In 1990, PPE was induced using pure fumonisin $B_1$ (Harrison *et al*., 1990). Field outbreaks of PPE have not occurred in the USA since the early 1990s, but reports of ELEM have persisted. In poultry, reduced performance associated with feeds contaminated with fumonisins has been reported, but the effects seldom involve increased mortality (WHO, 2000).

### 2.3 Ochratoxin A

In pigs, mycotoxic nephropathy was first described in 1928 in Denmark and was reproduced experimentally using mouldy barley or oats (Krogh, 1992). This disease has also been reported in Norway, Sweden, Ireland, Finland, Germany, Hungary, Poland, the former Serbia and Montenegro, and Bulgaria (Krogh, 1978a, 1978b; Marquardt and Frohlich, 1992; Stoev *et al*., 1998). The main clinical signs are renal dysfunction and oedema. In pigs, excessive thirst (polydipsia) and passage of large volumes of urine (polyuria) are characteristic signs of this disease under field conditions. The association of ochratoxin A (OTA) with field outbreaks of nephropathy in poultry was first reported in 1975 (Krogh, 1992) and has been reported in many countries since then (Marquardt and Frohlich, 1992). Outbreaks of mycotoxic nephropathy have also been reported in horses (Krogh, 1978b), and there are a few suspected cases in ruminants (Raisbeck *et al*., 1991). The first well-documented and confirmed case of ochratoxicosis occurred in young turkeys in the south-eastern USA (Hamilton *et al*., 1977). In 1976, about 80% of the marketed turkeys in North Carolina displayed signs of ochratoxicosis, and all cases involved maize contaminated with OTA (Schaeffer and Hamilton, 1986). In a turkey house with 16 000 turkeys, mortality was 59%. The first indication of intoxication was feed refusal (Schaeffer and Hamilton, 1986). Death was most commonly seen in younger birds, whereas older birds developed infections with *Escherichia coli* (air-sacculitis) that did not respond to antibiotics. Interestingly, in outbreaks in chickens, feed refusal did not occur, whereas poor pigmentation was common (Schaeffer and Hamilton, 1986). Other field cases in chickens have reported decreased egg production and eggshell quality, increased susceptibility to intestinal rupture during processing, decreased bone strength, and haemorrhagic episodes (Schaeffer and Hamilton, 1986). In all species, effects on the kidney were apparent.

### 2.4 Deoxynivalenol

Trichothecenes have been implicated in feed refusal by cattle, pigs, and chickens; however, pigs appear to be the most sensitive to deoxynivalenol (DON) (Rotter *et al*., 1996; Haschek *et al*., 2002). Outbreaks in animals of "red mould poisoning" from cereals in Japan as far back as 1890 (Miller, 2008) led to the discovery of DON. It was first isolated in Japan and was originally called Rd-toxin (Moorooka *et al*., 1972). It was given the name vomitoxin (Vesonder *et al*.,

1973) after its isolation from maize contaminated with *F. graminearum* because it is associated with emesis in pigs. Numerous confirmed cases have been reported of feed refusal or reduced feed intake in pigs consuming feeds contaminated with DON (Osweiler, 2000). The most frequently observed effect of DON in farm animals is feed refusal, which may explain why toxic effects are to a great extent self-limiting (Osweiler, 2000). There have been unconfirmed reports of feed refusal in dogs, cats, and rabbits consuming pet foods naturally contaminated with DON (Bohm and Razzazi-Fazeli, 2005). In ruminants, field disease outbreaks attributed to DON are rare (Raisbeck *et al*., 1991).

### 2.5 Zearalenone

Estrogenism in pigs consuming mouldy feed was first reported in 1928 (McNutt *et al*., 1928); since then, field outbreaks of reproductive problems, including vulvovaginitis and anestrus, and enlargement of the mammae in males have been attributed to consumption of feeds contaminated with zearalenone (ZEA) (Aucock *et al*., 1980; Raisbeck *et al*., 1991), which was previously called F-2 toxin (Mirocha *et al*., 1968). Field outbreaks of estrogenic syndrome in pigs have been reported in North America, Europe, Africa, Asia, and Australia (Christensen, 1979). ZEA-induced abortions in pigs have also been reported and are probably a result of embryo implantation failure (Osweiler *et al*., 1986). Suggestions that reproductive problems in ruminants can be attributed to ZEA are considered to be controversial (Raisbeck *et al*., 1991). The semisynthetic ZEA analogue zeranol is used in cattle as a growth promoter, and its estrogenic effects have caused reduced performance in bulls (Raisbeck *et al*., 1991). Reproductive problems have been reported in sheep grazing on grasses contaminated with ZEA (CAST, 2003), and ZEA was found to interfere with the induction of parturition by oxytocin in gilts and sows (Alexopoulos, 2001). In maize contaminated with ZEA, co-contamination with DON is likely.

### 2.6 Ergot alkaloids

Poisoning resulting from the consumption of foods contaminated by ergots, which are fungal sclerotia that replace grass seeds, has been known since the Middle Ages, but it was not until the 20th century that ergot alkaloids from *Claviceps purpurea* were determined to be the causative agent (Barger, 1931). Ergot alkaloids associated with or suspected to be involved in diseases of farm animals include ergoline alkaloids, which contain the lysergic acid ring structure, and ergopeptine alkaloids, which contain a tripeptide moiety. In farm animals, ergot poisoning is usually associated with grazing on seed heads of grasses contaminated with ergots of *Claviceps* spp. or grasses colonized by groups of fungi that occur as endophytic symbionts (*Neotyphodium* spp. and *Epichloë* spp.; Roberts *et al*., 2005) or with the consumption of feeds contaminated with ergot (CAST, 2003). Outbreaks of ergot poisoning have also been reported in pigs and cattle consuming sorghum-based feeds. Consumption of feeds based on small grains, such as rye and barley, or foraging on infected grasses contaminated with ergots has caused lameness or necrosis of ears, tails, and feet in cattle (CAST, 2003). Endophytes (*Neotyphodium* spp. and *Epichloë* spp.) that occur in pasture grasses such as tall fescue and ryegrass are known to produce ergot alkaloids, which have caused a diverse array of toxic syndromes in grazing animals, including reproductive problems and neurological effects in horses (staggers, "drunkenness", and sleep) and reduced reproductive performance, decreased milk production, and reduced growth in cattle (Cross, 2003; White *et al*., 2003). In cattle and sheep, increased body temperature due to peripheral vasoconstriction is often seen in animals that have ingested grasses infected by endophytes (Cross, 2003). Field cases of fescue foot (necrosis of the extremities) in cattle and laminitis in horses have been reported to be due to peripheral vasoconstriction caused by ergot alkaloids (Cross, 2003). Environmental conditions appear to influence the vasoconstrictive effects of ergot alkaloids in grasses; episodes of fescue foot are most common in autumn and early winter, and the condition known as summer slump is most common in warm weather (Raisbeck *et al*., 1991).

## 3. Toxicokinetics and metabolism

Mycotoxins are chemically diverse, and therefore their uptake, distribution, metabolism, and excretion are equally diverse. Galtier (1998) summarized the kinetics and biological fate of most of the economically important mycotoxins. Uptake from the gastrointestinal tract depends to a large extent on the water solubility or lipophilicity of the particular mycotoxin. Metabolism by microbes in the gut can also have a profound effect on uptake and toxicity. Some mycotoxins, like OTA, are well absorbed, whereas others, like fumonisin $B_1$, are very poorly absorbed. Binding of mycotoxins to plasma proteins can influence uptake, distribution, and the half-life in the blood. Again, some mycotoxins, including OTA, are tightly bound, whereas others, including fumonisin $B_1$, are not bound. Metabolism and excretion are important considerations

for determining whether residues will remain in tissues and also for developing biomarkers for exposure. For example, aflatoxin $B_1$ is extensively metabolized in the liver and elsewhere, and significant amounts can be excreted as metabolites in milk. For DON, most of the absorbed toxin is excreted in urine, whereas only very small amounts of fumonisin $B_1$ are excreted in urine. Fumonisin $B_1$ is not metabolized in the liver or other tissues and therefore is recovered intact or partially hydrolysed (probably by microbes in the gastrointestinal tract) in faeces. Although mycotoxin residues can be carried over into milk and eggs (Galtier, 1998), only aflatoxin $M_1$ and OTA have been detected naturally in cow's milk.

## 3.1 Aflatoxins

### 3.1.1 Absorption

Aflatoxin $B_1$ absorption, distribution, and elimination is rapid. Aflatoxin $B_1$ is well absorbed; it accumulates in the liver and is extensively metabolized in the liver and other tissues. The binding of metabolites to macromolecules, including proteins and nucleic acids, also occurs soon after absorption. Unbound water-soluble metabolites are excreted in urine and other fluids, and the parent compound is excreted in faeces; however, some metabolites bound to nucleic acids can persist for relatively long periods in tissues.

### 3.1.2 Gastrointestinal metabolism

Little published information is available about the ability of micro-organisms in the gastrointestinal tract to metabolize or bind aflatoxins. It is known, however, that certain bacteria can bind aflatoxin $B_1$ in vitro, and it has been suggested that they may aid intestinal excretion (Oatley *et al.*, 2000).

### 3.1.3 Bioavailability

Aflatoxin $B_1$ is rapidly absorbed from the small intestines, and the rate of absorption is much higher in suckling and young animals than in adults (Kumagai, 1985). The process of absorption from the small intestines is passive and complete. For example, the time needed to reduce aflatoxin $B_1$ in the intestinal lumen by 50% is about 7 minutes (Ramos and Hernández, 1996). Aflatoxins $B_1$ and $B_2$ are more rapidly absorbed than are aflatoxins $G_1$ and $G_2$, ensuring that their bioavailability is high (Ramos and Hernández, 1996). Aflatoxin $B_1$ is also absorbed slowly through the skin, and intratracheal absorption is more rapid than via the oral route (IARC, 1993a). Once absorbed, aflatoxin $B_1$ is non-covalently bound to albumin and is transported to other tissues.

### 3.1.4 Distribution

Early distribution studies focused on extraction of the parent compounds or non-polar aflatoxin metabolites and ignored the water-soluble metabolites (Busby and Wogan, 1981a). In sheep and other animals, aflatoxin $B_1$ is immediately transported to the liver after absorption from the gut (Wilson *et al.*, 1985). The half-life of aflatoxin $B_1$ in plasma after intravenous dosing is < 1 hour (Wong and Hsieh, 1980), whereas after intratracheal or oral dosing it is about 90 hours (Coulombe and Sharma, 1985). As with absorption from the small intestines, aflatoxin $B_1$ is rapidly taken up by the liver with a half-life of < 5 minutes (Busby and Wogan, 1981a). Aflatoxin $B_1$ is widely distributed in the body, with most accumulating in the liver, kidney, and lung. Maximal levels in tissues, and especially the liver, are reached quickly, and the relative retention of aflatoxin $B_1$ is greater than that of aflatoxin $B_2$ (Busby and Wogan, 1981a; IARC, 1993a). Low levels of aflatoxin $B_1$ and aflatoxin $M_1$ (< 1 µg/kg) were detected in the liver and kidneys of lactating cows fed diets containing 1250 µg/kg aflatoxin $B_1$ for 2 weeks (Busby and Wogan, 1981a). Pigs fed diets containing 100–400 µg/kg aflatoxin $B_1$ for 4 weeks had detectable levels of aflatoxins $B_1$ and $M_1$ in the liver, muscle, kidney, and blood. At 400 µg/kg aflatoxin $B_1$ in feed, the detected levels of aflatoxins $B_1$ and $M_1$ were as high as 4 µg/kg and 1.5 µg/kg, respectively (Busby and Wogan, 1981a). In chickens, maximal levels were attained in plasma and the liver within 6 hours after oral dosing, and levels declined rapidly thereafter (Hirano *et al.*, 1994). After either a single oral dose or daily oral dosing for 2 weeks, aflatoxin residues were detected in various organs and in muscle tissue in laying hens (Busby and Wogan, 1981a). Aflatoxins can cross the placenta and accumulate in developing fetuses (IARC, 1993a). Unmetabolized aflatoxin $B_1$ can accumulate in tissues rich in melanin pigment and the upper respiratory tract (Larsson and Tjälve, 1993).

### 3.1.5 Excretion

After intraperitoneal or intravenous dosing of aflatoxin $B_1$, excretion is rapid and most of the dose is eliminated in faeces and urine (Wong and Hsieh, 1980; Busby and Wogan, 1981a). The relative retention of aflatoxin $B_1$ is greater than that of aflatoxin $B_2$ due to greater urinary excretion of aflatoxin $B_2$ (Busby and Wogan, 1981a). A large percentage of the intraperitoneal dose is recovered in bile (Busby and Wogan, 1981a). In sheep, aflatoxin residues, mainly aflatoxin $M_1$, are excreted primarily in urine within 48 hours after oral dosing (Busby and Wogan, 1981a). Aflatoxin $M_1$ is the main unconjugated metabolite of aflatoxin $B_1$ excreted in the milk of sheep,

goats, and cows. In sheep and cattle, aflatoxin $B_1$ is the main component in faeces. In laying hens, aflatoxin $B_1$ excretion is primarily in faeces and is maximal 24 hours after a single oral dose, a finding similar to that seen in laboratory animals dosed intravenously (Wong and Hsieh, 1980). In laying hens, the body half-life of aflatoxin $B_1$ was 67 hours after a single oral dose (Busby and Wogan, 1981a).

### 3.1.6 Transmission

Approximately 1–15% (IARC, 2002a) of the aflatoxin $B_1$ dose is recovered as aflatoxin $M_1$ in the milk of sheep, goats, and cows (IARC, 1993a). The conversion rate is > 1% when the aflatoxin $B_1$ dose is low (IARC, 2002a). Extensive evidence has been found for the lactational transfer of aflatoxin $M_1$ and its accumulation in the livers and lungs of offspring (IARC, 1993a). Low levels of aflatoxin metabolites can be detected in the liver and other tissues several weeks after animals are exposed to high levels of aflatoxin $B_1$ (Busby and Wogan, 1981a; Coulombe and Sharma, 1985). However, the levels retained in edible tissues are generally low; feed-to-tissue ratios range from 800:1 to 14 000:1 (IARC, 2002a). Aflatoxin $B_1$ can be detected in eggs of laying hens fed diets containing aflatoxin $B_1$, with a feed-to-tissue ratio ranging from 2200:1 to 5000:1 (Oliveira *et al.*, 2000; IARC, 2002a). With the exception of milk, transmission to edible animal products should pose little health risk to consumers.

### 3.1.7 Metabolism

The metabolism of aflatoxin $B_1$ has been extensively reviewed (IARC, 1993a, 2002a; see also Chapter 6). Briefly, in the liver and other tissues, aflatoxin $B_1$ is metabolized in microsomal systems to aflatoxins $P_1$, $M_1$, and $Q_1$ and, most importantly, the highly reactive aflatoxin $B_1$-8,9-epoxide. In the liver, cytochrome P450 enzymes are responsible for activation of aflatoxins $B_1$, $M_1$, and $P_1$, all of which can form nucleic acid adducts or undergo conjugation to glutathione, conversion to dihydrodiols, or binding to serum proteins or other macromolecules. Aflatoxin $M_1$ is the main unconjugated metabolite in the urine of cows, pigs, and sheep. In rodents, and presumably in farm animals, aflatoxin $B_1$–nucleic acid adducts are also found in urine, and 80% of the depurinating adducts are excreted within 48 hours after dosing. A close correlation has been established between levels of adducts in urine and levels in the liver (IARC, 1993a), and correlations have also been observed between dietary intake and levels of adducts in urine and serum (IARC, 2002a).

The relative sensitivity of animals to the toxic effects of aflatoxin $B_1$ is closely linked to differences in metabolism among species (IARC, 2002a). The susceptibility of animals to aflatoxin $B_1$ toxicity and carcinogenicity depends to a large extent on the type of metabolites produced and the rate of formation and detoxification of the aflatoxin $B_1$-8,9-epoxide. Risk factors contributing to an individual's sensitivity to liver tumours and hepatotoxicity include level of exposure to aflatoxin $B_1$; expression of enzymes in the aflatoxin activation and detoxification pathways; nutritional status; co-exposure to other mycotoxins, especially fumonisin; and exposure to infectious agents (see Chapter 6). Evidence suggests that the critical factor determining species sensitivity is the rate at which the aflatoxin $B_1$-8,9-epoxide can be conjugated to glutathione by glutathione *S*-transferase (GST) (Eaton *et al.*, 2010). For example, domestic turkeys, one of the most susceptible species to aflatoxicosis, are known to be deficient in the GST that mediates detoxification of the aflatoxin $B_1$-8,9-epoxide (Klein *et al.*, 2000), and that deficiency results in high levels of hepatic aflatoxin $B_1$ epoxidation (Yip and Coulombe, 2006; Rawal *et al.*, 2009).

## 3.2 Fumonisins

### 3.2.1 Absorption

In most animals, fumonisin $B_1$ absorption, distribution, and elimination is rapid. Fumonisins are poorly absorbed, and although some evidence exists that fumonisins can be partially metabolized in the gut, metabolism by the liver or other tissues has not been convincingly demonstrated (WHO, 2000, 2001, 2012; IARC, 2002b; Shephard *et al.*, 2007; Voss *et al.*, 2007).

### 3.2.2 Gastrointestinal metabolism

Microbial metabolism most likely occurs in the gut of monogastric animals, because partially hydrolysed fumonisin $B_1$ (lacking one tricarballylic acid side chain) and, to a lesser extent, fully hydrolysed fumonisin $B_1$ (lacking both side chains) were recovered in faeces but not in bile of vervet monkeys (WHO, 2000). Most (60–90%) of the total fumonisin $B_1$ found in ruminant faeces was present as the partially hydrolysed form. In non-ruminants, the parent compound was the dominant species present (WHO, 2000). However, studies in pigs have reported significant amounts of fully hydrolysed and partially hydrolysed fumonisin $B_1$ in faeces and tissues (Fodor *et al.*, 2008). Whether the hydrolysed fumonisin $B_1$ was produced in the tissues was not determined.

### 3.2.3 Bioavailability

In all animals studied, including pigs, laying hens, turkey poults, ducks, and dairy cows, the fumonisin absorption that does occur is rapid and the quantity of fumonisin $B_1$ detected in plasma and tissues after oral administration is very low (negligible to < 4% of dose) (WHO, 2000, 2001, 2012; IARC, 2002b; Fodor *et al*., 2008; Tardieu *et al*., 2008, 2009). The bioavailability of fumonisin $B_2$ may be less than that of fumonisin $B_1$. Feeding studies have shown that, in the liver and kidney, fumonisin $B_1$ is accumulated to a much greater extent than expected based on the relative amounts in feed of fumonisin $B_1$, fumonisin $B_2$ (Riley and Voss, 2006; Fodor *et al*., 2008; Gazzotti *et al*., 2010), and fumonisin $B_3$ (Riley and Voss, 2006). Although diets containing predominantly fumonisin $B_2$ from culture material induced liver toxicity in both rats and horses (Riley *et al*., 1997; Voss *et al*., 1998), pure fumonisin $B_2$ did not induce liver toxicity in mice in one feeding study (Howard *et al*., 2002). In a cultured intestinal epithelial cell model, hydrolysed fumonisin $B_1$, but not fumonisin $B_1$, was found to cross the monolayer (primarily from basolateral to apical), suggesting a carrier-mediated efflux process (De Angelis *et al*., 2005).

### 3.2.4 Distribution

Although fumonisins are distributed to most tissues, the liver and kidney retain the highest concentrations of the absorbed material in all animals studied (reviewed in Voss *et al*., 2007). Fumonisin $B_1$ persists in the kidney much longer than in plasma or the liver, and in male Sprague Dawley (Riley and Voss, 2006) and Wistar rats (Martinez-Larranaga *et al*., 1999), the levels of fumonisin $B_1$ in the kidney can be 10 times the amount in the liver. Radiolabeled fumonisin has been detected in the brains of pigs (Prelusky *et al*., 1996a), but little or no fumonisin has been detected in the brain tissue of horses, although the brain is a known target organ (Haschek *et al*., 2002). Until recently it was believed that fumonisins could not cross the placenta and enter the developing embryo (WHO, 2001). However, recent studies have detected [$^{14}$C]fumonisin $B_1$ in embryos and placentas, an observation confirmed by the presence of elevated levels of free sphinganine, a biomarker for fumonisin inhibition of ceramide synthase (Gelineau-van Waes *et al*., 2005).

### 3.2.5 Excretion

After intraperitoneal or intravenous dosing of fumonisin $B_1$, initial elimination from tissues is rapid, with no evidence of metabolism (Shephard *et al*., 2007; Voss *et al*., 2007), but extensive enterohepatic circulation occurs (Prelusky *et al*., 1996a). After oral dosing, peak plasma levels occur within 1 hour to several hours. Several studies using different routes of exposure and different animal species have shown that fumonisins are excreted primarily in faeces, either unchanged or with loss of one or both of the tricarballylic acid side chains. Low levels of fumonisin $B_1$ can be detected in the urine of animals exposed experimentally to fumonisin, including rabbits (Orsi *et al*., 2009), rats (Cai *et al*., 2007), pigs (Fodor *et al*., 2008; Dilkin *et al*., 2010), horses (Tumbleson *et al*., 2003), and vervet monkeys (Shephard *et al*., 2007). In pigs, < 1% of the oral dose is recovered in urine (Prelusky *et al*., 1996a; Dilkin *et al*., 2010). It has been estimated that pigs exposed to dietary fumonisin $B_1$ at 2–3 mg/kg body weight (bw) in feed would require a withdrawal period of at least 2 weeks for the fumonisin $B_1$ to be eliminated from the liver and kidney (Prelusky *et al*., 1996a, 1996b). Several studies have confirmed this finding using the persistence of free sphinganine as a biomarker in the kidney and liver to show that although fumonisin $B_1$ is rapidly eliminated, the biomarker remains elevated for a much longer period. Although the half-life after oral dosing is not known, the oral half-life is probably between 8 hours and 48 hours based on what is known from the parenteral routes, the time required to reach peak levels in plasma (1–7 hours) after gavage, and the estimated time for complete clearance from the liver and kidney (2 weeks) (WHO, 2000).

### 3.2.6 Transmission

Little evidence exists to suggest significant transfer of fumonisins through milk (WHO, 2000). No fumonisin $B_1$ was detected in the milk of lactating sows fed diets containing nonlethal levels of fumonisin $B_1$, and no evidence was found of toxicosis in their suckling pigs. In a study with lactating cows administered fumonisin $B_1$ intravenously, the carry-over of fumonisin $B_1$ into the milk was either very small or not detected. The fact that very little fumonisin $B_1$ is retained in any tissue, milk, or eggs has led to the conclusion that fumonisin residues in food products derived from animals are insufficient to render them injurious to consumers (WHO, 2000). However, in a study in weaned piglets fed diets containing fumonisin $B_1$ at 10 mg/kg or 30 mg/kg diet, the livers contained 306 µg/kg or 830 µg/kg of fumonisin $B_1$, respectively, after 28 days (Dilkin *et al*., 2003). At the higher level of contamination, a 70 kg person would need to consume 170 g of liver to exceed the Joint WHO/FAO Expert Committee on Food Additives (JECFA) provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg bw/day. Pigs fed diets containing low levels (1.66 mg/animal/day) of fumonisins for 7 weeks

had detectable, but much lower, levels of fumonisins (15–43 µg/kg) in the liver (Gazzotti *et al*., 2010). Levels of fumonisin detected in the muscle of pigs or poultry orally dosed with fumonisin were either very low or below the detection limits (Prelusky *et al*., 1996a; Tardieu *et al*., 2008).

### *3.2.7 Metabolism*

Fumonisins do not appear to be metabolized in vitro or in vivo by animal tissues (WHO, 2001), even though they are clearly excreted in bile, and hydrolysed and partially hydrolysed fumonisin $B_1$ has been reported in tissues (Fodor *et al*., 2008). The source of hydrolysed fumonisins in tissues is unknown. However, formation of hydrolysed fumonisin during alkaline processing (nixtamalization) and by microbial metabolism in the gastrointestinal tract has been demonstrated (see Section 3.2.2). One study suggested that a cytochrome P450 isoform is capable of producing a fumonisin $B_1$ metabolite (Marvasi *et al*., 2006), but no convincing evidence has been reported of in vivo or in vitro metabolism by cytochrome P450, the microsomal esterase, or any other microsomal enzyme (WHO, 2001, 2012). However, studies have shown that cytochrome P450 activity can be altered as a result of the inhibition of the enzyme ceramide synthase by fumonisin (WHO, 2001). Both B fumonisins and their hydrolysed counterparts can react with ceramide synthase; hydrolysed fumonisin $B_1$ is a substrate for this enzyme, producing the compound *N*-palmitoyl-$AP_1$ (Seiferlein *et al*., 2007). Studies using hydrolysed fumonisin $B_1$ have shown that it is much less toxic than the parent compound (Howard *et al*., 2002; Collins *et al*., 2006; Voss *et al*., 2009; Grenier *et al*., 2012).

Even though little evidence has been found for metabolism of B fumonisins in tissues, chemical acylation of the free primary amino group prevents toxicity and reduces the ability of the fumonisin to inhibit ceramide synthase (Norred *et al*., 1997, 2001). The two tricarballylic acid side chains are also important for toxicity, as demonstrated by reduced or no toxicity, or evidence for disruption of sphingolipid metabolism, when fumonisin is hydrolysed (Howard *et al*., 2002; Collins *et al*., 2006; Voss *et al*., 2009; Grenier *et al*., 2012).

## *3.3 Ochratoxin A*

### *3.3.1 Absorption*

Comprehensive reviews of the pharmacokinetics and metabolism of OTA are available (Marquardt and Frohlich, 1992; WHO, 2001; Dietrich *et al*., 2005; Pfohl-Leszkowicz and Manderville, 2007). OTA is rapidly absorbed. The half-life in plasma depends on the extent of binding to plasma proteins. OTA is widely distributed; in pigs, it is accumulated in the kidney and other tissues and can occur in edible tissues. OTA and its metabolites are reabsorbed by the kidney and excreted in urine and also undergo enterohepatic circulation and excretion in faeces. The potential exists for extensive metabolism by microbes to less toxic metabolites in the gastrointestinal tract. Ochratoxins A and B can also be metabolized by cytochrome P450 enzymes in various tissues.

### *3.3.2 Gastrointestinal metabolism*

In cows and sheep, OTA is degraded by the rumen flora and protozoa (WHO, 2001). However, some portion is not metabolized and can accumulate in serum, tissues, and milk (IARC, 1993b; Höhler *et al*., 1999). The enzymes responsible for the metabolism are carboxypeptidase A and chymotrypsin. Antibiotics that inhibit the intestinal microbial population can also reduce the hydrolysis of OTA to its non-toxic metabolite, ochratoxin α, leading to increased blood concentrations of OTA (WHO, 2001). In pigs, ochratoxin B is metabolized much more efficiently to ochratoxin β than OTA is to ochratoxin α. The rate of disappearance of OTA from rumen fluids and its gastrointestinal metabolism depend on diet (Müller *et al*., 2001) and are affected by the amount of feed concentrates (feed additives with a high nutrient density) added to the diet (Höhler *et al*., 1999).

### *3.3.3 Bioavailability*

OTA is well absorbed from the gastrointestinal tract, presumably from the small intestines, and it is also well absorbed from the lungs, with a calculated bioavailability of 98% (WHO, 2001). Bioavailability from the oral route is reported as ranging from 40% to 70% in chickens, rabbits, and pigs. Absorbed OTA is rapidly and tightly bound to serum proteins and in that form is only slowly transferred from the bloodstream to the liver and kidney. The high affinity of OTA for serum albumin results in a higher concentration in plasma than in the gut (Kumagai, 1988). The maximal concentration in serum after a single oral dose is attained in < 1 hour in chickens and in 10–48 hours in pigs (WHO. 2001). The plasma half-life of OTA is quite variable, ranging from a few hours in chickens to 5 days in pigs.

### *3.3.4 Distribution*

In pigs, chickens, and goats, the relative tissue distribution of OTA is usually kidney > liver or muscle > fat (WHO, 2001; Biró *et al*., 2002; Dietrich *et al*., 2005). In rabbits, the relative distribution is kidney > liver > mammary gland > muscle (Ferrufino-Guardia *et al*., 2000). OTA has been shown to cross the blood–brain

barrier in rats and the placenta in rats and pigs (WHO, 2001). The rate of disappearance of OTA from the blood is much slower than that from the kidney or liver and other tissues, indicating the importance of binding to serum proteins and enterohepatic recirculation in the overall fate of OTA once it has been absorbed. In cells, OTA is accumulated via a multispecific organic anion transporter (O'Brien and Dietrich, 2005). The accumulation and persistence of OTA in the kidney is due to its reabsorption by the organic anion transporter (Zingerle *et al*., 1997; Welborn *et al*., 1998). The ability of the kidney to accumulate OTA plays an important role in its nephrotoxicity. In pigs fed OTA for 1 month, the half-life of residues in the kidney, liver, and muscle was 3.5–4.5 days (Busby and Wogan, 1981b).

### *3.3.5 Excretion*

Elimination of OTA is primarily via the urine, but significant amounts can also be excreted in faeces. OTA is eliminated slowly because it undergoes enterohepatic recirculation, is reabsorbed by the kidney, and binds tightly to serum protein (WHO, 2001). The extent of albumin binding also markedly decreases the uptake of OTA by transporters (Bow *et al*., 2006). The elimination half-life is much shorter for ochratoxins B and C than for OTA and its metabolites (Li *et al*., 1997).

### *3.3.6 Transmission*

OTA has been shown to be transferred efficiently to milk in rodents and rabbits (WHO, 2001). At high levels of exposure, OTA can accumulate in eggs of chickens and quail (WHO, 2001). Detectable levels have been reported in pig kidney and liver, blood products, and other meat products for human consumption (WHO, 2001; CAST, 2003).

### *3.3.7 Metabolism*

In addition to gastrointestinal degradation by microbes, microsomal preparations from rabbits and pigs containing various cytochrome P450 enzymes can oxidize OTA to less toxic hydroxyochratoxin A (WHO, 2001). At least 20 OTA derivatives have been identified after incubation with liver microsomes or cultured cells, and hydroxylated metabolites have been detected in pig kidney (Pfohl-Leszkowicz and Manderville, 2007). Metabolism by cyclo-oxygenases and other oxidative enzymes can produce reactive oxygen species, leading to oxidative damage to tissues. OTA has been suggested to induce DNA adducts (see Pfohl-Leszkowicz and Manderville, 2007, and Chapter 6).

## *3.4 Deoxynivalenol*

### *3.4.1 Absorption*

Depending on geographical location, the predominant trichothecenes may be DON, nivalenol (NIV), or one of their acetylated precursors. However, DON is the focus of this section. For more information specific to NIV and acetylated derivatives of DON and NIV, see Pestka (2010a) and Sugita-Konishi and Nakajima (2010).

Absorption, distribution, and elimination of DON are rapid after either oral or parenteral dosing. No evidence has been found for DON accumulation in tissues or transmission to eggs or milk at the DON levels normally encountered in animal feed (Prelusky *et al*., 1994). The potential for extensive metabolism in the gastrointestinal tract via de-epoxidation reactions results in the formation of de-epoxy DON (DOM-1) (reviewed in WHO, 2001; Pestka, 2010a, 2010b).

### *3.4.2 Gastrointestinal metabolism*

A great deal of information has been published about the ability of microorganisms in the gastrointestinal tract to metabolize DON. In some studies, incubation with cultures or extracts from the gastrointestinal tract has resulted in extensive conversion of DON to the de-epoxy metabolite, DOM-1 (WHO, 2001). Near-complete de-epoxidation has been shown using intestinal contents from pigs and chickens and bovine rumen fluid. Pigs lacking the ability to carry out intestinal de-epoxidation can acquire the ability through contact with faeces from pigs capable of making the de-epoxy metabolite (Eriksen *et al*., 2003). It has also been shown that de-acetylation of 3-acetylDON to DON occurs in the pig gastrointestinal tract before DON is absorbed. Whereas the de-epoxides of DON and NIV are less cytotoxic than the parent compounds, de-epoxidation, unlike de-acetylation, appears to occur primarily in the distal portion of the digestive tract, where DON absorption is low (Eriksen *et al*., 2003).

### *3.4.3 Bioavailability*

DON is rapidly absorbed from the gastrointestinal tract in sheep, cows, and pigs (WHO, 2001). In sheep, the bioavailability is low (< 10%). Bioavailability in cows also appears to be low, whereas one study estimated that the systemic bioavailability in pigs was 55%. After a single intragastric dose in pigs, the peak plasma level occurred at 15–30 minutes and the plasma half-life was 7.1 hours. The plasma half-life after intravenous dosing was 3.9 hours in pigs and 100–125 minutes in sheep. When pigs were fed diets containing 3-acetylDON for 3 days, DON was detected in plasma 20 minutes after the first feeding (Eriksen *et al*., 2003). The maximal plasma level occurred

2.8 hours after feeding, suggesting that absorption started in the stomach or the upper part of the duodenum.

### 3.4.4 Distribution

The little information available on the distribution of DON in tissues suggests that this compound is rapidly and widely distributed but is not accumulated. In chickens, 3 hours after a single oral dose of radiolabelled DON, the relative accumulation was bile >> kidney > blood/plasma > liver >> other tissues (WHO, 2001). At later time points (72 and 96 hours), very little or no DON was detected. In pigs, 3 hours after a single intravenous dose, DON was distributed to all tissues examined and the relative accumulation was kidney > plasma > liver > fat >> other tissues (WHO, 2001). At 24 hours after dosing, the levels in all tissues were reduced by > 90% relative to those at 3 hours after dosing.

### 3.4.5 Excretion

DON is rapidly eliminated in urine and faeces in chickens, sheep, and pigs (WHO, 2001). In chickens, 79%, 92%, and 98% of an oral dose could be accounted for in excreta at 24, 48, and 72 hours after dosing, respectively. In sheep, 36 hours after a single oral dose, 6.9%, 0.11%, and 64% of the dose was recovered in urine, bile, and faeces, respectively. In ewes, 91% and 6% of a single intravenous dose was recovered in urine and bile, respectively, after 24 hours, and a similar result was seen in pigs. Pigs consuming wheat naturally contaminated with DON excreted 50–62% of the DON in their urine (Goyarts and Dänicke, 2006). In a feeding study with 3-acetylDON, 45% and 2% of the dose was recovered in urine and faeces, respectively, 48 hours after the pigs were taken off the contaminated diets, and the remainder of the dose was unaccounted for (Eriksen *et al*., 2003).

### 3.4.6 Transmission

DON was not detected in milk from cows fed a diet containing maize naturally contaminated with DON at up to 12 mg/kg dry diet for 10 weeks (WHO, 2001). Low levels of DON (< 1.7 mg/egg) were detected in eggs from chickens fed diets containing DON at 5.5 mg/kg diet for 65 days. Other studies also indicate that transmission to milk is small in sheep and cows. Transmission of DON to edible animal products should pose little health risk to consumers.

### 3.4.7 Metabolism

No evidence has been found that DON is metabolized by microsomal enzymes. However, evidence for glucuronide conjugation in sheep and pigs is well documented (WHO, 2001; Eriksen *et al*., 2003). In some studies with sheep, the glucuronide conjugate of DON can account for a large percentage of the total plasma DON. In pigs fed diets containing 3-acetylDON, 42% and 33% of the DON in plasma and urine, respectively, was conjugated to the glucuronide (Eriksen *et al*., 2003). In that study, a significant portion of the DON in faeces was the de-epoxide.

## 3.5 Zearalenone

### 3.5.1 Absorption

ZEA is rapidly absorbed and eliminated. It is metabolized in the liver (and possibly the intestinal mucosa) and excreted in urine and faeces as the glucuronide after considerable enterohepatic recirculation. It is metabolized by rumen microbes. Accumulation in tissues is minimal. Comprehensive reviews of the toxicokinetics of ZEA in animals are available (Fink-Gremmels and Malekinejad, 2007; Zinedine *et al*., 2007).

### 3.5.2 Gastrointestinal metabolism

ZEA can be degraded in the rumen. In both sheep and cattle, rumen fluid reduces ZEA to its more easily excreted metabolites, α- and β-zearalenol (Raisbeck *et al*., 1991).

### 3.5.3 Bioavailability

ZEA is poorly absorbed from the gut of chickens (Christensen, 1979). For example, in chickens, 12 hours after oral dosing, 0.08% of the dose was recovered in tissues and organs, whereas 99% was recovered in excreta, bile, and the digestive tract plus its contents (Christensen, 1979). Peak plasma concentrations occurred 2–6 hours after a single oral dose in broilers (Bernhoft *et al*., 2001). Oral bioavailability may be much greater in other species; for example, in rats the oral bioavailability was reported as 2.7% of the dose (Shin *et al*., 2009). In pigs orally dosed with ZEA, the half-life in plasma was 87 hours (Biehl *et al*., 1993) due to extensive enterohepatic recirculation.

### 3.5.4 Distribution

In rats orally dosed with ZEA, only very low levels were detected in tissues (Christensen, 1979). The liver contained the highest levels of ZEA of the tissues examined (Christensen, 1979; Bernhoft *et al*., 2001). ZEA and its metabolite α-zearalenol can cross the placenta and enter the fetus (Bernhoft *et al*., 2001).

### 3.5.5 Excretion

The half-life of ZEA in pigs dosed either intravenously or orally was 87 hours (Biehl *et al*., 1993). When bile was removed, the half-life was reduced to 3 hours, indicating

that enterohepatic cycling of ZEA (principally as the glucuronide) is extensive in pigs. Excretion in bile is rapid in broilers; peak concentrations occur 2–6 hours after a single oral dose (Dänicke *et al*., 2001). In chickens orally dosed with ZEA, 75% of the administered dose was recovered in excreta after 24 hours (Christensen, 1979). Zearalenol can be detected as the glucuronide in urine and faeces of pigs and in excreta of chickens (Christensen, 1979; CAST, 2003). α-Zearalenol glucuronide appears to be the major metabolite detected in pig urine and serum after prolonged exposure to ZEA (Dänicke *et al*., 2005).

### *3.5.6 Transmission*

ZEA is poorly transferred to milk as zearalenol derivatives (< 1% of dose) and may induce signs of estrogenism in female piglets (Osweiler, 2000). In the liver, α-zearalenol is detected more frequently than β-zearalenol. Trace amounts of ZEA and its metabolites can be detected in muscle tissues of pigs fed oats contaminated with ZEA; however, residues do not persist (Zöllner *et al*., 2002).

### *3.5.7 Metabolism*

After considerable enterohepatic re-circulation, ZEA is metabolized in the liver and excreted in urine and faeces as the parent compound, the metabolites α-zearalenol and/or β-zearalanol, or their respective glucuronide conjugates (reviewed in Fink-Gremmels and Malekinejad, 2007; Zinedine *et al*., 2007). The enzymes responsible for the conversion of ZEA to α- and β-zearalenol are 3-α- and 3-β-hydroxysteroid dehydrogenase, respectively. These two microsomal enzymes are important in steroid metabolism, so ZEA has the potential to disrupt steroid metabolism because the substrates for these enzymes include natural steroid hormones (reviewed in Fink-Gremmels and Malekinejad, 2007). The intestinal mucosa may also actively reduce ZEA to α-zearalenol and mediate conjugation to glucuronic acid (Biehl *et al*., 1993). The rapid conversion of ZEA to the more easily excreted α- and β-zearalenol derivatives in cattle, along with microbial metabolism in the rumen, could explain the relative resistance of cattle to the reproductive effects of ZEA, compared with pigs (Raisbeck *et al*., 1991).

## *3.6 Ergot alkaloids*

### *3.6.1 Absorption*

The absorption and metabolism of ergot alkaloids may be quite different in monogastrics and ruminants. Water-soluble alkaloids appear to be more strongly absorbed than lipophilic ones, and rumen microbes appear to play an important role in the release and intestinal availability of soluble alkaloids in the rumen (Hill, 2005; Ayer *et al*., 2009). The more soluble alkaloids, such as lysergic acid, are excreted in urine, and the less soluble alkaloids can undergo enterohepatic recirculation and are excreted primarily in bile. Transmission is unlikely, and accumulation in tissues, if any, is low. Once absorbed, ergot alkaloids such as ergotamine can be metabolized by cytochrome P450 enzymes.

### *3.6.2 Gastrointestinal metabolism*

Studies in sheep and cattle suggest that rumen microorganisms can degrade plant material and release the more soluble ergoline alkaloids (Hill, 2005; Ayer *et al*., 2009). The small amounts of less soluble ergopeptine alkaloids (ergonovine and ergovaline) present in rumen fluids are quickly degraded by rumen microorganisms to metabolites such as lysergic acid (Moyer *et al*., 1993; Hill, 2005; Ayer *et al*., 2009). In cattle, the main ergot alkaloid found in urine is lysergic acid (Stuedemann *et al*., 1998). Gastrointestinal metabolism in monogastrics is probably limited (Hill, 2005).

### *3.6.3 Bioavailability*

Gastrointestinal absorption of ergot alkaloids is low (< 5%), and clearance is rapid (Haschek *et al*., 2002). However, physiological effects can be persistent, suggesting either that metabolites are tightly bound or that an undiscovered reservoir exists in the body. The bioavailability of ergot alkaloids appears to be a function of solubility. The ergopeptine alkaloids are less soluble than the ergoline alkaloids and when administered orally must be dissolved in lipophilic carriers or chemically modified to improve solubility (Hill, 2005). In sheep rumen and omasal tissue, transport of alkaloids appears to be an active process, and lysergic acid and lysergol are transported much more effectively than are ergopeptine alkaloids (Hill *et al*., 2001).

### *3.6.4 Distribution*

Ergot alkaloids are widely distributed, as evidenced by the fact that most of the physiological effects involve direct interaction with dopamine receptors in the peripheral vasculature and, in some cases, in the brain and other neuronal tissue (see Section 4.6).

### *3.6.5 Excretion*

Once ergot alkaloids are absorbed, clearance is very rapid. For example, intravenously administered ergovaline was shown to have a plasma half-life of 24 minutes in sheep (Jaussaud *et al*., 1998) and a half-life of 56 minutes in horses (Bony

*et al*., 2001). Ergopeptine alkaloids are excreted in bile and ergoline alkaloids in urine (Hill *et al*., 2001). In cattle grazing on tall fescue infected by endophytes, the urine contained approximately 96% of the total ergot alkaloid excreted, and after 2 days of grazing on grass free of endophytes, the urinary alkaloids were reduced to control levels (Stuedemann *et al*., 1998). The maximal levels of ergot alkaloids in urine were attained within 48 hours, and the main alkaloid was lysergic acid.

### *3.6.6 Transmission*

There are no reports that ergot alkaloids are transferred to milk or accumulate in tissues.

### *3.6.7 Metabolism*

Ergopeptine alkaloids are metabolized in the liver via cytochrome P450 enzymes such as CYP3A, and hydroxylated metabolites are excreted in bile (Moubarak and Rosenkrans, 2000; Haschek *et al*., 2002). Ergoline alkaloids (i.e. lysergic acid), which are more water soluble, are excreted in urine (Hill, 2005; Ayer *et al*., 2009) and faeces in amounts greater than that consumed, suggesting that ergovaline and possibly other ergot alkaloids are metabolized in tissues to lysergic acid (Schultz *et al*., 2006).

## 4. Toxicological effects

Only a few known mycotoxins pose a measurable health risk to farm animals, for several reasons. First, a fundamental tenet of toxicology is "the dose makes the poison". Thus, even though farm animals are exposed to mycotoxins every day through their feed, the dose is usually insufficient to make the contaminated feed acutely poisonous. Second, the doses and routes of exposure used in controlled laboratory experiments cannot model the uncontrolled exposure of farm animals to naturally contaminated feeds and foods in the field, where multiple factors contribute to the expression of disease. Thus, the potential for toxicity revealed in controlled experiments is often not predictive of the levels of exposure in feeds associated with suspected field outbreaks of disease. One explanation for the difficulty in equating dose–response in laboratory studies with dose–response in the field is the inability to identify all the environmental, nutritional, and genetic factors that contribute to disease expression. Nevertheless, information gained from in vitro studies and studies with laboratory animals is predictive of the possible contribution of mycotoxins in altering immune function (Bondy and Pestka, 2000), thereby contributing to unexplained animal diseases and performance problems in farm animals (Osweiler, 2000). Several excellent reviews have documented the toxicology of mycotoxins in farm animals and provided extensive descriptions of the clinical manifestations (Oltjen, 1979; Richard and Thurston, 1986; Raisbeck *et al*., 1991; WHO, 2001; Haschek *et al*., 2002; CAST, 2003; Cousin *et al*., 2005; O'Brien and Dietrich, 2005; Fink-Gremmels and Malekinejad, 2007; Pestka, 2007, 2010a, 2010b; Pfohl-Leszkowicz and Manderville, 2007; Voss *et al*., 2007; Zinedine *et al*., 2007; Steyn *et al*., 2009). In this section, we describe the main clinical signs in farm animals exposed to the levels of mycotoxins encountered in field outbreaks. We also present postulated responses to low levels of mycotoxins.

### *4.1 Aflatoxins*

The overt symptoms of aflatoxin poisoning are not definitive. Animals do not eat well and therefore have reduced weight gain and decreased feed efficiency. The effects on growth are dose-dependent and at low levels may be barely discernible. Other performance effects include decreased reproductive performance, abortion, and reduced egg or milk production. In turkeys, a sensitive species, reduced weight gain is seen at a dose of 125 µg/kg diet, impaired immune response and increased mortality at 250 µg/kg, and acute mortality at 500 µg/kg (Norred, 1986). A similar relative dose–response occurs in pigs but at higher levels of exposure because they are less affected by aflatoxins. In cattle and chickens, much higher levels are required to induce a decrease in performance, and in chickens impaired immune response can occur at levels that have no effect on the growth rate. In all species, aflatoxins are hepatotoxic, with fatty changes, hepatocyte degeneration, necrosis, and altered liver function. A common clinical sign is jaundice. Grossly, the liver appears pale and swollen or fatty with variable texture. In chickens, fatty liver syndrome is believed to be caused by aflatoxin (Norred, 1986), although OTA can also cause fatty liver in poultry (Trenholm *et al*., 1988). Liver damage ultimately can lead to coagulopathy, as evidenced by haemorrhaging and anaemia (Fig. 5.1). In poultry and pigs, coagulopathy contributes to the appearance of internal bruising during handling. In pigs, this may occur at levels as low as 150 µg/kg diet (Edds, 1979).

The mechanism of action of aflatoxins involves metabolism to reactive intermediates and their binding to macromolecules (nucleic acids and proteins) and consequent disruption of transcriptional and translational processes and regulatory pathways critical for repair of damaged DNA, for cell growth, death, and differentiation, and ultimately for toxicity and

carcinogenicity (Wild and Gong, 2010; Eaton *et al*., 2010; Kensler *et al*., 2011). Some evidence has been found that aflatoxin $B_1$ produces reactive oxygen species, resulting in oxidation of DNA bases (Guindon *et al*., 2007).

The response of an animal to aflatoxin depends to a large extent on the rate of metabolism and the type of metabolites that are produced (see Section 3.1.7 and Chapter 6). For example, quail and turkeys are sensitive to aflatoxin toxicity and have a high rate of epoxide formation and a low rate of glutathione conjugation. In resistant species, even if the rate of epoxidation is high, a high rate of glutathione conjugation is protective. However, the resulting clinical signs are similar although the dose dependence may be quite different. Aflatoxin adducts in urine and blood of farm animals may be very useful as a biomarker for exposure during suspected field outbreaks (Riley *et al*., 2011).

### *4.2 Fumonisins*

Consumption of feeds contaminated with fumonisins is a proven cause of two farm animal diseases and a suspected cause of others. ELEM is a fatal neurotoxic disease that occurs only in equids (horses and related species). The disease is characterized by the presence of liquefactive necrotic lesions in the white matter of the brain; the grey matter may also be involved (WHO, 2000). All aspects of the disease can be reproduced experimentally. The brain lesions are caused by vasogenic cerebral oedema (Haschek *et al*., 2002; Foreman *et al*., 2004) and are accompanied by increased protein in the cerebrospinal fluid and other changes consistent with vasogenic cerebral oedema (Smith *et al*., 2002; Foreman *et al*., 2004). Early symptoms include lethargy, head pressing, and decreased feed intake, followed by convulsions and death after several days. Early clinical signs include hindlimb ataxia, delayed forelimb placing reactions, and tongue paresis (Fig. 5.2), which are all mild signs of proprioceptive dysfunction (Foreman *et al*., 2004). Elevation in levels of serum enzymes indicative of liver damage occurs soon after elevation in levels of free sphingoid bases and sphinganine-1-phosphate in serum. The elevation in sphinganine levels is also seen in the liver and kidney (Riley *et al*., 1997) and other tissues (Tumbleson *et al*., 2003). Sphinganine-1-phosphate is also greatly elevated in serum of horses treated with pure fumonisin $B_1$ (Constable *et al*., 2005). The elevated serum and tissue levels of free sphingoid bases and sphinganine-1-phosphate are biomarkers for exposure to potentially toxic levels of fumonisins (Riley *et al*., 2011) and have been used in studies in horses, pigs, rabbits, poultry, and other farm animals (WHO, 2000). In horses, serum enzyme levels often return to near-normal concentrations but usually increase markedly immediately before, or at the first signs of, behavioural changes indicative of the onset of ELEM (WHO, 2000, 2001).

In addition to the brain lesions, histopathological abnormalities in the liver and kidney have been reported in horses, and these are also correlated with elevation in levels of free sphinganine (Tumbleson *et al*., 2003). ELEM concurrent with significant liver disease and fatal liver disease in the absence of any brain lesions have been observed in horses and ponies. The appearance of the clinical disease is likely to depend on multiple factors, including the length of exposure, level of contamination, individual animal differences, previous exposure, and pre-existing liver impairment.

In equids, the minimum toxic level of fumonisin $B_1$ in feed for inducing ELEM appears to be between 15 mg/kg and 22 mg/kg diet (WHO, 2000, 2001). Analysis of feeds from confirmed cases of ELEM indicated that a diet containing a fumonisin $B_1$ concentration of > 10 mg/kg diet presented an increased risk of developing ELEM (WHO, 2000, 2001).

Like ELEM, PPE syndrome is a rapid-onset disease that is often fatal to affected animals. Clinical signs typically occur 2–7 days after pigs start consuming diets containing large amounts of fumonisins. Clinical signs include decreased feed consumption, dyspnoea, weakness, cyanosis, and death. When animals are examined at necropsy, varying amounts of clear yellow fluid are seen in the pleural cavity together with varying degrees of interstitial and interlobular oedema, with pulmonary oedema and hydrothorax (Fig. 5.3). Toxic hepatosis usually occurs concurrently with pulmonary oedema, and in some animals that consume high levels of fumonisins, toxic hepatosis appears without signs of pulmonary oedema. Nodular hyperplasia has been observed in some pig livers (WHO, 2000, 2001).

As with ELEM, fumonisin concentration in maize screenings obtained from different farms was closely correlated with outbreaks of PPE in the USA in 1989–1990. The minimum dose necessary to induce this disease has not been clearly established, but in diets containing fumonisin $B_1$ from culture material, concentrations as low as 17 mg/kg diet induced pulmonary oedema in 5 days, whereas concentrations of 150–170 mg/kg diet for up to 210 days caused liver effects early on but no evidence of pulmonary oedema (WHO, 2000). Pigs fed a diet containing fumonisin $B_1$ at 45 mg/kg diet for 10 days developed mild signs of pulmonary oedema,

including the accumulation of fluid in the pleural cavity, which persisted in several animals 10 days after removal from the contaminated diets (Fodor *et al*., 2008). Pigs given a single oral dose of 5 mg/kg bw of fumonisin $B_1$ (equivalent to 83 mg/kg diet) did not develop pulmonary oedema but did show behavioural and clinical signs of toxicity suggestive of its onset (Dilkin *et al*., 2010).

In pigs, tissues other than the liver and lung that have been reported to be targets for fumonisins include the pancreas, heart, kidney, spleen, pulmonary intravascular macrophages, and oesophagus. Altered growth and changes in selected haematological parameters in pigs have been reported at dietary levels as low as 1 mg/kg diet (WHO, 2000). In weaned pigs, growth, attainment of sexual maturity, and sperm production were impaired in animals consuming diets containing fumonisin $B_1$ at > 5 mg/kg diet (Gbore, 2009). The physiological basis for performance problems induced in pigs by fumonisin is unclear; however, several in vitro and in vivo studies have shown that fumonisin exposure can have deleterious effects on intestinal integrity and function, which can lead to altered intestinal immune responses and possibly other effects on intestinal physiology (Bouhet *et al*., 2004; Bouhet *et al*., 2006; del Rio Garcia *et al*., 2007; Loiseau *et al*., 2007; Devriendt *et al*., 2009; Lessard *et al*., 2009). Oral exposure to feed contaminated with fumonisins also has effects on other immune responses, including sex-specific decreased antibody titres after vaccination and increased susceptibility to secondary pathogens (Halloy *et al*., 2005; Marin *et al*., 2006).

Several published reports suggest the involvement of *Fusarium verticillioides* in diseases of poultry, and by implication the presence of fumonisin contamination in feed.

**Fig. 5.1.** (a) Liver from a chick fed a diet containing 7.5 mg/kg of aflatoxin $B_1$; (b) a normal liver. Similar results are seen in turkey poults fed the same diets. Photograph courtesy of Timothy Phillips, Texas A&M University.

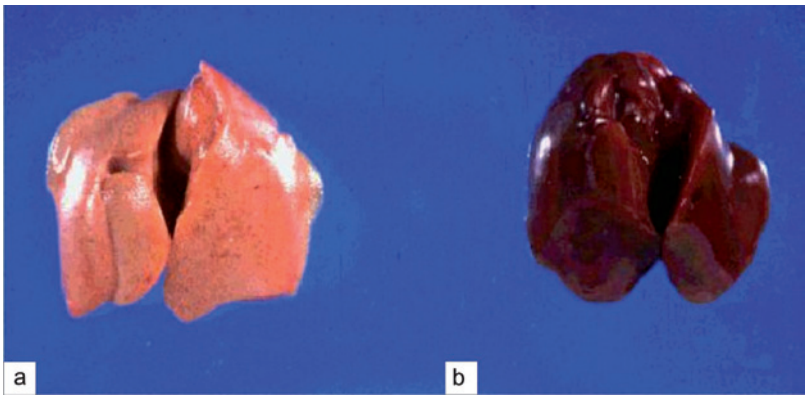
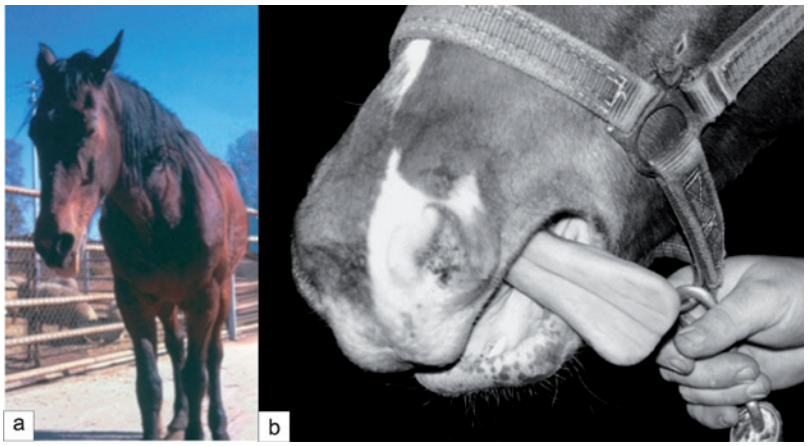
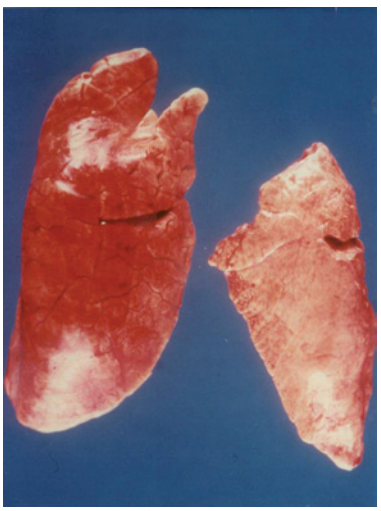



**Fig. 5.2.** (a) A horse in the initial stage of equine leukoencephalomalacia. Photograph courtesy of Walter F.O. Marasas, Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC Unit), South African Medical Research Council. (b) A weanling Appaloosa filly administered fumonisin $B_1$ intravenously at 0.20 mg/kg bw/day for 7 days. Note the severe displacement of the tongue after manual exteriorization by the examiner. From day 4 through day 7, this filly developed progressive tongue paresis, proprioceptive deficits, and ataxia in all four limbs, hind limb spasticity and paresis, and forelimb hypermetria. On the day of euthanasia, it kept its tongue out of its mouth for periods of up to 1 minute at a time. Source: Foreman *et al*. (2004); reproduced with the permission of the publisher.




Clinical features include diarrhoea, weight loss, increased liver weight, poor performance, and increased susceptibility to infectious diseases. Several studies have confirmed that *F. verticillioides*, *F. proliferatum*, fumonisin $B_1$, and moniliformin are toxic to poultry (broiler chicks, turkeys, turkey poults, quail, and ducklings). The levels of fumonisin used in many of the early studies were quite high (75–644 mg/kg diet) because poultry appeared to be unusually resistant to dietary fumonisins (WHO, 2000, 2001). However, toxicity and altered haematological parameters have been observed in broiler chicks fed diets containing only 10 mg/kg of pure fumonisin $B_1$ and 30 mg/kg of fumonisin $B_1$ from *F. verticillioides* culture material (WHO, 2000, 2001). Like other poultry, ducks are

**Fig. 5.3.** (Left) A lung with severe oedema from a pig with porcine pulmonary oedema after being fed fumonisin-contaminated feed and (right) a normal lung. Photograph courtesy of Wanda Haschek, University of Illinois.



relatively resistant to the toxic effects of fumonisins (Tran *et al*., 2005). However, increased mortality and toxicity have been observed in ducks force-fed diets containing 20 mg/kg of fumonisin $B_1$ (Tardieu *et al*., 2009). As in pigs, fumonisin can also alter immune response and susceptibility to infectious agents in poultry (Tessari *et al*., 2006; Deshmukh *et al*., 2007).

Other farm animals that have been studied using pure fumonisins, contaminated maize screenings, or maize culture material from *F. verticillioides* include carp, catfish, lambs, goats, trout, cattle, mink, and rabbits. Rabbits are especially sensitive to nephrotoxicity (Voss *et al*., 2001; Ewuola, 2009). In all cases where toxicity was evident, it involved the liver and/or kidney or the equivalent organs in fish.

Disruption of lipid metabolism appears to be the underlying mechanism by which fumonisins cause toxicity to animals (WHO, 2000, 2001). The initial mechanism of action of fumonisin is inhibition of ceramide synthase, a key enzyme in the de novo sphingolipid biosynthesis pathway (Wang *et al*., 1991). Fumonisins are structurally similar to sphingoid bases and especially 1-deoxysphinganine, which lacks a hydroxyl group on carbon 1 (Zitomer *et al*., 2009). Sphingoid bases are essential components of the chemical backbone of all sphingolipids in animals. When toxicity associated with fumonisins is observed in laboratory or farm animals, the onset and severity of the pathology is closely correlated with evidence of disrupted sphingolipid metabolism (Riley *et al*., 1996, 2001; WHO, 2000, 2001, 2012; Riley and Voss, 2006; Voss *et al*., 2011). Disrupted sphingolipid metabolism can also be evident at dosages that do not cause overt toxicity (NTP, 2001). This is especially true in resistant species such as ducks (Tardieu *et al*., 2006). The major biochemical and cellular consequences resulting from blockage of ceramide biosynthesis are the accumulation of free sphingoid bases and sphingoid base 1-phosphates (Riley and Voss, 2006; Zitomer *et al*., 2009), the depletion of more complex sphingolipids (Voss *et al*., 2009), and the global disruption of lipid metabolism (WHO, 2001). The changes in concentrations of important lipid mediators lead ultimately to perturbation of the signalling pathways and altered regulatory and physiological processes (Lemmer *et al*., 1999; Bondy *et al*., 2000; Merrill *et al*., 2001), which are the basis for the observed clinical signs associated with the diseases induced by fumonisins. ELEM is associated with alterations in cardiovascular function induced by sphingolipids, i.e. deregulation of cerebral arteries responsible for autoregulation of blood flow to the horse's brain (Haschek *et al*., 2002; Foreman *et al*., 2004). PPE is hypothesized to be a result of acute left-sided heart failure as a consequence of inhibition of L-type calcium channels induced by sphingoid bases (Haschek *et al*., 2002). The elevation of free sphingoid bases and sphingoid base 1-phosphates and the depletion of more complex sphingolipids in tissues, serum, and urine have proven to be useful biomarkers for exposure and the effects of fumonisin in farm animals (Riley *et al*., 2011).

### *4.3 Ochratoxin A*

The primary effect of OTA in all farm animals is nephrotoxicity. In pigs and poultry, the proximal tubules are mainly affected and the kidney is pale and grossly enlarged (Fig. 5.4).

Fatty liver can occur in poultry. The most sensitive indicator of acute ochratoxicosis in chickens is the reduction in total serum proteins and albumin. A decrease in phosphoenolpyruvate carboxykinase in the kidney is a sensitive and specific indicator in pigs (Krogh, 1992; Marquardt and Frohlich, 1992). In pigs, large increases in levels of proteins excreted in urine are indicative of glomerular proteinuria and correlate with histological observations of renal damage.

In poultry and pigs, exposure to OTA at lower levels can result in altered performance, including decreased feed consumption and reduced weight gain, and at higher levels can result in delayed response to immunization and increased susceptibility to infection (Stoev *et al*., 2000a, 2000b). Other effects in poultry include decreased egg production, coagulopathy (increased susceptibility to bruising during processing), decreased bone strength, decreased tensile strength of the large intestines, underpigmentation, and glycogen accumulation in the liver.

The mechanism of action in farm animals is unclear. However, the structural similarity of OTA to phenylalanine and the fact that it inhibits many enzymes and processes that are dependent on phenylalanine strongly suggest that OTA acts at least

**Fig. 5.4.** Examples of pig kidneys taken at slaughter, showing increasing degrees of mycotoxic nephropathy, with enlarged and mottled or enlarged and pale kidneys. Mycotoxic nephropathy in these animals was attributed to exposure to ochratoxin A and other mycotoxins (primarily, fumonisin and penicillic acid); however, mycotoxic nephropathy has also been reported in pigs exposed primarily to ochratoxin A (Elling and Moller, 1973; Krogh, 1974). Source: Stoev *et al.* (2010); reproduced with the permission of the publisher.

partially by disrupting phenylalanine metabolism (CAST, 2003; Riley *et al.*, 2011). Several studies have shown that supplementation of feed with L-phenylalanine or proteins protects against the toxic effects of OTA, including mortality (Marquardt and Frohlich, 1992; WHO, 2001). In addition to inhibition of protein synthesis via binding to phenylalanine-tRNA synthetase, recent studies have demonstrated the ability of OTA to induce oxidative stress, reduce cellular defence, and alter signalling pathways involved in various aspects of cellular and mitotic regulation (Mally and Dekant, 2009). The ultimate consequence of generalized disruption of these metabolic and regulatory pathways is increased cell death, and the kidney is the most sensitive target because of its ability to accumulate OTA to high levels. Because OTA binds tightly to albumin and serum proteins, serum OTA is a useful biomarker for exposure in pigs.

### *4.4 Deoxynivalenol*

Many reviews on the toxicity of DON have been published (Beasley, 1989; Prelusky *et al.*, 1994; Rotter *et al.*, 1996, WHO, 2001; Pestka, 2010a, 2010b). Although DON is not considered to be acutely toxic to farm animals, it is considered to be a major cause of economic losses due to reduced performance (Miller, 2008). In the field, concentrations as low as 1 mg/kg have been associated with feed refusal in pigs; however, more typically concentrations of > 2–5 mg/kg are required for decreased feed intake and reduced weight gain and concentrations of > 20 mg/kg for vomiting and feed refusal (Trenholm *et al.*, 1988; Haschek *et al.*, 2002; Fig. 5.5). Dogs and cats are also sensitive to the emetic effects of DON, and acetylated DON also induces emesis. Feed refusal and emesis appear to be due to neurochemical imbalances in the brain, and although the emetic centre is clearly involved, the mechanism appears to be indirect (Miller, 2008; Pestka, 2010a). In pigs, feed refusal occurs even when DON is administered intraperitoneally, so feed refusal cannot be due to taste or learned responses (Prelusky, 1997). Clinical signs include gastrointestinal problems, soft stools, diarrhoea, increased susceptibility to other diseases, and decreased performance. In pigs, mild renal nephrosis, reduced thyroid size, gastric mucosal hyperplasia, increased albumin-to-α-globulin ratio, and sometimes mild changes in other haematological parameters have been reported (WHO, 2001). Numerous studies in laboratory animals have demonstrated alterations in immune function induced by DON (Bondy and Pestka, 2000; Pestka, 2010a), but conclusive evidence that DON induces altered resistance to infectious diseases in the field is still lacking (Osweiler, 2000). Nevertheless, the mechanism of action in laboratory animals suggests, and controlled studies in the laboratory setting support, the potential for involvement by DON in altered immune response.

The mechanism of action of DON is complex. Disruption of ribosomal and endoplasmic reticulum function as a consequence of DON binding to ribosomes is clearly a key event in disease causation in animals (Pestka, 2010a, 2010b). Binding of DON to ribosomes is known to inhibit translation by preventing polypeptide chain initiation or elongation in animals (Ueno, 1984). However, several other ways exist in which DON can interfere with protein synthesis, all involving disrupted ribosomal function (Pestka, 2010a). The downstream cellular consequences of disrupted ribosomal function appear to be dose-dependent, with low doses being cytostimulatory and high doses cytotoxic (Pestka, 2010a). For example, DON is

**Fig. 5.5.** Reduced weight gain in pigs, an example of the effects of deoxynivalenol. These pigs are littermates, but the pig in the foreground was fed a diet containing deoxynivalenol at 5 mg/kg diet for 7 weeks after weaning. Note also the white, rat-like hair of the pig fed the deoxynivalenol-containing diet. Source: Trenholm *et al*. (1988), Fig. 3, p. 15; reproduced with the permission of the Minister of Public Works and Government Services Canada, 2012.

known to increase expression of pro-inflammatory cytokines, affect transcription factors for many genes related to immunity and inflammation, increase mRNA expression, increase mRNA stabilization, affect MAP kinase signalling, and induce the ribotoxic stress response, all of which contribute to its biological effects in animals (Pestka, 2010b). It has also recently been shown that DON disrupts the growth hormone axis; this finding provides a credible explanation for growth retardation in animals. Specifically, DON induces expression of pro-inflammatory cytokines, which leads to upregulation of suppressors of cytokine signalling, which leads ultimately to reduced levels of both insulin-like growth factor 1 and insulin-like growth factor acid-labile subunit in the blood (Amuzie and Pestka, 2010). The combined use of urinary DON glucuronide level as an exposure biomarker (Turner, 2010) and changes in plasma levels of insulin-like growth factor 1 and insulin-like growth factor acid-labile subunit as mechanism-based biomarkers (Amuzie and Pestka, 2010) could prove useful as markers of DON involvement in disease outbreaks in farm animals (Riley *et al*., 2011).

### *4.5 Zearalenone*

The species that is the most sensitive to the effects of ZEA is the pig. The observed effects of ZEA on pigs involve primarily the reproductive system (Fitzpatrick *et al*., 1989; Fink-Gremmels and Malekinejad, 2007; Zinedine *et al*., 2007). The sensitivity of the pig appears to involve the way in which this species metabolizes ZEA. Metabolism depends primarily on two hydroxysteroid dehydrogenases, which produce the two main metabolites α- and β-zearalenol. The α-zearalenol isomer has a much greater uterotropic activity than ZEA does, and in pigs the predominant form of hydroxysteroid dehydrogenase is the one that yields primarily α-zearalenol (Fink-Gremmels and Malekinejad, 2007). After binding of ZEA to estrogen receptors, the complex interacts with genes controlling both estrogen-like and antiestrogen-like responses (Boehme *et al*., 2009; Parveen *et al*., 2009). ZEA is also a ligand for the pregnane X receptor, which controls the expression of genes in some pathways that regulate biotransformation of endobiotics and xenobiotics (Ding *et al*., 2006). ZEA, and its metabolites and their conjugates, are detectable in urine and faeces and have been used as exposure biomarkers in animal studies (reviewed in Zinedine *et al*., 2007).

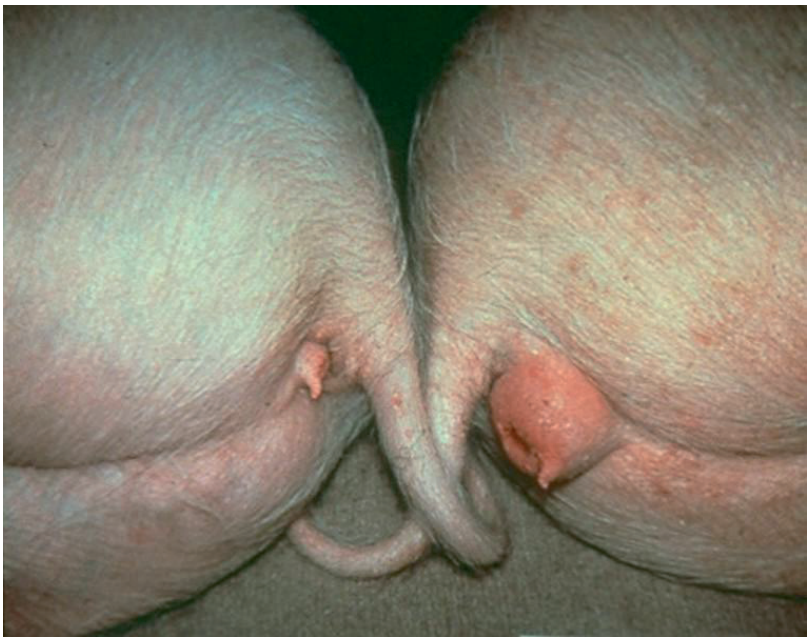
Clinical signs of oestrus can be induced in ovariectomized sows. Doses of ZEA as low as 1–5 mg/kg bw can induce vulvovaginitis, tenesmus, vaginal prolapse, and rectal prolapse in young female pigs (Osweiler, 1986; Fig. 5.6).

Effects on pre-pubertal boars have also been reported, and these include reduced libido, decreased plasma testosterone, and other effects (Osweiler, 1986). In sows, dietary levels of 3–10 mg/kg of ZEA can induce anoestrus, reduced litter size, fetal reabsorption, and implantation failure. Cattle are more resistant to the estrogenic effects; however, conception rates can be reduced.

### *4.6 Ergot alkaloids*

Based on major signs, ergotism in farm animals can be divided into the gangrenous form and the convulsive form (Robbins *et al*., 1985; Raisbeck *et al*., 1991). Livestock consuming feed grains containing small seed grasses or foraging on pasture grasses are at particular risk. Cattle are at greatest risk, and sheep and horses are less frequently affected. The symptoms of ergotism and toxic alkaloids (Oliver, 2005) include lameness, gangrene, agalactia, reduced weight gain, abortion, hypersensitivity, ataxia, convulsions,

**Fig. 5.6.** Vulvas of pre-pubertal gilts fed control diet (left) or zearalenone-contaminated feed (right). Note swelling and oedema due to zearalenone consumption. Photograph courtesy of Walter F.O. Marasas, Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC Unit), South African Medical Research Council.



and, in sheep, intestinal inflammation. In cattle grazing on tall fescue, syndromes known as fescue foot, summer slump, and fat necrosis have been described (Robbins *et al.*, 1985). Fescue foot is characterized by gangrene of the extremities (feet, ears, and tail) and is caused by vasoconstriction resulting in reduced blood flow and ischemia in the peripheral tissues (Oliver 2005; Fig. 5.7). This is usually accompanied by increased core body temperature in cattle. Necrosis and loss of the tip of the tail is a common sign. Cold weather aggravates the problem of fescue foot, whereas summer slump is commonly associated with warm conditions. Summer slump is characterized by reduced milk production, heat intolerance, rough hair coat, increased respiration rate, decreased feed intake, reduced serum prolactin levels, and reduced conception rates (Robbins *et al.*, 1985; Raisbeck *et al.*, 1991; Spiers *et al.*, 2005). A common sign in herds with summer slump is that the cattle spend much of their time seeking shade or standing in ponds (Raisbeck *et al.*, 1991). Fat necrosis is characterized by hard fat masses in the adipose tissue of the abdominal cavity. Fat necrosis is believed to require long-term consumption (for years) of tall fescue, whereas summer slump and fescue foot may occur within weeks of exposure (Raisbeck *et al.*, 1991). In all cases, it is the vasoconstrictive action of the ergot alkaloids and their metabolites (Hill *et al.*, 2001; Ayer *et al.*, 2009) that is the underlying cause of the three common fescue toxicities. In horses, reproductive problems resulting from consumption of ergot alkaloids in tall fescue include longer gestation, stillborn foals, agalactia, placental retention, and reduced breeding efficiency (Cross, 2003). Laminitis can also be caused by consumption of toxic tall fescue and, like fescue foot in cattle, it is believed to be caused by vasoconstriction in the extremities induced by ergot alkaloids (Cross, 2003).

## 5. Sensitive or target species and confounding factors

Although acute disease outbreaks from exposure to mycotoxins are not uncommon, definitively linking exposure to effects is not easy at the low levels of exposure most commonly encountered in the field. The observed physiological effects usually involve subtle changes in animal performance or behaviour and increased susceptibility to infectious disease, all of which are relatively nonspecific effects. Nevertheless, when changes in animal performance are observed and can be linked to the feed, exposure to low levels of mycotoxins in feed should be suspected as a contributing factor, at the very least. Young animals are more sensitive than adults to the adverse effects of mycotoxins, in part because of a higher metabolic rate. Factors that can influence susceptibility to performance problems and disease due to mycotoxins are sex, genetic strain, reproductive status, pre-existing conditions, nutritional factors such as vitamin- or protein-deficient diets, environmental stress, concurrent exposure to infectious agents, and exposure to other toxins, including mycotoxins. Target-organ specificity and species sensitivity are known largely from laboratory studies in which a single species has been studied using a single toxin. Comparative studies are quite rare, so dose–response relationships comparing species, sex, and exposure to multiple toxins at low levels – a scenario that is likely in field situations – are also rare. In this section, we briefly describe the susceptibility of farm and domestic animals to mycotoxins and the target organs for aflatoxins, fumonisins, OTA, DON, ZEA, and ergot alkaloids (summarized from WHO, 2001; Haschek *et al.*, 2002; CAST, 2003).

**Fig. 5.7.** A field case of fescue toxicity. The cow's tail switch has fallen off and a prominent ring has formed, produced by ergot alkaloids in tall fescue. In this animal, the foot is also affected (not shown) with reddening and swelling; this is common with gangrenous ergotism. Photograph courtesy of Charles Bacon, United States Department of Agriculture, Agricultural Research Service.

### 5.1 Aflatoxins

The liver is the primary target organ for aflatoxins, for both acute and chronic toxicity. The kidney can also be affected in pigs and goats. However, anecdotally the most commonly suspected indicator of exposure to aflatoxins is decreased performance and/or increased susceptibility to environmental and microbial stressors. Conversely, undernourished or stressed animals will be more sensitive to aflatoxin toxicity, and studies in rodents show that exposure to bacterial endotoxins augments liver injury induced by aflatoxin (Barton *et al*., 2000). Cyclopiazonic acid and aflatoxins frequently occur together in groundnuts and maize, and the original outbreaks of turkey "X" disease in the United Kingdom in 1960 may have involved both toxins (Cole, 1986). The most sensitive species for acute toxicity is the duckling (death), and the most sensitive species for chronic toxicity is the rainbow trout (liver cancer). Domestic turkeys and quail are also very sensitive. Reproductive effects have been reported in mink at low dietary levels (10 µg/kg), and dogs are also quite sensitive to acute toxicity. In poultry, the relative sensitivity is ducklings > turkey poults > goslings > chicks > quail. In other farm animals, the relative sensitivity is rabbits > young pigs > calves > mature cattle > sheep. However, these comparisons depend very much on the experimental conditions under which the studies were carried out. For example, young pigs are much more sensitive than older pigs. The susceptibility of a species to aflatoxin toxicity depends on its ability to metabolize aflatoxin efficiently and quickly to less toxic or nontoxic metabolites (see Section 3.1.7). Because maize is an important source, exposure to both aflatoxins and fumonisins is likely in animals consuming maize-based feeds.

### 5.2 Fumonisins

Fumonisins cause liver damage in all farm animals tested and also kidney damage in rabbits, cattle, and sheep. In addition, fumonisins induce species-specific toxicities, in the brain in horses and the lung in pigs, that are a consequence of cardiovascular dysregulation (WHO, 2001; Haschek *et al*., 2002). Because fumonisins inhibit sphingolipid biosynthesis, it is likely that receptors and processes that are dependent on sphingolipids are affected. For example, glycosphingolipids are necessary for the proper functioning of many membrane receptors, including those for some vitamins (i.e. folate) and for the recognition of numerous microbial pathogens and microbial toxins (i.e. Shiga-like toxins and cholera toxin) (Merrill *et al*., 2001). Studies have shown that pigs treated with fumonisins have increased susceptibility to intestinal infection with *Escherichia coli* (Oswald *et al*., 2003) and decreased specific antibody response during vaccination (Taranu *et al*., 2005). Fumonisins frequently co-occur with aflatoxins in maize and have been shown to promote aflatoxin-initiated liver tumours in rainbow trout (IARC, 2002b). Fumonisins also cause liver toxicity in catfish, poultry, mink, goats, and cattle (WHO, 2001). It has been suggested that mycotoxic nephropathy in pigs and chickens is a result of concurrent exposure to multiple mycotoxins, including fumonisin, OTA, and penicillic acid (Stoev *et al*., 2010). The relative sensitivity, based on the United States Food and Drug Administration (FDA) Guidance to Industry, is equids and rabbits > pigs and catfish > ruminants, poultry, and mink, and breeding animals are considered to be more sensitive than animals being raised for slaughter (FDA, 2001).

### 5.3 Ochratoxin A

The kidney and, to a much lesser extent, the liver are the main targets of OTA. Cattle (but not calves) are considered resistant due to metabolism of OTA to nontoxic ochratoxin α by rumen microbes. Other ruminants may also show resistance; however, dietary factors can affect the extent of rumen metabolism (Höhler *et al*., 1999). Pigs and dogs are the most sensitive domestic animals, and poultry are less sensitive than pigs. The relative sensitivity in poultry is chicks > turkeys > quail (Newberne and Rogers, 1981).

### 5.4 Deoxynivalenol

In farm animals, the primary target organ for DON toxicity is uncertain. The reason is that the most sensitive effects are reduced weight gain in the mouse, presumably due to reduced feed intake, and feed refusal and emesis in pigs (WHO, 2001). There is evidence that feed refusal and emesis are due to a central serotoninergic effect. Cattle, sheep, and poultry are resistant to the emetic effects of DON, but reduced feed intake is seen at 10–20 mg/kg diet in ruminants (Osweiler, 2000). Horses are resistant, but shrimp are very sensitive to the effects on body weight gain, whereas dogs and cats are also sensitive to the emetic effects of DON (WHO, 2001). DON and ZEA frequently occur together, making exposure to both likely.

### 5.5 Zearalenone

Pre-pubertal female pigs are the most susceptible farm animal to the estrogenic effects of ZEA. The sensitivity of pigs may be due to a higher affinity of their estrogen receptors for α-zearalenol (Fitzpatrick *et al*., 1989; Fink-Gremmels and Malekinejad, 2007). Cattle and sheep are much less sensitive than pigs, and poultry are considered to be resistant (Haschek *et al*., 2002). Cycling mares also appear to be relatively insensitive to the estrogenic effects of ZEA at low doses (equivalent to natural contamination) (Juhász *et al*., 2001). An interaction between ZEA and oxytocin (an ergot alkaloid derivative) has been reported in pigs (Alexopoulos, 2001). Grains contaminated with DON are frequently also contaminated with ZEA.

### 5.6 Ergot alkaloids

The vasoconstrictive properties of ergot alkaloids are the primary cause of problems in farm animals. Sensitive species are those that graze on pasture grasses (especially tall fescue) or consume feeds comprised of small seed grasses (such as ryegrass). Cattle are probably the most sensitive, followed by sheep and horses. Environmental factors (especially temperature) are very important in determining the nature of the observed effects in cattle (Raisbeck *et al*., 1991; Oliver, 2005).

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CHAPTER 6.

# Mycotoxins and human health


## Summary

Mycotoxins have been investigated in relation to a wide range of adverse human health effects, but the evidence for all but a small number of associations is limited. Thus, the full impact on human health of the widespread exposure to mycotoxins remains to be defined. The main exception is for aflatoxins; epidemiological, experimental, and mechanistic studies have contributed to establishing aflatoxins as a cause of human liver cancer, with a particularly elevated risk in people chronically infected with hepatitis B virus. In addition, acute aflatoxicosis after exposure to high dietary toxin levels has been demonstrated. The impairment of child growth by aflatoxin exposure early in life remains an important subject of study. More information is also required on the potential immune effects of aflatoxins, especially in vulnerable populations. For fumonisins, studies indicate a possible role in oesophageal cancer and in neural tube defects, although no definitive conclusions can be drawn at present. For deoxynivalenol and other trichothecenes, exposure has been linked to acute poisoning outbreaks in large numbers of subjects. For ochratoxin A and zearalenone, the human health effects remain undefined. The limited tools available to accurately assess human exposure to mycotoxins and the relative paucity of epidemiological studies need to be addressed if the full extent of the adverse effects of these common dietary contaminants is to be understood and adequate public health measures taken. In this respect, newly established biomarkers of exposure at the individual level are proving valuable in improving exposure assessment in epidemiological studies.


## 1. Introduction

This chapter covers the effects on human health of the major mycotoxins occurring in foods. This chapter also includes information on mechanisms of action of mycotoxins in humans where relevant to the adverse health effects under consideration. No attempt is made at a comprehensive review, but at appropriate points we refer to more extensive accounts.

The major source of human exposure to mycotoxins is consumption of contaminated foods. Exposure is highest when those foods are dietary staples, such as maize, groundnuts, or various other cereals. Exposures to metabolites or parent toxins may also occur by consumption of contaminated milk and milk products. We covered the dietary sources of mycotoxin exposure in some detail in Chapter 1. In this chapter, we also consider

occupational exposures in granaries and other food and feed processing due to mycotoxins contained in dusts from contaminated grains. The specific effects due to particular mycotoxins are discussed in Sections 2–6. Additional, more general information on health problems associated with mycotoxins in grain dusts is covered in Section 7.

The human health effects considered here encompass acute poisoning, cancer, other chronic diseases, and biological effects, including growth impairment and immunomodulation.

One of the major limitations in assessing the effects of mycotoxins on health has been the inability to accurately assess exposure at the individual level. The development of validated biomarkers for aflatoxins has greatly assisted epidemiological studies and allowed an evaluation of aflatoxins in relation to cancer, aflatoxicosis, child growth impairment, and immune effects (see Wild and Gong, 2010). Development of validated biomarkers for fumonisins (Wild and Gong, 2010; Van der Westhuizen *et al*., 2011) and deoxynivalenol (Meky *et al*., 2003; Turner *et al*., 2008a, 2008b, 2008c) also offers promise for future studies of the human health effects of these mycotoxins. However, the biomarker field for mycotoxins also offers a cautionary tale: an unvalidated biomarker for ochratoxin A in plasma or serum has been used to assess dietary exposure to this toxin, but subsequent careful duplicate diet studies have shown that this biomarker does not reflect intake at the individual level (Gilbert *et al*., 2001). Nevertheless, the availability of biomarkers to measure exposure to mycotoxins provides new opportunities for more systematic monitoring of exposure in populations as well as improved etiological studies.

## 2. Aflatoxins

Aflatoxins are produced in a wide range of commodities by *Aspergillus flavus* and *A. parasiticus*, and occasionally other *Aspergillus* species. The commodities most at risk are maize and groundnuts in tropical areas (see Chapter 1).

### *2.1 Mechanisms*

Until recently, attention on aflatoxins has been focused on their carcinogenic effects. For more detailed information, see the extensive reviews in IARC (2002), WHO (2002), and Wild and Gong (2010). Consideration is usually given to the naturally occurring aflatoxins in the diet – aflatoxins $B_1$ ($AFB_1$), $B_2$ ($AFB_2$), $G_1$ ($AFG_1$), and $G_2$ ($AFG_2$) – or to $AFB_1$ alone, or more rarely to aflatoxins $M_1$ ($AFM_1$) and $M_2$ ($AFM_2$), the hydroxylation products of $AFB_1$ and $AFB_2$, respectively, that occur in milk. This distinction between the type of aflatoxin exposures under consideration is important but is rarely considered, particularly when studying adverse health effects other than cancer.

Given the focus on mutagenicity and carcinogenicity, most studies have been of $AFB_1$, which, due to the presence of a double bond at the 8,9 position, can be metabolized to the reactive $AFB_1$-8,9-epoxide, which binds to cellular macromolecules including DNA (for more detail, see Wild and Turner, 2002). The major DNA adduct is $AFB_1$-N7-guanine, and this pro-mutagenic lesion commonly results in a G → T transversion mutation. $AFB_1$-N7-guanine can also be detected in the urine and used as an exposure biomarker in epidemiological studies. $AFB_2$ and $AFG_2$ are generally considered to be far less biologically active due to the absence of the 8,9 double bond. $AFG_1$ can be bioactivated to the 8,9-epoxide but is less mutagenic than $AFB_1$, reflecting the stearic chemistry of the respective epoxides; the $AFB_1$-8,9-epoxide intercalates more readily into the DNA double helix than does the equivalent $AFG_1$ molecule, resulting in higher levels of DNA adduct formation for a given dose. Minimal information exists about the importance of the reactive epoxide for the non-mutagenic actions of aflatoxins or indeed about the biological effects of aflatoxins independent of metabolic activation to the 8,9-epoxide. Unlike $AFM_2$, $AFM_1$ contains an 8,9 double bond and hence can be bioactivated to the reactive 8,9-epoxide.

A significant observation in terms of aflatoxin carcinogenicity is the association between exposure and a specific mutation in the *TP53* tumour suppressor gene in liver cancer (hepatocellular carcinoma [HCC]). In HCC tumours from patients who are from regions endemic for aflatoxin and who are chronically infected with hepatitis B virus (HBV), a high prevalence exists of a specific missense mutation in the gene, namely an AGG → AGT (Arg → Ser) point mutation at codon 249 (codon $249^{ser}$) (IARC, 2002; Hussain *et al*., 2007). This mutation is extremely rare in HCC associated with HBV in areas where aflatoxins are uncommon, but it is as yet unclear whether HBV infection influences occurrence of the mutation in HCC from aflatoxin-endemic areas.

The major human cytochrome P450 (CYP) enzymes involved in aflatoxin metabolism are CYP3A4, 3A5, and 1A2, and the predominant site of bioactivation is the liver, although CYP3A4 expression in the human intestine means that metabolism may also occur in that organ (Wild and Turner, 2002; Kamdem *et al*., 2006; Thelen and Dressman, 2009). The contribution of these enzymes to $AFB_1$ metabolism in exposed people will depend on both

the affinity and level of expression of the different enzymes; CYP3A4 appears to be the most important in generating the *exo*-8,9-epoxide, and the relative contribution of CYP3A5, which also produces the *exo*-8,9-epoxide, varies by individual (Kamdem *et al*., 2006). In fact, CYP3A5 expression is polymorphic and varies by ethnic group; for example, 40% of African Americans show no expression due to identified genetic polymorphisms. Such polymorphisms may affect sensitivity to the toxic effects of aflatoxins (Wojnowski *et al*., 2004). CYP1A2 leads predominantly to formation of the hydroxylated $AFM_1$ metabolite and the $AFB_1$-*endo*-8,9-epoxide, which does not form DNA adducts.

Given the fact that aflatoxin is known to cross the placenta, it is also of interest that CYP3A7, a major CYP in human fetal liver, can activate $AFB_1$ to the 8,9-epoxide (Kamataki *et al*., 1995; Wild and Turner, 2002). Indeed, aflatoxin adducts have been identified in cord blood (Wild *et al*., 1991; Turner *et al*., 2007), indicating that environmental levels of aflatoxin are bioactivated to the reactive metabolites in utero.

Detoxification of the aflatoxin *exo*- and *endo*-epoxides occurs mainly through glutathione *S*-transferase-mediated conjugation to reduced glutathione (Guengerich *et al*., 1998). The epoxides can also undergo rapid non-enzymatic hydrolysis to $AFB_1$-8,9-dihydrodiol, which in turn forms a dialdehyde phenolate ion with an opened ring. The dihydrodiol can react with the ε-amino group of lysine in serum albumin to form aflatoxin–albumin adducts, which are often used as exposure biomarkers (Wild and Gong, 2010). In a further metabolic step, aflatoxin aldehyde reductase catalyses the NADPH-dependent reduction of the dialdehyde phenolate ion to a dialcohol (Johnson *et al*., 2008).

An understanding of the metabolism, DNA damage, and induction of mutations in people exposed to aflatoxins in the diet has contributed to the overall assessment of their adverse health effects (Groopman *et al*., 2008; Wild and Gong, 2010). The major health effects linked to aflatoxin exposure are described briefly here.

### 2.2 Aflatoxicosis

Sporadic historical accounts of human poisoning with aflatoxins were reported, but these early studies were not definitive in assigning causation (Hall and Wild, 1994). In 1974, hepatitis cases due to aflatoxicosis in Western India (Krishnamachari *et al*., 1975) were associated with consumption of maize contaminated with *A. flavus*. Patients exhibited jaundice preceded by fever, vomiting, and anorexia, with subsequent progression to ascites and oedema in lower extremities. In maize from households where cases occurred, the aflatoxin levels were extremely high, 6.25–15.6 mg/kg, and the estimated daily ingestion of aflatoxins was 2–6 mg in adults.

In Kenya in 1981, another outbreak of acute hepatitis was associated with aflatoxin poisoning (Ngindu *et al*., 1982). Patients were diagnosed with jaundice preceded by abdominal discomfort, anorexia, general malaise, and low-grade fever; tachycardia and oedema were also observed. Maize from two affected households contained up to 3.2 mg/kg and 12 mg/kg $AFB_1$. An additional report came from an incident in Malaysia in 1988, where 13 children died from acute hepatic encephalopathy after consuming noodles (Lye *et al*., 1995).

In 2004, well-documented cases of aflatoxicosis occurred in Kenya, close to the locality of the cases reported in 1981 (Azziz-Baumgartner *et al*., 2005; Lewis *et al*., 2005). These outbreaks resulted in several hundred deaths associated with consumption of maize heavily contaminated with aflatoxin. A case–control study of aflatoxicosis, defined as acute jaundice of unknown origin, found that aflatoxin levels in foods from affected households were much higher than those in foods from unaffected households. Similar differences between cases and controls were found when aflatoxin biomarker levels in blood were examined (Azziz-Baumgartner *et al*., 2005; McCoy *et al*., 2008).

The association of aflatoxin contamination of maize with acute hepatitis and aflatoxicosis is well supported by the evidence, most notably by the observations in Kenya. It is of interest that aflatoxicosis has been reported only in communities where maize is the dietary staple. This reflects both high levels of aflatoxins in maize and high daily intakes (300–500 g) of this staple commodity. In addition, however, the role of co-contaminating mycotoxins, notably fumonisins, has not been assessed, and these may contribute to the acute toxicity observed.

The level of aflatoxin intake associated with aflatoxicosis and death has been estimated (Wild and Gong, 2010). The intake of total aflatoxins estimated to result in a risk of fatality was > 1 mg/day, i.e. > 20 µg/kg body weight (bw)/day in adults. It was considered that aflatoxicosis without fatality may occur with 5–10-fold lower doses. Further estimates suggested that the total intake of $AFB_1$ associated with half the exposed people dying (i.e. the median lethal dose [$LD_{50}$]) would be 0.54–1.62 mg/kg bw, a similar magnitude to the $LD_{50}$ value reported for rabbits, cats, dogs, pigs, and baboons (Wild and Gong, 2010). Therefore, daily exposure to staple foods consumed at several hundred

grams per day and contaminated with ≥ 5000 µg/kg of aflatoxins may lead to death in humans. Daily consumption of foods with > 1000 µg/kg may lead to aflatoxicosis.

It is of great concern that these contamination levels in maize that are associated with aflatoxicosis and death are only 10–100 times the levels that occur regularly in many parts of sub-Saharan Africa. Despite the demonstration that heavy contamination of maize with aflatoxins does lead to aflatoxicosis and death, these outbreaks continue to occur. Thus, in affected parts of the world, there is an urgent need for a rapid field test that, as part of a preventive strategy, can detect dangerously high levels (e.g. > 1000 µg/kg) of aflatoxins in cereals and nuts, as well as an emergency response analogous to those in place for outbreaks of infectious diseases.

### 2.3 Liver cancer

The International Agency for Research on Cancer (IARC) has classified naturally occurring mixtures of aflatoxins as Group 1, carcinogenic to humans (IARC, 2002) (Table 6.1). Before the 1990s, most studies of aflatoxins and HCC were either ecological correlation studies or case–control studies. The ecological studies did not always consider infection with HBV and hepatitis C virus, had relatively crude estimates of aflatoxin intakes, and had limitations in diagnosis and registration of HCC cases. Despite this, most showed a positive correlation between estimated aflatoxin intakes and HCC rates in a given region (IARC, 1993, 2002).

Prospective cohort studies of improved design, begun in the late 1980s and 1990s in South-East Asia, used biomarkers of exposure to both HBV and aflatoxins. These studies provided strong evidence of a more than multiplicative interaction between these two factors in relation to increased HCC risk (Qian *et al*., 1994; Wang *et al*., 1996; IARC, 2002). In a more recent follow-up of the cohort in Taiwan, China, Wu *et al*. (2009) conducted the largest nested case–control study to date and reported that the combined effect of $AFB_1$ exposure and HBV infection was more consistent with an additive model than with the multiplicative one observed in the original report of Wang *et al*. (1996). However, after examining the plasma of HCC patients, cirrhosis patients, and controls for the *TP53* gene mutation (codon $249^{ser}$; AGG → AGT), Kirk *et al*. (2005) showed that the increased risk associated with the presence of both the $249^{ser}$ mutation and HBV infection was consistent with a multiplicative effect of exposure to aflatoxin and chronic HBV infection.

The HCC risk from exposure to aflatoxins in the absence of chronic HBV infection is difficult to assess in populations where HBV infection is widespread. A review by Omer *et al*. (2004) reported 1.7–3.4-fold increased risks in individuals exposed to aflatoxins without chronic HBV infection. Wu *et al*. (2009) reported a similar magnitude of increased HCC risk in subjects positive only for aflatoxin exposure biomarkers, but occult HBV infections in some of the individuals in these studies cannot be ruled out.

The overall evidence from epidemiological studies shows a particularly elevated risk of HCC from aflatoxin exposure in individuals chronically infected with HBV and reasonable evidence that an increased risk also exists in individuals exposed to aflatoxins without chronic HBV infection. Given that > 350 million chronic HBV carriers exist worldwide, many living in aflatoxin-endemic areas, the need to reduce aflatoxin exposure remains highly relevant for cancer prevention.

### 2.4 Cirrhosis

To date, little information exists on the risk of liver cirrhosis in relation to aflatoxin exposure. A case–control study in The Gambia (Kuniholm *et al*., 2008) reported that increasing lifetime groundnut intake (a surrogate for aflatoxin consumption) was associated with a significantly increased risk of cirrhosis, approaching 3-fold with the highest level of consumption. The presence of the codon $249^{ser}$ mutation associated with aflatoxin was also associated with a similar magnitude of increased risk of cirrhosis. However, further studies are needed before any conclusions can be drawn about aflatoxin and cirrhosis. This is an area that merits more attention, given the large burden of cirrhosis worldwide.

### 2.5 Immune effects

The immunomodulatory effects of aflatoxins have been considered in experimental studies in cell models and animals as well as in observations of farm animals (IARC, 1993, 2002; WHO, 2002; Williams *et al*., 2004). However, only a few studies have considered the association between aflatoxin exposure and immune parameters in human populations. Two such studies have been reported from The Gambia (Allen *et al*., 1992; Turner *et al*., 2003). The first provided some evidence that children with higher aflatoxin exposure were more likely to have malaria parasitaemia, but no significant associations were observed with experience of malaria infection, antibody titre to asexual stages of *Plasmodium falciparum*, or lymphoproliferative responses. The second study investigated the effect of aflatoxin exposure on cell-mediated immunity (skin test), antibody titres (in response to rabies and pneumococcal vaccines), and salivary immunoglobulin A (IgA). No associations were found between

aflatoxin exposure and either the skin test or antibody titres, but higher aflatoxin exposure was associated with lower salivary IgA, suggesting that aflatoxin exposure could modulate mucosal immunity.

From Ghana, two studies have been reported that compared aflatoxin biomarker levels and subsets of peripheral blood cells in adults (Jiang *et al*., 2005, 2008). In the first, a higher aflatoxin biomarker level was associated with a lower percentage of CD3+ and CD19+ cells (B lymphocyte antigens) expressing the CD69+ activation marker and with lower percentages of CD8+ T cells expressing perforin and granzyme A. In the second study, a higher aflatoxin biomarker level was associated with lower percentages of CD8+ cells expressing perforin and of CD19+ cells expressing CD69+. In addition, HIV-positive individuals with higher aflatoxin biomarker levels had significantly lower percentages of CD4+ T regulatory cells and naive CD4+ T cells compared with HIV-positive individuals with lower aflatoxin biomarker levels.

Overall, the studies of immunomodulation do not permit conclusions to be drawn about the impact of environmental levels of aflatoxin exposure on human immunity and susceptibility to infectious disease. Nevertheless, the data suggest that immune parameters could be affected in populations exposed chronically to aflatoxins. If this were to be proven, the impact would add greatly to the burden of disease related to cancer and aflatoxicosis.

## 2.6 Child growth impairment

Children are chronically exposed to high levels of aflatoxins in areas where food contamination is endemic. Exposure begins in utero and continues throughout early life, although the breastfeeding period provides some respite from high daily intakes. Studies in several animal species indicate that aflatoxin exposure can severely affect growth and development. However, until recently such effects had not been considered in human populations.

Early studies explored the link between aflatoxin exposure and kwashiorkor (Hendrickse *et al*., 1982), but no firm conclusions could be drawn due to various weaknesses in study design (Hall and Wild, 1994). A study in rural Kenya in the 1980s linked aflatoxin detection in mothers' blood with significantly lower birth weights of female babies (De Vries *et al*., 1989). A more recent study in Kisumu District, Kenya, showed a significantly greater prevalence of wasting (low weight for height) in children fed cereals with high aflatoxin contamination, compared with those whose cereals had lower aflatoxin levels (Okoth and Ohingo, 2004).

A series of studies has been conducted in West African children exposed to aflatoxins early in life. In the first of these, a cross-sectional study of children aged 1–5 years in Benin and Togo, a striking inverse association was found between aflatoxin–albumin adduct level and growth (Gong *et al*., 2002). In a subsequent 8-month longitudinal study, a strong negative correlation was observed between aflatoxin–albumin adduct level and height increase (Gong *et al*., 2004). The highest quartile of aflatoxin–albumin adducts was associated with a mean reduction of 1.7 cm in height increase compared with the lowest quartile. Recently, an association was also found between exposure to aflatoxin in utero and impaired growth during the first year of life in children in The Gambia (Turner *et al*., 2007). This finding suggests that the consumption of aflatoxin-contaminated food during pregnancy may have effects on the child after birth.

In summary, growth faltering in West African children may occur at the time of introduction of solid foods, when high exposure to aflatoxin occurs. The dose–response relationships between aflatoxin biomarker levels and growth effects are also consistent with a causal effect. However, at this time other confounding factors cannot be excluded as explanations for these associations. The mechanisms of action by which aflatoxin may exert an effect on growth are currently unknown, although the possibility of a compromised intestinal integrity, through altered barrier function as a consequence of endothelial cell toxicity or immune suppression, is a valid hypothesis that should be explored further (Wild and Gong, 2010).

In areas where aflatoxin is common, namely sub-Saharan Africa and South Asia, 7.1 million children died under the age of 5 years in 2008. It is estimated that about 50% (3.55 million) of these deaths are related to undernutrition and poor growth (Black *et al*., 2003). If aflatoxin exposure were to be responsible for even a few per cent of these deaths, the total number would be tens of thousands per year.

## 2.7 Occupational exposures

$AFB_1$ concentrations of up to 612 µg/kg have been reported in airborne dusts during the handling of contaminated maize and groundnuts (Miller, 1994a; Sorenson, 1999). Most aflatoxin was contained in the < 7 µm and 7–11 µm particle size ranges. In grain dusts, a substantial fraction of the aflatoxin is contained in the spores of *A. flavus* and *A. parasiticus* (Miller, 1994a, 1994b).

Retrospective studies of feed processing workers in Denmark reported elevated risks of HCC, gall bladder cancer, and extrahepatic bile duct cancer in this population exposed occupationally, with a

2-3-fold increased risk after a 10-year latency period. Inhalation exposure to aflatoxin (170 ng/day) was reported to be the most likely explanation. Some evidence of elevated aflatoxin biomarker levels related to the handling of contaminated feeds was also reported (Olsen *et al*., 1988; Autrup *et al*., 1991, 1993). A risk assessment model suggested that exposure to $AFB_1$ in airborne dust may pose little significant risk during maize harvest and elevator loading/unloading but a relatively high risk during swine feeding and storage bin cleaning (Liao and Chen, 2005).

## 3. Fumonisins

Fumonisins occur in maize and, much less commonly, other cereals, as a result of infection with *Fusarium verticillioides* and related species (see Chapter 1). An Environmental Health Criteria document for fumonisin $B_1$ ($FB_1$) has been published (WHO, 2000a), and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established a provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg bw/day for $FB_1$, $FB_2$, and $FB_3$ alone or in combination (WHO, 2001, 2002). This PMTDI is based not on tumorigenicity data but on the no-observed-effect level (NOEL) for nephrotoxicity in rodents of 0.2 mg/kg bw/day, divided by a safety factor of 100 (WHO, 2001).

### *3.1 Mechanisms*

Fumonisins may exert their biological effects through several different mechanisms. $FB_1$ genotoxicity is somewhat unclear; negative results were obtained from several genotoxicity assays, but other in vitro studies reported that $FB_1$ induced micronuclei and chromosomal aberrations (Ehrlich *et al*., 2002; IARC, 2002). The DNA damage may be a result of stimulation of oxidative damage and lipid peroxidation (Stockmann-Juvala and Savolainen, 2008). This finding is consistent with increased oxidative DNA damage and malondialdehyde adducts in rat liver and kidney (Domijan *et al*., 2006) and lipid peroxidation (Abel and Gelderblom, 1998) in vivo after $FB_1$ treatment. Fumonisin-induced carcinogenesis in the liver proceeds through initiation and promotion stages in a manner similar to that for genotoxins and is dependent on the dose and time of exposure (Gelderblom *et al*., 2008a). Nevertheless, no evidence has been found for direct interaction of fumonisin with DNA nor for its metabolism to a reactive metabolite (WHO, 2001; IARC, 2002).

$FB_1$ disrupts de novo sphingolipid biosynthesis by inhibition of the enzyme ceramide synthase (Merrill *et al*., 2001), resulting in many effects on signalling pathways and cell functions that are dependent on ceramide, sphingoid bases, sphingoid base 1-phosphates, and complex sphingolipids (Dragan *et al*., 2001; Merrill *et al*., 2001). These include effects on apoptosis and mitosis, thus potentially contributing to carcinogenesis through an altered balance of cell death and replication (Stockmann-Juvala and Savolainen, 2008). Disruption of sphingolipid metabolism leads to changes in the sphinganine-to-sphingosine ratio, with increased sphinganine tissue concentrations, which correlate closely with the in vivo toxicity and carcinogenicity of fumonisins (Riley *et al*., 2001). Such changes were demonstrated in rat liver and mouse kidney at carcinogenic doses of $FB_1$ (Voss *et al*., 2002). Disruption of cholesterol, phospholipid, and fatty acid synthesis and interaction with ceramide have been proposed to play key roles in the differential growth patterns of altered hepatocytes during cancer promotion in the liver (Gelderblom *et al*., 2008b).

The role of fumonisins in immunomodulation has also been highlighted through changes in cytokine levels in vitro and in vivo in animal models (Sharma *et al*., 2000) and effects on antibody vaccine responses in pigs exposed to $FB_1$ (Taranu *et al*., 2005; Stockmann-Juvala and Savolainen, 2008).

Animal experiments have shown that fumonisins are rapidly excreted unmetabolized from the gut (Shephard *et al*., 1994a, 1994b; Martinez-Larranaga *et al*., 1999). It is not known whether the gut microflora metabolize fumonisins, although hydrolysed $FB_1$ has been detected in faeces of vervet monkeys (Shephard *et al*., 1994a, 1994b), pigs (Fodor *et al*., 2008), and ruminants (WHO, 2000b). Studies using radiolabelled fumonisins failed to show any metabolism by primary hepatocytes or hepatic esterases and lipases (Cawood *et al*., 1994).

Analyses of human faeces has revealed unmetabolized $FB_1$ and $FB_2$ (Chelule *et al*., 2000, 2001). The presence of $FB_1$, $FB_2$, and $FB_3$ in human hair has been demonstrated, suggesting that fumonisins are absorbed from the gut after ingestion of contaminated maize (Sewram *et al*., 2003). More recently, $FB_1$ in urine has been reported in individuals consuming large amounts of maize (Gong *et al*., 2008). Direct evidence therefore exists that human populations are exposed to fumonisins after absorption, although the actual levels and the possible risk this poses still need to be quantified.

Probable daily intake values of fumonisins, determined using a validated dietary assessment tool, showed that intakes of up to 10 times the PMTDI can occur in individuals in a rural, subsistence farming community where maize is the main dietary staple (Burger *et al*., 2010). Drinking home-brewed maize beer further increased the level of exposure. Estimates of daily

fumonisin intake in rural communities in Guatemala (Torres *et al*., 2007) have also shown that total fumonisin intake can potentially be > 10 times the recommended PMTDI.

### 3.2 Acute poisoning

No confirmed cases have been reported of acute human poisoning due to fumonisin exposure. Part of the difficulty in discerning a specific effect of fumonisins in acute poisoning is their co-occurrence with other mycotoxins, notably aflatoxins and trichothecenes. For example, one poisoning outbreak in India occurred where fumonisin contamination of foods was reported, notably in unleavened bread prepared from mouldy sorghum or maize, and where symptoms were characterized by abdominal pain, borborygmi, and diarrhoea (Bhat *et al*., 1997). However, assays of other mycotoxins potentially present were not reported.

An outbreak of human intoxication related to the consumption of maize gruel prepared from mouldy maize powder occurred in Guangxi Province, China, in 1989 (Li *et al*., 1999). In this case, co-occurrence with trichothecenes was found, and based on an average maize meal intake of 200 g/person (60 kg bw) per day, the total daily dietary intake of deoxynivalenol and $FB_1$ was 80 µg/kg bw and 2.3 µg/kg bw, respectively.

These incidents highlight the need for comprehensive analyses of mycotoxins in contaminated foods and in biological samples when acute poisoning outbreaks occur.

### 3.3 Cancer

Ecological studies in the former Transkei region of South Africa showed that both *F. verticillioides* and fumonisin contamination of maize were positively correlated with oesophageal cancer incidence rates (Marasas, 2001; IARC, 2002). Similar correlations have been reported in China (Sun *et al*., 2007). Other reports have associated maize consumption per se with high oesophageal cancer incidence rates but did not consider fumonisin exposure specifically (Franceschi *et al*., 1990). In these studies, other fungi and their mycotoxins were generally also present, and to date no analytical studies have been conducted that specifically link $FB_1$ to human cancer at any organ site (IARC, 1993, 2002; WHO, 2001).

Based on the disruption of sphingolipid biosynthesis mentioned above, the serum sphinganine-to-sphingosine ratio was used as an exposure biomarker in a nested case–control study of oesophageal cancer in China (Abnet *et al*., 2001), but no association was found between biomarker levels and cancer risk. One study conducted in China did report that the sphinganine-to-sphingosine ratio in urine was significantly increased in males estimated to have consumed > 110 µg/kg bw/day of $FB_1$ (Qiu and Liu, 2001). However, in all subsequent reports, no association was observed between sphingoid bases or sphinganine-to-sphingosine ratios in the plasma and urine and individual fumonisin exposure, suggesting that these biomarkers are not sufficiently sensitive for monitoring exposure in human populations (Nikièma *et al*., 2004; van der Westhuizen *et al*., 2010; Xu *et al*., 2010).

Many risk factors exist for the development of oesophageal cancer. These differ among geographical regions with respect to demography, ethnicity, genetic susceptibility, cultural practices, and socioeconomic and nutritional status. The use of home-grown maize as one of the main dietary staples, coupled with an underlying poor socioeconomic status, could implicate fumonisins as a contributing factor in the development of oesophageal cancer. However, confounding by other risk factors and the possible interactions of fumonisins with other mycotoxins should be considered in future studies.

Some experimental studies have reported a synergistic interaction between $AFB_1$ and $FB_1$ in the development of liver cancer (Carlson *et al*., 2001; Gelderblom *et al*., 2002). Perhaps due to the focus on oesophageal cancer, the role of fumonisins in cancer in other organs has been largely unexplored. However, given the interactions found experimentally, the co-contamination of crops by aflatoxins and fumonisins, and the fact that both toxins occur in populations with a high prevalence of HBV infection, a role for fumonisins in HCC is plausible. Some ecological correlation studies provide support for this hypothesis (Ueno *et al*., 1997; Li *et al*., 2001; Sun *et al*., 2007). The possible interaction between various mycotoxins (fumonisins, aflatoxins, trichothecenes) and the microcystins B (algal toxins) in the development of HCC merits more investigation. Interactions of fumonisins with different dietary constituents could also have an impact on the toxicological effects (Gelderblom *et al*., 2004).

IARC has concluded that there is inadequate evidence in humans for the carcinogenicity of toxins derived from *F. verticillioides* (as *F. moniliforme*), leading to a classification of Group 2B, possibly carcinogenic to humans (IARC, 1993). $FB_1$ was also classified as Group 2B (IARC, 2002) (Table 6.1).

### 3.4 Neural tube defects

Animal studies have demonstrated that fumonisin exposure can cause neural tube defects, possibly through the disruption of sphingolipid biosynthesis and consequent depletion of sphingolipids, which are critical for lipid raft functions, specifically folate processing via

the high-affinity folate transporter (Stevens and Tang, 1997; Sadler *et al*., 2002; Gelineau-van Waes *et al*., 2005). Neural tube defects are known to be associated with reduced folate levels, and cell membrane disruption induced by fumonisins could lead to reduced folate absorption through damage to the folate receptors on the membrane (Marasas *et al*., 2004). More recently, elevation in sphingoid base 1-phosphates induced by fumonisins has been implicated in the induction of neural tube defects in mice (Gelineau-van Waes *et al*., 2009).

A possible link between human neural tube defects and fumonisin consumption was suggested when a high rate of neural tube defects was recorded in babies of Mexican American women living in Texas who conceived during 1990–1991 (Hendricks, 1999), soon after the outbreaks of equine leukoencephalomalacia and porcine pulmonary oedema that occurred in 1989–1990 in the USA (Ross *et al*., 1991). In the border region of Texas, exposure to fumonisins may be elevated due to frequent consumption of contaminated maize.

In a case–control study in this region of Texas (Missmer *et al*., 2006), moderate tortilla consumption in the first trimester of pregnancy was associated with an increased risk of neural tube defects compared with low consumption. However, high consumption was not associated with increased risk. A similar result was found using estimates for fumonisin intake from tortillas, whereas an increased sphinganine-to-sphingosine ratio was associated with increased risk, apart from the highest category.

High incidence rates of neural tube defects have been recorded in rural areas of Mpumalanga Province, South Africa, and in the Umzimkulu district of the former Transkei region in Eastern Cape Province, South Africa (Ncayiyana, 1986; Venter *et al*., 1995); in Hebei Province, China (Moore *et al*., 1997; Marasas *et al*., 2004); and in Guatemala (Marasas *et al*., 2004) and Mexico, all areas where large quantities of maize are consumed.

### *3.5 Occupational exposure*

There are no reports of occupational exposure to fumonisin and adverse health effects.

## 4. Ochratoxin A

Human exposure to ochratoxin A (OTA) occurs principally in Europe and Canada, where it comes from eating foods made from barley and wheat in which *Penicillium verrucosum* has grown. Minor sources include meat, especially pork, from animals fed contaminated grain. In tropical and subtropical countries, OTA consumption is much lower, resulting from contamination due to growth of *Aspergillus carbonarius* and, less commonly, *A. niger* in coffee, cocoa and cocoa products, and dried fruit, and sometimes in cereals, including sorghum, maize, and millet (see Chapter 1).

OTA has been the subject of an Environmental Health Criteria document (WHO, 1990) and JECFA evaluations (WHO, 1991, 2001, 2002, 2007, 2008). JECFA noted that neither a conclusive association between OTA intake and human cancer nor the mechanism by which OTA is carcinogenic in animals has been established. JECFA has confirmed a provisional tolerable weekly intake (PTWI) for OTA of 100 ng/kg bw/week (WHO, 2001, 2008). It is noteworthy that risk assessment indicated that acute toxicity of OTA occurred in animals at lower levels than did long-term effects such as carcinogenicity, so this PTWI is based on acute toxicity.

### *4.1 Mechanisms*

Recent reviews have extensively summarized evidence on the absorption, distribution, metabolism, and mechanisms of action of OTA (Pfohl-Leszkowicz and Manderville, 2007; Marin-Kuan *et al*., 2008; Mally and Dekant, 2009). Wide species differences have been reported in the serum half-life of OTA in vivo. In humans, the elimination of OTA follows a two-phase pattern, a fast excretion followed by a slow clearing, with a calculated plasma half-life of 35 days. Even infrequent exposure (consumption of contaminated food once a week or even once a month) can result in persistent blood levels of OTA (Studer-Rohr *et al*., 2000). Blood samples from healthy people in European countries show OTA levels of 0.1–40 ng/mL (WHO, 2008). The parent molecule is the major compound found in blood, whereas ochratoxin α is the major component detected in urine (Studer-Rohr *et al*., 2000).

OTA is absorbed from the gastrointestinal tract in mammals and becomes strongly bound to plasma proteins (predominantly albumin) in blood, whereby it is distributed to the kidneys, with lower concentrations in liver, muscle, and fat. OTA is metabolized by several different CYP enzymes, depending on the species and tissue involved. In cells expressing human CYP enzymes, the main metabolite was 4(*R*)-hydroxy-OTA formed by CYP1A2, 2B6, 2C9, 2D6, and 2A6, whereas the 4(*S*)-hydroxy-OTA derivative was formed by only CYP2D6 and 2B6 (Pfohl-Leszkowicz and Manderville, 2007). Identified OTA metabolites include not only these two hydroxylated species but also 10-hydroxy-OTA and ochratoxin α, which is formed by hydrolysis of the peptide bond in OTA and thus lacks the phenylalanine moiety and consequently is non-toxic. OTA may also be metabolized

**Table 6.1.** IARC Monographs evaluations of carcinogenic hazards of mycotoxins to humans

| Mycotoxin | Monographs volume (year) | Degree of evidence of carcinogenicity | | Overall evaluation of carcinogenicity to humans[a] |
|---|---|---|---|---|
| | | In humans | In animals | |
| Aflatoxins, naturally occurring mixtures of | 56 (1993), 82 (2002) | Sufficient | Sufficient | Group 1 |
| Aflatoxin $B_1$ | 56 (1993) | Sufficient | Sufficient | |
| Aflatoxin $B_2$ | 56 (1993) | | Limited | |
| Aflatoxin $G_1$ | 56 (1993) | | Sufficient | |
| Aflatoxin $G_2$ | 56 (1993) | | Inadequate | |
| Aflatoxin $M_1$ | 56 (1993) | Inadequate | Sufficient | Group 2B |
| Toxins derived from *Fusarium verticillioides*[b] | 56 (1993) | Inadequate | Sufficient | Group 2B |
| Fumonisin $B_1$ | 82 (2002) | Inadequate | Sufficient | Group 2B |
| Fumonisin $B_2$ | 56 (1993) | | Inadequate | |
| Fusarin C | 56 (1993) | | Limited[c] | |
| Ochratoxin A | 56 (1993) | Inadequate | Sufficient | Group 2B |
| Toxins derived from *Fusarium graminearum, F. culmorum,* and *F. crookwellense* | 56 (1993) | Inadequate | | Group 3 |
| Deoxynivalenol | 56 (1993) | | Inadequate[d] | |
| Nivalenol | 56 (1993) | | Inadequate | |
| Zearalenone | 56 (1993) | | Limited[e] | |
| Citrinin | 40 (1986) | Inadequate | Limited[f] | Group 3 |
| Patulin | 40 (1986) | Inadequate | Inadequate | Group 3 |
| Toxins derived from *Fusarium sporotrichioides* | 56 (1993) | Inadequate (no data) | | Group 3 |
| T-2 toxin | 56 (1993) | | Limited[g] | |

[a] Group 1, carcinogenic to humans; Group 2A, probably carcinogenic to humans; Group 2B, possibly carcinogenic to humans; Group 3, not classifiable as to its carcinogenicity to humans; Group 4, probably not carcinogenic to humans.
[b] Formerly known as *Fusarium moniliforme*. Fumonisin $B_1$ is also produced by additional *Fusarium* species.
[c] Fusarin C caused marginal increases in incidences of papillomas and carcinomas of the oesophagus and forestomach when given by gavage to mice and rats.
[d] More recent carcinogenicity studies of deoxynivalenol in mice have been negative (Iverson *et al.*, 1995; Lambert *et al.*, 1995) and reinforce the conclusion of the 1993 IARC Working Group that there is inadequate evidence in experimental animals for the carcinogenicity of deoxynivalenol.
[e] Zearalenone caused increased incidences of hepatocellular and pituitary tumours in mice of both sexes when given in the diet, but no carcinogenic effect was seen in rats.
[f] Citrinin caused benign epithelial tumours (clear cell adenomas) of the kidney in male rats in one experiment. Whereas this study (Arai and Hibino, 1983) was unequivocally positive, other studies in other strains of rat at lower doses were negative (IARC, 1986). A single positive study with only benign tumours as findings is considered limited evidence of carcinogenicity by the IARC Monographs criteria.
[g] T-2 toxin caused increased incidences of liver cell tumours and lung tumours in male mice when given in the diet.

by cyclo-oxygenase, lipoxygenase, and epoxygenase, particularly in extrahepatic organs such as the kidney, to yield reactive oxygen species, which in turn may result in oxidative damage.

OTA competitively inhibits phenylalanine-tRNA ligase, resulting in inhibition of protein synthesis as well as RNA and DNA synthesis. In animals, acute toxic effects of OTA can be inhibited by co-administration of phenylalanine (FAO/WHO/UNEP, 1999).

The formation of DNA adducts by OTA and their potential role in cancer induction has been investigated (Mally and Dekant, 2009; Mantle *et al*., 2010), and hypotheses for the formation include direct adduct formation induced by OTA after metabolic activation via an OTA phenoxy radical and indirect DNA damage resulting from oxygen radical formation as mentioned above (Pfohl-Leszkowicz and Manderville, 2007).

The concentration of OTA-specific transporters in tissues has been proposed to explain the relative species, sex, and target organ sensitivities to OTA toxicity (reviewed in Dietrich *et al*., 2005). Another contributor to selective sensitivity is the extent of albumin binding, which markedly decreases the uptake of OTA by transporters (Bow *et al*., 2006). Mechanisms that account for the toxicity and carcinogenicity of OTA without invoking the production of OTA DNA adducts have also been proposed (reviewed in Mally and Dekant, 2009) and typically involve alterations in expression of genes regulating rates of cell proliferation and cell death. Potential biomarkers of effect in target tissues include the development of unique gene expression profiles specific to alterations of gene expression induced by OTA. Genes include those involved in cellular defence, cell proliferation, and cell death (reviewed in Marin-Kuan *et al*., 2008; Adler *et al*., 2009; Mally and Dekant, 2009) and in oxidative stress (Arbillaga *et al*., 2008; Cavin *et al*., 2009). Changes in urinary metabolite profiles, obtained using GC-MS, LC-MS, and [$^1$H]-NMR, have been used as a metabonomic approach to assess the potential of these changes for development of a predictive model for OTA toxicity (Sieber *et al*., 2009). Although the results were not specific for OTA, they were indicative of kidney damage and general toxicity, and this approach could prove to be of value for discovery of more specific mechanisms unique to OTA.

### 4.2 Acute toxicity

The kidney is the major target organ for adverse effects from OTA (Pfohl-Leszkowicz and Manderville, 2007; WHO, 2002). Short-term toxicity studies in mice, rats, dogs, and pigs have shown both time- and dose-dependent development of progressive nephropathy. Significant sex and species differences exist, as well as differences due to route of administration. Other toxic effects include cardiac and hepatic lesions in rats, lesions of the gastrointestinal tract and lymphoid tissues in hamsters, myelotoxicity in mice, and kidney lesions in chickens. Pigs appear to be the most sensitive species to the nephrotoxic effects; the lowest-observed-effect level (8 µg/kg bw) was used as the basis for establishing the PTWI.

A great deal of work has been undertaken recently to elucidate the likely mechanisms of toxicity. Degenerative changes in the proximal tubules of the kidney have been the most common effects seen in animal species studied. However, it has not been possible to determine just what acute effects, if any, OTA has on humans (WHO, 2008).

### 4.3 Cancer

Evidence for the carcinogenicity of OTA is principally from studies in experimental animals. OTA is carcinogenic to laboratory rats and mice, causing HCC in mice and kidney carcinomas in mice and rats. The mechanism of carcinogenic action has not been firmly established.

Reports of increased cancer risk in humans who consumed OTA have been limited to descriptive studies. No analytical epidemiological studies were available to IARC at the time of the evaluation of OTA (IARC, 1993). The descriptive studies generally focused on the co-occurrence of Balkan endemic nephropathy, a fatal chronic renal disease, and higher than expected rates of urinary tract tumours, including tumours of the kidney and urinary bladder, in Bulgaria and other Balkan countries. Available studies have not established that this increased cancer incidence was due to exposure to OTA.

IARC has concluded that there is sufficient evidence that OTA is carcinogenic in experimental animals but inadequate evidence that OTA increases cancer risk in humans. OTA has therefore been classified as Group 2B, possibly carcinogenic to humans (IARC, 1993) (Table 6.1).

### 4.4 Occupational exposure

OTA is found in the spores of *P. verrucosum* on grains and also *A. carbonarius* as well as some strains of *A. niger* on grapes and coffee beans. Some occupational studies in Europe reported elevated OTA levels in plasma in workers exposed to grain dust (Pfohl-Leszkowicz and Manderville, 2007). From what was inferred to be a massive exposure to OTA from working in a confined space with grain contaminated by

*A. ochraceus*, a farmer developed acute renal disease after temporary respiratory distress (Di Paolo *et al.*, 1993). Only limited estimates of inhalation exposure to OTA from occupational exposure are available (Mayer *et al.*, 2007).

## 5. Deoxynivalenol

Deoxynivalenol (DON) is produced in cereals, especially wheat, as the result of growth of *Fusarium graminearum* and related species (see Chapter 1). JECFA has established a PMTDI for DON of 1 µg/kg bw/day on the basis of the NOEL for body weight reduction in mice in a 2-year bioassay and a safety factor of 100 (WHO, 2001, 2011). The NOEL in mice was 100 µg/kg bw/day (Iverson *et al.*, 1995).

### 5.1 Mechanisms

DON is directly toxic via an epoxide moiety and thus does not require metabolic activation to exert its biological effects. Low-level trichothecene exposure in animal models has been shown to modulate the expression of several cytokines and chemokines that are key regulators of immune function (Pestka, 2008). Exposure to DON causes the upregulation of the mRNAs responsible for production of cytokines, chemokines, and other immune-related proteins and can also induce gene transcription. In addition, DON modulates numerous physiological processes controlled by mitogen-activated protein kinases (MAPKs). These include processes controlling cell growth, differentiation, and apoptosis, which are all crucial for signal transduction in the immune response (Pestka, 2008). Thus, in addition to altered cytokine expression, alterations in MAPK expression are likely to also contribute to the immune dysregulation and toxicity of DON and other trichothecenes. Also associated with MAPK activation by DON is the activation of processes leading to the ribotoxic stress response, which is induced by other translational inhibitors that, like DON, bind to or damage a specific region at the 3′ end of the 28S rRNA. The ribosome plays a key role in the ribotoxic stress response by serving as scaffolding for interactions between various MAPKs (Pestka, 2008).

DON toxicity studies have recently revealed several possible approaches for developing useful biomarkers of its effects. For example, DON exposure in mice results in upregulation of several suppressors of cytokine signalling. These suppressors are known to impair growth hormone signalling (Pass *et al.*, 2009). Impairment of the growth hormone axis precedes the growth retardation in the mouse induced by DON (Amuzie and Pestka, 2010). Oral DON perturbs the growth hormone axis by suppressing two growth-related proteins, IGFALS and IGF1. Thus, reduced expression of these two proteins in conjunction with elevated urinary DON levels could potentially serve as biomarkers of effect.

Detoxification varies by species; metabolism by gut microflora generates a de-epoxy metabolite (DOM-1), and conjugation to glucuronic acid is catalysed by UDP-glucuronyltransferase (reviewed by Pestka and Smolinski, 2005; Wu *et al.*, 2007). In humans, DON-glucuronide has been reported in urine samples in several studies (Turner *et al.*, 2008a, 2008b, 2008c). In contrast, information on DOM-1 is limited. An absence of de-epoxidase activity in a small series of human faecal samples was reported (Sundstøl-Eriksen and Pettersson, 2003), whereas, in apparent contrast, a study in France of farmers exposed to grain handling reported detection of DOM-1 in a proportion of subjects (Turner *et al.*, 2010).

### 5.2 Acute toxicity

In animals, DON has a wide range of proven toxicities, including feed refusal, decreased weight gain, gastroenteritis, cardiotoxicity, teratogenicity, and immunotoxicity (Rotter *et al.*, 1996; Meky *et al.*, 2001; Pestka *et al.*, 2004; Pestka and Smolinski, 2005; Gray and Pestka, 2007; Amuzie and Pestka, 2010).

DON can cause acute poisoning in humans, where severe gastrointestinal toxicity is the primary symptom. Consumption of cereals contaminated with DON has been associated with numerous poisoning incidents in China between 1961 and 1991 (see Pestka and Smolinski, 2005) and a major outbreak in India (Bhat *et al.*, 1989); in some of these episodes, tens of thousands of individuals were affected. In these outbreaks, symptoms were analogous to those observed in animals, notably a rapid onset, nausea, vomiting, abdominal pain, diarrhoea, headache, dizziness, and fever. In an episode in the Kashmir valley, DON levels in wheat ranged from 0.4 mg/kg to 8.4 mg/kg (Bhat *et al.*, 1989), and in China DON poisoning was linked to wheat contaminated at DON levels between 0.3 mg/kg and 100 mg/kg (Pestka and Smolinski, 2005). These data suggest that acute toxicity may occur at exposures estimated in the low µg/kg bw/day range.

### 5.3 Cancer

Minimal data are available on the carcinogenicity of DON in either humans or experimental animals.

A 2-year bioassay in B6C3F1 mice of both sexes fed DON in the diet at concentrations of 0, 1, 5, or 10 mg/kg showed no increase in the incidence of pre-neoplastic or neoplastic lesions in the liver or other tissues (Iverson *et al.*, 1995). DON was also tested for its ability to initiate or promote skin tumours when applied topically

to the skin of female SENCAR mice, with negative results (Lambert *et al*., 1995). No studies have reported on the carcinogenicity of DON in humans. Oesophageal cancer in humans has been anecdotally linked to consumption of grains infected with *Fusarium* species that produce DON and other mycotoxins, but no analytical epidemiological studies link DON to the occurrence of any human cancer (IARC, 1993).

IARC has concluded that there is inadequate evidence in both humans and experimental animals for the carcinogenicity of DON. DON and other toxins derived from *F. graminearum*, *F. culmorum*, and *F. crookwellense* have therefore been categorized as Group 3, not classifiable as to their carcinogenicity to humans (IARC, 1993) (Table 6.1).

### 5.4 Occupational exposure

Inhalation exposure to DON has been the subject of several health hazard evaluations. Fusarium head blight in wheat resulting from infection by *F. graminearum* or *F. culmorum* begins at the outside of the grain head and moves inward. As a result, most DON is found in the outer layers of the kernel and the chaff (Miller, 1994b; Snijders, 1994). Grain dusts can contain quite high concentrations of DON and sometimes of other mycotoxins, which are not always present in the kernels. For example, other fungi, including *F. sporotrichioides*, can grow on what becomes the chaff so that small amounts of T-2 toxin can be present.

Air samples collected in grain elevators in Canada contained a mean airborne concentration of 37 ng/m$^3$ DON and a maximum of 2.59 µg/m$^3$ DON. Airborne dust from the same source contained 0.5–5.8 mg/kg DON, 1 mg/kg T-2 toxin, and low levels of HT-2 toxin (De Mers, 1994). Concentrations of dusts, fungal spores, and DON associated with handing of grain on farms in Finland, including grain drying, milling, and cattle feeding, were similar to those reported from Canada (Lappalainen *et al*., 1996).

Studies of DON concentrations during grain handling in Germany reported a median concentration of 2 ng/m$^3$ and a maximum of 703 ng/m$^3$ (Mayer *et al*., 2007). In France, urinary biomarkers for DON (DON, DOM-1) were higher in active farmers, particularly those from larger farms, than in retired farmers, whose exposure was from diet (Turner *et al*., 2010).

Epidemiological studies have been conducted in Norway relating to occupational exposures of male and female farmers to mycotoxins. Norwegian grains (wheat, oats, and barley) are affected mainly by Fusarium head blight, and various trichothecenes and culmorins have been reported as common (Langseth and Elen, 1996; Ghebremeskel and Langseth, 2001). A longitudinal survey of farmers over more than two decades suggested a relationship between grain farming and mid-pregnancy deliveries in the families of farmers, possibly linked to mycotoxins (Kristensen *et al*., 1997). Small increased relative risks were observed in several cancers in female but not male farmers (Kristensen *et al*., 2000).

## 6. Zearalenone

Zearalenone (ZEA) is a non-steroidal estrogenic mycotoxin. It is produced principally by *F. graminearum* and related species, and consequently occurs wherever DON occurs, most notably as a contaminant of maize, wheat, barley, oats, rye, sorghum, millet, and rice. Distribution of ZEA is worldwide (Zinedine *et al*., 2007). Estimates of human exposure from dietary sources are generally in the range of 1–30 ng/kg bw/day (Zinedine *et al*., 2007). JECFA has established a PMTDI for ZEA of 0.5 µg/kg bw/day based on the NOEL for hormonal effects in pigs (WHO, 2001).

### 6.1 Mechanisms

ZEA is metabolized during absorption by the intestinal tissue in pigs. Metabolism involves reduction of the 6-keto group of ZEA and results in formation of α- and β-zearalenol as well as, upon further reduction, α- and β-zearalanol, all of which can be conjugated in turn to glucuronic acid (WHO, 2001). Few data are available in relation to metabolism in humans. Studies of liver microsomes in vitro have suggested a high rate of α-zearalenol production compared with that of β-zearalenol in pigs and humans, a point of importance because of the greater relative estrogenicity of α-zearalenol compared with ZEA (Fink-Gremmels and Malekinejad, 2007). In other words, the formation of α-zearalenol may be considered a bioactivation step contributing to the estrogenic effects of ZEA.

ZEA and its metabolites can bind to estrogen receptors, resulting in various changes consequent to binding to elements in the nucleus responsive to estrogens. In addition, however, ZEA is a competitive substrate for enzymes involved in steroid synthesis and metabolism and therefore has the potential to act as an endocrine disruptor. ZEA can activate the pregnane X receptor by displacement of a co-repressor and recruitment of co-activators (Ding *et al*., 2006). Thus, ZEA could have widespread effects on gene expression as a result of the modified activity of this nuclear transcription factor.

### 6.2 Acute toxicity

ZEA is considered to be of relatively low acute toxicity. No reports have appeared of acute poisoning due to ZEA in humans.

### 6.3 Cancer

ZEA resulted in an increased incidence of liver cell and pituitary tumours in mice, consistent with a hormonal mode of carcinogenic action (IARC, 1993). No carcinogenic effect was seen in rats, however, and overall animal carcinogenicity data for ZEA were considered limited (IARC, 1993). No studies of human carcinogenicity have been reported for ZEA (Table 6.1).

ZEA was measured in endometrial tissue from a small group of women with endometrial adenocarcinomas, endometrial hyperplasia, or normal proliferative endometria (Tomaszewski *et al*., 1998). ZEA in blood samples has also been investigated in some small studies of early onset of puberty in Hungary (Szuets *et al*., 1997) and in Italy (Massart *et al*., 2008). In the study in Italy, there was a suggestion that elevated serum ZEA and α-zearalenol levels were associated with early puberty in 6 of the 17 girls examined from a rural area, but no positive samples were seen in the 15 patients from an urban area.

### 6.4 Occupational exposure

No studies of occupational exposure of humans have been reported for ZEA.

## 7. Occupational exposures to grain and groundnut dusts

Grain dusts present an occupational hazard when protection of workers is inadequate, and several health consequences are possible, including the allergic disease hypersensitivity pneumonitis, endotoxicosis, and organic dust toxic syndrome (Rylander and Jacobs, 1994; Sorenson and Lewis, 1996). Endotoxicosis is a result of exposure to bacterial endotoxin, and organic dust toxic syndrome (also called pulmonary mycotoxicosis, toxic organic dust syndrome, or grain fever), as far as is known, is not caused by mycotoxins. Inhalation of silica, allergens, mycotoxins, and triple-helical glucans contained in airborne dusts is a potential health risk, rarely from systemic exposure but from effects on lung biology. Outdoor work is normally not a problem, except when handling the most damaged maize, small grains, and groundnuts. In contrast, handling contaminated grains, especially damaged grains, in any confined space (e.g. grain storage, storage bin cleaning, animal feeding in barns, indoors) carries a more significant risk.

The allergic disease hypersensitivity pneumonitis, also called extrinsic allergic alveolitis or farmer's lung, develops through repeated exposure to allergens. Dust from any mouldy crop, such as straw, maize, small grains, or groundnuts, can cause the condition. Symptoms may include shortness of breath, a dry cough, a sudden general feeling of sickness, fevers and chills, a rapid heart rate, and rapid breathing. The symptoms are serious, and once an allergic reaction begins, the person will always have the potential for symptoms with exposure to the offending fungi. Long-term exposure can cause permanent lung damage, physical disability, or even death (Sorenson and Lewis, 1996; Schenker *et al*., 1998; Girard *et al*., 2009).

The reproductive structures of many fungi are known to contain mycotoxins or low-molecular-weight toxic compounds, often in high concentrations (Sorenson, 1999). Most is known about the fungi that produce toxins important in agriculture. The conidia of *A. flavus*, *A. parasiticus*, *F. graminearum*, and *F. sporotrichioides* contain very high concentrations of toxins, particularly in the case of the species that produce aflatoxins. The spores of the two *Aspergillus* species have been reported to contain 100–1100 µg aflatoxin/g, or approximately $10^{-4}$ moles (Wicklow and Shotwell, 1983). Several interesting toxins have been found in sclerotia of various *Aspergillus* species, again at higher concentrations than occur either in culture or in affected crops (Gloer *et al*., 1988; Wicklow *et al*., 1988), some of which are thought to be present in conidia along with kojic acid and other *A. flavus* toxins. Spores of *F. graminearum* contained 30 µg/g of T-2 toxin and those of *F. sporotrichioides* 50 µg/g (both approximately $10^{-5}$ moles). The spores of many species of toxigenic fungi have been demonstrated to contain mixtures of the toxins associated with the species.

The high-molecular-weight toxic compound present in spores and in spore and mycelial fragments from the anamorphic *Trichocomaceae* (i.e. *Penicillium*, *Aspergillus*, and related hyphomycetes) is (1→3)-β-D-glucan in the triple-helical form (Rand *et al*., 2010). In the species tested so far, the concentration is 1–11 pg/spore (Foto *et al*., 2004; Iossifova *et al*., 2008).

Inhalation of intact spores leads to little net exposure because their relatively large size enables entrapment and removal by lung defence mechanisms. However, in outdoor air, exposure is primarily to spore and mycelial fragments (Green *et al*., 2012) and to dusts (particulate matter < 2.5 µm in diameter), which efficiently penetrate deep into the lung (Buczaj, 2008; see Miller *et al*., 2010). Inhalation of toxins affects macrophage function and other aspects of lung biology. Pure compounds tested for effects in macrophages include fumonisin, aflatoxin (Liu *et al*., 2002), T-2 toxin (Sorenson *et al*., 1986), and the aflatoxin precursor sterigmatocystin (Miller *et al*., 2010). DON has been tested for effects in peritoneal macrophages (Ayral *et al*., 1992).

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CHAPTER 7.

# Risk assessment and risk management of mycotoxins


## Summary

Risk assessment is the process of quantifying the magnitude and exposure, or probability, of a harmful effect to individuals or populations from certain agents or activities. Here, we summarize the four steps of risk assessment: hazard identification, dose–response assessment, exposure assessment, and risk characterization. Risk assessments using these principles have been conducted on the major mycotoxins (aflatoxins, fumonisins, ochratoxin A, deoxynivalenol, and zearalenone) by various regulatory agencies for the purpose of setting food safety guidelines. We critically evaluate the impact of these risk assessment parameters on the estimated global burden of the associated diseases as well as the impact of regulatory measures on food supply and international trade. Apart from the well-established risk posed by aflatoxins, many uncertainties still exist about risk assessments for the other major mycotoxins, often reflecting a lack of epidemiological data. Differences exist in the risk management strategies and in the ways different governments impose regulations and technologies to reduce levels of mycotoxins in the food-chain. Regulatory measures have very little impact on remote rural and subsistence farming communities in developing countries, in contrast to developed countries, where regulations are strictly enforced to reduce and/or remove mycotoxin contamination. However, in the absence of the relevant technologies or the necessary infrastructure, we highlight simple intervention practices to reduce mycotoxin contamination in the field and/or prevent mycotoxin formation during storage.


## 1. Introduction

This chapter covers two key topics related to mycotoxins in human food: risk assessment and risk management. Managing risks of food contaminants such as mycotoxins is of global importance. Indeed, two international policy-making bodies – the United Nations Environment Programme (UNEP) and the World Health Organization (WHO) International Programme on Chemical Safety (IPCS) – have declared that humans have a right to food free from mycotoxins that could cause significant health risk. In highly populated parts of the world, mycotoxins in staple crops remain the most significant foodborne risk for human health, animal health, and market access (FAO/WHO/UNEP, 1999).

Risk assessment and risk management of mycotoxins deserve much more global attention and action than

they have been given. Wild and Gong (2010) identified several reasons for current inaction in addressing mycotoxin risks in developing countries. First, knowledge about mycotoxins and the full range of their risks to health is incomplete. Second, risks are poorly communicated to policy-makers in regions where mycotoxins are most prevalent. Third, the perceived value of interventions to reduce mycotoxin risk is low compared with those of other medical interventions, such as vaccination programmes, malaria control, and improved sanitation. Fourth, intervention to control mycotoxins is required at multiple time points both before and after harvest. Fifth, regulation of mycotoxins has minimal effects on food quality for subsistence farmers. Finally, mycotoxin contamination is a problem that encompasses agriculture, health, and economics, fields that traditionally have not interacted at a research or policy-making level.

This chapter covers both the theoretical and quantitative aspects of risk assessment, to provide a background in how human health risks are assessed both experimentally and for decision-making purposes. Section 2 outlines the four steps in the risk assessment process and describes how risk assessments have been conducted for five mycotoxins: aflatoxins, fumonisins, ochratoxin A, deoxynivalenol, and zearalenone. Section 3, on risk management, is more practical and focuses specifically on how to manage mycotoxin risks in foods. Finally, Section 4 briefly describes the importance of risk communication and public education about mycotoxins in countries at risk.

## 2. Risk assessment

Risk assessment is the process of estimating the magnitude and probability of a harmful effect to individuals or populations from certain agents or activities. Four steps are involved in estimation of risk: hazard identification, dose–response assessment, exposure assessment, and risk characterization (NRC, 2008).

### *2.1 Hazard identification*

Hazard identification is the process of determining whether exposure to an agent can increase the incidence of a particular adverse health effect. A variety of different studies can help to determine whether an agent causes a specific health effect; these include studies of adverse effects in humans, animals, and in vitro systems, and mechanistic studies.

Epidemiological studies on the relationship between exposure to a mycotoxin and particular harmful effects obviously provide the best evidence for identifying a human health hazard. Studies that provide a quantitative estimate of association between a hazard and a disease include cohort and case–control studies. Other types of human studies, which may or may not provide a quantitative estimate of association, include studies that are descriptive (case reports), ecological (geographical comparison), and cross-sectional (observing disease prevalence across different populations at a single time point).

In a cohort study, an investigator selects a group of initially healthy individuals (a cohort) exposed to any number of potentially hazardous agents, and follows individuals in this group over time to determine the incidence of a particular disease that may be associated with particular exposures. Then, the incidence of the disease in exposed individuals is compared with the incidence in unexposed individuals, or the incidence in a highly exposed group may be compared with the incidence in a group exposed to lower levels. For example, it may emerge that over time, the incidence rate of a particular cancer is higher in a group of individuals exposed to higher levels of a particular mycotoxin than the rate in a group of individuals exposed to lower levels of that mycotoxin, or not exposed at all. A cohort study can be used to estimate the relative risk (RR) of a particular disease in exposed versus unexposed populations:

> RR = (Incidence of disease in the exposed population)/(Incidence of disease in the unexposed population).

In the exposed group, if the number of people who develop the disease is *a* and the number of people who do not develop the disease is *b*, then the incidence of disease in the exposed population is $a/(a + b)$. Likewise, in the unexposed group, if the number of people who develop the disease is *c* and the number of people who do not develop the disease is *d*, then the incidence of disease in the unexposed population is $c/(c + d)$.

In a case–control study, an investigator identifies a group of individuals with a particular disease (cases) and a comparable group of individuals without that disease (controls), and determines what proportion of the cases were exposed and what proportion were not (Gordis, 2009). The assumption is that if the proportion of individuals exposed to an agent is different between cases and controls, then exposure to the agent may be associated with an increased or decreased occurrence of the disease. A case–control study can be used to estimate an odds ratio (OR) to compare the odds that cases were exposed to the agent with the odds that controls were exposed:

> OR = (Odds that a case is exposed)/(Odds that a control is exposed).

Using the same variables described above to estimate RR, the

odds that a case is exposed is $a/(a + c)$ and the odds that a control is exposed is $b/(b + d)$.

More recently, attention has focused on whether it is possible to take advantage of the benefits of both of these study types by combining elements of both cohort and case–control approaches into a single study. For example, a case–control study can be initiated within a cohort study, i.e. the individuals within the cohort who develop a particular disease during the study period can be considered cases and those who do not develop the disease can be considered controls. In nested case–control studies, the controls are a sample of individuals who are at risk for the disease at the time each case of the disease develops (Gordis, 2009).

### *2.1.1 Human studies*

Human studies for the purpose of risk assessment can be problematic, for several reasons. First, epidemiological studies exist for only a few hazardous agents. Second, circumstances of human exposure levels and duration are difficult to measure precisely, and there is often a time interval between when exposure occurs and disease symptoms appear. Third, even well-planned studies cannot always show a clear causative association between an agent and a disease. Many potential confounders exist that can contribute to the disease etiology, such as exposure to multiple environmental disease agents, varied nutritional status, and genetic factors that modulate disease susceptibility. Finally, human studies may be susceptible to bias or systematic errors in the design, conduct, or analysis of the studies that result in mistaken estimates of the effect of an exposure on disease risk (Gordis, 2009). For example, there may be selection bias in how individuals are chosen for or respond to a study, or information biases such as those associated with interviewing, surveillance, recall, or reporting.

### *2.1.2 Animal studies*

Animal studies (in vivo) are often used in place of human studies for hazard identification, for reasons described above. The major concern with animal studies is that extrapolation from results in another species to results in humans is imperfect. However, animal experiments have several advantages: dose levels of the hazardous agent may be varied, other conditions may be kept constant across all groups to reduce confounders, and an animal group can specifically be kept unexposed as controls. Then, the incidence of a health effect can be compared across these groups.

### *2.1.3 Cell and tissue culture studies*

Cell and tissue culture assays, unlike human and animal studies, are in vitro assays, and these are increasingly important experiments for identifying hazards. Isolated cells or tissues (or microorganisms) are prepared and maintained in culture by methods that preserve some in vivo properties, and, after exposure to a hazardous agent, they can be tested for point mutations, chromosomal aberrations, DNA repair or damage, gene expression, cell transformation, and metabolic and other physiological effects. These tests may give information on potential mechanisms of the biological end-point (e.g. an adverse health effect).

Structure–activity relationship (SAR) models allow the toxicological activity of an unknown chemical or agent to be predicted on the basis of its chemical and/or structural properties. The relationships are computationally derived from information about agents of known toxicity. These predictive equations are most useful in setting priorities for further research, thereby reducing the number of animal experiments required. Requirements for SAR modelling thus include a database of chemicals of known toxicity or carcinogenicity, information about their chemical and spatial structure, and information about their physicochemical properties.

## *2.2 Dose–response assessment*

The second step in risk assessment, dose–response assessment, determines the relationship between the dose of a toxic agent and the occurrence of health effects. This relationship is often graphically represented in a dose–response curve, which shows the proportion of a given population that experiences an adverse health effect (on the vertical axis) at different doses of a toxic agent (on the horizontal axis). The shape of the dose–response curve is a critical component in policy-making to control human health risks.

Usually, dose–response data are gathered from animal and/or human studies. Animal studies are especially useful for dose–response assessment because the exact doses of a particular toxin can be carefully controlled. Different groups of animals are exposed at each dose level through food, water, air, or dermal contact, and one group of animals is not exposed to the toxin at all (control group). The prevalence of a health effect, or the lack thereof, is measured in each animal group at each dose level, and these results are plotted in a curve.

Dose–response assessment addresses toxic (non-carcinogenic) and carcinogenic effects separately and differently, even if an agent can cause both types of effects (e.g. aflatoxin). These effects are briefly described here.

### 2.2.1 Toxic (non-carcinogenic) effects

For toxic but non-carcinogenic effects caused by an agent, it is assumed that protective mechanisms must be overcome before the adverse health effect can occur. A threshold dose of the toxin may exist, i.e. humans and animals can be exposed to the toxin at doses below this particular threshold without experiencing the health effect in question. The threshold dose is referred to as the no-observed-effect level (NOEL), sometimes called the no-observed-adverse-effect level (NOAEL). In animal studies, the NOEL is the highest dose of a toxin at which no significant increases in the frequency or severity of adverse effects are observed when an exposed group is compared with an unexposed group. Sometimes a lowest-observed-effect level (LOEL), the lowest dose in the experiment at which an adverse effect can be observed, is used in place of or in addition to the NOEL.

The NOEL is one metric used to evaluate the toxicity of an agent. Another is the median lethal dose ($LD_{50}$), the dose of a toxin that, when administered to a group of animals over a specified period of time, is lethal to 50% of the animals. $LD_{50}$ values are used to rank toxicity across multiple agents. Other metrics include the 10% lethal dose ($LD_{10}$), the dose of a toxin lethal to 10% of test animals, and the median effective dose ($ED_{50}$), the dose that causes a particular health effect – not necessarily death – in 50% of test animals.

More recently, benchmark dose modelling – which uses the entire dose–response curve from an animal study – has been used to provide a different point of departure from the NOEL in calculating a tolerable daily intake (TDI). Benchmark dose modelling involves finding a model that best fits the overall shape of the dose–response curve in an animal study and then, from that model, finding the dose that corresponds to a proportion (usually 10% or 5%) of response in the test animals. This particular dose is called the benchmark dose. Then, the lower bound of the confidence interval around that dose (from uncertainties in the animal study itself) is calculated. This benchmark dose lower confidence limit ($BMDL_{10}$ or $BMDL_{05}$) is used as the point of departure for extrapolating to a TDI for humans.

To extrapolate animal toxicity data to humans, a provisional maximum tolerable daily intake (PMTDI), also called a reference dose (RfD) in some cases, is calculated. The PMTDI is the dose below which humans exposed to the agent in question are not expected to experience adverse health effects. The PMTDI is derived from the NOEL or BMDL by applying uncertainty factors, which can account for interspecies variability (extrapolating from animals to humans), intraspecies variability (to protect sensitive individuals), use of a LOEL instead of a NOEL, the chronicity of the study, and other terms reflecting the professional assessment of additional uncertainties in the data. The PMTDI is calculated by dividing the NOEL or BMDL by the product of the uncertainty factors:

PMTDI = NOEL/(product of uncertainty factors), or

PMTDI = BMDL/(product of uncertainty factors).

Often, the uncertainty factors for interspecies and intraspecies variability are each given the value of 10. Therefore, the human PMTDI for a non-carcinogen is usually 0.01 times the NOEL or BMDL found in animal studies. It is worth noting that for policy-making purposes, this type of extrapolation from an animal study to a human PMTDI has often caused controversy because of the arbitrary nature of choosing uncertainty factors. Practically speaking, the safety factor assumes that humans are 10 times as sensitive as the most sensitive animal species tested for a particular toxin and that the most sensitive human is 10 times as sensitive as the least sensitive human.

### 2.2.2 Carcinogenic effects

In contrast to most toxic effects, carcinogenesis may be regarded as a process in which the presumption of no threshold may be appropriate if the chemical is (directly acting) or its metabolites are (indirectly acting) reactive with genomic DNA (Klaunig and Kamendulis, 2008). For such carcinogens, theoretically even a single molecular event could evoke changes in genomic DNA leading to mutations, selective cellular proliferation, and cancer. Hence, it is assumed that there is no safe dose above zero. For example, it is assumed that there is no threshold of exposure to aflatoxin $B_1$ below which cancer would never occur, because aflatoxin $B_1$ has a reactive metabolite that interacts directly with DNA. Experiments in rats exposed to aflatoxin $B_1$ in their drinking-water showed that the level of DNA adducts in the liver was linear over 6 orders of magnitude after both single and chronic dosing; dose levels reached those seen in exposed human populations (Buss *et al*., 1990). Additional mechanisms of carcinogenicity may exist that do not involve genotoxicity, which could be relevant for other mycotoxins.

For policy-making purposes, carcinogens are evaluated in two parts. First, a weight of evidence (WOE) is designated, and second, a slope factor, or cancer potency factor, is calculated on the basis of the dose–response

curve. WOE is a term that refers to the strength of the evidence that a particular agent causes cancer in humans. Two organizations that provide WOE evaluations for carcinogens are the International Agency for Research on Cancer (IARC) and the United States National Toxicology Program (NTP). IARC classifies agents as to carcinogenicity to humans by considering the WOE (Table 7.1).

For example, IARC has classified naturally occurring mixtures of aflatoxins as Group 1, carcinogenic to humans, and fumonisin $B_1$ and ochratoxin A as Group 2B, possibly carcinogenic to humans.

For carcinogenic agents that are reactive with DNA, a slope factor, or cancer potency factor, is calculated by estimating the slope of the linearized dose–response curve. Sometimes, for policy-making purposes, the slope factor is actually the upper 95% confidence limit of the dose–response slope. In practical terms, the slope factor or cancer potency factor estimates the increase in probability of developing the particular cancer per unit dose of the agent over a human lifetime. Thus, the steeper the dose–response curve for a carcinogenic agent, the more potent it is in causing cancer. For carcinogens that do not directly damage DNA, the NOEL can be used for setting a PMTDI. For example, the long-term nephrotoxicity of fumonisin $B_1$ is a prerequisite for the renal carcinogenicity of fumonisin $B_1$ (Dragan *et al.*, 2001). In this case, the renal carcinogenicity is subsumed by the dose–response relationship for the nephrotoxicity, which is clearly a threshold event (Bolger *et al.*, 2001).

## 2.3 Exposure assessment

The third step in risk assessment, exposure assessment, is the process of estimating the intensity, frequency, and duration of human or animal exposures to an agent in the environment. Exposure assessment is also a critical component of all epidemiological studies and is often used to identify control options or technologies to reduce risk.

**Table 7.1.** IARC classification of agents as to carcinogenicity to humans based on the weight of evidence

| Category | Significance |
|---|---|
| Group 1 | Carcinogenic to humans |
| Group 2A | Probably carcinogenic to humans |
| Group 2B | Possibly carcinogenic to humans |
| Group 3 | Not classifiable as to its carcinogenicity to humans |
| Group 4 | Probably not carcinogenic to humans |

### 2.3.1 Calculating exposure

Three steps are involved in calculating exposure: (i) characterizing the exposure setting, (ii) identifying exposure pathways, and (iii) quantifying exposure.

To characterize an exposure setting, both the physical setting and the exposed populations must be understood. The physical setting includes factors such as climate, geographical setting, vegetation, soil type, and location of water. Potentially exposed populations include both humans and animals: those living nearest the risky agent; those with diets, water, or air supply containing the agent; those who come to an area near the risky agent for work or play; and any other demographic groups (e.g. based on age or sex) that would be disproportionately exposed to the agent.

Characterizing an exposure pathway involves identifying sources and points of contact, media that transport the agent to the population, ways in which the agent may react or change in transport media, and other physical and chemical properties of the agent that explain its fate as it moves along its pathway to a target population.

Finally, to quantify exposure, it is common to estimate an average daily dose (ADD) or intake. This term is usually expressed as the mass of substance in contact with the body per unit body weight (bw) per unit time, such as in mg/kg bw/day, for ingestion exposures, or as the mass of substance per cubic meter of ambient air, such as in $mg/m^3$, for inhalation exposures. If the agent being studied is a carcinogen, a lifetime average daily dose (LADD) is calculated, with an averaging time equal to the expected lifetime of the individual (e.g. the United States Environmental Protection Agency assumes an averaging time for cancer risk assessment of 70 years, although the average lifespan in the USA is now longer than this).

To calculate ADD or LADD, the exposure quantity $E$ must first be estimated. It is the concentration of an agent as a function of time $t$, over an exposure duration. The total exposure quantity during that given time is expressed as the integral (sum) of concentrations $C$ over the exposure duration:

$$E = \int C(t)\, dt.$$

However, the integrated concentration can be difficult to obtain, so instead one can estimate the arithmetic average of the concentration $C_{ave}$ over the exposure duration $ED$ to estimate the total exposure:

$$E = C_{ave} * ED.$$

The intake rate *IR* is the amount of the agent passing through the initial intake barrier (mouth, nose, skin) into the body over a period of time. This can be measured in mg/day or L/day, for example. Then, the ADD is calculated as:

$$ADD = (E * IR)/(BW * AT),$$

where *BW* is body weight and *AT* is the averaging time in days. LADD is calculated similarly; in this case, however, the exposure duration (used to calculate *E*) is the number of years that an individual is exposed to a carcinogen, and the averaging time *AT* is the expected lifetime in years.

### *2.3.2 Estimating human exposure to mycotoxins*

Many difficulties have been encountered in estimating mycotoxin exposure in human diets. Until recently, human exposure to mycotoxins was measured almost exclusively in one of two ways: by questionnaires or food diaries relying on recall of what and how much had been eaten; or by food samples collected from populations, which ideally were representative of true exposures. Both of these ways pose potential problems. Dietary recall is often inaccurate. It can be difficult in many cultures worldwide to take food samples without disturbing social contexts, and measurement may lead to abnormal eating behaviour during recording. In addition, snack foods may be a significant source of exposure to mycotoxins, but these may not be recalled and may not be measured (Hall and Wild, 1994).

In recent years, however, biomarkers to assess mycotoxin exposure, internal dose, and biologically effective dose have been developed and are increasingly being used to estimate human exposure (Groopman *et al*., 2008). Biomarkers can also be used to assess the effectiveness of interventions to reduce mycotoxin exposure. Measurement of biomarkers related to mycotoxin exposure typically requires samples of either urine or serum. As described in Chapter 6, the measurement of validated biomarkers for aflatoxin exposure in human populations has greatly assisted epidemiological studies. Validated biomarkers for fumonisins and deoxynivalenol have also been developed. However, a past occurrence of measurement of an unvalidated biomarker for ochratoxin A resulted in inaccurate representation of actual dietary intake (Gilbert *et al*., 2001). Moreover, collection of samples for measuring biomarkers in human populations may also pose cultural challenges.

Without accurate exposure data, quantitative risk assessments can be limited, because exposure is a major component of the calculations. Hence, it is important to ensure that measurements are carried out in the most accurate and reliable way possible.

## *2.4 Risk characterization*

Risk characterization, the final step in risk assessment, combines the information on exposure with that on toxicity and dose–response assessment to determine whether an individual or a population is experiencing a significant risk of illness or disease based on exposure to a hazardous agent. It translates the available data to describe this significance to a broad audience.

Risk characterization, like dose–response assessment and exposure assessment, is conducted differently for non-carcinogens and carcinogens. For toxic effects, the individual or population ADD of an agent is compared with the PMTDI of the agent. For carcinogenic effects, the individual or population LADD is compared with the dose–response information from the specific agent. Discussion of major assumptions, scientific judgements, and estimates of uncertainties should also be part of the final risk characterization.

### *2.4.1 Risk characterization of non-carcinogenic toxins*

Simplistically, determining whether an individual or a population may suffer a health risk from a hazard relies on knowing whether their average daily exposure is greater than the daily dose of the hazard that may cause adverse effects. To recapitulate: the PMTDI is estimated from dose–response assessments that determine a NOEL or BMDL of a particular hazardous agent. The PMTDI is obtained by dividing the NOEL or BMDL by uncertainty factors that take into account extrapolation from laboratory animals to humans and variation among humans. Thus, the PMTDI is an estimate of a daily exposure level for humans, including sensitive individuals, that is unlikely to cause adverse health effects. The ADD can be calculated based on human exposure to the hazardous agent.

If ADD > PMTDI, then the potential for health risk exists. One way to express this is the hazard quotient (HQ) used by the United States Environmental Protection Agency. HQ is calculated as the ratio of the ADD of a particular agent to the PMTDI of that same agent:

$$HQ = ADD/PMTDI.$$

If HQ > 1, then an individual or a population may suffer a health risk due to their levels of exposure to the hazardous agent. If HQ < 1, then the individual or population is unlikely to suffer a health risk from current exposure levels to the agent.

### *2.4.2 Risk characterization of carcinogens*

Rather than using a threshold, such as HQ in the evaluation of non-carcinogens, carcinogenic risk is estimated for an individual or a population over an expected lifetime. Policy-makers may then determine whether this expected lifetime risk is acceptable or whether the carcinogen should be regulated. The WOE, based on available studies as described above, also factors into policy decision-making about the carcinogenic agent in question.

To reiterate: the slope factor of a carcinogen is derived by taking the slope of the linearized dose–response curve. LADD is estimated based on an exposure of an individual to the carcinogenic agent. Then,

*Risk* = LADD * slope factor,

where *Risk* is a unitless probability of an individual developing cancer over a lifetime (for example, 3 in a million, or $3 \times 10^{-6}$) from being exposed to the carcinogenic agent.

## *2.5 Risk assessment of mycotoxins*

A detailed discussion of the human health risks associated with aflatoxins, fumonisins, ochratoxin A, deoxynivalenol, and zearalenone is given in Chapter 6. In this section, we go into more detail about risk assessment of each of these mycotoxins for the purposes of policy-making.

### *2.5.1 Aflatoxins*

The evidence for aflatoxins causing liver cancer (hepatocellular carcinoma [HCC]) in humans has been established from decades of epidemiological research. These studies have elucidated dose–response relationships from which quantitative cancer risk assessments can be conducted (WHO, 1998). Based on the WOE of the effects of aflatoxins in human, animal, and in vitro studies, IARC has classified naturally occurring mixtures of aflatoxins as Group 1, carcinogenic to humans (IARC, 2002). However, the carcinogenicity, or cancer potency, of aflatoxins differs in humans with and without chronic hepatitis B virus (HBV) infection. The risk of HCC attributable to aflatoxins is up to 30-fold higher in populations chronically infected with HBV than in uninfected populations (Groopman *et al*., 2008).

In 1998, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) undertook an aflatoxin and HCC risk assessment to estimate the impact on population HCC incidence of moving from a hypothetical total aflatoxin standard of 20 µg/kg to a stricter standard of 10 µg/kg (WHO, 1998; Henry *et al*., 1999). Assuming that all food that contained aflatoxin levels higher than the standard would be discarded and that enough maize and nuts would remain to preserve consumption patterns, JECFA determined that HCC incidence would decrease by about 300 cases per billion people per year if the stricter aflatoxin standard were applied in countries with an HBV prevalence of 25%. However, in countries where the HBV prevalence was 1%, using the stricter aflatoxin standard would decrease HCC incidence by only 2 cases per billion people per year. This assessment associated HCC risk with particular doses of aflatoxin. However, these doses do not correspond to actual doses in food in different parts of the world, and two hypothetical values for HBV prevalence were assumed: 1% and 25% (Liu and Wu, 2010).

Liu and Wu (2010) estimated the global burden of HCC induced by aflatoxin by using the quantitative cancer risk assessment described above and collecting national data on foodborne aflatoxin levels, consumption levels of maize and groundnuts, and HBV prevalence. The cancer potencies of aflatoxin for HBV-positive and HBV-negative individuals were considered, together with uncertainties in all variables. Liu and Wu estimated that of the 550 000 to 600 000 new HCC cases worldwide per year, about 25 200 to 155 000 may be attributable to aflatoxin exposure. In other words, aflatoxin may play a causative role in 4.6–28.2% of all global HCC cases. Most cases occur in sub-Saharan Africa, South-East Asia, and China, where populations suffer from both high HBV prevalence and largely uncontrolled exposure to aflatoxin in food.

### *2.5.2 Fumonisins*

Compared with the risk assessment of aflatoxins, risk assessments of fumonisins and ochratoxin A for risk management are more complex from the human health standpoint because of the lack of convincing evidence linking either mycotoxin to human disease (WHO, 2001b) and, in the case of ochratoxin A, the controversy over its mechanism of action (WHO, 2001b).

Gelderblom *et al*. (2001) estimated the NOAEL for HCC induction by fumonisin $B_1$ in male rats at 0.8 mg/kg bw/day, which translated to a TDI of 0.8 µg/kg bw/day when divided by a safety factor of 1000. The justification for the large safety factor is that although fumonisin does not directly damage DNA, it induces cancer in rats and mice (IARC, 2002) and is a cancer promoter (Carlson *et al*., 2001; Gelderblom *et al*., 2002). Adopting such a standard, however, could seriously affect the food supply and trade between producers and consumers (Steyn *et al*., 2008).

JECFA set and then retained a PMTDI for fumonisins of 2 µg/kg bw/day, based on one evaluation of a NOAEL for nephrotoxicity in male

rats and a safety factor of 100 (WHO, 2001b), and then more recently based on a benchmark dose ($BMDL_{10}$) that causes megalocytic hepatocytes in male mice and a safety factor of 100 (WHO, 2011). The safety factor of 100 was deemed appropriate because the mechanism of toxicity and carcinogenicity did not involve direct damage to DNA. IARC has classified fumonisin $B_1$ as Group 2B, possibly carcinogenic to humans (IARC, 2002).

### *2.5.3 Ochratoxin A*

The mechanism of carcinogenicity of ochratoxin A (OTA) remains unclear. Six hypotheses about this mechanism were listed by JECFA at its most recent evaluation (WHO, 2008). Positive results for genotoxicity were usually obtained only at high OTA exposure levels and were usually indicative of oxidative damage. JECFA concluded that a direct genotoxic mode of action remains unconfirmed and that several possible modes of non-genotoxic action could be involved in the formation of renal tumours induced by OTA. Non-carcinogenic effects may occur at lower levels that those inducing tumour formation, however. On the basis of a study on damage to renal function in pigs (Krogh *et al*., 1974) and a safety factor of 500, JECFA established a provisional tolerable weekly intake (PTWI) for OTA of 112 ng/kg bw/week (WHO, 1991). This finding was confirmed by JECFA in 1995, when the PTWI was rounded off to 100 ng/kg bw/week (WHO, 1995). For the most recent evaluation, JECFA modelled carcinogenicity data on OTA from rat bioassays performed by the United States National Toxicology Program (NTP, 1989). Six different models were used on data on carcinoma in the male rat kidney, the most sensitive sex, species, and target organ. Risk assessment indicated that acute toxicity occurred at lower levels than did long-term effects such as carcinogenicity, so the previous PTWI, set on the basis of nephrotoxicity before carcinogenicity was definitely established, was retained (WHO, 2008). IARC has classified OTA as Group 2B, possibly carcinogenic to humans (IARC, 1993).

The current average dietary exposure levels to OTA have been determined by JECFA to be 8–17 ng/kg bw/week (WHO, 2008). These levels are well below the PTWI; however, JECFA had previously determined (WHO, 2001b) that the 95th percentile for OTA consumption was about 84–92 ng/kg bw/week, approaching the PTWI. A recent reappraisal of the risk associated with OTA resulted in a reduced PTWI of 21 ng/kg bw/week, one fifth of the JECFA estimate, based on applying an even larger uncertainty factor to the study of Krogh *et al*. (1974) evaluated by JECFA (Kuiper-Goodman *et al*., 2010).

Dietary exposure is based primarily on data from Europe, where processed cereal foods often show high levels of contamination with OTA (Steyn *et al*., 2008; WHO, 2008). Contamination of cereals in Europe is due to *Penicillium verrucosum*, which is a fungus of cool climates and does not occur in the tropics. Although sometimes contaminated by *Aspergillus* species capable of producing OTA, cereals in tropical countries are not usually a major source. Coffee and cocoa are potential sources of OTA exposure in tropical countries (WHO, 2008).

### *2.5.4 Deoxynivalenol*

The toxicity of deoxynivalenol (DON) has been reviewed by WHO and IARC, and risk assessments including toxicological reviews of DON have been published for Canada, the Nordic Council, the Netherlands, and the European Union (WHO, 2001). IARC has categorized DON as Group 3, not classifiable as to its carcinogenicity to humans (IARC, 1993). A 2-year study of mice exposed to DON suggested no carcinogenic hazard. Although the weight of the mice exposed to DON was lower than that of the controls, the difference was not considered biologically significant. JECFA established a PTMDI for DON of 1 µg/kg bw/day, based on the NOEL (for decreased body weight at day 500) in this mouse study of 100 µg/kg bw/day and a safety factor of 100 (WHO, 2001a).

### *2.5.5 Zearalenone*

The toxicity of zearalenone (ZEA) was evaluated by JECFA in 2000. Reproductive and developmental effects, as well as estrogenic effects, were found in a variety of animal species, including rats, mink, and pigs (WHO, 2000). There is, however, little evidence for acute toxicity or carcinogenicity. In humans, ZEA was suspected to have caused premature thelarche in girls, but the evidence was inconclusive and other causative or contributing agents could not be ruled out (WHO, 2000). IARC has categorized ZEA as Group 3, not classifiable as to its carcinogenicity to humans (IARC, 1993). Using a safety factor of 80, JECFA set a PMTDI for ZEA of 0.5 µg/kg bw/day, based on the NOEL (for reversible increase in length of estrous cycle) of 40 µg/kg bw/day in a 15-day study in pigs (WHO, 2000).

## 3. Risk management

Risk assessments have confirmed health risks to human populations worldwide from several mycotoxins in food, including aflatoxins, fumonisins, ochratoxin A, and deoxynivalenol. Although several potential interventions exist by which to manage mycotoxin risks in food,

control is very difficult in practice. At this time, no single strategy enables risk from mycotoxins to be eliminated in any country.

Mycotoxin risks in food can be managed either by governmental regulations or by agricultural and public health interventions. Governments can impose food safety standards that specify a maximum tolerable limit of a particular mycotoxin in human food. At the same time, agricultural and public health interventions can be adopted to reduce mycotoxin levels in food or reduce the bioavailability of mycotoxins.

Mycotoxin standards have reduced foodborne mycotoxin risk in developed countries because enforcement is strong and because technologies and methods exist to successfully reduce or remove mycotoxin contamination. Commodities with moderately excessive levels of mycotoxins are removed from the food stream and used as feeds for animals that tolerate higher levels of mycotoxins, such as beef cattle, or as biofuels or fertilizers.

In low-income countries (LICs), the situation is quite different. In many parts of the world, regulatory standards for mycotoxins in food have little or no impact on actually reducing mycotoxin risk, for several reasons.

First, many rural farmers engage in subsistence farming, in which case food grown on farms is directly consumed by the families without ever undergoing a formal inspection process for mycotoxins. Second, even if regulatory standards exist for certain mycotoxins, there is often little to no enforcement of these standards in certain parts of the world. Third, if the regulatory standards are imposed by importing countries, farmers in LICs may export their best quality foods and keep the poorer quality for domestic use, inadvertently raising health risks related to mycotoxins in populations already vulnerable to disease because of poverty (Wu, 2004).

The risk of mycotoxin contamination of commodities and foods is greatest in LICs, where agricultural systems are often poorly equipped to handle food safety risks. Suboptimal field practices and poor storage conditions make the crops vulnerable to fungal infection and subsequent mycotoxin accumulation. The problem is exacerbated by the fact that maize and groundnuts, two of the food crops that are most susceptible to aflatoxin contamination, are staples in the diets of many people worldwide, and thus aflatoxin exposure is higher where dietary variety is difficult to achieve (Shephard, 2008). In good seasons, subsistence farmers and local food traders may be able to avoid eating obviously mouldy maize and groundnuts, but in drought seasons, or in situations of food insecurity, often people have no choice but to eat mouldy food or starve (Wu and Khlangwiset, 2010a). Indeed, 125 people died due to acute aflatoxicosis in rural Kenya in 2004 when food insecurity, caused by a variety of climatic and social factors, led to widespread consumption of maize contaminated with high levels of aflatoxins (Lewis *et al*., 2005).

One additional limitation of the setting of mycotoxin standards is that such standards are usually in the form of an allowable mycotoxin concentration in a particular food commodity, such as maize. Such a standard does not take account of the fact that some populations consume much more of the food commodity than other populations in the world; hence, those populations could be much more exposed even if regulatory standards were enforced.

For example, regulations on allowable fumonisin levels in maize may sufficiently protect populations that do not typically consume large amounts of maize, but the allowable levels may be too high in many parts of sub-Saharan Africa, where maize is a dietary staple. There, populations may be exposed to potentially dangerous amounts of fumonisin from maize even if that maize meets regulatory standards. Table 7.2 highlights how people who have a very high maize intake can ingest dangerous levels of fumonisin even if the maize itself is considered relatively clean by regulatory standards, whereas people who consume very small amounts of maize could ingest a much more contaminated commodity without having significant fumonisin exposure (Gelderblom *et al*., 2008).

Hence, regulatory standards for mycotoxins in food sometimes have no impact, or even potentially adverse impacts, on human health in LICs (Wu, 2004; Shephard, 2008; Williams, 2008). Instead, the focus for risk management should be on technologies and public health interventions to reduce mycotoxin risk, infrastructures to support these technologies, and public education (Fig. 7.1).

Mycotoxins can be managed at various points along the food production chain from the field to the plate. In pre-harvest, or field, conditions, using good agricultural practices, such as choosing appropriate cultivars for the geographical region, can reduce the risk of fungal infection and subsequent mycotoxin accumulation. Post-harvest interventions involve careful sorting, cleaning, drying, storage, transportation, and processing to reduce the risk of further mycotoxin accumulation, or to lower mycotoxin levels directly.

Much of the work that has been done on dietary methods to reduce the bioavailability of mycotoxins has pertained specifically to aflatoxin. However, one simple dietary intervention that applies to all mycotoxins is, where feasible, to consume less of the foodstuffs that contain the mycotoxins. In the case of aflatoxin, that would mean consuming less maize and groundnuts if

possible, in favour of other food crops that have significantly lower aflatoxin contamination, such as rice, sorghum, or pearl millet. An example is the recent economic growth in China that has led to reduced maize consumption, and hence reduced aflatoxin exposure (Wild and Gong, 2010). Where it is not easy to make such a dietary shift (e.g. where maize and groundnuts have traditionally been staples), other dietary interventions may prove helpful. These interventions are described in greater detail in Chapter 9.

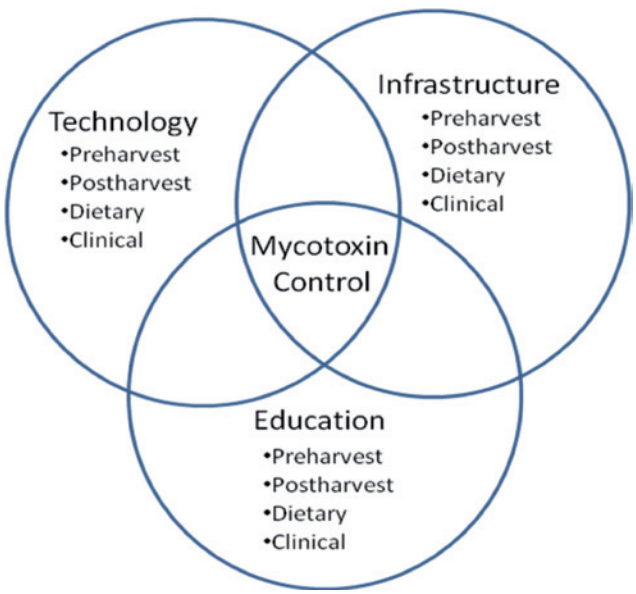
One public health intervention does not result in lower mycotoxin levels but can reduce the adverse effects associated with HCC induced by aflatoxin: vaccination against HBV. A regular practice now in developed countries, HBV vaccination in children still requires wider implementation. Vaccinating children against HBV over the past three decades has resulted in significantly decreased HBV infection rates in several countries and regions, including Europe (Williams *et al*., 1996, Bonanni *et al*., 2003); Taiwan, China (Chen *et al*., 1996); and Thailand (Jutavijittum *et al*., 2005). This vaccine may have significant impacts on HCC incidence, particularly in Africa and Asia, where the current prevalence of chronic HBV infection is relatively high. To date, a reduction in HCC incidence resulting from HBV vaccination has been demonstrated in children and adults in Taiwan, China, after vaccination was introduced in 1984 (Chang *et al*., 2009). By lowering the prevalence of chronic HBV infection, this vaccination should prevent the synergistic interaction between HBV and aflatoxin in inducing HCC (Khlangwiset and Wu, 2010; Liu and Wu, 2010).

**Table 7.2.** Probable daily intake of fumonisins (µg/kg bw/day) as a function of maize intake and fumonisin contamination levels in food

| Fumonisin level (mg/kg) | Maize intake (g/person [60 kg]/day)[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | 10 | 50 | 100 | 150 | 200 | 400 | 500 |
| 0.2 | 0 | 0.2 | 0.3 | 0.5 | 0.7 | **1.4** | **1.7** |
| 0.5 | 0.1 | 0.4 | 0.8 | **1.3** | **1.7** | ***3.4*** | ***4.2*** |
| 1 | 0.2 | 0.8 | **1.7** | ***2.5*** | ***3.3*** | ***6.6*** | ***8.3*** |
| 2 | 0.3 | **1.7** | ***3.3*** | ***5.0*** | ***6.7*** | ***13.4*** | ***16.7*** |
| 3 | 0.5 | ***2.5*** | ***5.0*** | ***7.5*** | ***10.0*** | ***20.0*** | ***25.0*** |
| 4 | 0.7 | ***3.3*** | ***6.7*** | ***10.0*** | ***13.3*** | ***26.6*** | ***33.3*** |

[a] The shaded values are those intakes closest to the Joint FAO/WHO Expert Committee on Food Additives (JECFA) provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg bw/day for fumonisin $B_1$ ($FB_1$), $FB_2$, and $FB_3$ alone or in combination.
Adapted, by permission of the publisher, from Gelderblom *et al.* (2008).

**Fig. 7.1.** Mycotoxin control in developing countries. In the absence of enforcement of mycotoxin standards, technology, appropriate infrastructure, and public education are required to implement strategies that reduce mycotoxin risk.



## 4. Mycotoxin risk and public education

There is no question that mycotoxins in food pose a significant public health risk in many parts of the world. Risk assessments conducted using human, animal, and in vitro studies and exposure assessments have confirmed the reality of this global health problem. HCC is a leading cause of cancer deaths worldwide (Ferlay *et al*., 2008), and an HCC risk assessment reveals that aflatoxin may play a causative role in more than one quarter of HCC cases (Liu and Wu, 2010). Epidemiological studies have also linked aflatoxin exposure with stunted growth in children, acute aflatoxicosis, and liver cirrhosis and have suggested fumonisin exposure as a possible risk factor for oesophageal cancer and neural tube defects (Wild and Gong, 2010).

In developed countries, mycotoxin exposure in the diet is controlled by good agricultural practices, good storage and processing, and control of excess levels of the major mycotoxins by standards and enforced regulation. However, many countries where the risk is particularly high have neither the technologies nor the infrastructures to reduce mycotoxin exposure. Moreover, suboptimal field practices and poor storage conditions make the crops vulnerable to fungal infection and subsequent mycotoxin accumulation (Williams, 2008). Maize and groundnuts, two crops susceptible to mycotoxin contamination, are staples in the diets of many people in Africa, Asia, and Latin America. Therefore, interventions should focus on reducing mycotoxin risk in simple and cost-effective ways.

It is important to remember, however, that public health interventions must be readily accepted by their target populations to have any meaningful impact, and must have financial and infrastructural support to be feasible in the parts of the world where they are most needed (Wu and Khlangwiset, 2010b). A critical component to implementing any or all of these methods is public education (Wild and Gong, 2010). Educational efforts should include not only how to use the intervention properly to achieve maximum benefit for mycotoxin risk reduction but also why the interventions are important from the perspectives of public health and food markets, so that users have incentives to continue with the interventions.

Public and governmental education on mycotoxin risk is crucial to provide incentives to adopt interventions. Even if an intervention to reduce mycotoxin risk is cost effective in terms of lives saved and improved quality of life (Wu and Khlangwiset, 2010a), no incentive to implement it may exist unless the effects of mycotoxins on public health and food markets are fully understood. It is worth noting that in Ghana aflatoxin exposure has been shown to be significantly correlated with farmers' knowledge of aflatoxin risk (Jolly *et al*., 2006), whereas in Benin farmers' knowledge of aflatoxin risk has been shown to be correlated with the motivation to implement interventions to reduce aflatoxin formation (Jolly *et al*., 2009).

Education must take place on at least three different levels. First, government policy-makers must receive information about the burden of mycotoxin-induced disease in their countries – in terms of effects on both public health and food markets – as well as information about possible interventions, their cost-effectiveness in reducing mycotoxins, and their technical feasibility requirements. Supplying the appropriate information will be one step in sensitizing governments to provide the finances and other resources necessary to initiate the interventions. Second, depending on the intervention characteristics, the farmers, the consumers, or both these groups must receive education on why mycotoxins are a concern and how to implement the intervention in question. Third, international health and agricultural organizations must be informed about the extent to which mycotoxins can affect both food markets and public health. This will provide incentives to aid countries in which foodborne mycotoxins are still a significant public health risk.

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CHAPTER 8.

# Economics of mycotoxins: evaluating costs to society and cost-effectiveness of interventions


## Summary

The economic impacts of mycotoxins to human society can be thought of in two ways: (i) the direct market costs associated with lost trade or reduced revenues due to contaminated food or feed, and (ii) the human health losses from adverse effects associated with mycotoxin consumption. Losses related to markets occur within systems in which mycotoxins are being monitored in the food and feed supply. Food that has mycotoxin levels above a particular maximum allowable level is either rejected outright for sale or sold at a lower price for a different use. Such transactions can take place at local levels or at the level of trade among countries. Sometimes this can result in heavy economic losses for food producers, but the benefit of such monitoring systems is a lower risk of mycotoxins in the food supply. Losses related to health occur when mycotoxins are present in food at levels that can cause illness. In developed countries, such losses are often measured in terms of cost of illness; around the world, such losses are more frequently measured in terms of disability-adjusted life years (DALYs). It is also useful to assess the economics of interventions to reduce mycotoxins and their attendant health effects; the relative effectiveness of public health interventions can be assessed by estimating quality-adjusted life years (QALYs) associated with each intervention. Cost-effectiveness assessment can be conducted to compare the cost of implementing the intervention with the resulting benefits, in terms of either improved markets or improved human health. Aside from cost-effectiveness, however, it is also important to assess the technical feasibility of interventions, particularly in low-income countries, where funds and infrastructures are limited.


## 1. Introduction

Two important topics are central to a discussion of the economics of mycotoxins: (i) the overall economic impact of mycotoxins on society, and (ii) the benefits and costs of strategies to control mycotoxins in food.

A common misunderstanding about the overall economic impact of mycotoxins on society is that only market impacts – losses from food lots rejected due to excessively high mycotoxin levels, as well as losses related to livestock and poultry – are included in this estimation. In estimating the economic impact of mycotoxins, human health impacts

matter just as much, if not more so, particularly in low-income countries (LICs). Since the 1990s, the field of health economics has developed sufficiently that now improved methods exist to evaluate human health impacts of diseases and conditions, including those associated with mycotoxin exposure in food. To derive an estimate for the total cost to society of mycotoxins in food and feed, both market impacts and health impacts must be included in the calculation.

Strategies to control mycotoxin contamination should also be subject to economic analysis. Multiple strategies have been developed to reduce mycotoxin risks before harvest (in the field), after harvest (in storage, transportation, or processing), in diets, and in clinical settings (see Chapter 9). If these mycotoxin control strategies are to be adopted in the parts of the world where they are most needed, then their expected benefits, or effectiveness – in terms of both market and health outcomes – should exceed their costs. Moreover, their capital costs should not be so high that LICs would find it impossible to adopt the strategies. Low-tech strategies may prove the most economically feasible option to control mycotoxins in LICs. Finally, cultural acceptability of the interventions is crucial, to ensure long-term adoption and effectiveness in mycotoxin reduction.

In addition to the overall economic impact of mycotoxins and the cost-effectiveness of control strategies, another important economic consideration is the technical feasibility of these strategies, which includes risk assessment of potential health and environmental impacts. These issues are also discussed in this chapter.

It is important to remember that values for the different variables in economic models can change substantially with time. Hence, when the models are used at any point in time, the results should not be overinterpreted, to avoid the danger of making long-term decisions based on analyses of current (limited) information.

## 2. Market and trade impacts of mycotoxins

The primary way in which mycotoxins affect markets is to lower the value of the commodity being traded. The price paid for a particular lot of food or feed is reduced, or the lot is rejected entirely, or the lot must be treated at additional cost before being sold at a higher price. This can occur at multiple different levels of trade, from local all the way to international. Depending on the demands of the buyer, the stakeholder group that bears the burden of mycotoxin cost can be individual farmers, handlers, processors, distributors, consumers, or government.

### *2.1 Dynamics of market supply and demand due to mycotoxin contamination*

Microeconomic theory explains how the overall market and trade costs of mycotoxins can be evaluated. Simply, mycotoxin contamination decreases the available supply of acceptable food to be sold or bought. Fig. 8.1 illustrates the dynamics of supply and demand for food for human consumption when supply is decreased. The demand curve represents the quantity of a particular food that consumers are willing to buy at a particular price. At very high prices, less demand will exist for the food, whereas at lower prices, demand will be higher. The supply curves (labelled $S_0$ and $S_1$) represent the quantity of food that producers will provide at different prices per unit of food. Hence, the original equilibrium of quantity of food supplied, $Q_0$, and price per unit of food, $P_0$, is the intersection of the demand curve with the original supply curve, $S_0$. However, when the supply curve is shifted left, to $S_1$ (as happens when food supply is decreased due to excessively high mycotoxin levels), a new equilibrium is reached, represented by the intersection of the demand curve with the new supply curve, $S_1$. The reduced quantity of units of food sold, $Q_1$, demands a higher price per unit of food, $P_1$.

Thus, both producers and consumers bear costs associated with mycotoxins. Producers sell less food and thus have reduced revenue, and consumers must buy the food at a higher price. Specifically, the decrease in food producers' welfare due to mycotoxin contamination is represented by the shaded area in Fig. 8.1. This area represents the difference between the producers' initial welfare (area of triangle bounded by $P_0$, $Q_0$, and the demand curve) and their resulting welfare (area of triangle bounded by $P_1$, $Q_1$, and the demand curve). The decrease in consumers' welfare because of mycotoxins is represented by the difference between their initial welfare (area of triangle bounded by $P_0$, $Q_0$, and supply curve $S_0$) and their resulting welfare (area of triangle bounded by $P_1$, $Q_1$, and supply curve $S_1$).

In practical terms, producers of commodities vulnerable to mycotoxin contamination may suffer market losses directly, if the buyers monitor and enforce limits for mycotoxins. Consumers suffer market losses indirectly, by facing a reduced supply of the commodity and therefore a higher price. (In contrast, consumers may suffer health-related losses directly, as discussed in Section 3.) Often, this cost to consumers is marginal, particularly in developed countries, where the bulk of the cost of food is made up of food processing rather than the commodity itself. But in LICs, reducing the supply of food by removing heavily contaminated commodities can result in food shortage crises. Moreover, for

**Fig. 8.1.** The impact of a strict food quality standard on supply and subsequent price. Source: Wu (2008); reproduced with the permission of the publisher.

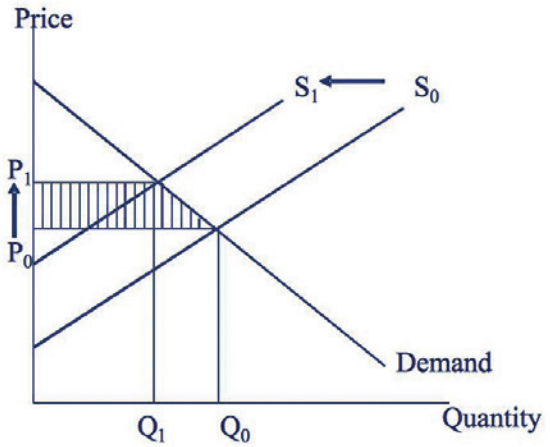
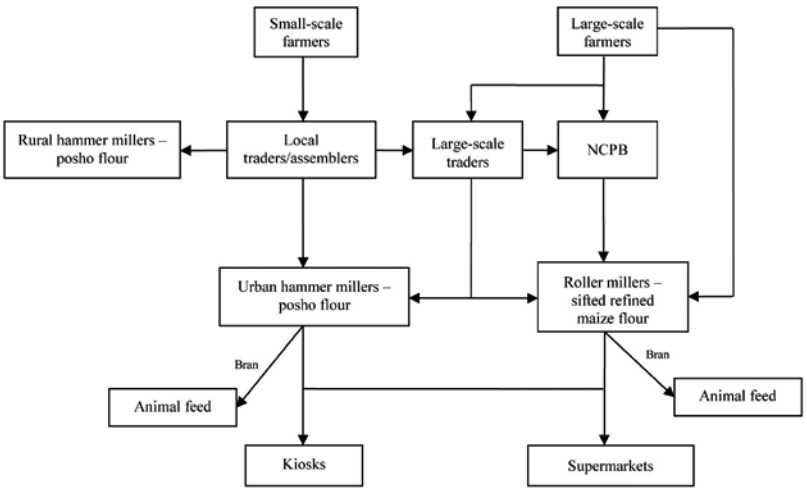



**Fig. 8.2.** Complexity of the value chain of maize in Kenya, and points at which aflatoxin control strategies could be implemented. NCPB, National Cereals and Produce Board of Kenya. Source: Dr Jonathan Hellin, International Maize and Wheat Improvement Center (CIMMYT), personal communication.




staple food items such as maize, the price elasticity of demand is usually very low, which means that demand will not change significantly in response to a change in price; consumers will purchase staples even if the price becomes much higher, because staple foods are a necessity.

## *2.2 Mycotoxin costs in local and regional markets*

The complexity of local and regional commodity markets varies considerably among countries, and thus the extent to which mycotoxins may impose market costs varies. In the USA, for example, crop value chains are relatively simple. Farmers may sell their crops to grain elevators (e.g. maize, wheat) or to shellers or other handlers (e.g. groundnuts, tree nuts), who may then further process the crop and sell it to animal operations or to food processors for human consumption. After processing, the food is sold to distributors, who then sell it to retail markets to be purchased by consumers.

For the USA, mycotoxin action levels (for aflatoxin, specifically) or industry guidelines (for fumonisin and deoxynivalenol) set by the United States Food and Drug Administration are enforced at different levels, such as at grain elevators or by food handlers. Crops with the lowest mycotoxin levels can be sold for human food or for feed to the most sensitive animal species at a higher price, whereas crops with higher mycotoxin levels can be sold for animal feed at a lower price or are rejected outright. Within each commodity, different stakeholder groups are affected differentially by mycotoxins. In the USA, producers of grain commodities generally bear the burden of costs related to mycotoxins, whereas shellers and handlers, not growers, bear the largest burden of mycotoxin-related costs associated with groundnuts and tree nuts (Wu *et al.*, 2008).

In other parts of the world, local commodity markets are far more complex. The value chain of maize in Kenya is illustrated in Fig. 8.2, to provide an example of trade of one commodity in one country. Maize growers (labelled in Fig. 8.2 as "Small-scale farmers") may sell their maize to a wide variety of different local maize traders, who may travel from household to household to purchase maize. These traders, in turn, may sell the maize to large-scale traders or to a variety of millers. Each step further in the value chain of maize provides different opportunities for both buyers and sellers.

This complexity of the value chain of crops in Africa means that it can be very difficult to implement mycotoxin control strategies that would have widespread effects. The control strategies would have to be implemented at multiple points, and these points would have to be coordinated. In the event of an outbreak of mycotoxicosis, interventions would need to be distributed, and communication among different stakeholders is absolutely crucial. Depending on available communications infrastructures, implementing interventions may be a difficult task.

### *2.3 Mycotoxin costs in international markets*

The issue of mycotoxin control is becoming increasingly important for LICs as international trade becomes more prominent in a world of increasing demand for crops (to be used for food, animal feed, or even fuel). Hence, mycotoxin costs must also be considered in the context of international trade.

More than 100 countries have established maximum tolerable levels for aflatoxins in human food (FAO, 2004), whereas relatively few countries have established these levels for other mycotoxins such as fumonisin, ochratoxin A, and deoxynivalenol. Table 8.1, which lists maximum tolerated levels for foodborne aflatoxins in selected countries and regions as of August 2010, shows that aflatoxin standards vary greatly among countries, even for a small sample of countries; this difference may cause food trade barriers. Indeed, the Council for Agricultural Science and Technology states that one key goal for the 21st century is to "develop uniform standards and regulations for mycotoxin contamination" (CAST, 2003). These standards have implications not just for the country that imposes the standard but also for countries that attempt to export foods there.

From an international trade standpoint, mycotoxin contamination inflicts heavy economic burdens. It reduces the price paid for crops and can cause disposal of large amounts of food. In the USA, losses from mycotoxins – in the hundreds of millions of US dollars annually – are usually associated with these market costs rather than with health effects because enforcement of mycotoxin standards and pre-harvest and post-harvest control methods have largely eliminated harmful exposures in food in the USA (Wu, 2004).

In LICs, impacts of mycotoxins are far more severe. Many individuals are not only malnourished but also chronically exposed to high aflatoxin levels in their diet, resulting in deaths from aflatoxicosis, cancer, and other conditions (Wild and Gong, 2010). LICs often lack the resources, technology, and infrastructure necessary for routine tests of mycotoxin levels in food. Further complicating the problem in the case of aflatoxin is that for a given level of aflatoxin exposure, cancer risk may be more severe in LICs than in developed countries because of higher prevalence of chronic hepatitis B virus (HBV) infection, which synergizes with aflatoxins to significantly increase the risk of liver cancer (hepatocellular carcinoma [HCC]) (Groopman *et al*., 2008).

Globalization of trade has exacerbated food losses due to mycotoxins in three ways. First, strict mycotoxin standards mean that LICs will export their best quality foods and keep more heavily contaminated foods for domestic consumption, resulting in higher mycotoxin exposure in LICs (Cardwell *et al*., 2001). Second, even the best quality foods produced in LICs may be rejected for export, resulting in millions of US dollars in losses (Wu, 2004; Wu *et al*., 2008). Third, the cost of a rejected food shipment is enormous (about $10 000 per lot in transportation, storage, and dockage fees; Wu *et al*., 2008), even if the lot can be returned to the country attempting to export the food.

These dilemmas led former United Nations Secretary-General Kofi Annan to recognize the magnitude of the problem of setting appropriate aflatoxin standards worldwide. At the Third United Nations Conference on the Least Developed Countries, held in Brussels in 2001, he commented, "The European regulation on aflatoxins costs Africa $670 million each year in exports. And what does it achieve? It may possibly save the life of one citizen of the European Union every two years. Surely a more reasonable balance can be found."

Annan had based his statement on a report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (WHO, 1998), which assessed the effect of aflatoxin regulations on HCC incidence, depending on HBV prevalence. JECFA developed two scenarios to determine the effect of moving from an enforced aflatoxin standard of 20 µg/kg to a stricter standard of 10 µg/kg in two hypothetical countries: one with an HBV prevalence of 1% and another with an HBV prevalence of 25%. In the first country, tightening the aflatoxin standard would yield a drop in the estimated population HCC incidence by 2 cases per billion people per year. In the second country, tightening the aflatoxin standard would yield a drop in the estimated population HCC incidence of 300 cases per billion people per year. Hence, in high-income importing countries with low HBV prevalence, tightening the aflatoxin standard would reduce HCC incidence by an amount so small as to be undetectable (Henry *et al*., 1999; Wu, 2004).

Now that a global push exists to harmonize mycotoxin standards

**Table 8.1.** Maximum tolerated levels for aflatoxins in human food in selected countries and regions[a]

| Country or region | Total allowable level of total aflatoxins in human food (µg/kg) |
|---|---|
| Australia[b] | 5 or 15 |
| China | 20 |
| European Union[c] | 4, 10, or 15 |
| Guatemala | 20 |
| India | 30 |
| Kenya | 20 |
| Taiwan, China | 50 |
| USA | 20 |

[a] A more complete list can be found in FAO (2004).
[b] The Australian standard for maximum allowable aflatoxins in groundnuts is 15 µg/kg, more permissive than those for other foods.
[c] The European Union standard for maximum allowable aflatoxins is 4 µg/kg for cereals and all products derived from cereals, except maize to be subjected to sorting, which has a standard of 10 µg/kg. The standard for groundnuts, almonds, hazelnuts, and pistachios "ready to eat" is 10 µg/kg, whereas the standard for groundnuts, almonds, hazelnuts, and pistachios intended for further processing is 15 µg/kg.

(CAST, 2003), it is important to consider on a global scale what the economic impacts would be of harmonizing different standards, which range from relatively strict to relatively permissive. Wu (2004) provided a framework for assessing losses related to markets, as outlined below.

Given a particular internationally imposed mycotoxin standard, the total national export loss of a particular food crop can be calculated as the product of the price of the food crop per unit weight on the world market, the total amount of that crop exported, and the fraction of the export crop that is rejected as a result of that mycotoxin standard (Wu, 2004):

$$\text{Export loss}_{i,j,k} = P_i * W_{i,j} * r_{i,j,k},$$

where $i$ is the crop (e.g. maize, groundnuts); $j$ is the country; $k$ is the international mycotoxin standard (e.g. for fumonisin or aflatoxin); $P_i$ is the world price for food crop $i$ per unit weight; $W_{i,j}$ is the total export amount (in metric tons) of crop $i$ from country $j$; and $r_{i,j,k}$ is the fraction of export volume of crop $i$ from country $j$ rejected at international mycotoxin standard $k$.

A sensitivity analysis on $k$ reveals how export losses for food crops in a particular country change as a function of the harmonized standard chosen. Values for $r_{i,j,k}$ are calculated by fitting probability density functions $PDF_{i,j,k}$, based on the relevant literature, of concentrations of fumonisin and/or aflatoxin in crop $i$ in country $j$. The particular country $j$ to study is chosen by looking at the most important exporting countries of crop $i$. Cumulative distribution functions are estimated from the probability density functions of the percentage of the crop having mycotoxin levels at or lower than a given concentration. Then, the fraction of export volume rejected at that concentration is:

$$r_{i,j,k} = 1 - \int PDF_{i,j,k}\, dk,$$

where $PDF_{i,j,k}$ is the probability density function of the percentage of crop $i$ from country $j$ having mycotoxin levels at or lower than standard $k$, and its integral over $k$ is the cumulative distribution function. Then, export losses calculated for each country are summed across major food exporting countries to derive a total global burden of export loss at different mycotoxin standards.

## 3. Health economic impacts of mycotoxins

Looking at the market and trade impacts of mycotoxins is only one side of the story. The other important facet to consider is the public health impact of setting different mycotoxin standards worldwide, assuming they could be enforced. The human diseases and conditions caused by mycotoxin exposure must first be evaluated.

Evaluating the human health economic impacts of mycotoxins is crucial to understanding their total economic impact because mycotoxins primarily affect LICs, where trade-related losses are not nearly as prominent as adverse health effects from consuming food contaminated with mycotoxins. Subsistence farmers and local food traders occasionally have the luxury of discarding obviously mouldy food, but in conditions of drought or food insecurity, poor people often have no choice but to eat the contaminated food or starve.

Until recently, it was difficult to put an economic value on health effects. Fortunately, the field of health economics has made great strides in the past two decades. Health economics strives to quantify health benefits and costs in such a way as to be comparable with monetary benefits and costs. Otherwise, it can be difficult to understand how much a particular risk affects human society, especially if death is not a significant

outcome, or, conversely, how much a public health intervention benefits human society, if no direct market outcomes exist. Putting monetary values on these health outcomes helps decision-makers to understand how important a risky agent or a disease is, how useful an intervention might be, and how to compare the relative importance of risks and the relative effectiveness of interventions (Wu and Khlangwiset, 2010a).

Much of the literature in health economics focuses on medical treatments, with relatively few applications in food and agriculture. Some examples of health economic assessments of food additives include studies of the potential cost-effectiveness of transgenic golden rice in reducing vitamin A deficiency (Stein *et al*., 2008) and the cost-effectiveness of biofortifying foods in reducing micronutrient deficiency (Meenakshi *et al*., 2007). Havelaar (2007) provided a notable example of estimating the health costs of foodborne zoonoses, such as those caused by *Campylobacter*, *Salmonella*, or *Cryptosporidium* in Europe.

It is also important to consider the health economic impacts of mycotoxins, which can impose an enormous socioeconomic cost. As stated above, in developed countries it is relatively straightforward to estimate the costs of mycotoxins because these costs are primarily related to markets. Commodities that contain mycotoxins at levels exceeding regulatory guidelines for human food or animal feed are discarded or sold at a lower price for a different use (Wu *et al*., 2008). One can estimate the cost of mycotoxins to a particular commodity group by estimating how much of the commodity must be discarded or discounted due to mycotoxin contamination. In LICs, in contrast, health-related costs are usually much higher than market-related costs, and health economic impacts are more difficult to evaluate.

The burden of human diseases, such as those caused by mycotoxin consumption, can be calculated in two primary ways. The first is cost of illness (COI), which is more appropriate in developed countries because a large portion of the estimate is health-care cost. The second is disability-adjusted life years (DALYs), which is appropriate for both developed and developing countries. A third metric, quality-adjusted life years (QALYs), is more often used to estimate the relative effectiveness of different public health interventions in improving overall quality of life.

### 3.1 Cost of illness

For an individual or for a particular population, COI caused by a disease or condition is calculated as the sum of three factors: direct health-care costs (DHC), direct non-health-care costs (DNHC), and indirect non-health-care costs (INHC):

$$COI = DHC + DNHC + INHC.$$

DHC are costs associated with medical services. These include general practice consultations, consultations with specialists, hospitalization, any surgery or treatments required, drugs, supplements (e.g. intravenous fluids), and rehabilitation. DNHC are costs associated with the disease that do not relate to the medical system. These include travel costs to medical centres, costs of childcare, and co-payments by patients for medicines.

INHC are defined as the value of production lost to society due to the disease or condition, as a result of temporary absence from work, permanent or long-term disability, or premature mortality (Havelaar, 2007; Wu and Khlangwiset, 2010a). To the extent that they can be evaluated, the costs of pain and of suffering associated with the condition would also be included in this category.

### 3.2 Disability-adjusted life years

The DALY, like COI, is a measure of the overall burden of disease. It extends the concept of potential years of life lost due to premature mortality to include equivalent years of healthy life lost in states of less than full health, broadly termed disability (Havelaar, 2007; Wu and Khlangwiset, 2010a). One DALY can be thought of as one lost year of healthy life. The total DALYs associated with a particular disease are calculated as follows:

$$DALYs = YLL + YLD,$$

where YLL is the years of life lost due to premature mortality from the disease and YLD is the years lost due to disability. YLD is estimated as the number of years lived with the disability multiplied by a weighting factor, between 0 and 1, that reflects the severity of the disability.

The World Health Organization (WHO), among other organizations, has estimated DALYs for many diseases and conditions in different parts of the world. DALYs for any given disease are estimated separately for high-income, middle-income, and low-income countries. This stratification is based on assumptions about how many years individuals will live with a disability in different parts of the world and what resources are available to alleviate disability (Wu and Khlangwiset, 2010a).

### 3.3 Quality-adjusted life years

The QALY is used to assess the value for money of a medical or public health intervention. It is based on the estimate of the number of years of life that would be added by the intervention, and hence is used

to rank the relative effectiveness of different interventions for a particular condition. Every year of "perfect health" is assigned a value of 1, whereas a year not lived in perfect health is assigned a value between 0 and 1 that reflects the quality of life (similar to the weighting factor for YLD in DALYs).

QALYs are calculated as follows. Individuals with a serious, life-threatening condition (as is often the case with excessive exposure to aflatoxin) can receive a standard treatment (which, in LICs, may be no treatment at all) that will allow them to live for *X* more years with a quality of life of *A*. However, if they receive a new treatment instead, they will live for *Y* more years with a quality of life of *B*. The difference between the new and the standard treatment in terms of QALYs gained is

$$\text{QALYs gained} = Y * B - X * A.$$

To assess the relative effectiveness of an intervention, the cost of the new treatment must also be taken into account. The difference in treatment costs divided by the QALYs gained is used to estimate the cost per QALY, i.e. how much would need to be spent to provide one additional QALY.

### *3.4 Challenges to evaluating health economic impacts of mycotoxins*

To use either the cost of illness or the DALYs method to calculate socioeconomic costs, one must first identify human health end-points, i.e. diseases or conditions, to assess. Otherwise, it is impossible to gather data on the necessary factors to assess the economic impact: mortality and morbidity, incidence, duration, and severity associated with the disease.

The challenge in calculating the socioeconomic costs of mycotoxins is that, with the exception of aflatoxins, specific human health end-points are difficult to attribute quantitatively or even qualitatively to a particular mycotoxin. For example, fumonisins have been associated with oesophageal cancer and neural tube defects in humans, but these associations are not clearly established and there are no human dose–response data, i.e. doses of fumonisins causing particular levels of disease incidence, by which to perform a reliable quantitative risk assessment. Further complicating the issue is that each mycotoxin may have multiple health end-points, including cancer, acute toxicity, and immunomodulation. Even if quantitative relationships could be established for each end-point, an analyst would need to ensure that every possible human health outcome of a mycotoxin was included in the calculation, to derive an accurate health economic estimate.

With aflatoxins, more progress has been made in quantifying the link between exposure and disease incidence. Aflatoxins cause a multitude of conditions, including acute aflatoxicosis and HCC, and are believed to contribute to immunosuppression and stunted growth in children. In an aflatoxicosis outbreak in Kenya in 2004 (Strosnider *et al.*, 2006), it was possible to estimate the total number of cases, the number of deaths, and the concentrations of aflatoxins in the contaminated maize that caused the toxicoses. Decades of work have likewise established dose–response relationships between aflatoxins and HCC in HBV-positive and HBV-negative individuals, from which aflatoxin cancer potency factors can be derived for quantitative cancer risk assessment (WHO, 1998). The immunosuppressive effects of aflatoxins cannot yet be quantified in humans, but limited quantitative data are available to assess the link between aflatoxin exposure and stunted growth in children (Gong *et al.*, 2002).

## 4. Evaluating total economic impacts of mycotoxins

If both the market and the health economic impacts of mycotoxins can be estimated, the cost-effectiveness of different interventions to reduce mycotoxin risk can then be assessed.

Various studies have attempted to quantify the potential market losses associated with mycotoxins in crops. In the USA, Vardon *et al.* (2003) estimated the total annual losses due to three mycotoxins – aflatoxin, fumonisin, and deoxynivalenol – to reach as high as US$ 1 billion. Almost all of this loss was borne by maize, groundnut, and wheat growers. However, a small portion of this loss was estimated to be suffered by livestock producers due to adverse animal health effects.

In three Asian countries – Thailand, Indonesia, and the Philippines – the total estimated annual loss due to aflatoxin was about 1 billion Australian dollars (Lubulwa and Davis, 1994). This loss was a combination of market impacts, through rejected lots with excessively high mycotoxin levels, and adverse health effects – specifically the impacts of HCC in these populations.

Wu (2004) estimated the market impacts to the world's top maize-exporting and groundnut-exporting countries and regions of conforming to hypothetical harmonized standards for fumonisin in maize and aflatoxin in groundnuts. If the current United States Food and Drug Administration (FDA) total fumonisin guideline of 2 mg/kg were adopted worldwide, the total annual maize export losses for the USA, Argentina, and China would be US$ 100 million, whereas if a fumonisin standard of 0.5 mg/kg were adopted worldwide, those total annual losses would increase to US$ 300 million. If the current FDA total aflatoxin action level of 20 µg/kg were adopted worldwide, total annual

groundnut export losses for the USA, Argentina, China, and Africa would be US$ 92 million, whereas if an aflatoxin standard of 4 µg/kg were adopted worldwide, those total annual losses would increase to US$ 450 million.

Liu and Wu (2010) and Liu *et al*. (2012) estimated the global burden of aflatoxin-related HCC using two different approaches: quantitative cancer risk assessment and population attributable risk (PAR), respectively. The quantitative cancer risk assessment methodology (Liu and Wu, 2010), relying on dietary surveys and cancer potency factors, yielded an estimate of 5–28% of total global HCC cases attributable to aflatoxin. Similarly, the PAR approach (Liu *et al*., 2012), making use of a systematic review and meta-analysis of human biomarker studies on aflatoxin-related cancer, yielded an estimate of 21–24% of global HCC cases attributable to aflatoxin. Because the total number of new HCC cases worldwide is hundreds of thousands each year and each HCC case is associated with 13 DALYs (Wu and Khlangwiset, 2010a), aflatoxin-related HCC alone may cause > 2 million DALYs each year.

## 5. Assessing cost-effectiveness of interventions to control mycotoxins

Multiple public health interventions exist by which to control mycotoxins or their burden in the human body. Interventions to reduce illness induced by mycotoxins can be roughly grouped into three categories: agricultural, dietary, and clinical. Agricultural interventions are methods or technologies that can be applied either in the field (pre-harvest) or in drying, storage, and transportation (post-harvest) to reduce mycotoxin levels in food. Agricultural interventions can thus be considered primary interventions because they directly reduce mycotoxin levels in food. Dietary and clinical interventions can be considered secondary interventions. They cannot reduce actual mycotoxin levels in food, but they can reduce mycotoxin-related illness either by reducing the bioavailability of mycotoxins (e.g. through enterosorption) or by ameliorating damage induced by mycotoxins (e.g. through inducing phase 2 enzymes that detoxify metabolites of mycotoxins). These control strategies are described in greater detail in Chapters 7 and 9.

In developed countries, it is relatively straightforward to estimate the cost-effectiveness of controlling mycotoxins because the costs and benefits are primarily market-related (Wu *et al*., 2008). The cost of a particular mycotoxin to a particular commodity group is calculated by assessing how much of the commodity must be discarded or discounted due to contamination. Then, measuring the benefit accrued from a particular intervention requires estimating how much levels of the mycotoxin are reduced as a result of the intervention and how much more of the commodity can thus be sold. The difference between the total market value of the commodity with and without the intervention is a rough estimate of the cost-effectiveness of that intervention.

To calculate the cost of mycotoxin contamination in developed countries, three market economic factors need to be considered: the expected cost of mycotoxin contamination to growers or handlers in the absence of any interventions, the cost of purchasing and applying an intervention, and the expected net benefit of applying the intervention in terms of mycotoxin reduction. The cost per hectare, *C*, of mycotoxin contamination to growers in the absence of agricultural interventions can be expressed as

$$C = Y * P * R,$$

where *Y* is the crop yield per hectare, *P* is the price differential for high-quality use (low mycotoxin levels required) versus other uses, and *R* is the percentage of the crop with mycotoxin levels above the limit for high-quality use.

This cost *C* is compared with the benefits and costs of applying a mycotoxin control method. The net benefit per hectare, *B*, of applying the intervention can be expressed as

$$B = (E * C) - A,$$

where *E* is the percentage efficacy in reducing mycotoxins to levels that allow growers a premium, *C* is the cost per hectare associated with mycotoxin contamination (as shown in the previous equation), and *A* is the total cost of purchasing and applying the mycotoxin control strategy.

In LICs, however, interventions to reduce mycotoxins have both market and human health importance. How can the cost-effectiveness of a health intervention be determined if no direct market benefits exist? The WHO Commission on Macroeconomics and Health has provided the following guideline for thresholds of cost-effectiveness (WHO, 2001). An intervention is considered very cost effective if the monetary amount spent on the intervention per DALY saved is less than the per capita gross domestic product (GDP) of the country in which the intervention is applied. An intervention is considered moderately cost effective if the monetary amount spent on the intervention per DALY saved is less than 3 times the per capita GDP. An intervention is considered not cost effective if the monetary amount spent on the intervention per DALY saved is greater than 3 times the per capita GDP.

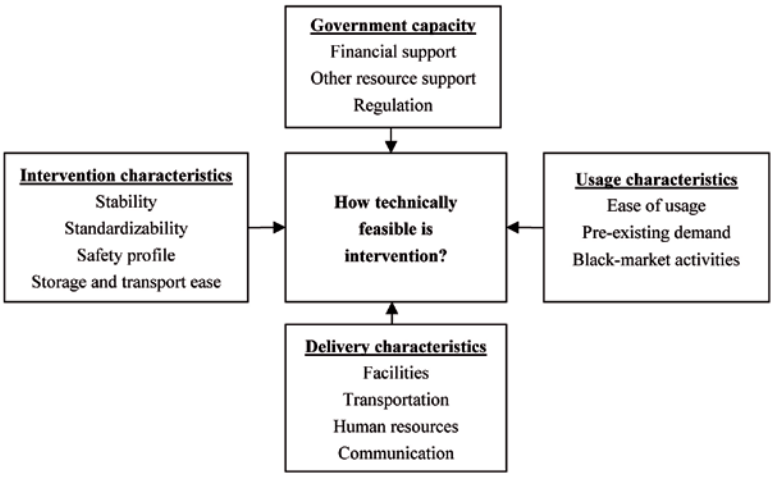
As described in Section 3.4, the health effects of the mycotoxin in question must be identified to estimate DALYs for this cost-effectiveness calculation. DALYs for any given disease are estimated separately for high-income, middle-income, and low-income countries. The DALYs estimate for each kind of country is based on assumptions about how many years individuals will live with a disability in different parts of the world and what resources are available to alleviate disability.

This WHO guideline is not without controversy. First, the cut-off of 3 times the per capita GDP for the cost-effectiveness of an intervention is a debatable metric. Second, using the average GDP in the estimation can be controversial in itself, especially for countries in which income is distributed bimodally rather than normally (i.e. average GDP can be meaningless in a country with very rich versus very poor populations and no middle class). Third, DALYs can be a controversial measure, particularly in selecting a weighting factor associated with each illness or condition. Finally, cost-effectiveness is but one component of the feasibility of aflatoxin reduction strategies in LICs. Many other factors are important, including the technical feasibility of the intervention, which is described next.

## 6. Technical feasibility of interventions to control mycotoxins

The cost-effectiveness of a mycotoxin control strategy is not enough to ensure its successful adoption in the parts of the world where it is needed. More questions must be addressed. Does the strategy entail countervailing health or environmental risks? What would the delivery mechanism be, and would local infrastructures support that mechanism? Do governmental regulations inhibit or promote the intervention? Is the intervention culturally appropriate and easily adopted by the target population? If an intervention to reduce mycotoxins fails in any or all of these points, then it is not likely to be adopted on the large scale, no matter how cost effective it may be.

**Fig. 8.3.** Framework for assessing the technical feasibility of public health interventions. Source: Wu and Khlangwiset (2010b); reproduced with the permission of the publisher.




A conceptual framework has been developed for evaluating the technical complexity – and hence the feasibility – of public health interventions for LICs with limited resources (Gericke *et al.*, 2005). The framework has four relevant dimensions (see Fig. 8.3): intervention characteristics, delivery characteristics, government capacity, and usage characteristics. Each of these is discussed below.

### *6.1 Intervention characteristics*

What aspects of the intervention itself make it more or less feasible for large-scale adoption? Gericke *et al.* (2005) pointed out that one of the most important aspects of the feasibility of an intervention, once it has been proven to have some level of efficacy in a field or clinical trial, is that it has the potential to be implemented on a much larger scale. This is crucial both spatially and temporally, to allow maximum effectiveness of the intervention in a target population. To achieve large-scale implementation, the following characteristics of the basic intervention would influence feasibility: (i) the stability of the product, including its usable lifetime and its risk of degradation or destruction; (ii) the degree to which the intervention can be standardized for production and sale; (iii) the safety profile of the intervention, in terms of both adverse health and environmental effects and risk associated with inappropriate use; and (iv) the ease of storage and transportation of the intervention.

In considering safety, it is important to recognize that implementing certain mycotoxin control interventions in LICs may result in health or environmental risks that would be less likely to occur in developed countries. Items to be considered include occupational hazards associated with producing or implementing the intervention, health risks to workers, quality control of production and application, immune status of the target population, local ecologies, and potential side-effects of dietary interventions (Wu and Khlangwiset, 2010b).

### 6.2 Delivery characteristics

How will a particular intervention be delivered to a target population? First, the target population must be identified, which can be a challenge in countries where food is grown in smallholdings. Communication of a need to those able to respond may pose difficulties. In addition, there are requirements for facilities, transportation, human resources, and communication (Gericke *et al*., 2005). Proper facilities are necessary to store and to administer the intervention and must be distributed widely enough within the target population so that most people have reasonably easy access to the intervention. Transportation may necessitate specific infrastructure (e.g. cold storage in vehicles for vaccines, and power to maintain cold temperatures). Transportation issues may also make a significant difference in cost if the intervention needs to be imported rather than produced locally. Human resources and communication are crucial when the public, or any subgroup thereof (such as farmers or food storage handlers), must be educated on proper use of the intervention and why it is important for health and economic reasons.

### 6.3 Government capacity

How would national or local governments either support or inhibit adoption of the intervention? Governmental financial support and other resource support, such as staff support and outreach activities, would be crucial for at least the start-up phase of an intervention. Moreover, governmental regulations can determine whether an intervention can be adopted broadly in a region. If, for example, regulations have been enacted against genetically modified organisms, certain food additives, or certain chemical or microbial agents, then particular interventions related to agriculture and food safety cannot be implemented on a scale that achieves widespread public health benefits (Wu and Khlangwiset, 2010b).

### 6.4 Usage characteristics

Generally, the more readily a target population can use or adopt an intervention, the more likely it is to be adopted with a frequency that actually makes a difference to public health (i.e. long-term use) and the more likely people are to adopt it (i.e. breadth of use). Gericke *et al*. (2005) identified three crucial dimensions of usage: (i) the ease of usage; (ii) the pre-existing demand for the intervention; and (iii) the risk of diminished effectiveness and efficiency because of illicit trade activities, such as counterfeit products.

Ease of usage includes the need for consumer information and education or training on how to use the mycotoxin control strategy effectively and safely. If no pre-existing demand exists for the intervention, adoption might be more difficult and would require more time. Finally, illicit trade activities can pose dangers, especially in the case of dietary interventions.

Understanding constraints on the feasibility of mycotoxin control interventions helps scientists and policy-makers to think beyond efficacy, and even beyond material costs. For interventions to succeed in LICs, governments, scientists, international organizations, farmers, and consumers must work collaboratively to overcome challenges in implementing the intervention – challenges in terms of human resource needs; equipment, technology, and transportation requirements; financial aid; and user adoption constraints. Feasibility analyses can indicate research and development priorities to increase the likelihood of adopting interventions that can improve public health and market outcomes (Wu and Khlangwiset, 2010b).

## 7. Conclusions

For developed countries, it is relatively easy to estimate the cost to human society of mycotoxins as well as to assess the cost-effectiveness and technical feasibility of interventions to control them. This is because the costs of mycotoxins and the benefits of interventions are largely restricted to the marketplace; human health effects can largely be considered negligible. Moreover, in developed countries, the feasibility of mycotoxin control becomes an issue only if the intervention is extremely expensive, in which case another, less expensive intervention is usually available.

For LICs, including the impact of mycotoxins on human health results in much more complicated economic analyses. Health economic tools from the past two decades have improved the ability to place monetized values on human health effects. This, in turn, aids cost-effectiveness analysis because the monetary cost of interventions can be compared with the human health benefits of implementing the interventions. Evaluating the technical feasibility of interventions is still complex because the many impeding factors and external risks and benefits need to be considered.

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CHAPTER 9.

# Practical approaches to control mycotoxins


## Summary

Control strategies to minimize mycotoxin levels in food comprise several broad categories, including good agricultural practice, good manufacturing practice, and hazard analysis and critical control point principles. In general, intervention strategies include pre-harvest, post-harvest, and dietary approaches, depending on the specific mycotoxins and the food commodity likely to be contaminated. This chapter describes practical interventions, which are arranged by the major groups of mycotoxins and are described according to their stage of development, efficacy, geographical regions in which they have been tested or applied, simplicity or complexity, and breadth of usefulness. Typical pre-harvest interventions include the breeding of resistant plant cultivars, good agricultural practice, and biocontrol using non-toxigenic strains. Post-harvest interventions include the removal of infected and/or insect-damaged food components by sorting, maintaining correct drying and storage conditions, and chemical deactivation such as nixtamalization. Dietary interventions include reducing mycotoxin bioavailability or modulating metabolism in ways that reduce the harmful effects of reactive metabolites. Cost-effective and simple intervention methods, predominantly at the population level, should be emphasized in developing countries, where resources are limited and sophisticated technologies are lacking.


## 1. Introduction

This chapter provides detailed information about interventions and practices that can help to reduce mycotoxin risks in a variety of settings: before harvest (in the field), after harvest (in storage, transportation, or processing), and in diets. The sections are organized by mycotoxin and the interventions that have been developed to control them, as follows: aflatoxins, fumonisins, ochratoxin A, and deoxynivalenol and zearalenone (treated together because they are produced by the same fungi).

For each intervention, information about each of the following aspects is listed.

Description. A description of the intervention.

Stage of development. How well developed and tested is the intervention in terms of controlling

the mycotoxin? Has it undergone testing under laboratory, field, or epidemiological conditions? How broad is the current adoption?

Efficacy. By how much can the intervention reduce mycotoxin risk, compared with conditions where there is no intervention?

Geographical regions. Where has this intervention been tested or adopted around the world?

Simplicity/complexity. How simple or complex is the intervention to implement? Is it so complex that individuals must have advanced education and training, or can it be made sufficiently simple for farmers or even the general public to adopt effectively?

Population/individual. Is the intervention tailored, or can it be tailored, to a population-level approach (e.g. so that a government can broadly implement the intervention), or does the intervention rely on individuals for implementation?

Useful in emergencies. Is this intervention suitable for use in a time of emergency, e.g. when mycotoxin levels are known to be high in available foods, or in the event of acute poisoning?

Locality of resources. Can the intervention be manufactured using local resources and drawing on local expertise, or does it require importation of resources and/or trained operators?

Accessibility. Is the intervention applicable in low-income countries (LICs) and among subsistence farmers with respect to access, cost, and feasibility?

References. For further reading on the topic.

Before individual interventions for each mycotoxin are discussed, three broad categories of interventions are described: good agricultural practice, good manufacturing practice, and the hazard analysis and critical control point system. These categories of interventions apply for general control strategies across multiple mycotoxins.

### *1.1 Good agricultural practice*

Good agricultural practice (GAP) involves good farm management. Many definitions exist, depending on local conditions, but in general GAP means maintaining healthy crops and sustainable agriculture by, among other activities: (i) planting with optimal row and seed spacing for local conditions, especially water availability, to reduce plant stress; (ii) maintaining adequate water supplies, by irrigation where practicable; (iii) reducing erosion by contouring, ditching, or hedging; (iv) controlling weeds, and mulching crops to reduce moisture stress; (v) controlling insects that damage developing grains or nuts and permit entry of the fungi that produce mycotoxins; (vi) rotating crops to reduce insect infestation and fungal infection, which are exacerbated by monoculture; (vii) applying fertilizers at appropriate times and concentrations, to benefit the crop but limit run-off of nutrients such as nitrogen and phosphorus; (viii) harvesting crops at or before full maturity, because overmature crops are liable to increased risk from insect damage and water stress and hence mycotoxin production; (ix) drying crops rapidly and completely, as soon as possible after harvest; and (x) maintaining good storage conditions on the farm (storage facilities should be soundly constructed to prevent water ingress, with raised floors to prevent moisture migration from soil; properly dried crops should be stored in closely woven sacks that permit air exchange; and rodents and insects should be controlled).

Drying of crops is a critical process in reducing development of mycotoxins. Grains and nuts are often dried in the field, with consequent poor control over conditions. In subtropical and temperate regions, the weather is usually drier at harvest time and field drying is effective. In tropical countries, groundnuts are frequently harvested and left to dry in stacks in the field, or are separated from plants at harvest and dried on the ground, on some form of matting, or on plastic sheets. Maize is sometimes shelled wet and then dried mechanically by middlemen. Storage practices in developed countries normally prevent development of mycotoxins after drying. However, less than ideal storage conditions in LICs may permit increases in moisture content, leading to increases in production of aflatoxins or ochratoxin A. Storage conditions can be improved by using dry, well-ventilated rooms with protection from sunlight (to prevent moisture migration) and control of insects and rodents. This is true not just for maize, cereals, and groundnuts (the major sources of mycotoxin exposure for humans) but also for tree nuts such as pistachios, for which there have been dramatic improvements in aflatoxin reduction in Iran over the past decade due to improved drying and storage conditions (Wu, 2008).

For further information about GAP, see FAO (2002).

### *1.2 Good manufacturing practice*

Good manufacturing practice (GMP) involves a wide range of practices that maintain the quality of foods, in developed countries often through legislation. In the context of mycotoxins, GMP includes practices that prevent fungal growth and hence reduce mycotoxin formation and that reduce or remove mycotoxin contamination in crops after harvesting and drying. On the farm, this most commonly involves removal of defects, including immature nuts or grains and also weed seeds, sticks, stones, earth, husks, and so on,

by hand sorting, winnowing, and gravity separation or other methods. Sorting out obviously mouldy nuts or kernels by hand and eye has proven a particularly effective method of removing a large proportion of the mycotoxin contamination in the food (Turner *et al*., 2005; Van der Westhuizen *et al*., 2011). Practices downstream, involving middlemen, cooperatives, and factories, depend on the crop and the mycotoxin. These include practices such as extrusion (Bullerman and Bianchini, 2007) and nixtamalization (described in more detail in Section 2.2.2), both of which have been shown to reduce levels of multiple mycotoxins in food.

### *1.3 The hazard analysis and critical control point system*

The hazard analysis and critical control point (HACCP) system for food safety management involves controlling critical points in food handling (FAO, 2001) and is important in managing the problem of mycotoxins in the food supply (Bryden, 2009; Chulze, 2010). Adopting guidelines of the United Nations Codex Alimentarius Commission (Codex Alimentarius Commission, 1995), the Food and Agriculture Organization of the United Nations (FAO) has outlined the following seven principles of HACCP for food safety (FAO, 2001). First, identify potential hazards associated with food production at all stages, assess the likelihood of hazard occurrence, and identify preventive measures for control. Second, determine points, procedures, and operational steps that can be controlled to eliminate, or reduce the likelihood of, hazards. These are the critical control points (CCPs). Third, establish critical limits that must be met to ensure that CCPs are under control. Fourth, establish a system to monitor control of CCPs by scheduled testing or observations. Fifth, establish corrective actions when monitoring indicates that certain CCPs are not under control. Sixth, establish procedures for verification to confirm that the HACCP system is working effectively. Seventh, establish documentation concerning all procedures and records appropriate to these principles and their application.

HACCP control for mycotoxins is an integrated approach (Bryden, 2009), which includes GAP and GMP (described above) as complementary approaches (Aldred *et al*., 2004). In developed countries, several HACCP programmes have been developed for aflatoxin in a variety of commodities as well as for ochratoxin A in coffee; these programmes rely on rapid diagnostic tools to monitor fungal occurrences and application of methods to quantify mycotoxins.

In LICs, some HACCP processes may not yet be technically and economically feasible (Bryden, 2009), necessitating other strategies to reduce mycotoxins. However, it can be useful to adopt HACCP principles as a way of thinking, regardless of economic and technical constraints. A 1999 conference on mycotoxins emphasized that GAP and GMP overlap and are prerequisites for HACCP. HACCP will ensure and improve food quality in a controlled environment; hence, such systems must be kept simple, practical, and understandable for those who use them to reduce mycotoxin risk (FAO/WHO/UNEP, 1999).

**Table 9.1.** Risk management strategies for major mycotoxins in pre-harvest, post-harvest, and dietary settings

| Setting | Mycotoxins | | | |
|---|---|---|---|---|
| | **Aflatoxins** | **Fumonisins** | **Ochratoxin A** | **Deoxynivalenol and zearalenone** |
| Pre-harvest | GAP<br>Developing drought-resistant cultivars<br>Biocontrol<br>Forecasting aflatoxin formation<br>Timely harvesting | GAP<br>Ensuring that cultivars are adapted to local environments<br>Breeding for insect resistance<br>Transgenic Bt maize<br>Forecasting fumonisin formation<br>Timely harvesting | GAP<br>Timely harvesting | GAP<br>Breeding for host plant resistance<br>Using cultivars that mature over a range of dates<br>Transgenic Bt maize<br>Using fungicides at anthesis or silking<br>Forecasting toxin formation |
| Post-harvest | GMP, HACCP, sorting, drying, nixtamalization | GMP, HACCP, sorting, drying, washing, nixtamalization | GMP, HACCP | GMP, HACCP, sorting, drying |
| Dietary | Enterosorbents (e.g. organic clays)<br>Chlorophyll and chlorophyllin | | | |

GAP, good agricultural practice; GMP, good manufacturing practice; HACCP, hazard analysis and critical control point.

**Table 9.2.** Likelihood of mycotoxin contamination in major commodities and current stage of development of potential or actual interventions

| | Commodity | | | | |
|---|---|---|---|---|---|
| | **Maize** | **Groundnuts** | **Tree nuts** | **Small grains** | **Others** |
| **Mycotoxin** | | | | | |
| Aflatoxins | X | X | X | | Figs, copra, spices, cottonseed |
| Fumonisins | X | | | | Sorghum, millet, soybeans, asparagus |
| Ochratoxin A | X | | | X | Dried vine fruits, wine, coffee, cocoa, chocolate |
| Deoxynivalenol and zearalenone | X | | | X | |
| **Relevant intervention** | | | | | |
| GAP | Practice | Practice | Practice | Practice | Practice |
| GMP | Practice | Practice | Practice | Practice | Practice |
| HACCP | Practice | Practice | Practice | Practice | Practice |
| Biocontrol via non-toxigenic strains | Pilot | Practice | Pilot | | Practice (cottonseed) |
| Fungicides | | | Practice | Practice | |
| Plant breeding (conventional and transgenic) | Practice | Pilot | Pilot | Practice | |
| Sorting | Practice | Practice | Practice | Practice | Practice |
| Nixtamalization | Practice | | | | |
| Enterosorbents | Practice (animal feeds in the USA) | Practice (animal feeds in the USA) | Practice (animal feeds in the USA) | Practice (animal feeds in the USA) | |
| Chlorophyllin | Promising; needs more research | Promising; needs more research | Promising; needs more research | Promising; needs more research | |
| Dietary chemoprevention | Promising; needs more research | Promising; needs more research | Promising; needs more research | Promising; needs more research | |

GAP, good agricultural practice; GMP, good manufacturing practice; HACCP, hazard analysis and critical control point; Pilot, studied or tested in experimental studies or on a pilot scale, but not in commercial use; Practice, currently being used by growers or producers to control a particular mycotoxin.

The tables organize several key characteristics of the interventions presented here. Table 9.1 summarizes the information on applicable interventions by mycotoxin and by type of intervention, i.e. pre-harvest, post-harvest, or dietary. Although hepatitis B virus vaccination, described in Chapter 7, is a clinical intervention that may reduce the potency of aflatoxin in causing liver cancer, it is not included in this chapter because it does not control mycotoxin levels directly. Likewise, food replacement – sourcing clean food from another region to a region suffering high foodborne mycotoxin contamination – is useful in emergency situations; however, it is also not included in the table because it does not directly reduce mycotoxin levels in the original food supply.

Table 9.2 provides information about which food commodities are likely to be contaminated by which mycotoxins, and whether particular interventions are common agricultural practice or have only been tested in pilot experiments. Table 9.3 describes usage characteristics of each intervention: the local availability of resources needed to develop it, the technical simplicity of implementing the intervention,

**Table 9.3.** Usage characteristics of interventions: local availability of intervention materials, ease of implementation, usefulness in emergencies, and whether the intervention is applicable at the population or individual level

| Intervention | Usage characteristic | | | |
|---|---|---|---|---|
| | Intervention materials available locally | Implementation technically simple | Useful in emergencies | Population or individual level |
| GAP | Yes | Yes | No | I, P |
| GMP | Yes | Yes | No | P |
| HACCP | Yes | Yes | No | P |
| Biocontrol via non-toxigenic strains | Yes (fungal strains and substrate) | Yes (after development) | No | P |
| Fungicides | Yes | Yes | No | P |
| Plant breeding | No | No | No | P |
| Sorting | Yes | Yes | Yes | I, P |
| Nixtamalization | Yes | Yes | Yes | I |
| Enterosorbents | No | No | Promising; needs more research | I, P |
| Chlorophyllin | No | No | Promising; needs more research | I |
| Dietary chemoprevention | No | No | Needs more research | I |

GAP, good agricultural practice; GMP, good manufacturing practice; HACCP, hazard analysis and critical control point; I, individual; P, population.

its usefulness in emergencies, and whether the intervention is applicable at the population or individual level.

## 2. Aflatoxins

### *2.1 Pre-harvest interventions*

#### *2.1.1 Conventional breeding for host plant resistance*

Description. Breeding methods have been explored to improve resistance to drought, insect herbivory, or other environmental stressors that would predispose groundnuts and maize to pre-harvest formation of aflatoxin. This has included work on identifying resistant crop lines and identifying biochemical and genetic resistance markers in crops. Sequencing of the *Aspergillus flavus* genome has been completed. Genes that potentially encode for enzymes involved in aflatoxin production have been identified, so that genomics as a tool for understanding aflatoxin biosynthesis has gained much ground (Yu *et al.*, 2008). It is hoped that these findings may have practical application in the future; for now, they have improved our understanding of the regulation and biosynthesis of aflatoxins. Also, recent proteomic studies involving the generation of > 20 000 expressed sequence tags from developing groundnut plants under drought stress have yielded several proteins potentially associated with resistance to aflatoxin production (Wang *et al.*, 2010).

Stage of development. Pilot. Much research has been conducted on resistance, but potentially resistant varieties lack other characteristics necessary for commercial application. For groundnuts, characteristics that confer drought tolerance may reduce pre-harvest aflatoxin accumulation. In addition, breeding for characteristics to reduce environmental stressors on maize has shown some efficacy in reducing aflatoxin.

Efficacy. Suggestive evidence of efficacy.

Geographical regions. Tested in the USA for maize. Several centres of the Consultative Group on International Agricultural Research (CGIAR) have conducted research.

Simplicity/complexity. High degree of complexity. Other strategies, such as giving germ plasm of improved open-pollinating lines of maize to indigenous farmers, have been proposed and possibly deserve reconsideration.

Population/individual. Population.

Useful in emergencies. No.

Locality of resources. Local crop lines should preferably be identified for suitability for each geographical region.

Accessibility. Likely to be accessible if appropriate hybrids are devel-

oped for different regions of the world and prices are established that are affordable for small-scale farmers.

References. Gorman and Kang (1991), Brown *et al*. (2001), Cleveland *et al*. (2003), Maupin *et al*. (2003), Menkir *et al*. (2006), Chen *et al*. (2007), Yu *et al*. (2008), Arunyanark *et al*. (2010), Girdthai *et al*. (2010), Wang *et al*. (2010), Warburton *et al*. (2011).

### 2.1.2 Biocontrol

Description. Biocontrol of aflatoxins relies on competitive exclusion. High numbers of spores of a non-toxigenic strain of *A. flavus* (or, less commonly, *A. parasiticus*) are introduced into the soil where crops are being grown, where they compete with existing toxin-producing spores for sites on the developing crop, thus reducing aflatoxin production. Usually the selected strain is introduced to the field on a carrier substrate that permits growth of the fungus with consequent production of high numbers of spores.

Stage of development. Commercial in the USA, and pilot.

Efficacy. Depending on climatic factors that affect biocontrol agent growth, and concentration of toxigenic spores in a given field, aflatoxin reductions may range widely, from no reduction at all to high levels (0–80%).

Geographical regions. Used commercially in cottonseed and groundnut fields in the USA. Tested in pilot studies on maize in the USA, Nigeria, Kenya, and Thailand. Tested in pilot studies on tree nuts in the USA.

Simplicity/complexity. Selecting and maintaining non-toxigenic strains is a specialist undertaking; producing substrates for field inoculation requires a feed mill or similar factory with suitable protection for workers because *A. flavus* is a known human pathogen. Application of the product onto fields can be carried out by trained farmers.

Population/individual. Individual in current commercial applications; possibility for population-level approach.

Useful in emergencies. No.

Locality of resources. Appropriate fungal strains must be sourced locally for each region where the process is used. Different substrates may be more economical in various regions; economic barriers for subsistence farmers.

Accessibility. Unlikely to be highly accessible, unless local soil samples are tested and facilities are built and maintained to develop biocontrol strains appropriate for the geographical region.

References. Dorner *et al*. (1999), Bandyopadhyay *et al*. (2005), Pitt and Hocking (2006), Cotty *et al*. (2007), Dorner and Horn (2007), Atehnkeng *et al*. (2008).

### 2.1.3 Forecasting

Description. AfloMan is a forecasting system for the formation of aflatoxin in groundnuts, in use in Queensland, Australia.

Stage of development. Commercial.

Efficacy. The model accounts for up to 95% of the variation in aflatoxin accumulation in groundnut crops at harvest. As with all models of this type, it is highly dependent on the reliability of weather and crop development data. Similar systems have been explored in the USA, but not beyond the conceptual stage.

Geographical regions. South Burnett region of Queensland, Australia.

Simplicity/complexity. Requires access to reliable climatic data and sophisticated mathematical modelling, but AfloMan is used daily by South Burnett groundnut growers via the Internet.

Population/individual. Population.

Useful in emergencies. No.

Locality of resources. Not applicable.

Accessibility. High in geographical regions where predictive models have been developed and access to computers is readily available to growers. Low in other regions.

References. Henderson *et al*. (2000), Chauhan *et al*. (2010), DEEDI (2010).

## 2.2 Post-harvest interventions

### 2.2.1 Sorting, drying, and storage

Description. Post-harvest control methods are based on GMP. Some specific methods that apply to aflatoxins follow: community-based approaches and industrial sorting methods.

At the level of communities, basic visual hand sorting can remove a large proportion of nuts or kernels that are significantly contaminated with aflatoxins. Additional community-based methods to keep aflatoxin levels low in post-harvest settings include proper drying, storage in bags that allow for air circulation, and use of well-ventilated storage facilities that control for pests.

Industrial sorting methods vary with the crop.

Maize. Primary sorting of maize kernels is by examination using an ultraviolet (UV) light (365 nm) after cracking; grains containing appreciable aflatoxin fluoresce, enabling sorting of lots. Fluorescence is due to plant peroxidase enzyme reacting with kojic acid produced by *A. flavus*. Some *A. flavus* strains do not produce kojic acid and therefore do not cause fluorescence. Further, tropical temperatures induce isomerization of kojic acid, preventing the fluorescence reaction, so sorting under UV light is ineffective in the tropics.

Groundnuts. When fungi invade groundnuts, enzymatic changes cause nut discolouration; thus, sorting out discoloured nuts also removes those that contain aflatoxin. Colour sorting should be followed

by aflatoxin assays. UV sorting of groundnuts is possible using a somewhat higher wavelength. In severe circumstances, groundnuts are blanched and roasted, which increases discolouration, enabling more effective colour sorting, which again should be followed by aflatoxin assays. Blanched and roasted nuts are susceptible to oxidative rancidity and thus should be packed under nitrogen in gas-tight packaging.

Pistachios. Pistachios have been very difficult to sort by UV or discolouration and have traditionally not been sorted. However, recent studies have shown that sorting by fluorescence and discolouration may be potentially useful. Aflatoxin in pistachios results from shells of nuts opening before the nut is dry, permitting ingress of *A. flavus* spores. Because shell opening is a desirable characteristic, control has involved the development of cultivars with later shell opening.

Almonds. Aflatoxin in almonds is usually caused by insect damage, so control relates to insect control. UV light can be used for sorting almonds containing aflatoxins.

Brazil nuts. Brazil nuts are infected by *A. flavus* when allowed to remain for extended periods on the forest floor before harvest (by picking them up). Infection by *A. nomius*, which also produces aflatoxins, apparently occurs before harvest. No control measures, other than aflatoxin assays, exist for Brazil nuts at this time. Some studies have suggested that sorting by size or physical appearance may be useful, but more research is needed.

Figs. Aflatoxin in figs results from *A. flavus* infection carried by insects during pollination. Control is by examination of individual dried or fresh fruit under UV light to detect the presence of aflatoxin.

Stage of development. Nut-producing regions of developed countries use the control measures described above.

Efficacy. A pilot test in Guinea of a post-harvest intervention package for groundnuts (including education on how to sort and dry nuts, natural fibre drying mats and storage bags, wooden pallets on which to store groundnut bags, and pesticide for storage floors) achieved a 70% reduction in aflatoxin levels in groundnuts after 5 months of storage compared with untreated groundnuts, and a 57% mean reduction in aflatoxin–albumin adduct levels in individuals who implemented the post-harvest intervention package compared with controls.

Geographical regions. Control measures outlined above are of universal applicability.

Simplicity/complexity. Control measures vary in complexity, but many can be applied in all regions.

Population/individual. Individual; possibility for population-level approach.

Useful in emergencies. No.

Locality of resources. Most elements of post-harvest control methods can be obtained or manufactured locally.

Accessibility. Community-based approaches are generally accessible among subsistence farmers, but education is necessary. Industrial sorting approaches are accessible in developed countries on the scale of large commercial farming.

References. Pearson and Slaughter (1996), Hadavi (2005), Turner *et al.* (2005), Kabak *et al.* (2006), De Mello and Scussel (2007), Magan and Aldred (2007), Wagacha and Muthomi (2008), Pacheco and Scussel (2009), Khlangwiset and Wu (2010), Pacheco *et al.* (2010).

### 2.2.2 Nixtamalization

Description. Alkaline cooking of maize in a solution of ash, lime, or other materials containing inorganic calcium. This process is useful for reducing concentrations of both aflatoxins and fumonisins (which are described in Section 3).

Stage of development. This process has been used for centuries to produce masa (a dough made from ground maize), by indigenous populations in regions throughout Latin America, especially Mexico and Central America, where maize is produced.

Efficacy. Under laboratory and commercial conditions, nixtamalization by the traditional process can reduce aflatoxin levels by up to 90%.

Geographical regions. Mexico, Central America.

Simplicity/complexity. Simple process; adequate clean water is required.

Population/individual. Individual; possibility for population-level approach.

Useful in emergencies. Yes.

Locality of resources. The activated lime used in nixtamalization is widely available. However, this process appears to be culturally acceptable only in the Americas. Water can sometimes be difficult to obtain, which can cause problems if washing is inadequate after alkaline steeping. In addition, use of polluted water for steeping can present a different set of risks.

Accessibility. Likely to be accessible in geographical regions where this practice is accepted and where clean water is readily accessible.

References. Torres *et al.* (2001), Elias-Orozco *et al.* (2002), De La Campa *et al.* (2004), Méndez-Albores *et al.* (2004), Bullerman and Bianchini (2007).

## 2.3 Dietary interventions

### 2.3.1 NovaSil clay

Description. NovaSil is a dioctahedral smectite clay that can bind aflatoxin in the gastrointestinal tract and aid in its elimination. NovaSil can be included in food or feed or taken separately during mealtimes.

Stage of development. Commercial in animal feed; pilot in humans.

Efficacy. In a pilot study in humans in Ghana, after 3 months of treatment, NovaSil clay added to diets achieved a 59% reduction in aflatoxin $M_1$ levels, and a 25% reduction in aflatoxin–albumin adduct levels, in treated individuals compared with controls.

Geographical regions. USA (as an anticaking agent in animal feed), Ghana (humans).

Simplicity/complexity. Currently, NovaSil clay comes only from one mine in the USA, so although the material is cheap, importation costs must be considered. Inclusion in the diet, in bread or in maize or groundnut meal, should be reasonably simple. Studies indicate some limitations, including the risk of vitamin and mineral binding in a nutritionally compromised population. Further, distribution under government supervision will be essential because imitation clay materials, similar in appearance to NovaSil but without the potential benefit, are readily available.

Population/individual. Individual; possibility for population-level approach.

Useful in emergencies. Promising; needs more research.

Locality of resources. So far, NovaSil clay has been mined only in the USA; would have to be exported to countries in need.

Accessibility. Will depend on cultural acceptance and cost.

References. Pimpukdee *et al.* (2004), Afriyie-Gyawu *et al.* (2008), Phillips *et al.* (2008), Wang *et al.* (2008).

### *2.3.2 Chlorophyll and chlorophyllin*

Description. Chlorophyll and its derivative chlorophyllin, which are natural constituents of green vegetables, can sequester aflatoxin in the gastrointestinal tract and impede its absorption. In addition, these compounds may have enzyme-inducing properties that contribute to mechanisms of detoxification.

Stage of development. Pilot; clinical trials.

Efficacy. A clinical trial in humans in Qidong, China, achieved a 55% reduction in aflatoxin-N7-guanine levels in treated individuals compared with controls.

Geographical regions. Clinical trials have been carried out in the USA and China.

Simplicity/complexity. The intervention was administered as a chemopreventive pill, which requires regular and continued administration.

Population/individual. Individual.

Useful in emergencies. Promising; needs more research for effects in situations of very high exposure to aflatoxin in vivo.

Locality of resources. Depends on availability of the chemopreventive pills.

Accessibility. Will depend on the availability of the medication and cost.

References. Dashwood *et al.* (1998), Egner *et al.* (2001), Simonich *et al.* (2007, 2008), Groopman *et al.* (2008), Jubert *et al.* (2009).

### *2.3.3 Naturally occurring dietary constituents*

Description. Green tea polyphenols, sulforaphane derived from cruciferous vegetables, and lactic acid bacteria.

Stage of development. Pilot.

Efficacy. In rat studies, green tea polyphenols have been shown to inhibit initiation of liver cancer induced by aflatoxin. In humans, inverse associations between green tea consumption and overall cancer risk have been observed. Sulforaphane, metabolized from glucoraphanin in cruciferous vegetables such as broccoli and cabbage, induces phase 2 enzymes such as the glutathione-*S*-transferases that prevent DNA damage induced by aflatoxin. In human studies, those individuals who converted more glucoraphanin to sulforaphane had lower aflatoxin-N7-guanine levels. Lactic acid bacteria from fermented vegetables, fruits, and dairy products have the ability to bind aflatoxin $B_1$ in laboratory tests; this has not yet been tested in animals or humans.

Geographical regions. China (green tea polyphenols, sulforaphane).

Simplicity/complexity. These interventions would be simple in the parts of the world where the foods or drinks containing these dietary constituents are already common in diets. They could be complex where this is not the case already. Optimized consumption patterns to modify aflatoxin metabolism over extended periods would need to be developed.

Population/individual. Individual; possibility for population-level approach.

Useful in emergencies. No.

Locality of resources. Common.

Accessibility. Readily accessible in developed countries where these dietary constituents are affordable; less accessible in LICs, where diets are much less varied.

References. Haskard *et al.* (2000), Fujiki *et al.* (2002), Kensler *et al.* (2005), Yates and Kensler (2007), Hernandez-Mendoza *et al.* (2009), Gao *et al.* (2010), Gross-Steinmeyer *et al.* (2010).

## 3. Fumonisins

### *3.1 Pre-harvest interventions*

### *3.1.1 Breeding for host plant resistance*

Description. Breeding and selection methods have been used for centuries to improve maize resistance to fungal and insect infection or other environmental stressors, stressors that have been discovered recently to predispose plants to fumonisin contamination. This has included work on developing resistant inbred crop lines and identifying biochemical and genetic resistance markers in crops.

Stage of development. It is reliably known that improved insect and

drought tolerance results in reduced risk for fumonisin accumulation. This approach is being applied to the extent that hybrids suitable for particular areas have become available.

Efficacy. In years of high insect pressure and drought, such resistant hybrids can increase the percentage of the crop suitable for human consumption.

Geographical regions. USA, Europe.

Simplicity/complexity. Although the commercial breeding process involves significant expertise and expense at first, seeds resistant to fungal development and fumonisin formation can be disseminated as readily as other types of seeds, within the area of adaptation.

Population/individual. Population.

Useful in emergencies. No.

Locality of resources. Local inbred maize lines can be identified for suitability to the geographical region; questions of access and affordability for LICs.

Accessibility. Likely to be widely accessible after local varieties with improved traits are developed.

References. Miller (2001), Clements *et al.* (2004), Afolabi *et al.* (2007), Henry *et al.* (2009), Loeffler *et al.* (2010), Parsons and Munkvold (2010).

### *3.1.2 Transgenic Bt maize*

Description. Transgenic Bt maize contains a gene from the soil bacterium *Bacillus thuringiensis* that results in the accumulation of proteins toxic to key insect pests of maize. Insect damage predisposes maize to fumonisin contamination by facilitating fungal infection.

Stage of development. Commercial.

Efficacy. Depending on the severity of insect infestation in a given year, fumonisin reductions afforded by Bt maize can greatly increase the percentage of the crop acceptable for human consumption.

Geographical regions. USA, Canada, Argentina, Brazil, Uruguay, South Africa, Honduras, Philippines, Hungary.

Simplicity/complexity. Although the commercial breeding process involves significant expertise and expense at first, Bt seeds can be disseminated as readily as other types of seeds, within the area of adaptation.

Population/individual. Individual; possibility for population-level approach in countries with commercial agriculture.

Useful in emergencies. No.

Locality of resources. Highly variable; requires reliance on biotechnology companies to permit small-scale farmers access to seed at prices under fair conditions.

Accessibility. High in developed countries that have permitted Bt maize planting and commercialization; low elsewhere worldwide.

References. Munkvold *et al.* (1999), Bakan *et al.* (2002), Hammond *et al.* (2004), de la Campa *et al.* (2005), Papst *et al.* (2005), Wu (2007), Folcher *et al.* (2010).

## *3.2 Post-harvest interventions*

### *3.2.1 Sorting and washing*

Description. Hand sorting of obviously contaminated kernels of home-grown maize, and washing before consumption.

Stage of development. Traditional hand and eye sorting methods are well developed; optical sorting using two wavelengths is possible but requires expensive equipment.

Efficacy. Sorting and washing maize kernels can reduce fumonisin contamination by > 84% in maize grains and by > 60% in maize porridge.

Geographical regions. Traditional sorting methods are used in many maize-producing regions.

Simplicity/complexity. Sorting and washing techniques are simple to implement; education can improve confidence in the results.

Population/individual. Individual; possibility for population-level approach.

Useful in emergencies. Yes.

Locality of resources. These post-harvest control methods can be carried out locally.

Accessibility. Generally accessible techniques worldwide.

References. Desjardins *et al.* (2000), Pearson *et al.* (2004; 2010), Fandohan *et al.* (2005), Afolabi *et al.* (2006), Kimanya *et al.* (2009a, 2009b), Van der Westhuizen *et al.* (2010, 2011).

### *3.2.2 Nixtamalization*

Description. Alkaline cooking of maize in a solution of ash, lime, or other materials containing inorganic calcium.

Stage of development. This process has been used for centuries to produce masa, by indigenous populations in regions throughout Latin America, especially Mexico and Central America, where maize is produced.

Efficacy. Nixtamalization can reduce fumonisin $B_1$ levels in fried tortilla chips by up to 80%.

Geographical regions. All maize-growing areas in Latin America.

Simplicity/complexity. Simple process; adequate clean water is required.

Population/individual. Individual; possibility for population-level approach.

Useful in emergencies. Yes.

Locality of resources. The activated lime used in nixtamalization is very widely available. However, this process appears to be culturally acceptable only in the Americas. Water can sometimes be difficult to obtain, which can cause problems if washing is inadequate after alkaline steeping. In addition, use of polluted water for steeping can present a different set of risks.

Accessibility. Likely to be accessible in geographical regions where this practice is accepted and where clean water is readily accessible.

References. Dombrink-Kurtzman *et al*. (2000), Voss *et al*. (2001, 2009), Palencia *et al*. (2003), De La Campa *et al*. (2004), Bullerman and Bianchini (2007), Torres *et al*. (2007), Burns *et al*. (2008).

## 4. Ochratoxin A

### *4.1 Pre-harvest interventions*

Little evidence exists that any of the important fungi producing ochratoxin A (OTA) invade crops before harvest, so plant breeding is of little value in the control of OTA formation. The one exception is that the invasion of grapes by *Aspergillus carbonarius* (and *A. niger*) takes place before harvest. The evidence is that these fungi cannot infect intact grapes, so entry is dependent on infection by fungal pathogens such as powdery mildews or *Rhizopus stolonifer*, mechanical damage, or skin splitting due to rain. Some cultivars are more susceptible to skin splitting, so plant breeding is of value in that area.

Pre-harvest control of OTA production in grapes, and hence dried vine fruits and wine, is based on limiting damage by the powdery mildews (by fungicidal spray programmes) and by *Rhizopus* (by a defoliant spray to increase exposure of grapes to sunlight, which limits growth of *Rhizopus*).

### *4.2 Post-harvest interventions*

Fungi producing OTA invade crops after harvest. Types of control vary with the crop.

Small grains. OTA is produced after harvest, during drying in small grain cereal crops (wheat and barley) in cool temperate zones, by *Penicillium verrucosum*. The problem is widespread, and the only effective control measure consists of rapid drying, where this is possible. Because *P. verrucosum* does not grow in warmer climates, OTA production in small grains is not a problem in warm temperate or tropical crops. OTA occurs in maize but appears to be a minor problem compared with aflatoxins, fumonisins, or deoxynivalenol.

Dried vine fruits and wines. As noted above, infection in grapes by *A. carbonarius* and *A. niger* may occur before harvest. In winemaking, fungal growth and toxin production cease when fermentation commences. Wine-making reduces the level of OTA in wine by up to 80%. In grape drying, mechanical damage during harvest and drying pretreatments increases the possibility of infection of grapes by *A. carbonarius* and *A. niger*, so rapid drying will reduce OTA formation.

Coffee. In coffee, OTA infection occurs immediately after harvest, when green coffee cherries are handled, hulled, and dried. The process is often slow because in many regions coffee is grown under climatic conditions that favour mist and rain at harvest time. Poor storage is also a documented factor causing increases in OTA levels. Partial control can be achieved during the early stages of manufacture, where defective cherries, which frequently contain most OTA in a sample, are sorted out. Roasting of coffee reduces OTA levels by amounts that vary with the severity of the roasting process, e.g. from a 50% reduction for a light roast (12 minutes at 180 °C) to a > 90% reduction for a dark roast (8 minutes at 240 °C).

Cocoa and chocolate. OTA occurs in cocoa, and hence in chocolate, but levels are usually low. Rapid and adequate drying of cocoa beans is the important control step.

References. Taniwaki *et al*. (2003), Leong *et al*. (2006), Copetti *et al*. (2010), Ferraz *et al*. (2010).

## 5. Deoxynivalenol and zearalenone

### *5.1 Pre-harvest interventions*

#### *5.1.1 Breeding for host plant resistance in small grains*

Description. Breeding methods have been explored to improve host plant resistance either to fungal infection or to other environmental stressors that would predispose the plants to accumulation of deoxynivalenol (DON) and, secondarily, zearalenone (ZEA). This has included work on identifying resistant cultivars and identifying biochemical and genetic resistance markers in cultivars. Some regions (notably Germany and Ontario, Canada) have stringent registration rules that place specific weight on eliminating the worst cultivars in trials each year.

Stage of development. Commercial.

Efficacy. Efficacy varies, depending on climatic conditions by year and on agronomic factors, including the topology of the field. Current efforts are focusing on developing high-yielding cultivars with good quality characteristics and moderate resistance to Fusarium head blight and DON production.

Geographical regions. Cultivars with reasonable efficacy are in use in the USA, Canada, and Europe.

Simplicity/complexity. Although the breeding process involves significant expertise and expense at first, resistant seeds can be disseminated as readily as other types of seeds, within the area of adaptation. Because small grains are open-pollinated, seeds can be saved from year to year.

Population/individual. Population.

Useful in emergencies. No.

Locality of resources. Local lines can be identified for suitability to the geographical region through the International Maize and Wheat

Improvement Center (CIMMYT) shuttle breeding program.

Accessibility. Likely to be widely accessible after local varieties with improved traits are developed.

References. Mesterhazy *et al*. (1999), Boutigny *et al*. (2008), Foroud and Eudes (2009), Snijders (2004), Müller *et al*. (2010).

### 5.1.2 Breeding for host plant resistance in maize

Description. Breeding methods have been explored to improve host plant resistance either to fungal infection or to other environmental stressors that would predispose the plants to accumulation of DON and, secondarily, ZEA. This has included work on identifying resistant cultivars and identifying biochemical and genetic resistance markers in hybrids. Some regions (e.g. Ontario, Canada) have stringent registration rules that place specific weight on eliminating the worst hybrids in trials each year.

Stage of development. Commercial.

Efficacy. Moderate resistance has been achieved.

Geographical regions. Cultivars with moderate resistance are in use in the USA, Canada, and Europe.

Simplicity/complexity. Although the breeding process involves significant expertise and expense at first, resistant seeds can be disseminated as readily as other types of seeds, within the area of adaptation.

Population/individual. Population.

Useful in emergencies. No.

Locality of resources. Local inbred varieties can be identified for suitability to the geographical region through the CIMMYT shuttle breeding program.

Accessibility. Likely to be widely accessible after local varieties with improved traits are developed.

References. Boutigny *et al*. (2008), Loeffler *et al*. (2010) and references therein.

### 5.1.3 Transgenic Bt maize

Description. Transgenic Bt maize contains a gene from the soil bacterium *Bacillus thuringiensis* that encodes for proteins toxic to key insect pests of maize. Insect damage predisposes maize to DON and ZEA contamination by facilitating fungal infection.

Stage of development. Commercial.

Efficacy. Reductions of DON levels by up to 59% and significantly lower ZEA levels in animal feed made from Bt maize compared with maize lacking the Bt gene.

Geographical regions. USA, Canada, Argentina, Brazil, Uruguay, South Africa, Honduras, the Philippines, Hungary, China.

Simplicity/complexity. Although the transgenic breeding process involves substantial initial expertise and expense, Bt seeds, once developed, can be disseminated as easily as other types of seeds.

Population/individual. Individual; possibility for population-level approach.

Useful in emergencies. No.

Locality of resources. Not local; requires reliance on biotechnology companies to permit small-scale farmers access to seed at prices under fair conditions.

Accessibility. High in developed countries that have permitted Bt maize planting and commercialization; low elsewhere worldwide.

References. Munkvold *et al*. (1999), Aulrich *et al*. (2001), Magg *et al*. (2002), Schaafsma (2002), Wu (2007), Burachik (2010), Folcher *et al*. (2010), Loeffler *et al*. (2010).

### 5.1.4 Fungicides

Description. Chemicals that control fungal species; azoles, commonly used to control Fusarium head blight, inhibit sterol biosynthesis in *Fusarium* species. However, an important issue for high efficacy of fungicides is the correct timing of application during anthesis, the optimal time for *F. graminearum* infection and DON accumulation.

Stage of development. Commercial.

Efficacy. Varies depending on fungal infection risk by year.

Geographical regions. Worldwide.

Simplicity/complexity. Simple to implement, although basic education is required.

Population/individual. Individual; possibility for population-level approach.

Useful in emergencies. No.

Locality of resources. Widely available in developed countries, much less so in LICs; cost implications for subsistence farmers.

Accessibility. Accessible in developed countries, much less so in LICs.

References. Hollingsworth *et al*. (2008), Odenbach *et al*. (2008), Paul *et al*. (2008), Zhang *et al*. (2009).

### 5.1.5 Forecasting

Description. The most evolved models for forecasting the formation of DON in small grains were developed in Ontario, Canada. Only preliminary work has been reported on a model for DON in maize.

Stage of development. Commercial in Ontario, Canada, and in Europe.

Efficacy. Analyses of data from maize produced a model with initial validation, i.e. data for enough years of variation in two countries to explain 70% of variation in fumonisin accumulation. A different model, developed in Italy, has had only limited validation thus far.

Geographical regions. These models have been applied in Uruguay, in France, and (since 2009) throughout Europe.

Simplicity/complexity. These models are used to enable management decisions, e.g. harvest time, whether foliar fungicides would be useful in a given year, and crop segregation. These models must be tested and validated in many areas, not only to

make the models more widely useful but also to test their robustness to climatic variation. As with all predictive models, these require reliable climatic and agronomic data and some sophisticated mathematics.

Population/individual. Population.

Useful in emergencies. No.

Locality of resources. To date, Canada and Europe.

Accessibility. High in regions of the world for which forecasting models have been developed (Canada, Europe); low elsewhere worldwide.

References. de la Campa *et al*. (2005), Hooker and Schaafsma (2005), Schaafsma and Hooker (2007), Maiorano *et al*. (2009), Müller *et al*. (2010), Van der Fels-Klerx *et al*. (2010).

### *5.2 Post-harvest interventions*

Because *Fusarium* species do not grow at water activities below about 0.90, DON and ZEA are not produced in grains that have been even partially dried. Post-harvest treatments are not applicable to these mycotoxins in wheat or maize.

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