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MYCOTOXINS AND THEIR CONTROL: CONSTRAINTS AND OPPORTUNITIES

NATURAL RESOURCES INSTITUTE
The University of Greenwich
ODA

Mycotoxins and their control: constraints and opportunities

R. D. Coker

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Natural Resources Institute
The University of Greenwich

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Summaries

SUMMARY

Mycotoxins are '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). Mycotoxins occur in a wide variety of foods and feeds and have been implicated in a range of human and animal diseases. Exposure to mycotoxins can produce both acute and chronic toxicities ranging from death to deleterious effects on the central nervous, cardiovascular and pulmonary systems, and on the alimentary tract. They may be carcinogenic, mutagenic, teratogenic and immunosuppressive. The ability of some mycotoxins to compromise the immune response and consequently, to reduce resistance to infectious disease, is now widely considered to be their most important effect. Furthermore, it is the opinion of some that the elimination of mycotoxins would be the single most effective and beneficial change which could be made in human diets around the world.

The mycotoxins attract world-wide attention because of the significant economic losses associated with their impact on human health, animal productivity and both domestic and international trade. It has been estimated that annual losses in the US and Canada arising from the impact of mycotoxins on the feed and livestock industries, for example, are in the order of US$ 5 billion. In developing countries where food staples (e.g. maize and groundnuts) are susceptible to contamination, there are likely to be significant additional losses amongst the human population because of morbidity and premature death associated with the consumption of mycotoxin-contaminated food.

The contraints and opportunities associated with the control of moulds and mycotoxins are addressed by adopting a 'systems' approach. The Mycotoxicology System is described in terms of interacting commodity, spoilage, mycotoxin, socio-economic and control subsystems; the main components of the control subsystem are considered under the titles (a) idendification of quality constraints (b) prevention (c) identification and segregation of contaminated material and (d) detoxification. The metabolic fate of selected mycotoxins is discussed in detail as a background to the toxicity and biomonitoring of mycotoxins; the chemical detoxification of aflatoxin is also discussed in depth.

The concept of Process Quality Management (PQM) which utilizes a Process-Commodity Matrix and a PQM Cycle to identify contraints and opportunities for interventions, and test the efficacy of the interventions, is introduced.

Future research needs are also discussed.

RESUME

Les mycotoxines sont des 'métaboliques fongiques qui, lorsqu'ils sont ingrérés, inhalés ou absorbés par la peau, entraînent une performance réduite, la maladie ou le décès chez les humans ou les animaux, les oiseaux y compris' (Pitt, 1996). Les mycotoxines se trouvent dans une grande variété d'aliments pour humains et pour animaux et ont été impliquées dans une gamme de maladies humaines et animales. L'exposition aux mycotoxines peut à la fois produire des toxicités aigües et chroniques, allant du décès à des effets délétères chroniques sur le système nerveux central, les systèms cardiovasculaire et pulmonaire et sur l'appareil digestif. Elles peuvent être carcinogènes, mutagènes, tératogènes et immunodépressives. La capacité de certaines mycotoxines à compromettre la réponse immune et, par conséquent, à réduire la résistance aux maladies infectieuses, est actuellement considerée comme leur effet le plus important. En outre, l'élimination des mycotoxines pourrait, selon certains avis, être la modification la plus efficace et bénéfique à elle seule qui pourrait être apportée aux régimes alimentaires humains de par le monde.

Les mycotoxines attirent l'attention dans le monde entier à cause des pertes économiques significatives qui sont associées a leur effet sur la santé des humains, la productivité des animaux et le commerce intérieur et international. Il est estimé que les pertes annuelles aux Etats-Unis et au Canada dues à l'en des mycotoxines sur les industries des aliments pour ani-

maux et de l'élevage, par exemple, sont de l'ordre de 5 milliards de dollars E-U. Dans les pays en développement, ou les aliments de base (ex: maïs et arachide) sont sensibles à la contamination, il est probable que les petres supplémentaires parmi la pupulation humaine soient significatives à cause de la morbidité et de la mort prématurée associées à la consommation d'aliments contaminés par les mycotoxines.

Les contraintes et les possibilités associees au contrôle des moisissures et des mycotoxines sont abordées au moyen d'une approche de 'système'. Le Système de mycotoxicologie est décrit en termes de l'interaction des sous-systèms de la denrée, de la détérioration, des mycotoxines, de contrôle de celles-ci et des facteurs économiques. Les éléments principaux de la sous-système du contrôle sont examinés sous les rubriques (a) identification des contraintes à la qualite (b) mesures de prévention (c) identification et ségrégation du matériel contaminé, et (d) désintoxication. Le sort métabolique de mycotoxines sélectionnées est abordé de façon détaillee en tant que contexte pour la toxicité et la surveillance biologique des mycotoxines; la désintoxication chimique de l'aflatoxine est également discutée de façon approfondie.

Le concept de Gestion de la qualité du processus (PQM), qui utilise une matrice de la denrée du processus et un cycle de PQM afin d'identifier les contraintes et les possibilités d'interventions et de tester l'efficacité de ces interventions, est présenté.

Les besoins futurs en matière de recherche sont aussi examinés.

RESUMEN

Las micotoxinas son 'metabolitos fungales que, al ser ingeridos, inhalados o absorbidos a través de la piel, producen una reducción en la capacidad funcional, síntomas de enfermedad y aun la muerte de seres humanos y animales, incluyendo las aves' (Pitt, 1996). Las micotoxinas se encuentran en una amplia variedad de productos alimenticios y piensos y han sido relacionadas con diversos tipos de enfermedad, tanto en seres humanos como en animales, que pueden llevar aun a la muerte del individuo afectado. La exposición a mictoxinas puede reultar en toxicidad aguda y crónica, con repercusiones nocivas tales como problemas crónicos del sistema nervioso central y de los sistemas cardiovascular y pulmonar, así como sobre el tubo digestivo. Su efecto puede ser carcinogénico, mutagénico e inmunosupresivo. En la actualidad, se considera como su efecto más importante la capacidad de algunas mictoxinas para afectar la reacción de inmunidad del organismo y, consiguientemente, para reducir su resistencia a enfermedades infecciosas. Además, en opinión de algunos científícos, la eliminación de las micotoxinas sería la transformación individual más eficaz y beneficiosa que podría ocurrir en la dieta del hombre en el mundo entero.

Las micotoxinas han llamado la atención por todo el mundo como resultado de las importantes pérdidas exonómicas asociadas con su impacto sobre la salud humana, productividad animal y comercioi nacional e internacional. Se ha calculado que, en los Estados Unidos y Canadá, las pérdidas anuales atribuibles a las micotoxinas en el sector ganadero y de los piensos asciende a unos USA$ 5000 milliones. En los países en desarrollo, en donde productos alimenticios básicos tales como el maíz y el cacahuete se hallan expuestos a contaminación, es probable que las micotoxinas reporten pérdidas adicionales de importancia entre la población humana, como resultado de la morbilidad y fallecimiento prematuro asociados con el consumo de productos alimenticios contaminados con micotoxinas.

Tanto las oportunidades como los impedimentos asociados con el control de los mohos y micotoxinas requieren la adopción de un planteamiento de 'sistemas'. El Sistema Micotoxicológico se describe en términos de la interacción de subsistemas tales como el producto, su deterioro, las micotoxinas y los aspectos socioeconómicos y de control; estudiándose los principles componentes de la subsistema control bajo los títulos siguientes: (a) identificación de las restricciones de calidad; (b) prevención; (c) identificación y segregación del material contaminado y (d) desintoxicación. A manera de información de fondo sobre la toxicidad y biosupervisión de las micotoxinas, se examina minuciosamente la suerte metabólica de micotoxinas seleccionadas. También se estudia con gran detalle la desintoxicación química de la aflatoxina.

El artículo presenta el concepto de la gestión de los procesos (PQM), en el que se utiliza una matriz de procesos-productos y un Ciclo PQM para identifcar las restricciones y oportunidades de intervención y para probar la eficacia de las intervenciones.

Finalmente, se examinan las exigencias de nuevos trabjos de investigación.

What is a mycotoxin?

Pitt (1996) recently defined mycotoxins as 'fungal metabolites which, when ingested, inhaled or absorbed through the skin, cause lowered performance, sickness or death in man or animals, including birds.'

It is likely that mycotoxins have plagued mankind since the beginning of organized crop production. For example, ergotism (St Anthony's Fire), which is caused by the consumption of rye contaminated with the 'ergot alkaloids', is discussed in the Old Testament, and reached epidemic proportions in many parts of Europe in the 10th century (Beardall and Miller, 1994). It has also been surmised that the severe depopulation of western Europe in the 13th century was caused by the replacement of rye with wheat, an important source of *Fusarium* mycotoxins (Miller, 1991). The development of the *Fusarium* toxins in over-wintered grain was responsible for the deaths of thousands of people, and the decimation of entire villages, in Siberia during World War Two. The mycotoxicosis latterly known as 'alimentary toxic aleukia' (ATA) produced vomiting, acute inflammation of the alimentary tract, anaemia, circulatory failure and convulsions.

Mycotoxins occur in a wide variety of foods and feeds and have been implicated in a range of human and animal diseases (Council for Agricultural Science and Technology, 1989). Exposure to mycotoxins can produce both acute and chronic toxicities ranging from death to deleterious effects on the central nervous, cardiovascular and pulmonary systems, and on the alimentary tract. Mycotoxins may be carcinogenic, mutagenic, teratogenic and immunosuppressive. The ability of some of them to compromise the immune response and consequently reduce resistance to infectious disease, is now widely considered to be their most important effect. Some are of the opinion that the single most effective and beneficial change which could be made in human diets around the world would be the elimination of mycotoxins (Miller, 1996).

The mycotoxins attract world-wide attention because of the significant economic losses associated with their impact on human health, animal productivity and both domestic and international trade. It has been estimated, for example, that annual losses in the US and Canada arising from the impact of mycotoxins on the feed and livestock industries are in the order of US$ 5 billion (Miller, personal communication). In developing countries where the food staples (e.g. maize and groundnuts) are susceptible to contamination, significant additional losses amongst the human population are likely because of morbidity and premature death associated with the consumption of mycotoxins (*see* p. 11).

Mycotoxicology – a Systems Approach

THE MYCOTOXICOLOGY SYSTEM

A 'systems' approach allows a given situation (the system) to be viewed as a set of interacting components (subsystems) in which the interactions are just as important as the components themselves (after Open University Business School, 1987). A proposed systems approach to mycotoxicology is outlined (*see* Figures 1–4 and Figures 9–11) as a number of proposed conceptual models of interactions between, and within, Commodity, Spoilage, Mycotoxin, Socio-Economic and Control Systems. Within a system, the subsystems can freely interact; in other words, activity within one subsystem can influence events in one or more other subsystems. In each figure, the system is contained within the rectangular box and the interacting subsystems are represented by the ellipses.

In Figure 1, the Mycotoxicology System is represented as interacting Commodity, Spoilage, Mycotoxin, Socio-Economic and Control Systems; each subsystem in Figure 1 is itself considered as a system in Figures 2–4 and Figure 9.

It can be strongly argued that a better comprehension of both the interactions and the components associated with these systems assists in understanding the aetiology of mycotoxin production, and in formulating appropriate interventions for the control of mycotoxins and mycotoxicoses (*see* The Control System, p. 21)

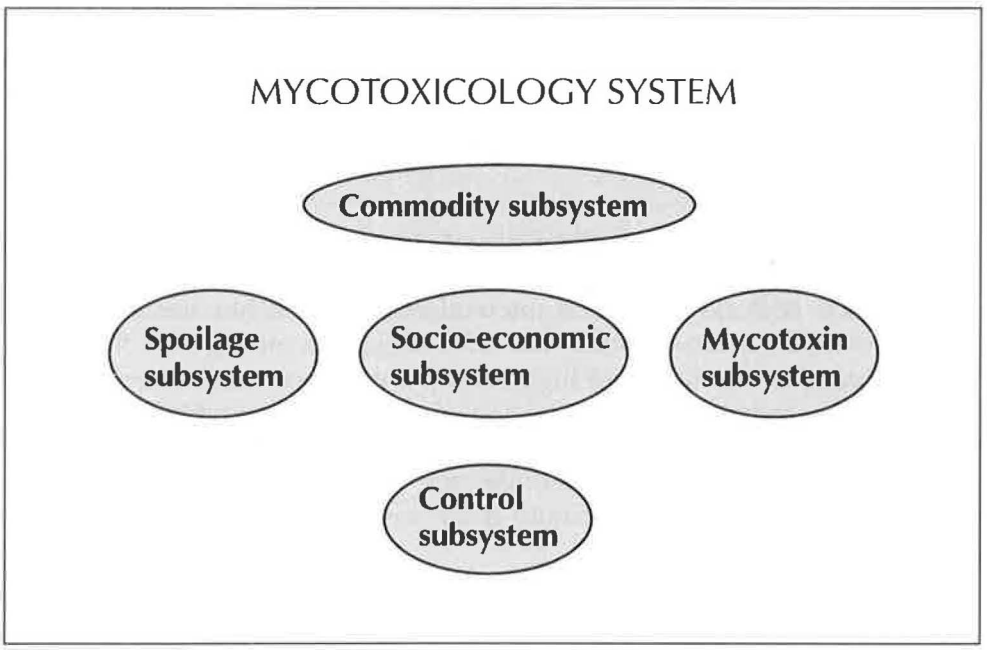


Figure 1 A Systems approach to Mycotoxicology: Conceptual Models

THE COMMODITY SYSTEM

Any Commodity System (involving the production, marketing and utilization of the commodity) is composed of numerous interacting technical and socio-economic 'processes' including, for example, pest and disease control, harvesting, drying, processing, marketing, credit and pricing policies, and cultural issues. A generalized, simplified Commodity System is shown in Figure 2; selected processes are represented as interacting subsystems.

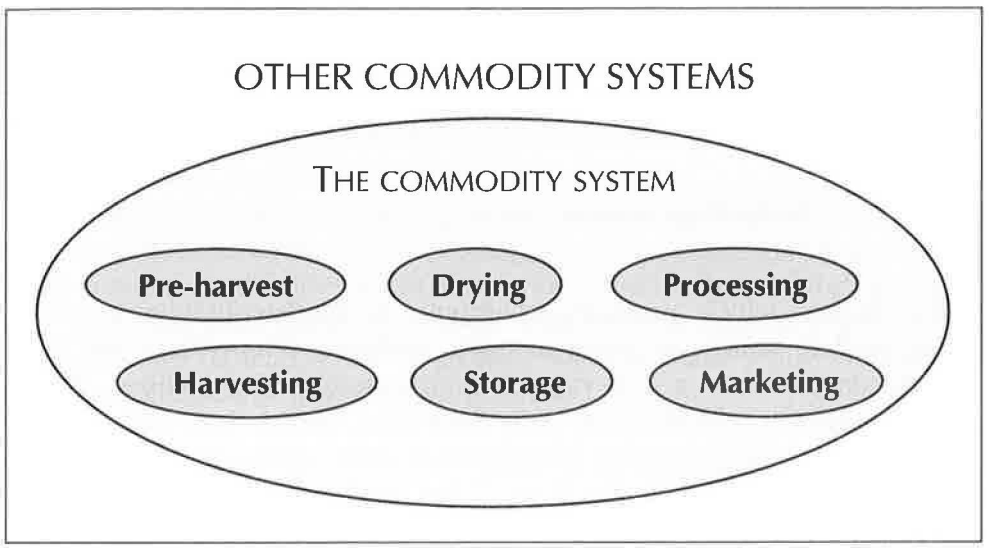


Figure 2 The Commodity System

At any point within the Commodity System, the condition of the commodity is determined by a complex milieu involving a multitude of interactions between the crop, the macro- and micro-environment and a variety of biological, chemical, physical and socio-economic factors. A change within any one process will invariably bring about changes in one or more of the other processes. For example, the current trend towards the dismantling of centralized, quasi-government grain stores and the concomitant increase in privately operated stores may, at least temporarily, reduce the overall standard of medium-scale storage. This situation may, in turn, both increase the need for the careful drying of grain before storage, and encourage processors to seek either recently harvested material and/or increase their own capacity for the storage of grain. Alternatively, processors may begin dealing directly with the farmers themselves, possibly influencing both pre- and immediate post-harvest practices.

Similarly, since it is very rare for a single commodity system to exist in isolation within a given agroclimatic region, it should be remembered that activities within one system can significantly effect events in other systems (*see* Figure 2).

THE SPOILAGE SYSTEM

Biodeterioration is the net result of numerous interacting spoilage agents which may be broadly described as biological, chemical, physical, macro-environmental and micro-environmental (*see* Figure 3). However, the relative impact of these agents will often be determined largely by the nature and extent of human intervention as described by the Socio-Economic System (*see* p. 21).

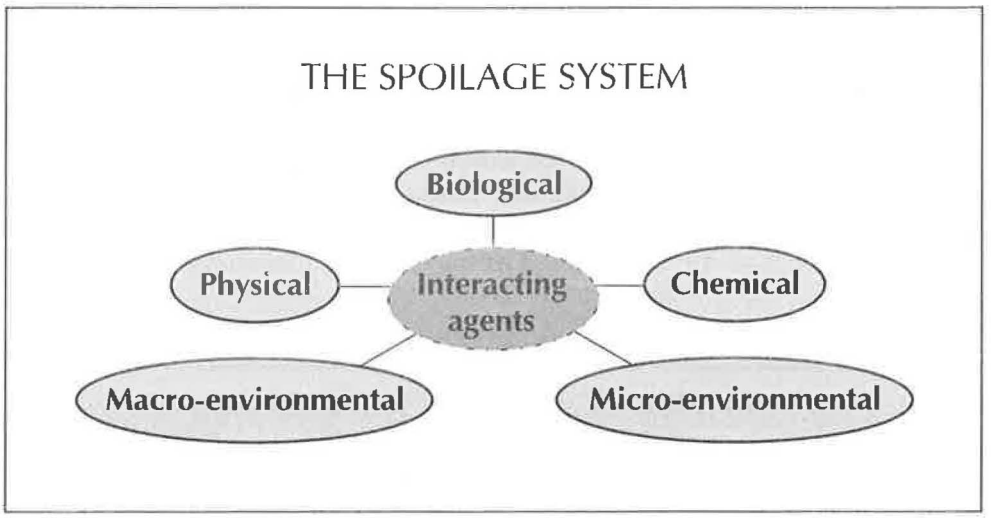


Figure 3 The Spoilage System

The factors which primarily contribute to biodeterioration (including mould growth) within an ecosystem are: moisture, temperature and pests. Moulds can grow over a wide range of temperatures and, in general, the rate of mould growth will decrease with decreasing temperature and available water. In grains, moulds utilize inter-granular water vapour, the concentration of which is determined by the state of the equilibrium between free water within the grain (the grain moisture content) and water in the vapour phase immediately surrounding the granular particle. The inter-granular water concentration is described either in terms of the equilibrium relative humidity (ERH, %) or water activity (a_w). The water activity describes the ratio of the vapour pressure of water in the grain to the vapour pressure of pure water at the same temperature and pressure; the ERH is equivalent to the water activity expressed as a percentage. For a given moisture content, different grains offer a variety of water activities and consequently support differing rates and type of mould growth. Typical water activities necessary for mould growth range from 0.70 to 0.99.

The interaction between grain temperature and moisture content also affects the extent of mould colonization. The passage of water from the grain into the vapour phase is encouraged by an increase in temperature. Consequently, for a given moisture content, the water activity and the propensity for mould growth will increase with temperature. Maize, for example, can be relatively safely stored for one year at a moisture level of 15% and a temperature of 15 °C; however, the same maize stored at 30 °C will be substantially damaged by moulds within three months.

Insects and mites (arthropods) can also contribute significantly to the biodeterioration of grain through the physical damage and nutrient losses caused by their activities, and their complex interaction with moulds and mycotoxins. In general, insects do not infest grain below 17 °C, but mite infestations can occur between 3 and 30 °C and above 12% moisture content. The metabolic activity of insects and mites increases both moisture content and temperature of the infested grain. Arthropods also act as carriers of mould spores, and their faecal material can be utilized as a food source by moulds. Moulds can provide food for insects and mites although in some cases, they may also act as pathogens.

Another important factor which can affect mould growth is the proportion of broken kernels in a consignment of grain. Broken kernels, caused by general handling and/or insect damage, predispose the exposed endosperm to mould invasion. It has been estimated that increasing the proportion of broken grains by 5% will reduce the storage-life of that consignment by approximately one order of magnitude, i.e., from perhaps 150 to 15 days (Steel *et al.*, 1969).

Mould growth is regulated by the proportions of oxygen, nitrogen and carbon dioxide in the inter-granular atmosphere. Many moulds will grow at very low oxygen concentrations; linear growth, for example, will be halved only if the oxygen content is reduced to less than 0.14%. Interactions between the gases and the prevailing water activity also influence mould growth.

The interactions described above, within granular ecosystems, will support the growth of a succession of micro-organisms, including toxigenic moulds, as the nutrient availability and micro-environment changes with time. In the field, grains are predominantly contaminated by those moulds requiring high water activities (at least 0.88) for growth, whereas stored grains will support moulds which grow at lower moisture levels.

Any activity which disturbs the stability of an ecosystem will increase the production of secondary metabolites, including mycotoxins. Such activities include the widespread use of fertilizers and pesticides, high-yielding plant varieties, and the cultivation of a limited number of plant species with restricted genetic variation. The normal practices of harvesting, drying, storage and processing also, of course, significantly disturb those ecosystems which have been established before harvest.

It is well recognized that water activity and temperature are the main factors influencing the production of mycotoxins. However, given the complexity of the ecosystems supporting the production of mycotoxins, the conditions under which toxigenic moulds produce mycotoxins are still poorly defined; they have recently been comprehensively reviewed (International Commission on Microbiological Specifications for Foods, 1996).

THE MYCOTOXIN SYSTEM

The Mycotoxin System (*see* Figure 4) may be considered in terms of four interacting subsystems: toxicology, metabolism, health and productivity, and wealth. Following exposure (by ingestion, inhalation or skin contact), the toxicity of a mycotoxin is determined by a sequence of events (metabolism) involving the administration, absorption, transformation, pharmacokinetics, molecular interactions, distribution, and excretion of the toxin and its metabolites. In turn, the toxicity of a mycotoxin will be manifested by its effect on the health and productivity of crops, humans and animals; these effects will influence the production of wealth associated with human endeavour and agricultural and livestock products.

The Mycotoxin System may be used as a conceptual model for a discussion of: (a) mycotoxins of world-wide importance; (b) mycotoxins of regional importance; and (c) mycotoxins of possible importance.

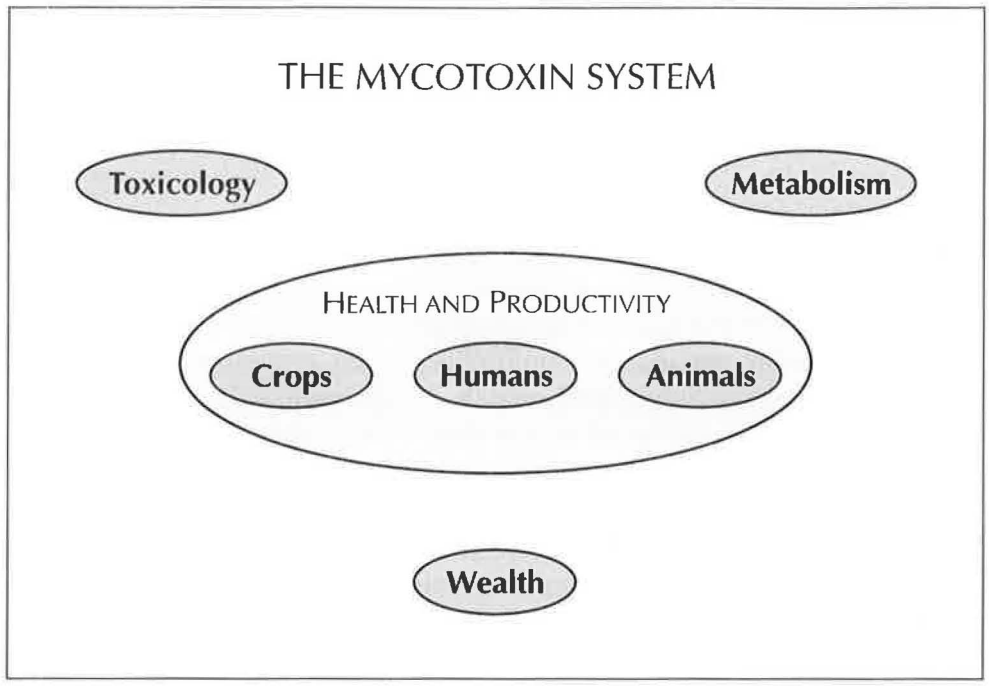


Figure 4 The Mycotoxin System

Mycotoxins of world-wide importance

Table 1 and Figure 5 show those moulds and mycotoxins currently considered to be of world-wide importance (Miller, 1994).

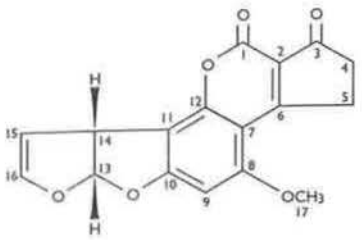
An 'important' mycotoxin will have demonstrated its capacity to have a significant economic impact on the exposed population. The frequency and level of occurrence are obviously major factors when considering the 'importance' of a mycotoxin. A mycotoxin which is highly toxic, but which occurs only in isolated regions, should be considered as 'important' if the mycotoxicosis clearly associated with the toxin has a significant economic impact within the affected region.

Table 1 Moulds and mycotoxins of world-wide importance

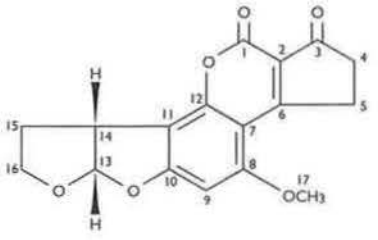
Mould species	Mycotoxins produced
Aspergillus parasiticus	Aflatoxins B_1, B_2, G_1, G_2
A. flavus	Aflatoxins B_1, B_2
Fusarium sporotrichioides	T-2 toxin
F. graminearum	deoxynivalenol (or nivalenol in some areas) zearalenone
F. moniliforme	fumonisin B_1
Penicillium verrucosum	ochratoxin A
A. ochraceus	

The aflatoxins

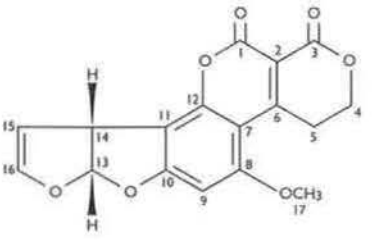
The optimal water activity for growth of *A. flavus* is high (about 0.99). The maximum is at least 0.998 but the minimum is still ill-defined; Pitt and Miscamble (1995) report a minimum of approximately 0.82. Data on the effect of water activity on the production of the aflatoxins B_1 and B_2 are inconsistent. In general, production of toxins appears to be favoured by high water activity. *A. flavus* is reported to grow within a temperature range of



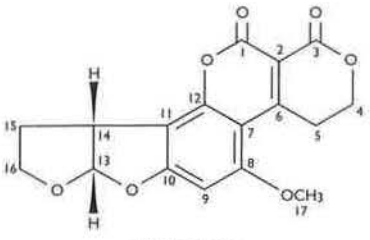
Aflatoxin B_1



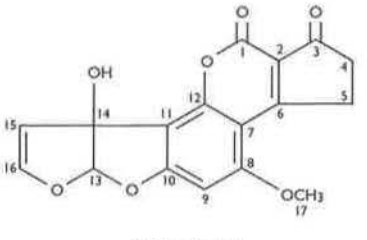
Aflatoxin B_2



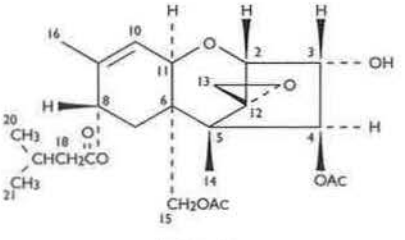
Aflatoxin G_1



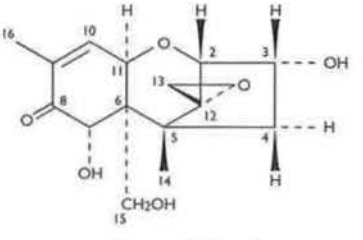
Aflatoxin G_2



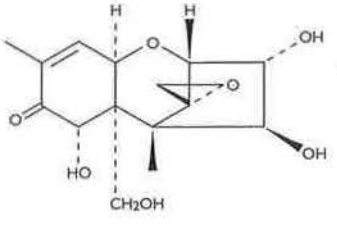
Aflatoxin M_1



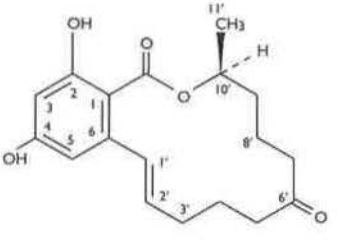
T-2 toxin



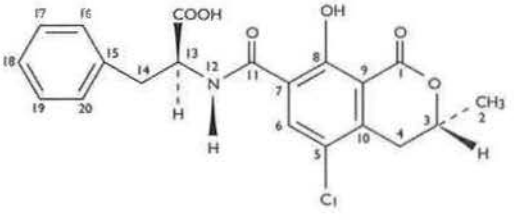
Deoxynivalenol



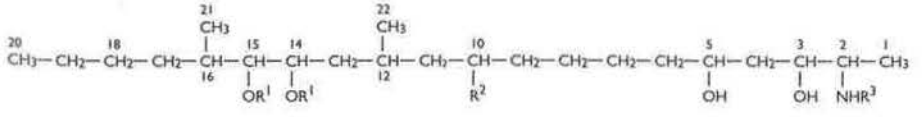
Nivalenol



Zearalenone



Ochratoxin A



Fumonisins

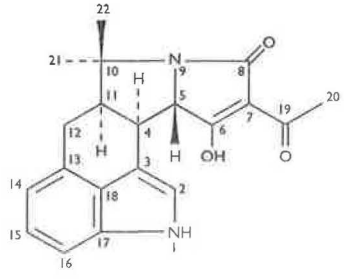
A_1: $R^1 = COCH_2CH(CO_2H)CH_2CO_2H$; $R^2 = OH$; $R^3 = C^{23}OC^{24}H_3$
A_2: $R^1 = COCH_2CH(CO_2H)CH_2CO_2H$; $R^2 = H$; $R^3 = COCH_3$
B_1: $R^1 = COCH_2CH(CO_2H)CH_2CO_2H$; $R^2 = OH$; $R^3 = H$
B_2: $R^1 = COCH_2CH(CO_2H)CH_2CO_2H$; $R^2 = R^3 = H$

Figure 5 Mycotoxins of world-wide importance

10–43 °C; optimal growth occurs at a little above 30 °C, reaching as much as 25 mm/day. The aflatoxins are produced by *A. flavus* over a temperature range of at least 15–37 °C; it is not possible to specify an optimum temperature, although production between 20 and 30 °C is reported to be significantly greater than at higher and lower temperatures.

The effect of water activity and temperature on the behaviour of *A. parasiticus* is similar to that described for *A. flavus*. Pitt and Miscamble (1995) have reported a minimum water activity for growth of about 0.83 and a minimum for aflatoxin production of about 0.87. Data on the effects of temperature on growth of *A. parasiticus* and the production of the aflatoxins are limited. Optimal growth and toxin production reportedly occur at approximately 30 and 28 °C, respectively.

The term 'aflatoxins' was coined in the early 1960s when the deaths of thousands of turkeys ('Turkey X' disease), ducklings and other domestic animals were attributed to the presence of *A. flavus* toxins in groundnut meal imported from South America (Austwick, 1978). (Although the aflatoxins are the major toxins associated with this mycotoxicosis, another mycotoxin, cyclopiazonic acid (*see* Figure 6), has been implicated in the aetiology of Turkey X disease (Bradburn *et al.*, 1994).) The chronic effects of low dietary levels (parts per billion) of aflatoxin on livestock are also well documented and include decreased productivity and increased suceptibility to disease (Council for Agricultural Science and Technology, 1989).



Cyclopiazonic acid

Figure 6 Cyclopiazonic acid

The aflatoxin-producing moulds occur widely in sub-tropical and tropical climates throughout the world. The aflatoxins may be produced on many foods and feeds, especially oilseeds, edible nuts and cereals, both before and after harvest (Coker, 1979).

Aflatoxin B_1 is a human carcinogen (IARC, 1993a) and one of the most potent hepatocarcinogens known. Human fatalities have also occurred from acute aflatoxin poisoning; in India, for example, unseasonal rains and a scarcity of food in 1974 prompted the consumption of heavily contaminated maize (Krishnamachari *et al.*, 1975). If the immunosuppression shown in livestock is similarly manifested in humans, it is possible that the aflatoxins (and other mycotoxins) could play a significant role in the aetiology of human disease in developing countries where high levels of exposure have been reported.

Lubulwa and Davis (1994) studied the economic losses in South-East Asian countries (Thailand, Indonesia and the Philippines) attributable to the occurrence of aflatoxin alone in maize and groundnuts. They found that about 66% of the total loss could be attributed to contaminated maize, and that spoilage and the deleterious effects on human and animal health accounted for 24, 60 and 16% of the total loss, respectively. However, the study only considered losses associated with morbidity and premature death due to cancer, and it is likely that when the additional effects on human health caused by the immunotoxic effect of aflatoxin (and other mycotoxins) are included, the percentage loss associated with the human sector is significantly higher.

The trichothecenes

Surprisingly little is known about the effects of water activity and temperature on the behaviour of the *Fusarium* moulds and the production of mycotoxins.

The temperature limits for growth of *F. graminearum* have not been reported, although the optimal temperature has been estimated at 24–26 °C. The minimum water activity for growth is 0.9 and the maximum recorded limit is more than 0.99. No information is available on the effect of water activity and temperature on the production of deoxynivalenol, nivalenol and zearalenone.

The minimum water activity for the growth of *F. moniliforme* is 0.87 and, again, the maximum limit exceeds 0.99. The minimum, optimal and maximum temperatures for growth are 2.5–5.0, 22.5–27.5 and 32–37 °C, respectively. There is no information on the conditions required for the production of fumonisin B_1.

The minimum water activity for the growth of *F. sporotrichioides* is 0.88 and the maximum limit greater than 0.99. The minimum, optimal and maximum temperatures for growth are −2.0, 22.5-27.5 and 35 °C, respectively. As with the other *Fusarium* moulds, there is no information on the conditions required for the production of T-2 toxin.

T-2 toxin and deoxynivalenol (*see* Figure 5) belong to a large group of structurally-related sesquiterpenes known as the 'trichothecenes'. T-2 toxin is produced on cereals in many parts of the world and is associated particularly with prolonged wet weather at harvest. It is the probable cause of 'alimentary toxic aleukia' (ATA), a disease which affected thousands of people in Siberia during the Second World War and led to the elimination of entire villages (IARC, 1993b). The symptoms of ATA include fever, vomiting, acute inflammation of the alimentary tract and a variety of blood abnormalities. T-2 toxin is responsible for outbreaks of haemorrhagic disease in animals and is associated with the formation of oral lesions and neurotoxic effects in poultry. The most significant effect of T-2 toxin (and other trichothecenes) is the immunosuppressive activity which has been clearly demonstrated in experimental animals; this activity is probably linked to the inhibitory effect on the biosynthesis of macromolecules. There is limited evidence that T-2 toxin may be carcinogenic in experimental animals.

Deoxynivalenol (DON) is probably the most widely occurring *Fusarium* mycotoxin, contaminating a variety of cereals, especially maize and wheat. Low concentrations of DON are commonly found in grains in North America, Japan and Europe; higher levels may occur intermittently in some developing countries. The outbreaks of emetic (and feed refusal) syndromes amongst livestock, caused by the presence of DON in feeds, has resulted in the trivial name of vomitoxin.

The ingestion of DON has caused outbreaks of acute human mycotoxicoses in India, China and rural Japan (Luo, 1988; Bhat *et al.*, 1989; IARC, 1993c). The Chinese outbreak in 1984–85 was caused by mouldy maize and wheat; symptoms occurred within 5 to 30 min. and included nausea, vomiting, abdominal pain, diarrhoea, dizziness and headache.

To date, nivalenol-producing isolates of *F. graminearum* have only been observed on rice and other cereals in Japan and have been associated with the occurrence of red mould disease ('Akakabi-byo'). Symptoms include anorexia, nausea, vomiting, headache, abdominal pain, diarrhoea and convulsions (Marasas *et al.*, 1984).

Zearalenone

Zearalenone is a widely distributed oestrogenic mycotoxin occurring in low concentrations, mainly in maize, in North America, Japan and Europe (Paucod, 1990). However, high concentrations can occur in developing countries, especially when maize is grown under more temperate conditions in, for example, highland regions.

Zearalenone is co-produced with DON by *F. graminearum* and has been implicated, with DON, in outbreaks of acute human mycotoxicoses.

Exposure to zearalenone-contaminated maize has caused hyperoestrogenism in livestock, especially pigs, characterized by vulvar and mammary swelling and infertility (Udagawa, 1988). There is limited evidence for the carcinogenicity of zearalenone in experimental animals.

The fumonisins

The fumonisins are a group of recently characterized mycotoxins produced by *F. moniliforme*; this mould occurs throughout the world and is frequently found in maize (IARC, 1993d). Fumonisin B_1 has been reported in maize (and maize products) from a variety of agroclimatic regions including the US, Canada, Brazil, South Africa, Austria, Italy and France. The toxins occur particularly when maize is grown under warm, dry conditions.

Exposure to fumonisin B_1 (FB_1) in maize causes leukoencephalomalacia (LEM) in horses and pulmonary oedema in pigs. LEM has been reported in many countries including the US, Argentina, Brazil, Egypt, South Africa and China. FB_1 is also toxic to the central nervous system, liver, pancreas, kidney and lung in a number of animal species.

The presence of the fumonisins in maize has been linked with human oesophageal cancer in the Transkei, southern Africa and China. The relationship between exposure to *F. moniliforme* in home-grown maize and the incidence of oesophageal cancer was studied in the Transkei from 1976 to 1986 (Rheeder *et al.*, 1992). In the high risk cancer area, the percentage of kernels infected with *F. moniliforme* was significantly greater throughout the entire study period, and FB_1 and FB_2 occurred at significantly higher levels in mouldy maize obtained from these areas in 1986.

There is sufficient evidence in experimental animals for the carcinogenicity of cultures of *F. moniliforme* containing significant amounts of the fumonisins. However, the evidence for the carcinogenicity of fumonisin B_1 is limited (IARC, 1993d).

Ochratoxin A

A. ochraceus grows more slowly than either *A. flavus* or *A. parasiticus*, but it can grow at a water activity as low as 0.79. Growth has been reported within a temperature range of 8 to 37 °C, with an optimum of 25–31 °C. Ochratoxin A is produced within the temperature range 15–37 °C, with optimal production at 25–28 °C.

P. verrucosum grows within the temperature range 0–31 °C and at a minimum water activity of 0.79. Ochratoxin A is produced over the whole temperature range. Significant quantities of toxin can be produced at a temperature as low as 4 °C and at a water activity as low as 0.86.

Exposure to ochratoxin A (OA) seems to occur mainly in wheat and barley growing areas in temperate zones of the northern hemisphere (IARC, 1993e). The levels reported range from trace amounts to 6000 µg/kg in Canadian wheat. In the UK, levels of 5000 and 2700 µg/kg have been reported in barley and wheat, respectively. OA also occurs in maize, rice, peas, beans and cowpeas; developing country origins include Brazil, Chile, Egypt, Senegal, Tunisia, India and Indonesia.

The ability of OA to transfer from animal feeds to animal products has been demonstrated by its presence in Europe in pig blood and retail pork products.

It has been suggested that pork products are significant human dietary sources of OA; it has been found in blood (and milk) from individuals in a variety of European countries, including France, Italy, Germany, Denmark, Sweden, Poland, the former Yugoslavia and Bulgaria (IARC, 1993e). One of the highest reported levels is 100 ng/ml OA in blood from the former Yugoslavia (Fuchs *et al.*, 1991); 6.6 ng/ml OA has been recorded in milk in Italy (Micco *et al.*, 1991).

There are existing or proposed regulations for OA in at least 11 countries; the permitted levels range from 1 to 50 µg/kg in foods, and from 100 to 1000 µg/kg in feeds. In Denmark, the acceptability of pork products from a specific carcass is determined by analysing the OA content of the kidney; meat and certain organs can be consumed if the OA content of the kidney is no more than 25 and 10 µg/kg, respectively (van Egmond, 1991).

A provisional tolerable weekly intake of OA of 112 ng/kg body weight has been recommended by a WHO/FAO Joint Expert Committee on Food Additives (WHO, 1991).

Ochratoxin A has been linked to the human disease Balkan endemic nephropathy, a fatal, chronic renal disease occurring in limited areas of Bulgaria, the former Yugoslavia and Romania. OA causes renal toxicity, nephropathy and immunosuppression in several animal species, and is carcinogenic in experimental animals.

The co-occurrence of mycotoxins

The complex ecology of mould growth and mycotoxin production can produce mixtures of mycotoxins in foods and feeds, especially in cereals. The co-occurrence of mycotoxins can affect both the level of mycotoxin production and the toxicity of the contaminated material (Miller, 1991). The production of aflatoxins in stored grains, for example, may be enhanced by the presence of trichothecenes, whereas the toxicology of naturally occurring combinations of trichothecene mycotoxins is reportedly determined by syner-

gistic interactions in experimental animals (Schiefer *et al.*, 1986; Bhavanishankar *et al.*, 1988). In a study with swine, the effect of DON on weight gain and feed conversion was synergized by T-2 toxin. Interactions involving non-toxic fungal metabolites have also been reported, including the potent synergism of non-toxic *F. graminearum* metabolites (culmorin, dihydroxycalonectrin and sambucinol) with DON (Dowd, 1989). Too little is currently known about this particularly important area of mycotoxicology.

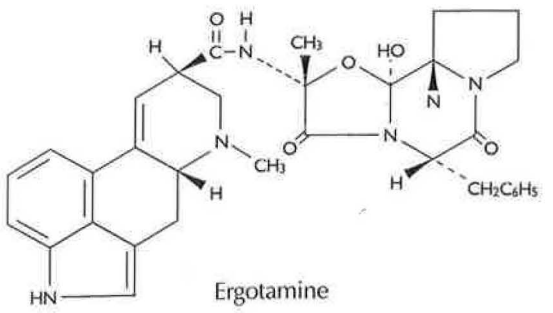
Although the moulds and mycotoxins listed in Table 1 are undoubtedly of world-wide importance, they reflect the mycotoxin problem as perceived, primarily, within the agricultural and livestock sector in the developed world, especially North America. Here, the widespread application of intensive farming practices tends to amplify the effects of mycotoxin exposure which, in turn, leads to the initiation of comprehensive studies on the occurrence and toxicity of the mycotoxin(s) in question. For example, although aflatoxin B_1 (*see* Figure 5) is now recognized as a potent human carcinogen, interest in it was actually initiated in the early 1960s because of its severe impact on the poultry industry in the UK. Similarly, studies on DON began in Canada in 1980 when substantial levels of the toxin were found in Ontario soft wheat and Quebec hard wheat. The major problem faced by the North American livestock industry is poor performance arising from low level consumption of DON, T-2 toxin and nivalenol.

Typically, once a livestock-related mycotoxicosis has been identified in the developed world, the acquisition of further toxicological data and the development of improved analytical methods mean that studies can be carried out on the effect of the toxin on human health in both developed and developing countries. For example, during the late 1980s, the ingestion of DON was implicated in outbreaks of acute human mycotoxicoses in India, China and Japan (Luo, 1988; IARC, 1989; Bhat *et al.*, 1989). However, this scenario militates against the identification and study of mycotoxins associated primarily with the developing world.

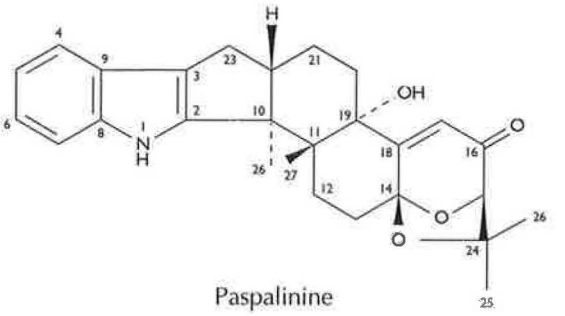
Mycotoxins of regional importance

Several mycotoxicoses which are not listed in Table 1 are of importance to the exposed populations in the affected regions. The mycotoxicoses in this category (*see* Table 2) include those associated with moulds occurring in both growing and stored forage crops. The moulds and mycotoxins include those which have been associated with a variety of livestock diseases such as ergotism, paspalum staggers, ryegrass staggers, facial eczema, fescue foot, lupinosis, slobber syndrome and stachybotryotoxicosis (*see* Figure 7) (Lacey, 1991).

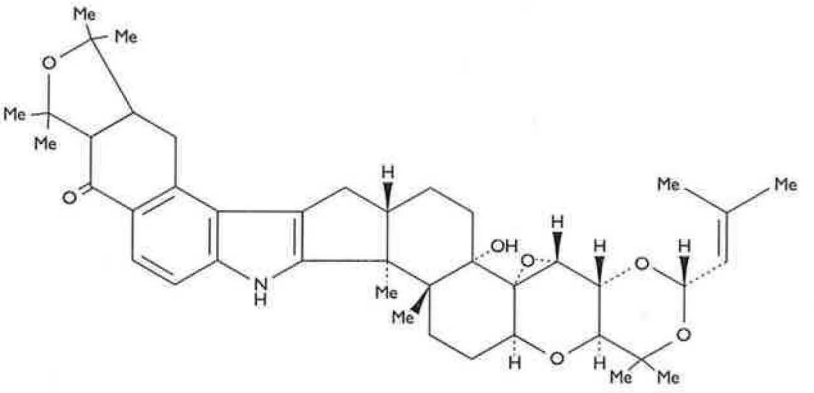
Most farm animals consume pasture crops either by grazing on the living pasture or by eating hay or silage. The crops can be colonized by moulds throughout this period, the development of the moulds and the production of fungi being dependent on the prevailing ecosystem. Growing crops provide a variety of micro-environments. For example, the uppermost leaves of a plant will be subjected to extreme fluctuations of temperature and relative humidity, whereas those leaves towards the base will present a more shaded, moderate, humid environment; the surface texture of the leaf will also affect the micro-environment.



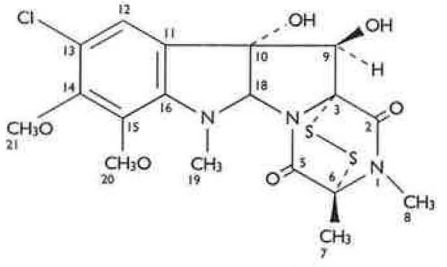
Ergotamine



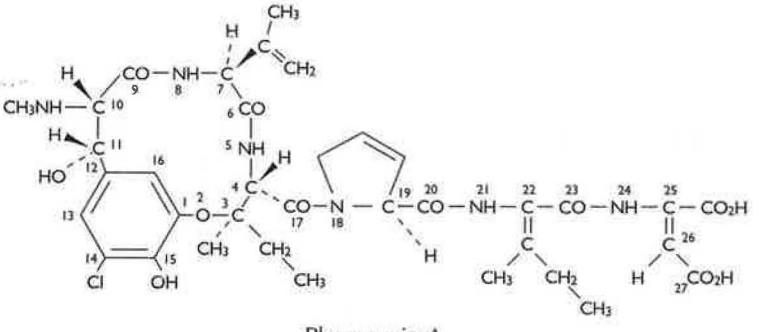
Paspalinine



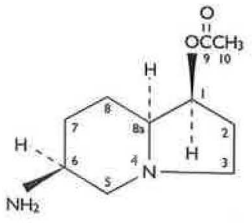
Lolitrem B



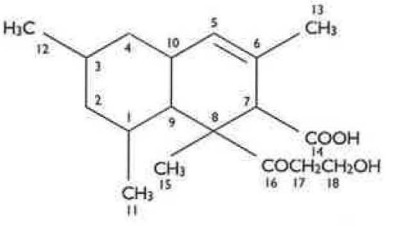
Sporidesmin A



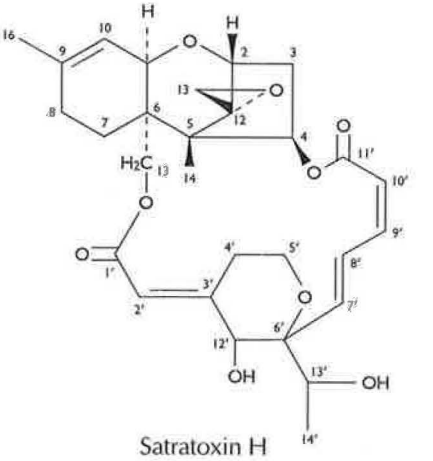
Phomopsin A



Slaframine



Diplodiatoxin



Satratoxin H

Figure 7 Mycotoxins of regional importance

Table 2 Moulds and mycotoxins of regional importance

Mould species	Mycotoxins produced	Mycotoxicosis
Claviceps purpurea	Ergotamine alkaloids	Ergotism
C. fusiformis	Clavine alkaloids	Ergotism
C. paspali	Paspalinine	*Paspalum* staggers
Acremonium loliae	Lolitrem	Ryegrass staggers
Balansia spp.?	Alkaloids?	Fescue foot
Pithomyces chartarum	Sporidesmin	Facial eczema
Phomopsis leptostromiformis	Phomopsin	Lupinosis
Rhizoctonia legumicola	Slaframine	Slobber syndrome
Stachybotrys atra	Satratoxins	Stachybotryotoxicosis
Diplodia maydis	Diplodiatoxin	Diplodiosis

Ergotism and *Paspalum* staggers

A wide range of grasses and small-grain plants (especially rye and barley) are infected during flowering with ascospores of the genus *Claviceps*. After infection, the ovary is replaced by a whitish mycelial mass which produces conidia within a sweet, sticky liquid (honeydew); this, in turn, may be transferred by insects, rain-splash and grazing animals to other plants. Eventually, the mycelial mass hardens into a seed-like sclerotium (the ergot) which can germinate in the soil, eventually producing ascospores to repeat the infection process. *C. purpurea* and *C. paspali* are the species most often reported as the causes of animal disease, particularly in the southern US, South America, southern Africa, Australia and New Zealand. Generally, *C. paspali* occurs in warmer climates than *C. purpurea*.

Ergotism

Moisture is an important factor in the aetiology of ergotism, influencing the germination of the *C. purpurea* sclerotia, the dispersal of the ascospores and the infection of the plant. Germination of the sclerotia is enhanced by damp conditions, whereas rain or heavy dew will promote the explosive discharge of ascospores. Dew accumulated at the base of the style will enhance the infection of the plant by encouraging the ascospores to germinate and penetrate the ovary. The presence of ryegrass (*Lolium perenne*) can significantly increase the infection of neighbouring wheat crops, and increasing use of fertilizers is reported to enhance the production of sclerotia.

Although the careful grading of grains has largely eliminated ergotism as a human disease, it is still a significant, sporadic animal mycotoxicosis. The main alkaloids produced by *C. purpurea* are reported to be ergotamine, ergostine, ergocristine, ergocryptine and ergocornine. However, the toxins produced by specific sclerotia have only rarely been recorded and may well depend on the fungal isolate, the host plant and presumably, both the macro- and micro-environments.

Ergotism occurs in three forms: a gangrenous form, a convulsive form, and a form associated with gastro-intestinal disorders. The gangrenous form of ergotism was a major human disease in mediaeval Europe and resulted from the consumption of ergot-contaminated rye grain. Early symptoms include swollen limbs with a simultaneous burning sensation (St Anthony's Fire), followed by necrosis and finally, the loss of appendages. In the 20th century, outbreaks of ergotism have occurred in Russia (1926), Ireland (1929), France (1953), India (1975) and Ethiopia (1978) (Beardall and Miller, 1994). The Indian outbreak was attributed to the consumption of pearl millet (*Pennisetum typhoideum*) infected with *C. fusiformis* (Krishnamachari and

Bhat, 1976). The mycotoxic alkaloids produced by *C. fusiformis* belong to the clavine group (e.g. agroclavine, elymoclavine, chanoclavine, penniclavine and setoclavine) which cause gastro-intestinal disorders (nausea, vomiting, diarrhoea, giddiness and somnolence) rather than the gangrenous and convulsive symptoms caused by ergotamines (the primary *C. purpurea* alkaloids).

Both the gangrenous and convulsive forms of ergotism occur in animals; the gangrenous form particularly affects the ears, tails and feet whereas the convulsive form causes ataxia, convulsions and paralysis. Productivity can also be affected and result, for example, in reductions in weight gain, milk production and reproductive efficiency. In the US, ergotism is primarily associated with the mid/northwestern states.

Paspalum *staggers*

Paspalum staggers, caused by *C. paspali*, occurs only as a nervous and convulsive disorder which closely resembles ryegrass staggers. The neurotoxic metabolites believed to be responsible for the symptoms are paspalinine and its derivatives, known collectively as the paspalitrems (Lacey, 1991).

Ryegrass staggers

Perennial ryegrass (*Lolium perenne*) staggers is a neuro-muscular disorder caused by the lolitrem-producing mould *Acremonium lolii*; it mainly affects sheep and cattle in New Zealand. (It should not be confused with annual ryegrass toxicity caused by infection of pasture with a nematode and bacterium.) In New Zealand, perennial ryegrass staggers occurs primarily during the warm, dry periods of summer and autumn when animals graze to the base of pastures, ingesting lolitrem-rich tissue. In sheep, the first symptoms are trembling followed by staggering and finally, collapse. Recovery is rapid, the main losses resulting from disorientated animals falling over cliffs or into water courses.

Fescue toxicity and fescue foot

The perennial grass, tall fescue (*Festuca arundinacea*), is associated with both fescue toxicity and fescue foot. In the US, it is grown on approximately 40 million acres and accommodates about 22% of the nation's cattle. Annual losses in excess of US$ 800 million have been attributed to reduced calving rate and reduced body weight of weaned calves caused by fescue toxicity. A minority of the herd may also suffer from fescue foot which is characterized by dry gangrene in the back legs and sloughing of the hooves.

It is not clear which moulds and mycotoxins cause fescue toxicity, although alkaloid-producing *Balansia* spp. have been linked to fescue foot.

Facial eczema

Facial eczema, which affects mainly sheep, is the most frequently reported form of mycotoxic photosensitization; it is caused by the accumulation of phylloerythrin (a porphyrin pigment) as a consequence of mycotoxic liver damage. It is mainly associated with New Zealand but also occurs in Australia, South Africa and South America. The causal fungus, *Pithomyces chartarum*, occurs widely in the tropics and has also been reported in the UK and US. In New Zealand, the litter from ryegrass/white clover pastures is the main substrate for fungal growth, with optimal development occurring at 24–26 °C.

The mycotoxic liver damage associated with facial eczema is caused by the sporidesmin mycotoxins, primarily sporidesmin A which is produced in greatest abundance at about 20 °C. On highly toxic pastures, sheep can develop liver damage after only 3 h of grazing, with severe liver damage occurring after 48 h and photosensitivity after 96 h. Although the severity of the disease varies from year to year, annual losses can amount to NZ$ 100 million.

Geeldikkop is a similar photosensitization disease of sheep occurring in the South Africa Karoo; it is associated with the consumption of *P. chartarum*-infected *Tribulus terrestris,* an annual pasture which grows widely in semi-arid regions. It has been reported that *T. terrestris* enhances the toxicity of sporidesmin.

Lupinosis

Lupinosis is a hepatoxicosis of sheep, cattle and other animals caused by the ingestion of forage crops of *Lupinus* spp. infected with the mould *Phomopsis leptostromiformis.* Phomopsin A is the major mycotoxin. *P. leptostromiformis* can remain viable in infected seed for at least two years. The spores are primarily dispersed by rain-splash over short distances, and probably by ascospores over longer distances, following rain or heavy dew. The disease is most common in sheep in Australia, New Zealand and South Africa; it has not been widely reported in the US. Outbreaks in Western Australia often occur one to two weeks after cyclonic rainfall of at least 50 mm over three days.

Slobber syndrome

Slobber syndrome is characterized by excessive salivation and decreased lactation in horses and cattle. It is caused by the consumption of red clover (*Trifolium pratense*) hay, occasionally by grazing the growing crop, or by the consumption of silage. Other clinical signs associated with the disease include diarrhoea, increased lachrymation, feed refusal and in severe cases, respiratory failure. It is caused by *Rhizoctonia legumicola* which produces the parasympathomimetic alkaloid, slaframine. The liver metabolite of slaframine (a ketoimine) has an affinity for muscarinic receptors of the nervous system, and slaframine has a particularly high affinity for receptors in the gastrointestinal tract. *R. legumicola* does not produce spores but spreads rapidly when the relative humidity is high and the temperature is in the range 25 to 29 °C.

Slobber syndrome has caused serious economic losses in the Midwest of the US and has resulted in a drastic reduction in the use of red clover as a forage crop.

Stachybotryotoxicosis

Stachybotryotoxicosis affects mainly horses in eastern Europe following exposure to straw and hay infected with the mould *Stachybotrys atra. S. astra* was first isolated from wallpaper in Prague over 150 years ago. Its toxic metabolites include the macrocyclic trichothecenes verrucarin J and roridin E, and the satratoxins F, G and H. The satratoxins G and H have been identified in isolates of *S. atra* from Hungary, the former Czechoslovakia and Finland.

Although the disease affects mainly horses, it has also been reported in cattle, sheep, pigs, poultry and man (as a result of inhaling airborne spores). In acute cases, animals given contaminated straw for one to two weeks may die within a few days. The more typical chronic form is characterized by

necrosis of the mucous membranes in the mouth leading to inflammation of the whole head and, finally, internal haemorrhage. Stachybotryotoxicosis also occurs in the US, and an outbreak in South Africa in 1979 caused high mortality in sheep.

Diplodia toxicity

Diplodia maydis can cause extensive spoilage of maize cobs during wet periods in the late growing season in parts of southern Africa. In these regions, diplodiosis (a neurotoxicosis) is often the most common bovine mycotoxicosis, the symptoms progressing from inco-ordination to paralysis and death. Although toxigenic isolates of *D. maydis* have been identified in South Africa, the US and South America, field outbreaks of the toxicosis appear to be restricted to southern Africa. Diplodiatoxin is the only metabolite which has been isolated from *D. maydis,* but it is not considered to be the major neurotoxin.

Mycotoxins of possible importance

It is probable that a number of mycotoxins (known and unknown) produced by commonly occurring moulds are 'waiting in the wings' to become 'important' (*see* Table 3 and Figure 8).

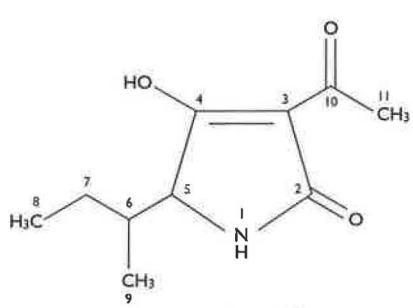
Penicillium toxins

The *Penicillium* mycotoxins citreoviridin and penitrem A may fall into this category. Citreoviridin is produced by *P. citreonigrum* (synonyms *P. citreoviride* and *P. toxicarium*); in Japan, it is reported to be responsible for acute cardiac beri beri caused by the consumption of mouldy 'yellow rice'(Ueno and Ueno, 1972). *P. citreonigrum* is widely distributed and occurs on rice after harvest, particularly in the more temperate rice-growing areas when the moisture content reaches 14.6%. Although a ban on the sale of yellow rice imposed in Japan in 1910 resulted in the disappearance of the disease, it is difficult to imagine that the toxin (or a related compound) is not being produced, and consumed, in other climatic zones similar to those which prevail in Japan.

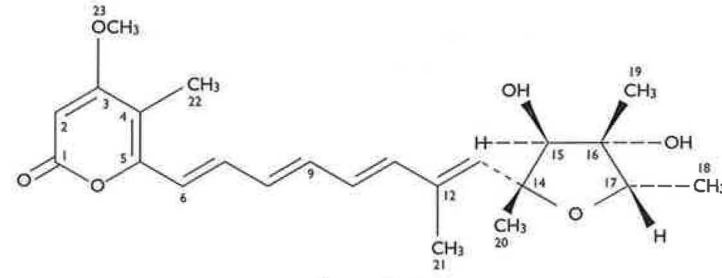
Table 3 Mycotoxins of possible importance

Mould species	Mycotoxins produced	Mycotoxicosis
Penicillium citreonigrum	Citreoviridin	Acute cardiac beri beri
P. crustosum	Penitrem A	Neurotoxicosis
Fusarium spp. (in fruit and vegetables)	*Fusarium* toxins	?
Alternaria spp. (in fruit and vegetables)	*Alternaria* toxins	?

Penitrem A is produced mainly by *P. crustosum* which occurs with a high frequency in foods and feeds; Pitt and Hocking (1985) isolated it from most of the cereal and animal feed samples they examined over a period of more than 10 years. The minimum, optimum and maximum temperatures for toxin production are less than 17, 20–26 and 30 °C, respectively, and the corresponding water activities are 0.92, 0.995 and 0.999. Penitrem A is a potent tremorgenic neurotoxin which has been associated with death and severe brain damage in sheep, cows, horses and dogs. The precise role of this mycotoxin and other fungal neurotoxins in human and livestock disease has yet to be determined.



Tenuazonic acid



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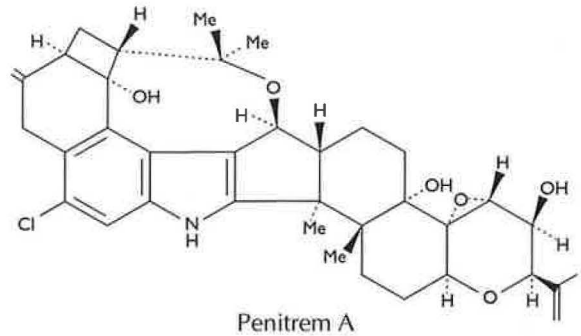
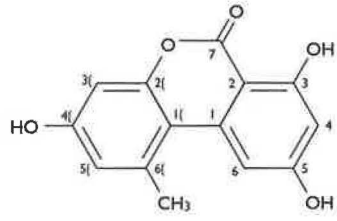
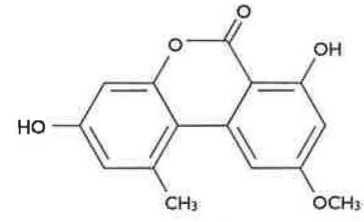


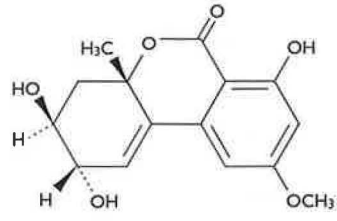
Penitrem A



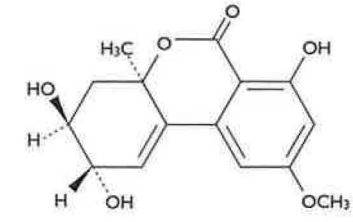
Alternariol



Alternariol methylether



Altenuene



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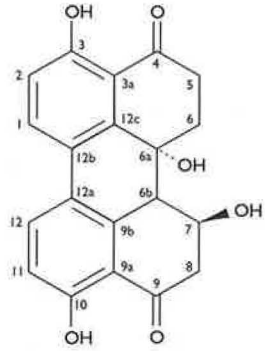
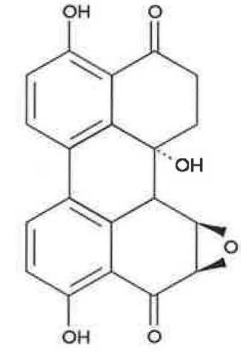


ATX-I



ATX-II

Figure 8 Mycotoxins of possible importance

Alternaria and *Fusarium* toxins in fruit and vegetables

The role of fruit and vegetables as sources of mycotoxins has not been properly studied. Although potentially toxigenic strains of *Fusarium* (including *F. graminearum*, *F. moniliforme*, *F. equiseti* and *F. sporotrichioides*) have been reported in a variety of fruit and vegetables, little or no work has been done on them (Blaney, 1991). Similarly, the significance of the reported occurrence of a variety of *Alternaria* moulds in fruit and vegetables, and of the effect of the *Alternaria* toxins (including alternariol, altenuene, isoaltenuene, altenuene monomethylether, the altertoxins and tenuazonic acid) (*see* Figure 8) on human health, has still to be elucidated (Blaney, 1991).

THE SOCIO-ECONOMIC SYSTEM

The Socio-Economic System describes those social (e.g. cultural, political) and economic (macro- and micro-) factors which exert an important influence on events within the Mycotoxicology System (*see* Figures 1 and 9). These factors should be thoroughly addressed when attempting to control the production of moulds and mycotoxins. In some instances, given the complexity and unpredictability of human behaviour, it can be very difficult to intervene successfully within the Socio-Economic System. However, whenever efforts are made to improve the quality of foods and feeds, it should be clearly established that there is a need for a better quality product, and that any associated increase in the cost of the product can be borne by the community.

THE CONTROL SYSTEM

Process-Quality Management

The Process-Commodity Matrix

A single commodity system (e.g. an edible nut system) may be considered in terms of numerous interacting processes (agricultural, 'technical' and socio-economic). Some of these are shown in the left-hand column of the *Process-Commodity Matrix* (*see* Figure 9) which may be considered as a more comprehensive version of Figure 2 (*The Commodity System*). The quality of the system will be determined by the combined qualities of the individual processes; a single process of poor quality will have a deleterious effect on the quality of the complete system. The successful management of the processes within a commodity system requires the inputs of an **inter**-disciplinary **commodity team** where the potential advantages arising from the dynamics of the team are realized by fully exploiting the **interactions** between the skills, disciplines and backgrounds of the individual team members.

However, wherever a variety of commodities is produced within a process-commodity matrix (*see* Figure 9), it is highly likely that (a) the process skills (e.g. on-farm storage) can be transferred across commodity systems and (b) that the commodity systems themselves are interactive.

The transfer of process skills across commodity systems requires the establishment of **specialist process teams** interacting with the appropriate **commodity teams**. (It is recognized that specialist process teams will often also be inter-disciplinary; the on-farm storage process, for example, requires a variety of disciplines.)

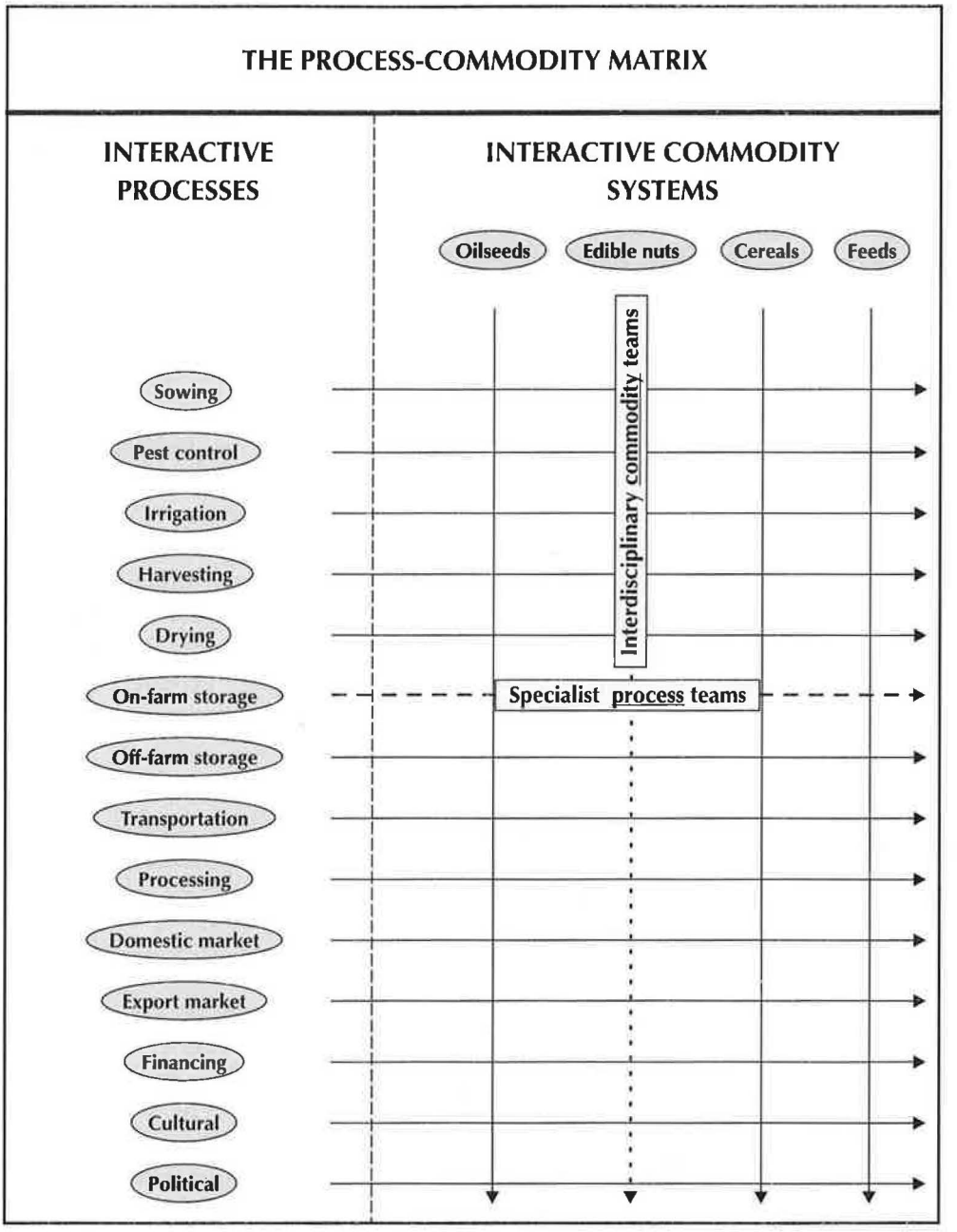


Figure 9 The Process-Commodity Matrix

The interactive nature of 'adjacent' commodity systems may be illustrated by considering the oilseed, edible nuts, cereals and feeds systems specified in Figure 9. In the production of groundnuts and maize, it is evident that both commodities can be considered in terms of both food and feed systems, and that activity within one system will influence events in the other. An increase in demand for (and in the price of) animal feed, for example, may encourage producers to consign a greater proportion of their maize crops to the feed sector, and to utilize more of the groundnut crop for the production of oil and cake. In turn, the increase in the availability of groundnut oil may influence the demand for other edible oils.

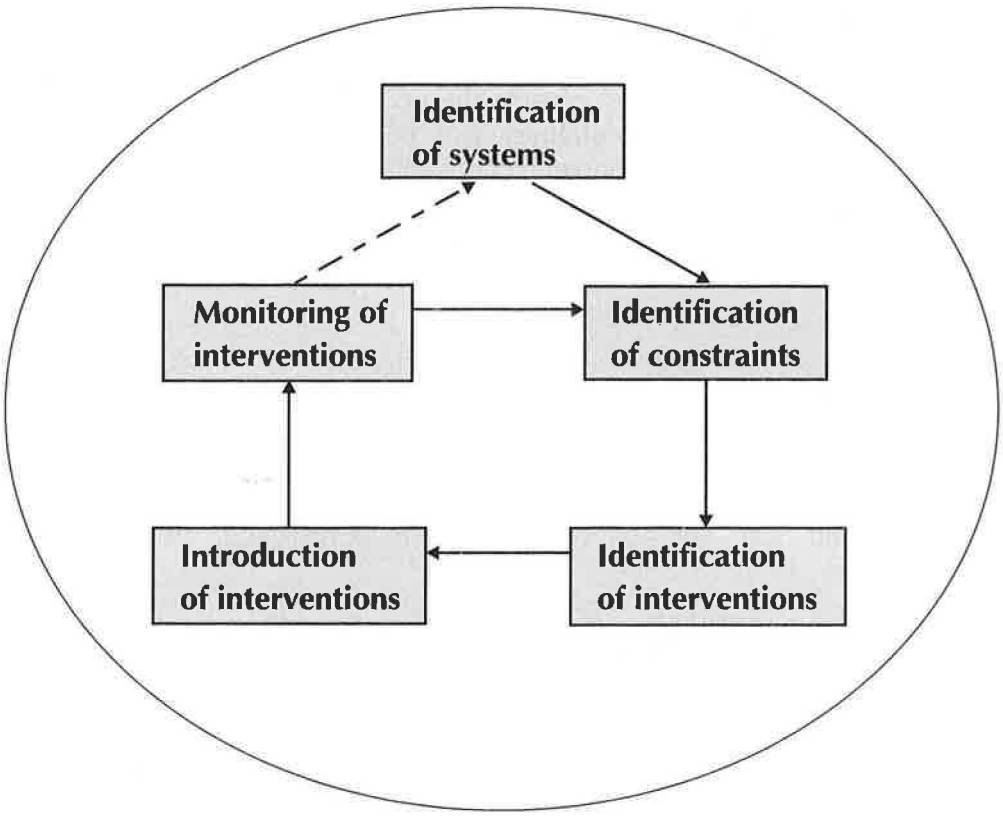


Figure 10 The Process-Quality Management cycle

The dynamics of the process-commodity matrix will determine the quality of any process, or combination of processes; the same milieu can be exploited via the concept of the *Process Quality Management* (PQM) cycle (*see* Figure 10) to ensure that quality is maintained as fully as possible throughout the matrix. Briefly, this process involves: (a) the identification of the contributing systems and the interactions within and between the systems; (b) the identification of those cells within the process-commodity matrix which are constraining the quality of the systems; (c) the design of appropriate interventions which will remove the quality constraints; (d) the introduction of the interven-

tions; and (c) the monitoring of the effectiveness of the interventions. If necessary, quality management can be further refined by referring to the feedback obtained from the monitoring process, and repeating the PQM cycle beginning at 'identification of constraints'. (In exceptional circumstances, there may also be a need to re-identify the selected systems.)

The Control System (*see* Figure 11) illustrates the three main types of intervention which are likely to be introduced for the control of mycotoxins after the quality constraints have been clearly identified. The interventions most likely to be employed may be considered in terms of three interacting subsystems: (i) prevention; (ii) identification and segregation of contaminated material; and (iii) detoxification.

Identification of quality constraints

Those factors which are compromising the quality of the commodity system(s) under investigation and causing the occurrence of moulds and mycotoxins (i.e., the quality constraints) can be identified by implementing carefully designed surveys. Surveillance studies will provide technical data (including data generated by observation, the completion of questionnaires, interviews, and the analysis of samples), socio-economic data, and analytical data obtained by the monitoring of biological samples (e.g. blood, urine, milk) collected from individuals.

It is beyond the scope of this Bulletin to describe the constantly evolving approaches available for the collection and analysis of surveillance data. However, the reader is referred to the numerous publications which address this topic, including those associated with participatory rural appraisal (PRA), agroecosystems, commodity systems assessment methodology, and a variety of 'hard' and 'soft' decision support systems (Checkland, 1990; Conway, 1990; La Gra, 1990; Chambers, 1993).

Prevention of contamination

Preventative measures which militate against the onset of spoilage and, subsequently, the production of moulds and mycotoxins, may be introduced throughout the Commodity System. Spoilage may be prevented both before and after harvest by manipulating those interacting agents (biological, physical, chemical, macro- and micro-environmental) of bioterioration which primarily contribute to the Spoilage System (*see* Figure 3). However, the opportunity to manipulate the Spoilage System will vary throughout the Commodity System and will be determined by the prevailing climate and the resources available. For example, the pre-harvest control of biodeterioration is somewhat compromised by man's inability to control the climate! Either insufficient or excessive rainfall during critical phases of crop development can lead to mould contamination and mycotoxin production. The very substantial economic losses attributed to mycotoxins on the North American continent clearly demonstrate the difficulties associated with the pre-harvest prevention of contamination, even in wealthy developed nations.

After harvest, it is important that the crop is dried to a 'safe' moisture level (which will not support the production of moulds and mycotoxins) as quickly as possible. The resource-rich farmer in the developed world can achieve this through mechanical drying whereas the resource-poor farmer will have to depend on an adequate supply of sun and wind, and an absence of rain. Similarly, richer communities can control the temperature of stored crops by refrigeration, but poorer populations do not have this option. The importance

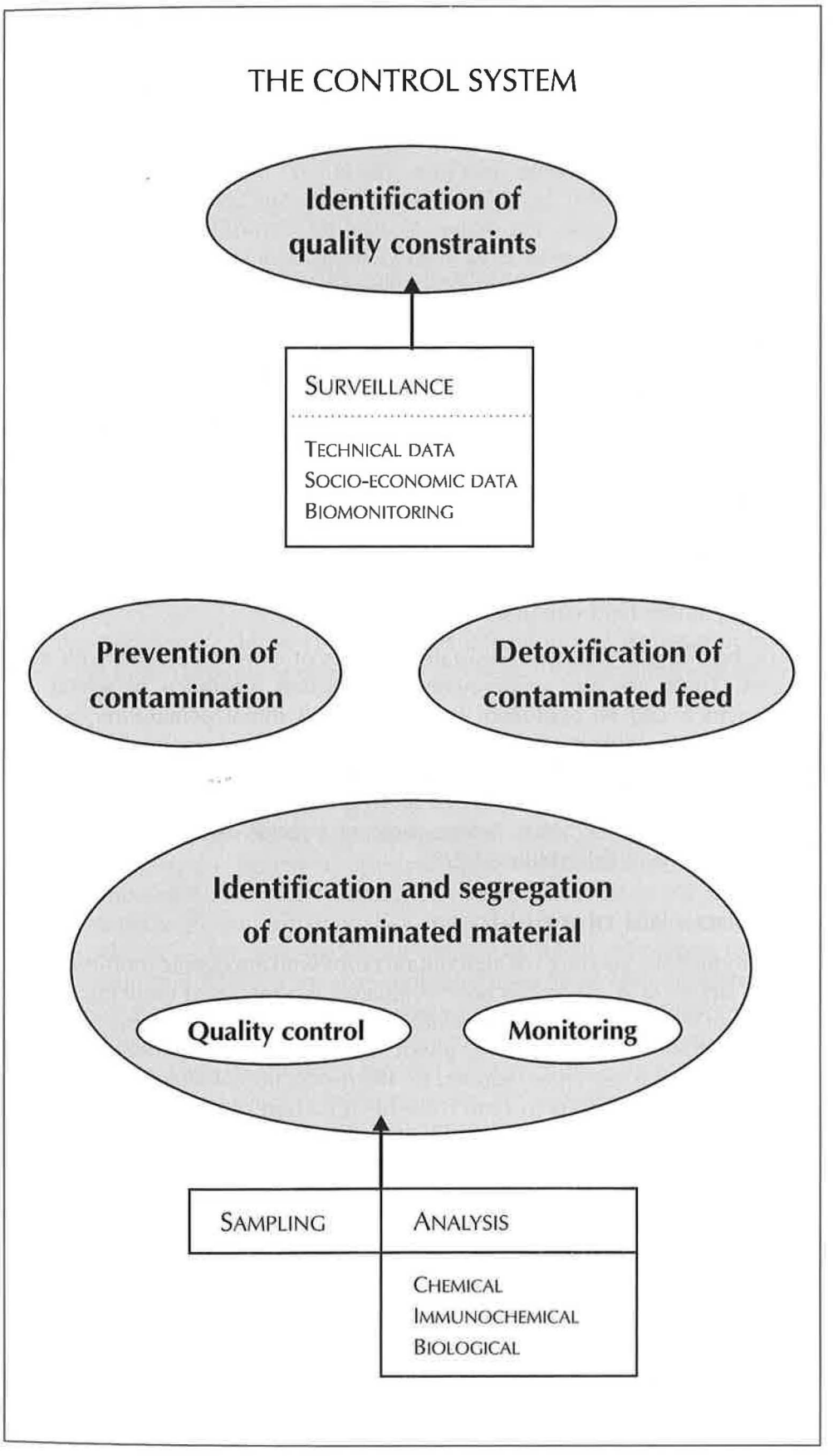


Figure 11 The Control System

of good storage practices in the prevention of spoilage cannot be overemphasized. In rural communities, traditional storage methods are the product of decades, or even centuries, of development and are normally ideally suited to the prevailing agro-climatic conditions. However, if a community is forced to move to a new agro-climatic zone, it may be many years before the necessary adjustments can be made to the storage procedures. Communities may also be forced to accommodate changes to traditional methods if, for example, the materials used to construct the stores become less readily available, or if new, high-yielding varieties of the stored commodity are introduced; these high-yielding varieties are often more susceptible to insect attack than traditional varieties, a situation which can be exacerbated by the introduction of exotic insect pests through trade or aid.

Attempts have been made to prevent mycotoxin contamination by (a) breeding for resistance to moulds and (b) 'biocontrol', involving the introduction of atoxigenic strains of fungi.

Breeding for resistance to moulds

To date, although much effort has gone into the development of varieties of groundnuts and maize which are resistant to *A. flavus*, none has performed effectively under field conditions.

The breeding of *Fusarium*-resistant varieties of cereals has met with more success. There are two components of *Fusarium* resistance in wheat and maize which can be exploited: (i) resistance to initial penetration; and (ii) resistance to the distribution of the pathogen in the host tissue. A third component to be considered is the effect of the host-pathogen relationship on mycotoxin production and/or degradation. However, it has been estimated that it will be 10–20 years before resistant hybrids and cultivars become commercially available (Miller, 1995).

The biocontrol of moulds

The introduction ('seeding') of agricultural crops with atoxigenic (non-toxin producing) strains of *A. flavus* has been evaluated as a means of reducing the toxigenicity of the resident fungal population (Cotty *et al.*, 1994). Atoxigenic strains reduce aflatoxin contamination by physically excluding the resident strains and competing for the resources required for the production of aflatoxins. The most comprehensive field tests to date have been carried out on cotton grown in Arizona, US. A significant reduction in the aflatoxin content of the mature crop was reported when an atoxigenic strain of *A. flavus* was seeded, on colonized wheat grain, into a field of developing cotton prior to flowering (Cotty *et al.*, 1994). However, further studies are required on the long-term efficacy and safety of biocontrol strategies before they can be accepted for routine use.

Although prevention is better than cure, and despite the best efforts of the agricultural community, mycotoxins will continue to be present in a wide range of foods and feeds in the foreseeable future. Consequently, strategies are required for the removal of mycotoxins from crops. Two approaches are currently available: (a) identification and segregation of contaminated material; and (b) detoxification of contaminated feeds (*see* Figure 11).

Identification and segregation of contaminated material

The identification and segregation of contaminated material may be pursued through quality control and monitoring procedures.

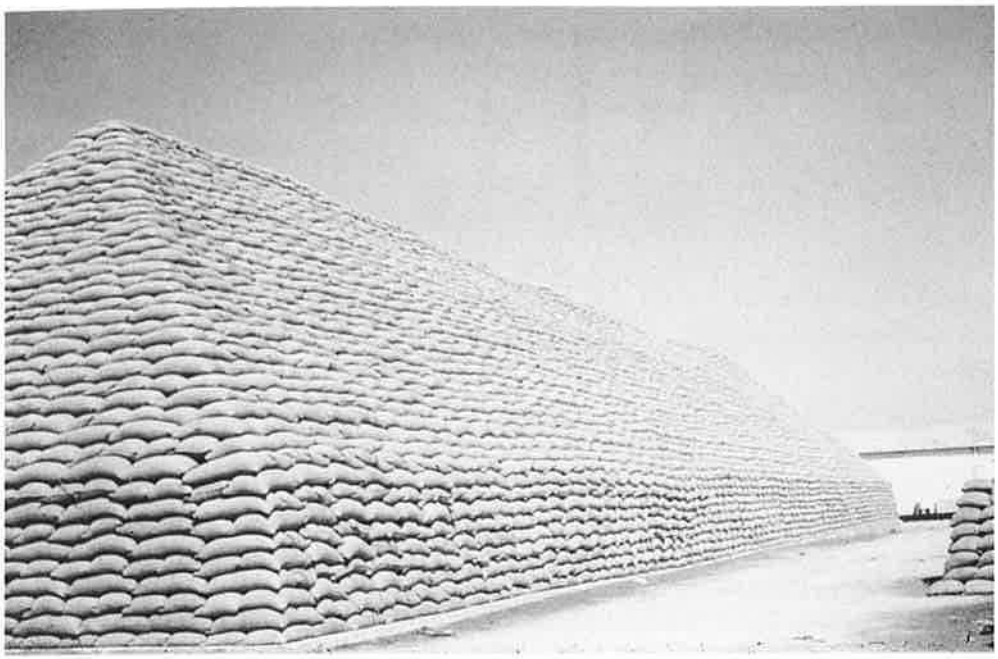


Figure 12 A large sack-stack of groundnuts

It is evident from Figure 11 that the identification and segregation of contaminated material requires efficient methodologies for **sampling** and **analysis**.

Sampling

Most attempts to develop an effective procedure for the collection of representative samples from food or feed lots have focused on the aflatoxins, since the majority of current regulations are concerned specifically with this group. However, the design of effective sampling procedures has been seriously hampered by the highly positively-skewed distribution of the aflatoxins, problems with the withdrawal of the samples (e.g. when sampling an oilseed cake in sacks), and significant difficulties when attempting to collect samples from throughout a lot. The problems associated with the sampling of large lots of stored material are self-evident (*see* Figure 12). Wherever possible, samples should be collected when the lot is mobile and therefore readily accessible. The large lot shown in Figure 12, for example, can only be properly sampled when the stack is being constructed or dismantled. Ideally, samples should be collected automatically from a moving stream of material.

Although tremendous progress has been made in the development of analytical methods for mycotoxins, attempts to develop internationally accepted sampling protocols have met with little success. Recently, the Food and Agriculture Organization (FAO) outlined options for sampling plans linked to maximum permissible levels (guidelines) of 5, 10, 15, 20 and 30 µg/kg aflatoxin B_1 in maize and groundnuts, but they fell short of a firm and internationally-binding endorsement (FAO, 1993).

The mycotoxin contamination of foods and feeds has attracted global attention. It has had a significant impact on the international trade of important foods and feeds, including those which originate in developing countries. Importing nations, frequently in the developed world, protect their populations from exposure to mycotoxins by introducing legislation which strictly limits the levels of mycotoxin (particularly aflatoxin) permitted in imported products. When adopting such a strategy, importing nations have a responsi-

Figure 13 A subsampling mill

bility to ensure that the permitted levels strike a fair balance between what is achievable by the producers in practice and the level of protection required by the consumers.

Representative samples of foods and feeds typically weigh between 3 and 20 kg (Coker, 1989; Coker, 1997). It is therefore essential that sample preparation procedures should allow the production of laboratory samples which are representative of the original sample. Laboratory samples may be produced by successively comminuting and dividing the sample, using either static or rotary dividers. Ideally, the comminution and sample-division steps should be performed simultaneously using a sub-sampling mill. The device illustrated in Figure 13 converts large samples of particulate materials into comminuted, representative sub-samples in a single operation.

Figure 14 A large shipment of animal feed

Those responsible for the production, processing and marketing of foods and feeds need to monitor the quality of the commodity constantly, either by implementing a traditional quality assurance programme or, ideally, through a Hazard Analysis Critical Control Point (HACCP) System. Again, a variety of contamination levels and quantities of materials will need to be addressed from, for example, a 50 000 tonne shipment of animal feed (*see* Figure 14) to a 50 g packet of roasted peanuts. Sampling plans will be needed which can be applied to a wide variety of situations. Ideally, the critical points identified by surveillance as contributing to the spoilage of the commodity and to the production of mycotoxins, should be integrated into quality control schemes and/or regulatory monitoring programmes.

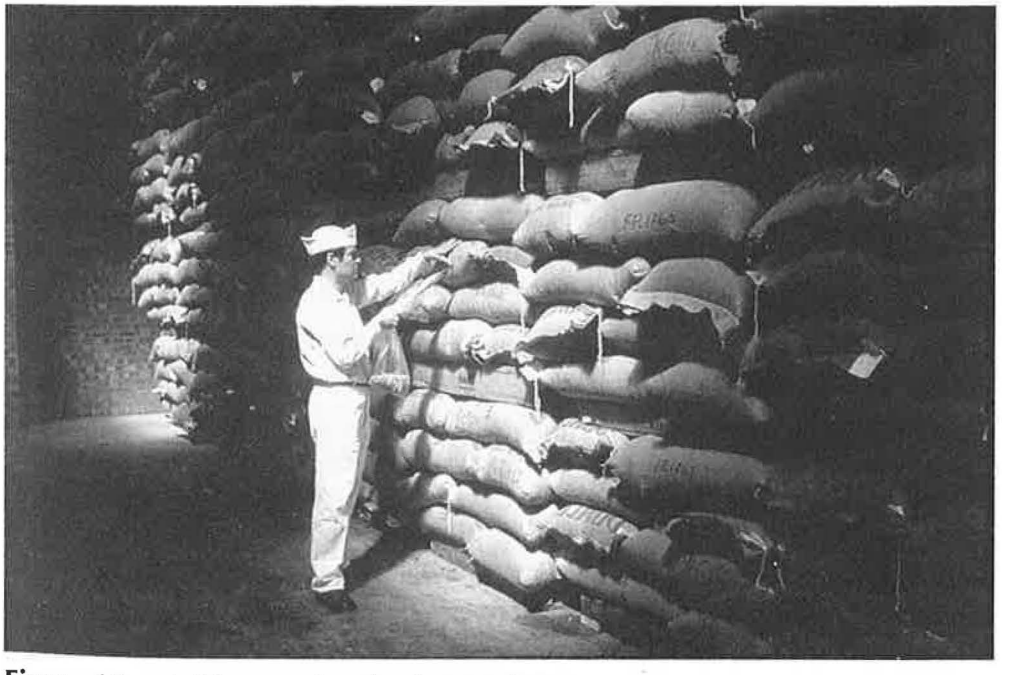


Figure 15 A 20-tonne batch of groundnuts

More than 50 countries currently regulate mycotoxins (especially the aflatoxins) in foods and feeds. There are usually specific regulations for both unprocessed and processed material. In the UK for example, a lot of unprocessed edible peanuts is acceptable if it contains no more than 10 µg/kg of total aflatoxins, whereas a retail pack (a small unit of food purchased by the consumer) of peanuts must contain no more than 4 µg/kg. In this example, attention is focused on a lot of unprocessed peanuts (typically around 20 tonnes in weight; *see* Figure 15) and on a small, retail pack (of around 100g) of processed nuts. Sampling plans which can be applied to both these situations are therefore required by the regulators.

The analysis of mycotoxins

The method of analysis will be determined by the nature of the job. If a large number of samples is to be analysed, an automated procedure such as HPLC, HPTLC or ELISA will be advantageous. However, automated methods offering high sample throughput, accuracy and precision require the purchase of expensive equipment. Such an investment would be justified in a busy quality control or research laboratory staffed by skilled analysts.

However, **rapid** methods, which are appropriate for the analysis of intermittent samples requiring a quick decision by relatively unskilled operators, are also required. Such methods must be simple to perform and must require relatively unsophisticated equipment.

Sample clean-up methods

The efficiency of chromatographic methods of analysis will be largely determined by the effectiveness of the sample clean-up step (i.e., the removal of interfering components from the initial, crude sample extract). Two recent developments have improved the precision of the clean-up procedure and facilitated the automation of this step.

The first development, known as solid phase extraction (SPE), involves the use of liquid phases bonded to an inert support, contained within a plastic cartridge (Coker and Jones, 1988). SPE utilizing a phenyl bonded-phase is used routinely at the Natural Resources Institute (NRI) for the clean-up of sample extracts of groundnut, cottonseed, corn, copra and oil-palm prior to the quantification of aflatoxin. A small volume of the crude sample extract (normally in aqueous acetone) is applied to the SPE cartridge which is then washed with water. After drying by the passage of air, the aflatoxins are eluted with chloroform and the eluate dried by passing through a sodium sulphate cartridge. Liquid handling equipment is now available which facilitates the automation of the SPE clean-up procedure.

After SPE clean-up, quantification may be performed by HPTLC, HPLC or another method.

At NRI, a combination of SPE (phenyl) clean-up and HPTLC quantification has been successfully applied to the analysis of aflatoxin in a variety of commodities including corn, cottonseed, oil-palm, copra and peanut butter (Bradburn *et al.*, 1988; Tomlins *et al.*, 1989; Bradburn *et al.*, 1990; Dell *et al.*, 1990; Nawaz *et al.*, 1992; Nagler, personal communication).

SPE clean-up methods have also been used in conjunction with HPLC quantification for the analysis of aflatoxin in groundnuts, cottonseed and corn (McKinney, 1981; Hutchins and Hagler, 1983; Hurst *et al.*, 1987; Coker and Jones, 1988).

The second development in improved clean-up methodology is the immunoaffinity cartridge. In this approach, the crude extract is passed through a cartridge containing monoclonal mycotoxin antibodies adsorbed on to an inert support. The retained mycotoxins are eluted from the affinity column with methanol prior to quantification using a method of choice. Affinity cartridges are commercially available for the analysis of the aflatoxins B_1, B_2, G_1, G_2 and M_1, and for the determination of fumonisin B_1, OA, DON and zearalenone.

After immunoaffinity clean-up, quantification may be performed by conventional HPLC methods, or by using a simple fluorometer for example.

Immunoaffinity clean-up has been used in conjunction with a simple fluorometer for the analysis of total aflatoxins in corn (Anon., 1990). In this procedure, the methanolic eluate from the affinity cartridge is treated with bromine solution to enhance the fluorescence of the aflatoxins B_1 and G_1. This method has been adopted by the USDA Federal Grain Inspection Service.

Rapid analysis methods

The utilization of sophisticated HPLC, HPTLC, GLC, ELISA and mass spectrometric procedures has been comprehensively reviewed elsewhere (Coker, 1984; Shephard, 1986; Coker and Jones, 1988; Beaver, 1989; Morgan, 1989; Coker *et al.*, 1993).

Effective quality control protocols require simple, rapid, efficient analysis methods which can be handled by relatively unskilled operators. Recently developed rapid methods include those which utilize immunochemical technology (*see* above) or selective adsorption agents.

Immunochemical technology has been applied to the development of rapid ELISA methods for a variety of mycotoxins including the aflatoxins, OA, fumonisins, zearalenone, T-2 toxin and DON.

A rapid ELISA method for estimating aflatoxin in groundnuts, cottonseed, corn, rice and mixed feeds has been subjected to a collaborative study and recommended for First Action Approval by the Association of Official Analytical Chemists (AOAC) (Anon., 1989).

Solid-phase ELISA kits have been developed for estimating aflatoxin, zearalenone, OA and T-2 toxin in a variety of commodities. An 'immunodot' cup test in which the antibody is immobilized on a disc in the centre of a small plastic cup, has been approved by the AOAC as an Official First Action screen for aflatoxin in groundnuts, corn and cottonseeds (Trucksess *et al.*, 1990).

Card tests have also been developed in which the antibody is immobilized within a small indentation on a card similar in size to a credit card. These tests have been developed for estimating aflatoxin, zearalenone, OA and T-2 toxin in corn.

The reported analysis (extraction, filtration and estimation) time for solid-phase ELISA kits is about 5–10 min. Doerner and Cole (1989) compared rapid ELISA and solid-phase ELISA methods with a conventional HPLC procedure for aflatoxin in groundnuts. There was a good agreement between the methods when the aflatoxin concentration of the sample was greater than 10ug/kg.

Mini-columns containing selective adsorption agents have been developed for aflatoxin/zearalenone (single test) and DON (Gordon and Gordon, 1990). In the aflatoxin/zearalenone test, the extracted mycotoxins are partitioned into toluene which is added to the mini-column. Selective adsorption agents remove interfering compounds and retain the mycotoxins as two discrete bands at the lower tip of the column. Designated levels of the mycotoxins can be observed under long-wave UV light.

The Federal Grain Inspection Service (FGIS) has evaluated eight commercially available, rapid tests for aflatoxin in corn. FGIS-approved kits include rapid ELISA, immunoaffinity cartridge, solid-phase ELISA and selective adsorbent mini-column procedures (Emnett, 1989).

Accredited methods

Although a number of procedures for the determination of the aflatoxins has been adopted by the AOAC and similar organizations, the only other mycotoxins represented by 'official' methods for foods and feeds are DON in wheat (two methods), OA in barley and green coffee, zearalenone in corn (two methods), patulin in apple juice and sterigmatocystin in barley and wheat. Also, the unavoidable delay between the development and validation of a new method, and the considerable cost of organizing collaborative studies, often result in the use of considerably out-dated 'official' methods. Current AOAC methods for the aflatoxins, for example, include procedures developed between 1968 and 1989; six of the eight 'official' methods were developed before or during 1980. The methods for DON and OA were developed in 1986 and 1973, respectively, and the 'official' procedures for zearalenone were developed in 1976 (TLC) and 1985 (HPLC).

This situation militates against the use of state-of-the-art procedures for the surveillance, monitoring and quality control of mycotoxins in foods and feeds. Given the current trend towards the accreditation of analytical methods within individual laboratories, an alternative to the use of 'official' methods may be to develop an accredited method which fulfils defined efficiency criteria (limit of detection, precision and accuracy) when implemented by an individual laboratory. National laboratories would be responsible for the administration of the proposed method accreditation scheme, including the organization of check sample studies to confirm that analytical laboratories are meeting the required efficiency criteria. Inter-country harmonization would be of paramount importance. Harmonization within Europe, for example, could be pursued through the EC Measurement and Testing Programme.

The concept of utilizing accredited methods for the determination of mycotoxins has been endorsed by the EC expert committee, 'Agricultural Contaminants'. The use of Certified Reference Materials (CRMs) provided by the Community Bureau of Reference (BCR) Measurement and Testing Programme for the quality assurance of analytical procedures, has been supported by the Comité Européan de Normalisation (CEN).

A similar approach to method accreditation has already been adopted by the AOAC for the validation of immunodiagnostic kits. In 1991, the AOAC Research Institute was established to review independently manufacturer-generated data and laboratory evaluations of test kits. The manufacturers of accredited kits are licenced to use the Institute's 'Performance Tested' certification mark.

The AOAC has recently established a Peer-Verified Methods (PVM) Advisory Committee to promote the rapid validation of newly developed methods. AOAC members are encouraged to seek PVM status for their methods so that more recently developed procedures can be used in conjunction with the AOAC Official Methods.

Bioassays

Even in a well-equipped laboratory, the chemical/immunochemical determination of mycotoxins will be limited to about 20 compounds; the simultaneous analysis of every sample for all possible mycotoxins will be prohibitively expensive and time-consuming. In addition, chemical analysis will not detect the presence of previously unknown mycotoxins.

Bioassays for the detection of mycotoxins can be defined as tests on biological systems designed primarily to confirm the presence of biologically-active compounds, the identity and concentration of which can be determined by chemical analysis.

In the first instance, a bioassay should be non-specific, highly sensitive, rapid and robust. Ideally, specific bioassays are also required to assist with the identification of the mycotoxin(s) under investigation.

The development of a bioassay requires the choice of a sensitive biological indicator and the establishment of a dose-response relationship which is sigmoid in shape but linear over a wide range of doses. The experimental protocol should also accommodate variations in species, age, sex, method of toxin administration, nutritional and disease status, and environmental factors, which can influence the effect of mycotoxins on animal species.

The use of bioassays for the detection of mycotoxins has been reviewed by Panigrahi (1993); test organisms include terrestrial and aquatic, animal and plant species.

A bioassay has been developed which exploits the sensitivity of the yeast, *Kluyveromyces marxianus,* to a range of mycotoxins (Dell, 1993; Engler, 1996). Two approaches have been used. First, measurement of the retardation in growth rate has facilitated the detection of a range of mycotoxins using an automatic microtitre plate-based procedure for recording the optical density of the yeast suspension. The second simple colorimetric approach exploits the inhibition of the induction of galactosidase enzyme by toxins.

It is clear that a combination of bioassay, chemical and immunochemical methods should be used as an integrated approach towards the effective determination of mycotoxins in foods and feeds. Such an approach requires a battery of bioassays which, given those methods currently available, could include plant-based, brine shrimp larvae, *Tetrahymena,* chick embryo, tissue culture and yeast bioassay systems.

Detoxification

Numerous oxidizing agents, aldehydes, acids and bases (inorganic and organic) have been investigated as potential chemical detoxification agents (Park *et al.*, 1988). Patented procedures exist which utilize ammonia, calcium hydroxide, hydrogen peroxide, methylamine and a mixture of calcium hydroxide and methylamine. Commodities which have been experimentally treated in this way include groundnut meal (ammonia, hydrogen peroxide, formaldehyde, calcium hydroxide plus methylamine, sodium hydroxide), cottonseed meal (ammonia, sodium hydroxide, calcium hydroxide), corn (ammonia,

sodium bisulphite, calcium hydroxide), copra (calcium hydroxide) and poultry feed (sodium hydroxide). Detoxification equipment has included large plastic silage bags (ammonia/corn, cottonseed), pelleters (sodium hydroxide/poultry feed; calcium hydroxide/copra), pressurized reaction vessels (ammonia/groundnut and cottonseed meal), unpressurized reaction vessels (calcium hydroxide plus formaldehyde/groundnut cake) and screw press extruders (calcium hydroxide plus methylamine/groundnut meal).

Ammonia detoxification

The chemical detoxification reagent which has attracted the widest interest is ammonia, both as an anhydrous vapour and an aqueous solution. Consequently, many studies have been performed within the EC and elsewhere, on the efficiency of ammonia detoxification and the nutritional and toxicological properties of ammoniated feeds.

Ammonia was first used for the detoxification of aflatoxin-contaminated cottonseed meal in the US in the late 1960s (Park *et al.*, 1988). Temporary Food and Drug Administraton (FDA) approval was obtained for using ammoniated cottonseed meal in feedstuffs for non-lactating ruminants and laying hens. However, although numerous nutritional and toxicological studies have since been performed under the auspices of the National Cottonseed Growers Association, formal FDA approval of the ammoniation process is still awaited.

Ammoniation has, however, been approved by some states including Arizona, California, Georgia, North Carolina and Alabama. Arizona and California allow the treatment of cottonseed products, and in Georgia, North Carolina and Alabama, corn is ammoniated.

The procedures used in Arizona are either atmospheric pressure/ambient temperature (APAT), or elevated temperature and pressure. The APAT procedure involves spraying cottonseed with aqueous ammonia and storing it for approximately two weeks in large, white silage bags. The elevated temperature/pressure procedure utilizes a large, rotating pressure vessel. Ammonia (4% w/w) and steam are added to the cottonseed to produce a pressure of 40 psi [270 kPa] and a temperature of 100 °C. A dwell-time of 30 min. is used. Although the APAT process has not been approved by the Californian authorities, a high temperature/pressure treatment is used which involves 1.5% ammonia, 14% moisture and a pressure of 30 psi [207 kPa].

A procedure for the detoxification of corn involves the successive addition of water (to 17.5% moisture) and ammonia to 29-tonne batches of corn held in special bins (Coker *et al.*, 1985). A mixture of ammonia and air is recycled through the corn for 48 h. The treated material is held at a mean temperature of approximately 36 °C for a further 11 days. The ammoniated corn is then transferred to a drying bin where it is deodorized and dried using hot air to a moisture content of about 10%.

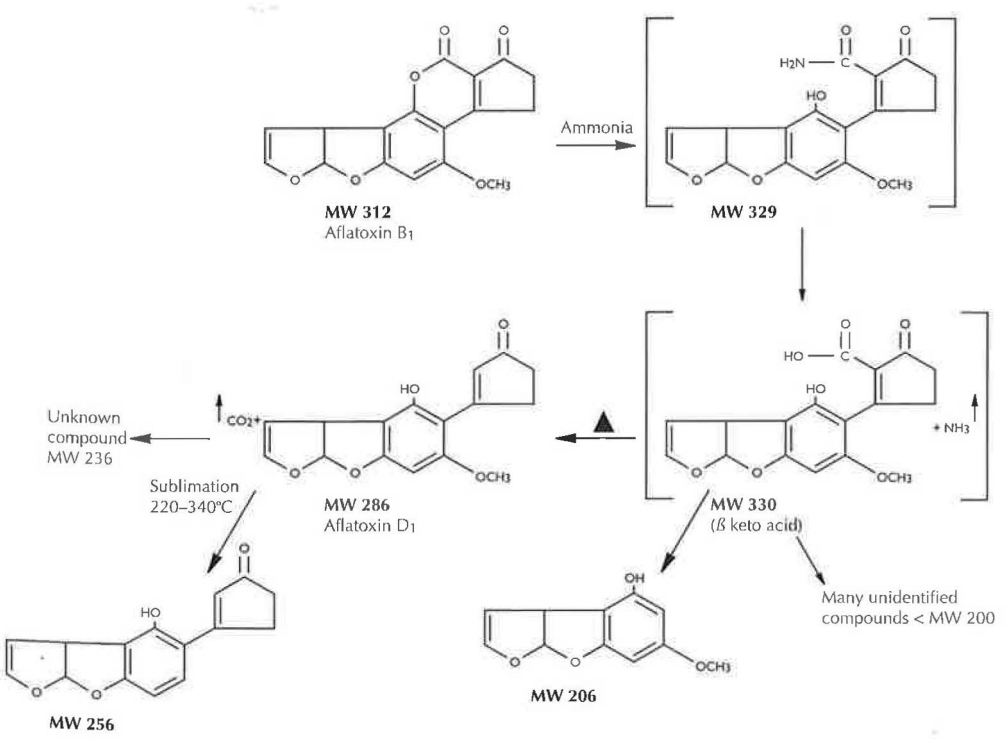
Commercial ammoniation plants for the treatment of groundnut meal are currently operating in Senegal, France and the UK.

The chemistry of ammoniation

The nature of the reaction products produced by the ammoniation of aflatoxin is still poorly defined. Most studies have focused on the reaction products of aflatoxin B_1 produced under a variety of conditions, including the treatment of: (a) pure toxin, *in vitro*; (b) pure toxin on an inert carrier; (c) feedstuffs spiked with pure toxin; and (d) naturally contaminated feedstuffs.

In vitro ammoniation of pure aflatoxin B_1 yields four compounds of molecular weights (MW) 286, 256, 236 and 206, and many unidentified compounds of MW less than 200. The compound of MW 286 has been characterized as the decarboxylated derivative (aflatoxin D_1) of aflatoxin B_1. The compound of MW 206 lacks the cyclopentenone ring of aflatoxin D_1. The loss of the methoxy group from aflatoxin D_1 produces the compound of MW 256. The reaction product of MW 236 has yet to be identified (*see* Figure 16).

When aflatoxin B_1 was treated with ammonium hydroxide at 100 °C, approximately 30% of the crude reaction product was attributed to aflatoxin D_1 (Lee *et al.*, 1974). In a similar experiment, the reaction product contained between 3 and 26% of compound MW 206, depending on the duration of the reaction. An increase in the concentration of this compound was accompanied by a decrease in the concentration of aflatoxin D_1 (Cucullu *et al.*, 1976).



From Park, 1988

Figure 16 The *in vitro* ammoniation of aflatoxin B_1

When pure radio-labelled aflatoxin B_1, on an inert carrier, was treated with ammonia at 40 psi [270 kPa] and 100 °C, 20% of the label was lost as volatile compounds while less than 1% remained as unreacted aflatoxin B_1. Although the reaction product of MW 206 was identified, no aflatoxin D_1 was detected (Lee *et al.*, 1984).

Further studies were carried out using groundnut meal spiked with labelled aflatoxin B_1. Treatment at 75 °C produced an ammoniated product which contained only 45-50% of the label. Unreacted aflatoxin B_1 and aflatoxin D_1 accounted for only 0.3 and 0.2% of the reaction product respectively. Some activity was also detected in the volatile fraction (Lee *et al.*, 1979).

Similar, larger scale studies involved the ammoniation of a 28 kg batch of naturally contaminated cottonseed meal additionally spiked with labelled aflatoxin B_1. Treatment at 40 psi [270 kPa] and 100 °C yielded an ammoniated product which accounted for about 86% of the radioactivity; 37% of the activity remained in the meal after solvent extraction and chemical and enzymic treatments (Park *et al.*, 1984). Subsequent mass spectral analysis of the ammoniated product confirmed the absence of both aflatoxin D_1 and compound MW 206 (Lee *et al.*, 1985). However, aflatoxin D_1 was detected by tandem mass spectrometry in very heavily aflatoxin-contaminated corn (175 ppm) after ammoniation at 37 °C for 21 days. The yield of aflatoxin D_1 was estimated to be substantially less than 10% (Grove *et al.*, 1984).

Ammoniation and the nutritional quality of feed

Ammonia will interact with a variety of compounds which determine the nutritional quality of feedstuffs. The nature of this interaction will be governed by the precise conditions of ammoniation and by the composition of the feedstuff. It may be difficult to predict fully the effect of ammoniation on compound feeds of variable composition.

Ammoniation produces changes in protein patterns accompanied by a reduction in, for example, the levels of cystine, methionine and available lysine. The nitrogen solubility index is also reduced, indicating that some degree of thermal/chemical denaturation of protein has occurred (Park *et al.*, 1988). Residual ammonia increases the level of non-protein nitrogen in ammoniated feeds. Although small increases of this nature can be accommodated by ruminants, excessive residual ammonia is highly undesirable in monogastric feeds. No significant changes have been reported in the levels of starch and lipid components (Peplinski *et al.*, 1983).

In vitro studies have shown that ammoniation increases amino acid digestibility and amyloglucosidase activity ((Adrian, 1976; Brekke *et al.*, 1978).

In general, ruminants showed improved weight gains and feed efficiency when fed ammoniated diets. Non-ruminants either showed no effect, or suffered a decrease in these parameters.

Undoubtedly, the attractiveness of ammoniated commodities as animal feedstuffs will be determined by the net result of a complexity of interactions.

Ammoniation and feed toxicity

The interaction of ammonia with both aflatoxin and the nutritional components of feedstuffs has been outlined in the two previous sections. The resultant composition of these reaction products will determine the effect of

ammoniation on both the nutritional and toxicological properties of the treated commodity. These properties will, in turn, determine the productivity of animals fed ammoniated feeds and the acceptability of animal products (milk, meat and eggs) as human food.

Toxicity of ammoniation reaction products

The toxicity of the reaction product, aflatoxin D_1, has been compared with that of aflatoxin B_1 using: (a) the Ames test (*Salmonella* mutagenicity); (b) the DNA covalent binding index (CBI); and (c) the chick embryo bioassay, as indicators of toxicity. Aflatoxin D_1 caused a 450-fold decrease in mutagenic potential, at least a 300-fold decrease in the DNA CBI (46), and a 20-fold decrease in toxicity to the chick embryo (Lee *et al.*, 1981). The reaction product, MW 206, was more than 600 times less mutagenic than aflatoxin B_1 (Haworth *et al.*, 1989).

Animal feeding trials

Very extensive feeding trials with ammoniated feeds have been carried out in a variety of animals including trout, rats, poultry, swine and beef and dairy cattle. The effect of ammoniation on the toxicity of aflatoxin-contaminated feeds has been determined by monitoring animal growth and organ weights, and by examining haematological, histopathological and biochemical parameters.

A 90-day feeding trial in male and female weaning Fischer 344 rats was performed using low pressure ammoniated (1.5% ammonia, 10 psi [69 kPa], 90 °C) groundnut cake (Manson and Neal, 1987). Four diets were prepared, each containing 75% groundnut cake and 25% powdered rat diet. Two diets (at 600 ppb) contained naturally aflatoxin-contaminated groundnut cake while the remaining diets were uncontaminated. One of each of the contaminated and uncontaminated diets was ammoniated. (The contaminated diet contained 40 ppb aflatoxin after ammoniation.) The hepatotoxic and potential hepatocarcinogenic effects of the diets were assessed by assaying the activity of γ-glutamyl transferase (GGT) in the rat livers. The results indicated that ammoniation of the aflatoxin-contaminated cake eliminated the development of focal GGT-positive (preneoplastic) lesions in both male and female animals. However, in the female rat, histochemical GGT staining of periportal hepatocytes was increased by all the experimental diets (including the ammoniated uncontaminated diet) compared with the untreated, uncontaminated diet. Ammoniated diets also decreased body weight gains.

The toxicity of low pressure ammoniated groundnut cake was also assessed by feeding ducklings five diets for 22 days (Coker *et al.*, 1983). The diets consisted of a control (zero aflatoxin) and feeds containing 5 and 25% ammoniated or untreated aflatoxin-contaminated cake. The ducklings which received the diet (300 ppb total aflatoxin) containing 25% untreated cake showed very low growth rates; all birds in this group had died by day 14 of the trial. No significant difference in growth was apparent between those ducklings receiving a control diet (<2.0 ppb aflatoxin) and those receiving 5% ammoniated cake (5 ppb). The performances of birds fed diets containing 5% untreated cake (50 ppb) and 25% ammoniated cake (15 ppb) were slightly reduced compared to the other groups. (The high natural biological variation within each group of ducklings made an accurate assessment of the significance of small differences in performance extremely difficult.) Microscopic examination showed that there were no abnormal lesions in the livers of ducklings which had received a diet containing 5% ammoniated

groundnut cake, but five of the nine livers from ducklings fed on the diet containing 25% ammoniated groundnut cake showed slight centriacinar bile duct proliferation; there was also evidence of single cell necrosis in one liver. All nine birds receiving a diet containing 25% untreated cake had marked bile duct proliferation, with five of the livers also showing single cell necrosis. Of the nine ducklings on 5% untreated cake, the livers of eight showed slight bile duct proliferation; the liver of the remaining duckling showed a marked proliferation of the bile duct. There was no evidence of centriacinar fibrosis in the livers of ducklings which had received diets containing ammoniated groundnut cake.

Rat feeding trials were carried out over a two-year period (Park *et al.*, 1988). The diets contained uncontaminated or aflatoxin-contaminated cottonseed meal, either ammoniated or untreated, at inclusion rates of 20%. None of the male rats on untreated, contaminated diets survived the trial. Female survival was also significantly reduced compared to rats fed uncontaminated diets. Organ weights in male rats were similar for all the ammoniated diets and were significantly lower than those in rats fed uncontaminated feed. Rats receiving either uncontaminated or ammoniated diets did not exhibit liver neoplasms.

The safety of meat and eggs produced by animals receiving diets containing ammoniated feeds has been evaluated by the USDA and the FDA (Park *et al.*, 1988). Diets containing uncontaminated or aflatoxin-contaminated corn (either ammoniated or untreated) were fed to poultry, pigs and beef cattle. Meat and eggs from these animals were then fed to rats (as 20% of the diets) for a 15-18 month period. No toxic or carcinogenic effects were reported. (A reduction in the survival of male rats receiving eggs was attributed to nutritional deficiencies in the diet.) Multi-generation studies on rats have been carried out by the USDA using poultry and beef tissue derived from animals fed ammoniated, aflatoxin-contaminated corn (Food and Drug Administration, 1982). A teratological evaluation indicated that foetal development was not affected by the consumption of tissue (as 20% of the diet) derived from poultry and cattle fed ammoniated corn for periods of 15-18 months.

The toxicity of milk derived from dairy cattle fed ammoniated cottonseed meal has been evaluated in rainbow trout (Sinnhuber, unpublished material). The aflatoxin content of contaminated cottonseed meal (about 4000 ppb, total) was reduced to <5.0 ppb aflatoxin by ammoniation. Ingestion of the treated meal (about 3.6 kg/cow/day) yielded milk with no detectable aflatoxin M_1. The freeze-dried milk was fed to rainbow trout as 25% of the diet for 12 months. The low incidence of observed hepatomas was similar to that induced by low dietary levels of aflatoxin.

Ammoniated corn has been fed directly to trout as 25% of the diet (Brekke *et al.*, 1977). There was no significant difference in the incidence of hepatomas between those fish fed uncontaminated corn and those receiving the ammoniated diet.

Poultry The effect of ammoniated feeds on the productivity, disease resistance and biochemical parameters in poultry has been examined.

Feeding trials with day-old ducklings, using ammoniated groundnut cake produced by a low pressure ammoniation process, are described above. Studies with day-old chicks have also been reported elsewhere (Delort-Laval *et al.*, 1980). Diets containing ammoniated groundnut cake did not affect feed intake, although a slight reduction in weight gain and feed conversion efficiency was observed. These reductions, however, could be reversed by adding methionine to the diet.

The effect of ammoniation on the effectiveness of vaccination against Newcastle Disease has been evaluated by feeding White Leghorn layer-breeders with ammoniated corn (Boulton *et al.*, 1981). No reduction in haemagglutination-inhibition (HI) titres was observed after vaccination in birds which had received either ammoniated/contaminated or ammoniated/uncontaminated corn. A significant reduction in HI titres was exhibited by those birds receiving untreated diets contaminated with 500 ppb aflatoxin.

The effect on egg production and quality has also been studied in White Leghorn layer-breeders fed ammoniated diets (Hughes and Jones, 1979; Hughes *et al.*, 1979). Ammoniation had no effect on egg production, egg weight, fertility or hatchability. However, reductions in feed consumption and weight gain were reported for those birds fed ammoniated diets.

Pigs Ammoniated corn has been fed to weanling pigs (Keyl and Norred, 1978). No reductions in weight gain or feed efficiency were observed after a period of 120 days.

Dairy cattle The effect of ammoniated feeds on dairy cattle, and on the concentration of aflatoxin M_1 in milk, has been extensively studied (Park *et al.*, 1988). Three feeding trials which used ammoniated groundnut cake and ammoniated cottonseed meal will serve to illustrate the typical results obtained.

Ammoniated cottonseed meal and whole cottonseed were fed to lactating Holstein cows for 14 days. No aflatoxin M_1 could be detected in the milk, but 3-5 ng/litre could be detected after feeding untreated, contaminated feed; this is equivalent to a daily intake of approximately 6.0 (cottonseed) and 1.0 (meal) µg aflatoxin B_1 (McKinney *et al.*, 1973).

The effect of feeding ammoniated groundnut cake at 5-17 ppb aflatoxin B_1 to Holstein Friesian cows over a 16-month period has also been investigated (Fremy *et* al., 1987). No aflatoxin M_1 levels in excess of 0.01µg/litre were observed. The contaminated, untreated diet containing 20% contaminated groundnut cake, had approximately 220 ppb aflatoxin B_1.

The effect of ammoniation at low and medium pressure on the carry-over of residual aflatoxin B_1 into milk was studied (Coker *et al.*, 1995). Six groups of 10 Holstein cows were fed the diets shown in Table 4. There were no statistically significant differences between diets B, C and D, and it was concluded that the incorporation of ammonia-detoxified groundnut meal into dairy feed does not enhance the carry-over of aflatoxin from feed to the milk.

Table 4 The percentage carry-over of aflatoxin B_1 into milk as aflatoxin M_1

Diet	Aflatoxin B_1 in feed (µg/kg)	Treatment	Percentage carry-over
A	42.5	Untreated groundnut meal (GNM) at 615 µg/kg	0.86
B	4.1	Low pressure detoxified GNM at 43 µg/kg	1.20
C	3.4	Medium pressure detoxified GNM at 21 µg/kg	1.72
D	3.0	Uncontaminated GNM + GNM at 615 µg/kg	1.59

The presence of aflatoxin in feedstuff is causing severe economic losses and threatening human health throughout the world, and the imposition of legislation by importing countries within the developed world is highlighting the aflatoxin problem within the exporting countries of the Third World.

The chemical detoxification of contaminated feeds has a major role in the prevention of economic loss and human disease. Although the use of ammonia as a detoxification reagent has been very widely investigated and commercially exploited, the use of ammoniated commodities as animal feeds has not been widely accepted. This acceptance will not be forthcoming until general agreement has been reached on the toxicological implications of ammoniation. The US FDA's Center for Veterinary Medicine has established a Public Master File as a repository for information on the toxicology of ammoniated feeds to enable data on this matter to be effectively reviewed.

THE METABOLISM OF MYCOTOXINS

An examination of the metabolic fate of aflatoxin B_1, ochratoxin A and fumonisin B_1 will illustrate the importance of the metabolic process in determining toxicity, and in determining exposure to mycotoxins, by measuring (a) mycotoxin-macromolecular conjugates (b) the parent mycotoxin and (c) biochemical change initiated by the mycotoxin.

The metabolism of aflatoxin

Numerous animal studies (*in vivo,* and using animal tissues *in vitro*) have been carried out on the metabolic fate of the aflatoxins, primarily aflatoxin B_1. There have also been limited studies on the measurement of aflatoxin B_1 and its metabolites in the blood, urine, milk and isolated tissues of humans.

The metabolic fate of the aflatoxins may be considered under the headings of administration, absorption, transformation (activation and detoxification), distribution and excretion.

Administration

Under natural conditions, exposure to the aflatoxins may occur orally (by food ingestion) and by tracheal and bronchial absorption (by the inhalation of contaminated dust). In addition to these 'natural' routes, intraperitoneal (ip), intravenous (iv) and dermatitic administration have been used under experimental conditions.

Absorption

Studies using radio-labelled aflatoxin B_1 in rats and monkeys have demonstrated that there is little difference in the distribution and excretion of the toxin after either oral or ip administration; this suggests that absorption following oral exposure is complete (Wogan *et al.*, 1967; Dalezios *et al.*, 1973). Further studies with sheep and rats have shown that after absorption the aflatoxins are transported via the blood and not via the lymphatic system (Wilson *et al.*, 1985; Kumagai, 1989).

Aflatoxin B_1 can also be absorbed rapidly, by passive diffusion, from the small intestines (especially the duodenum) into the mesenteric venous blood. Given the lipophilic nature of aflatoxin B_1, the composition of the intestinal epithelium is an important criterion. Hsieh and Wong (1994) found that over 50% of radio-labelled toxin disappeared from the duodenum within 1 h, appearing in the venous blood as water-soluble metabolites, protein adducts and free toxin. Protein adducts (in which the aflatoxin is linked to the lysine component of serum albumin) represented over 50% of the total radioactivity; the water-soluble metabolites occurred in only minor quantities.

Free aflatoxin B_1 represented less than 33% of the labelled material, the concentration absorbed decreasing with time, presumably as a result of gastro-intestinal metabolism. Although the liver is regarded as the main site of aflatoxin transformation, gastro-intestinal metabolism will reduce the exposure of the liver to aflatoxin B_1 and, in terms of hepatic toxicity, is an important means of detoxification. Importantly, the administration of low doses (comparable to human exposure) of toxin to rat duodenum resulted in the absence of free aflatoxin B_1 from the circulating blood.

No evidence has been found for the reabsorption of aflatoxin metabolites resulting from entero-hepatic recirculation.

Transformation

The transformation of aflatoxin B_1 results in both the activation and the detoxification of the toxin and may be considered as occurring in two phases: the first phase is the transformation of the toxin to a selection of metabolites; the second is the conversion of some of these metabolites to either water-soluble conjugates or macromolecular adducts. The transformation process is modulated by numerous factors including the genetic make-up of the species, nutritional and health status, and exposure to metabolic modifiers in foodstuffs.

The major metabolites of aflatoxin B_1 include: aflatoxin B_1-8,9-epoxide, -8,9-dihydro-8,9-diol; the aflatoxins B_{2a}, P_1, M_1 and Q_1; and aflatoxicol, aflatoxicol H_1 and aflatoxicol M_1 (*see* Figure 17) (Essigmann *et al.*, 1982). However, not all metabolites have been identified in all species. Their role in the toxicity of aflatoxin B_1 will now be discussed under the sub-headings activation and detoxification.

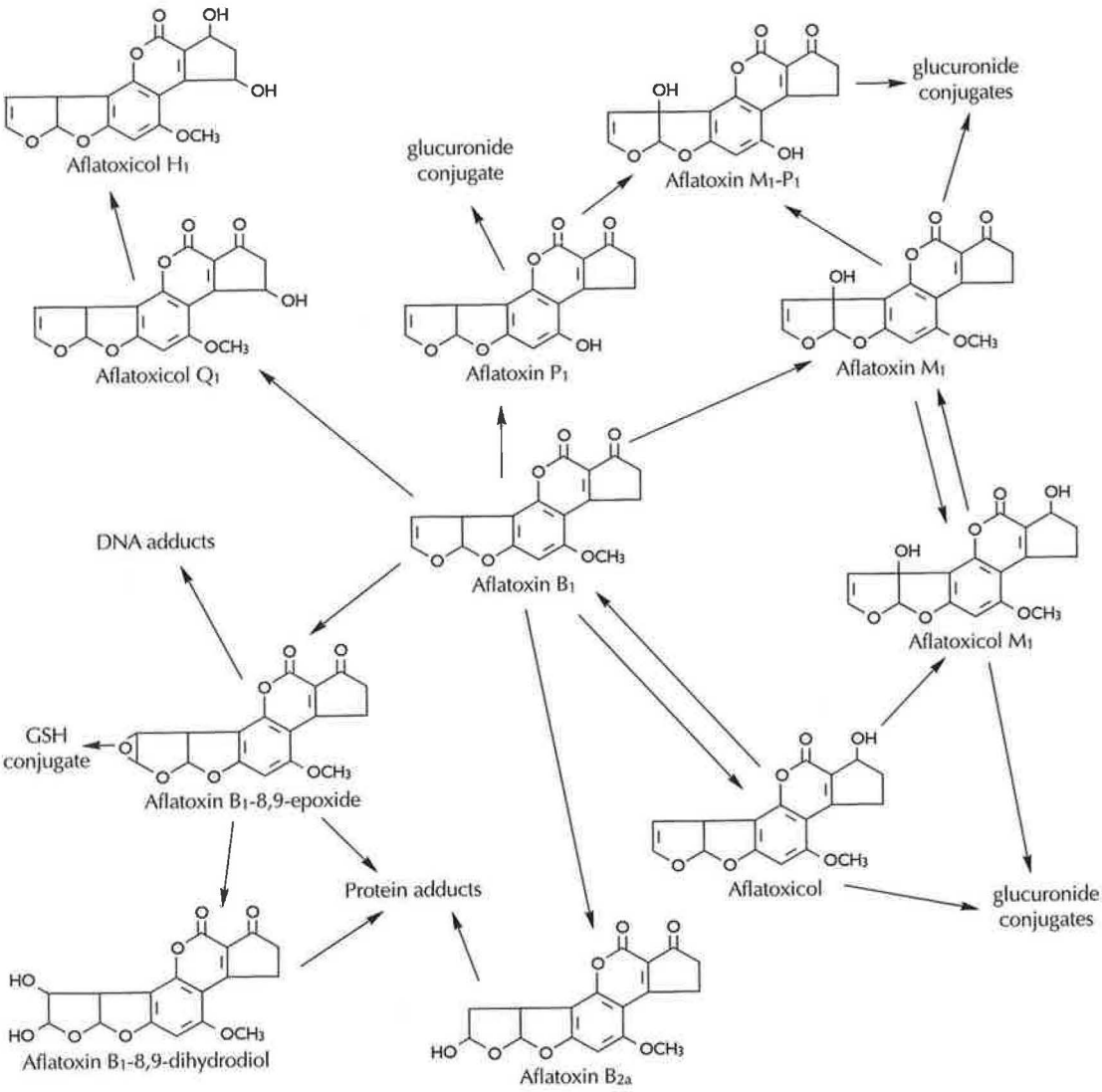
Activation

In the liver, aflatoxin B_1 may interact with both DNA and protein to elicit the carcinogenic and acutely toxic effects of aflatoxin, respectively. Initially, aflatoxin B_1 is converted by P450 cytochromes to the highly reactive aflatoxin B_1-8,9-epoxide which, in turn, may be converted to aflatoxin B_1-dihydrodiol (*see* Figure 17) (Baertschi *et al.*, 1989).

The carcinogenicity of aflatoxin B_1 arises from its interaction with the guanine moiety of DNA to produce the aflatoxin-N7-guanine adduct (Baertschi *et al.*, 1989), whereas the acute toxicity of aflatoxin B_1 is believed to stem from interaction between the dihydrodiol and protein amino groups to produce Schiff base adducts (Appleton *et al.*, 1982).

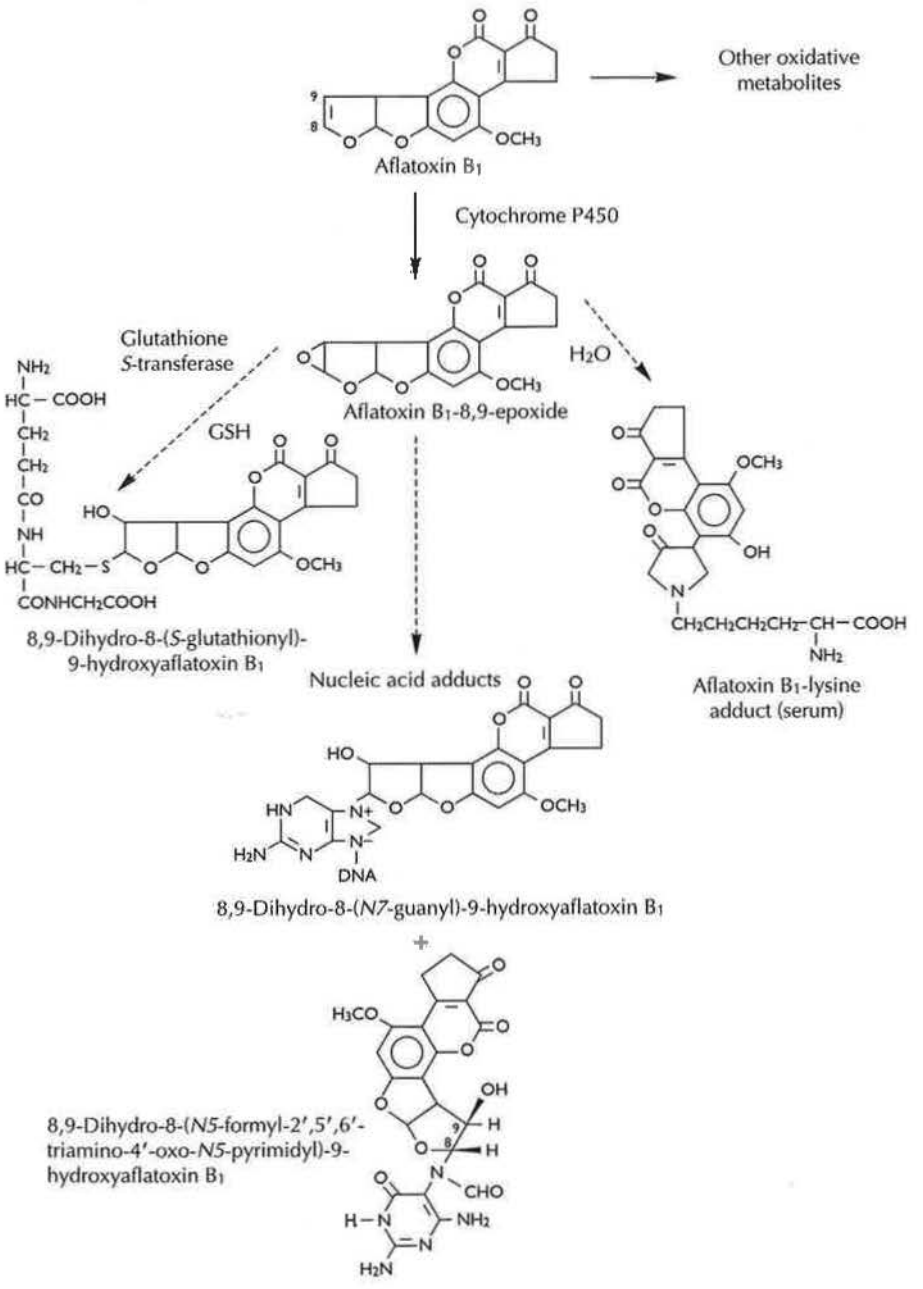
The aflatoxin metabolites M_1 and P_1 can also form DNA adducts (Essigmann *et al.*, 1983). It has been hypothesized that aflatoxin B_2 can be reduced to B_1 which, in turn, undergoes epoxidation and binding to DNA (Groopman *et al.*, 1981). Similarly, there is evidence that aflatoxin G_1 can bind to DNA (Garner *et al.*, 1979).

Studies using guinea pig kidney microsomes have indicated that the two enzyme systems, cytochrome P450 and prostaglandin H synthase (PHS), are equally active in the formation of DNA adducts (Liu *et al.*, 1990). However, prostaglandin H synthase was only responsible for 1-2% of DNA-binding in guinea pig liver microsomes, reflecting the higher P450/PHS ratio in the guinea pig liver.



From Eaton, 1994

Figure 17 The biotransformation of aflatoxin B_1



From Essigman *et al.* (1982)

Figure 17 *continued*

Aflatoxin B_1 is also converted to a DNA-binding species by the human bronchus and colon in culture. The bronchus is more active than the colon (Autrup *et al.*, 1979).

Aflatoxicol is a major aflatoxin B_1 metabolite in rat plasma (Wong and Hsieh, 1978). Post-mitochondrial fractions of liver from a variety of species have been shown to have relatively high activity for aflatoxicol formation *in vitro* (Loveland *et al.*, 1979). A cytosolic reductase (believed to be 17-keto-steroid dehydrogenase) which catalyses the conversion of aflatoxin B_1 to aflatoxicol has been isolated from chicken liver (Chen *et al.*, 1981; Neal, personal communication). Aflatoxicol is reported to have carcinogenic potency equivalent to that of aflatoxin B_1 and about 70% the mutagenicity (Schoenhard *et al.*, 1981; Coulombe *et al.*, 1982). Since it can be readily converted back to aflatoxin B_1, it has been proposed that aflatoxicol may act as a 'reservoir' for aflatoxin B_1 *in vivo*, thereby prolonging the lifetime of the toxin in the body.

Detoxification

The detoxification process occurs *via* the conversion of aflatoxin B_1 to less active hydroxylated metabolites, such as the aflatoxins M_1, Q_1 and P_1, and *via* the formation of the glutathione conjugate and glucuronides (*see* Figure 17).

However, whereas aflatoxin Q_1 is much less acutely toxic, mutagenic and carcinogenic than aflatoxin B_1, aflatoxin M_1 is a relatively potent carcinogen and only slightly less acutely toxic than aflatoxin B_1 (Hsieh *et al.*, 1974; Hendricks *et al.*, 1980; Coulombe *et al.*, 1982). The glutathione (GSH) conjugate of aflatoxin B_1-epoxide has been identified as the major metabolite in the bile of rats (Holeski *et al.*, 1987). GSH conjugation is mediated by cytosolic glutathione S-transferases (GSTs) and is an important reaction when determining the suceptibility of species to the toxic effects of aflatoxin (Ramsdell and Eaton, 1990a).

The important role played by GSTs in the detoxification process can be clearly illustrated by comparing the susceptibility of the mouse and rat to tumour induction. Although the mouse has a very high microsomal epoxidation activity, it is very resistant to the carcinogenicity of aflatoxin B_1, presumably because of its very high level of simultaneous GST activity (Hayes *et al.*, 1991). Of particular significance is the occurrence, in the mouse, of an alpha class GST (YC_2) which is highly active towards aflatoxin B_1-epoxide, and which is not found to any significant extent in adult rats or humans (Neal, personal communication).

One particular GST (the mu form M1a-1a) has shown considerable activity towards the aflatoxin B_1 endo-epoxide (Raney *et al.*, 1992). However, although human microsomes produce both endo- and exo-epoxides, it appears that the exo-epoxide binds primarily to DNA. The identification of the most important GSTs, and their distribution within and between populations, is crucial to understanding the manifestation of aflatoxin toxicity. (About 50% of Caucasians, for example, possess a gene deletion for the GST mu form M1a-1a.)

The glucuronides of aflatoxicol and aflatoxins M_1, P_1 and Q_1 have been identified as biliary metabolites of aflatoxin B_1 (Metcalfe and Neal, 1983; Loveland *et al.*, 1984; Eaton *et al.*, 1988). Current data suggest that conjugation occurs more readily at the aromatic hydroxyl group in aflatoxin P_1 than at the aliphatic hydroxyls in the aflatoxins M_1 and Q_1, although the importance of M_1 and Q_1 glucuronidation has still to be resolved (Eaton *et al.*, 1994).

An index of species susceptibility to aflatoxin B_1 has been produced using the activation/inactivation (detoxification) ratio in which activation = (rate of B_1-epoxide formation)/Σ(rates of B_1-epoxide and aflatoxins M_1, P_1 and Q_1 formation), and inactivation = (rate of cytosolic B_1-epoxide conjugation with GSH)/(rate of microsomal B_1-epoxide formation) (Degen and Neuman, 1981). The susceptibility indices for the rat, mouse, monkey and man are shown in Table 5; they are based on data produced *in vitro* using hepatic microsomes and cytosol. The high activation/inactivation index (18.00) for humans results mainly from the very low inactivation ratio (0.008) compared to the other species. Conversely, the low activation/inactivation index (0.13) for the mouse can be attributed to a high inactivation ratio (2.9).

Table 5 The activation/inactivation index for a variety of species

Species	Activation	Inactivation	Activation/inactivation
Rat	0.42	0.068	6.20
Mouse	0.39	2.9	0.13
Monkey	0.09	0.033	2.80
Human	0.14	0.008	18.00

Any evaluation of species susceptibility must also consider the kinetics of the metabolic pathways involved in the activation and detoxification of aflatoxin. For example, when comparing rat, mouse, monkey and man, the patterns of liver microsomal oxidation of aflatoxin B_1 vary significantly with changes in substrate concentration. In rat and human microsomes, the proportion of aflatoxin B_1 converted to the epoxide increases with decreasing B_1 concentration, indicating that humans may be relatively susceptible to aflatoxin carcinogenesis at the low exposure levels typically experienced (Ramsdell and Eaton, 1990b).

Non-nutritive components of foods of plant origin, including cruciferous vegetables such as broccoli, Brussels sprouts and cabbage, can modulate the biotransformation, DNA-binding and carcinogenesis of aflatoxin B_1. Ramsdell and Eaton (1988) reported that broccoli appears to enhance the detoxification of the aflatoxin epoxide in rats. Plants of the *Allium* genus have also shown anti-carcinogenic effects. Organosulphur compounds in garlic, and capsaicin in chilli peppers, are reported to decrease aflatoxin-DNA binding *in vitro* (Tadi *et al.*, 1991; Teel, 1991).

Distribution

After absorption from the intestine, aflatoxin B_1 rapidly enters the liver through the hepatic portal vein. The toxin is heavily concentrated in the liver after oral, ip and iv administration. Much less aflatoxin accumulates in the kidney. When radio-labelled aflatoxin B_1 was administered ip to rats, approximately 17% of the label was present in the liver within 30 min.; the kidneys and the eviscerated carcass contained about 5% and 27%, respectively. Traces (<0.5%) of labelled material were present in the adrenal glands, brain, heart, pancreas, spleen, thymus and testes (Wogan *et al.*, 1967). The radioactivity diminished rapidly, only 10% remaining in the liver after 2 h. In a similar experiment, Holeski *et al.* (1987) found that 2 h after administration, 70% of the residual toxin was in the form of covalently bound adducts. An orally-administered monkey still retained about 1% of the dose five weeks after administration (Dalezios *et al.*, 1973).

Excretion

Excretion of aflatoxin B_1 occurs primarily through the biliary pathway and to a lesser extent, the urinary pathway, and by excretion into the milk of lactating animals.

Biliary excretion When radio-labelled aflatoxin B_1 was fed to rats, the plasma half-life for radioactivity was 91.8 h; 23 days after dosing, 70% of the radioactivity had been excreted, 55% in the faeces and 15% in the urine (Coulombe and Sharma, 1985). In a further study, the rate of biliary excretion peaked at 30 min.; the major metabolites were the aflatoxin-glutathione adduct (about 53% of the total biliary radioactivity) and aflatoxin P_1 glucuronide (about 10%).

Urinary excretion Approximately 15% of radio-labelled aflatoxin B_1 was excreted in urine 20–24 h after ip administration to rats. The major metabolites were the aflatoxins M_1 (45% radioactivity) and P_1 (<10%), and aflatoxin B_1-N7-guanine (16%). The latter is the major degradation product of hepatic B_1-DNA adducts (Groopman *et al.*, 1994). Eighty per cent of excreted B_1-guanine was measured in the urine during the 48 h period following dosing, and a dose-dependent correlation between B_1 and B_1-guanine was observed in male rats (Bennett *et al.*, 1981; Essigmann *et al.*, 1982).

Aflatoxin M_1 was also the major urinary metabolite following oral administration of labelled aflatoxin B_1 to monkeys (Dalezios *et al.*, 1973).

Aflatoxin P_1 is the major metabolite in the mouse. The mouse is the only species known to excrete aflatoxin Q_1 in urine (Wei *et al.*, 1985).

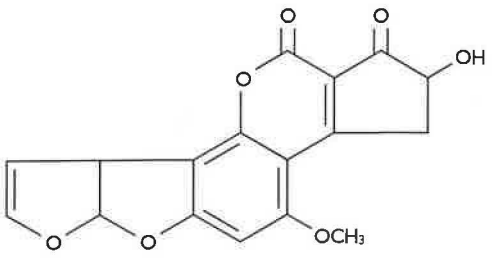
Analysis of urine collected from humans in the Guangxi region of China showed that the same metabolites were present as in rats (the aflatoxins M_1, P_1 and aflatoxin B_1-guanine); aflatoxin M_1 was the major metabolite (Groopman *et al.*, 1985). Males excreted about 1-2 % of ingested B_1 whereas females excreted lower levels.

Excretion through cow's milk Aflatoxin B_1 in dairy feed can be metabolized and transferred to cow's milk in the form of aflatoxin M_1. The percentage carry-over rate typically lies within the range of 1 to 5%, depending, for example, on the level of aflatoxin in the feed and the productivity of the cow. Generally, the carry-over rate increases as the feed contamination level decreases and as the cow productivity increases. However, the carry-over rate varies significantly from cow to cow and, on an individual cow basis, from day to day. Coker *et al.* (1995) reported a mean carry-over rate of 1.5% when cows were fed diets (including ammonia-treated diets) containing approximately 3.0 µg/kg aflatoxin B_1 (*see* Table 4). In the same study, a diet containing approximately 40 µg/kg aflatoxin B_1 resulted in a carry-over rate of 0.9%.

When goats were dosed with very low levels of radio-labelled aflatoxin B_1, aflatoxin Q_1 and aflatoxicol were identified in the milk, in addition to aflatoxin M_1 (Helferich *et al.*, 1986).

A hydroxy derivative of aflatoxin M_1 known as aflatoxin M_4 (*see* Figure 18) has been reported to co-occur with aflatoxin M_1 (at levels up to 16% of aflatoxin M_1); the toxicity and carcinogenicity of aflatoxin M_4 is reported to be greater than that of the aflatoxins B_1 and M_1 (Lafont and Lafont, 1987).

Excretion through human milk The presence of aflatoxins in human breast milk has also been reported. In Africa (the Sudan, Ghana, Kenya and Nigeria), the aflatoxins M_1, M_2, B_1, B_2, G_1 and G_2 have all been found in breast milk (Lamplugh *et al.*, 1988; Maxwell *et al.*, 1989). Aflatoxin M_1 was the major metabolite, occurring at concentrations ranging from 20 to 1800 ng/litre in Ghana.



Aflatoxin M_4

Figure 18 Aflatoxin M_4

The metabolism of ochratoxin A

According to an IARC evaluation, OA causes renal toxicity, nephropathy and immunosuppression in several animal species (IARC, 1993e). However, although there is sufficient evidence in experimental animals for the carcinogenicity of OA, evidence for its carcinogenicity in humans is inadequate.

The possible role of OA in the aetiology of human disease, including the possible linkage between exposure to OA and both Balkan endemic nephropathy and urinary tract tumours, has led to studies of the metabolic fate and toxicity of the parent toxin and its metabolites.

No data are available on the absorption, distribution, metabolism and excretion of OA in humans. However, it has been reported that *in vitro*, OA binds with extremely high affinity to unidentified macromolecules in human plasma (saturation occurring at the low level of 10–20 ng/ml OA in serum) and to plasma proteins ((Stojkovic *et al.*, 1984; Hult and Fuchs, 1986; Hagelberg *et al.*, 1989).

The DNA adducts induced in various mouse organs after treatment with the toxin show that OA is a genotoxic carcinogen. Pfohl-Leszkowicz *et al.* (1993) compared DNA adducts observed in OA-treated mice with DNA adducts from patients with urinary tract tumours (and with a suspected past exposure to OA). The analysis of ^{32}P post-labelled autoradiograms suggested that some of the DNA adducts from OA-treated mouse kidney co-migrated with those detected in humans. Although it is not yet known if the adducts retain the OA moiety or one of its metabolites, the identification of DNA adduct patterns provides a potential tool for the molecular dosimetry of OA exposure in humans.

OA administered to rats by gavage at about 4 ppm was found in the fat, small intestine, testis, kidney, liver, heart, lung, spleen, stomach, muscle and brain (in decreasing order of concentration) after 24 h. After 48 h, the level in fat had increased, whereas all other levels had decreased (Kane *et al.*, 1986).

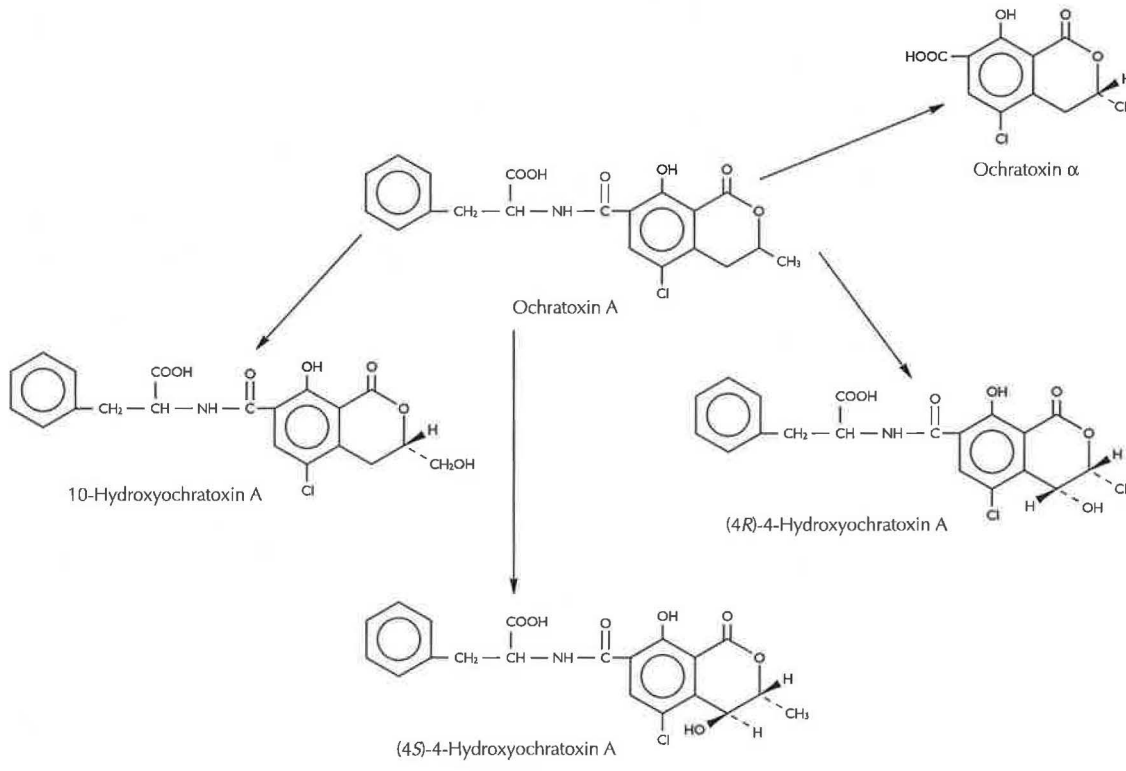
In ruminants, low levels of OA are rapidly hydrolysed by the ruminal flora (Galtier and Alvinerie, 1976; Hult *et al.*, 1976). However, OA was detected in the kidney, milk and urine of cows when a high dose of OA was ingested (Ribelin *et al.*, 1978; Shreeve *et al.*, 1979).

The plasma elimination half-time of OA varies very significantly between animal species. Hagelberg *et al.* (1989) report that it varies from 0.68 h in fish, to 120 h in rats and 510 h in monkeys.

OA can be metabolized *in vitro* to ochratoxin α, (4R) and (4S)-4-hydroxyochratoxin A, and 10-hydroxyochratoxin A (*see* Figure 19). The incubation of OA with rat, rabbit, pig and human liver microsomes produced both the 4R and 4S-hydroxy derivatives, the ratio between the two epimers depending on the animal species (Størmer *et al.*, 1981, 1983). The transformations were P450-dependent (Oster *et al.*, 1991). Rabbit liver microsomal treatment also produced 10-hydroxyochratoxin A. Incubation of OA with primary rat hepatocytes produced the 4R-hydroxy epimer as the major metabolite (Hansen *et al.*, 1982).

Fink-Gremmels *et al.* (1995) reported the formation of five different phenobarbital-inducible metabolites when pig microsomes were exposed to OA. Six stable metabolites were detected in the supernatant of primary cultures of pig hepatocytes, representing 50% of the initial OA concentration. The major metabolite has been characterized as the 4-hydroxy-derivative of OA (although the precise epimer has not been reported).

OA is hydrolysed to ochratoxin α by, for example, homogenates of duodenal, ileal, pancreatic and caecal tissues (Suzuki *et al.*, 1977; Galtier, 1978).



From Støren *et al.* (1982); Størmer *et al.* (1983)

Figure 19 Metabolites of ochratoxin A

The formation of (4R) and (4S)-4-hydroxyochratoxin A has been linked with cytochrome P450 forms which exhibit several similarities with rat CYP450 2B1 (Fink-Gremmels *et al.*, 1995). The same workers have also demonstrated that CYP450 isoenzymes belonging to the subfamilies 1A1, 1A2, 2C9 and 3A4 (expressed in genetically engineered 3T3 fibroblasts) are able to convert OA into mutagenic derivatives. However, further work is necessary on the fate of the Phase 1 metabolites of OA described above, and the role of glutathione conjugation in the transformation of OA has yet to be determined.

The metabolites of OA are less acutely toxic than the parent compound (Chakor *et al.*, 1988). However, Creppy *et al.* (1983) reported that the immunosuppressive activity of (4R)-4-hydroxyochratoxin A is similar to that of the parent toxin. Furthermore, the metabolites obtained by the treatment of OA with isolated hepatocytes are mutagenic when subjected to the *Salmonella*/microsome assay (Hennig *et al.*, 1991). The non-mutagenic activity of the microsomal metabolites of OA is believed to be the result of the lower metabolic capacity of microsomal incubations, rather than a qualitative difference in metabolic products.

When OA was administered to mice by gavage, 4-hydroxyochratoxin A was detected in the intestine, liver and bile. Up to 68% of biliary OA was in a conjugated form after 24 h (Chakor *et al.*, 1988).

The biliary excretion of OA is very efficient. For example, when radiolabelled (^{3}H) OA was administered to rats and mice either orally or intramuscularly, entero-hepatic circulation resulted in the detection of radio-label in the intestinal contents and serum (Fuchs *et al.*, 1988; Breitholtz-Emanuelsson *et al.*, 1992).

The relative contribution of each excretory route for OA varies with the species under study (Kuiper-Goodman and Scott, 1989). In albino rats for example, approximately 25% of the toxin was excreted in the urine as ochratoxin α, whereas 6% and 1–1.5% was excreted as OA and (4R)-4-hydroxyochratoxin A, respectively.

The concentration of specific OA metabolites in the urine was also strain-dependent. The ratio of OA to 4-hydroxyochratoxin A in the urine of dark Agouti female rats, for example, was two to five times higher than in Lewis rats (Castegnaro *et al.*, 1989).

The metabolism of fumonisin B_1

The fumonisins B_1 (FB1) and B_2 (FB2), which are the most commonly occurring in nature, are reviewed with the emphasis on fumonisin B_1.

Oral administration of FB1 to male rats produced hepato-cellular carcinomas, inducing the formation of foci (γ-glutamyltranspeptidase-positive) hepatocytes (IARC, 1993d). No data are available on the carcinogenicity of FB2.

FB1 causes outbreaks of leukoencephalomalacia (LEM) in horses and pulmonary oedema in pigs. It is toxic to the central nervous system, liver, pancreas, kidney and lung in a number of animal species. FB2 is hepatotoxic in rats (IARC, 1993). However, neither FB1 or FB2 were mutgenic to *S. typhimurium*, nor did they induce unscheduled DNA synthesis in rat hepatocytes, either *in vitro* or *in vivo*.

When FB1 was administered to rats by multiple ip injection, the effects included dehydration, nephrotoxicity and immunotoxicity (Bondy *et al.*, 1995). Suzuki *et al.* (1995) reported that urine volume, proteinuria, enzymuria and ion transport were sensitive indicators of early FB1-induced nephrotoxicity.

FB1 also altered sphingolipid metabolism and disrupted the barrier function of endothelial cells in culture (Ramasamy *et al.*, 1995). Injury to vascular endothelial cells, and the concomitant leakage of fluid into underlying tissues, could be a predisposing factor in both LEM in horses and pulmonary oedema in pigs.

Although the molecular mode of action of the fumonisins is not known, they appear to bear a remarkable structural similarity to sphingosine, the long-chain backbone of sphingomyelin and other sphingolipids. (Sphingolipids are believed to be involved in the regulation of cell growth, differentiation and neoplastic transformation.) Consequently, the ability of FB1 to inhibit the formation of the sphingolipids has been studied *in vitro* and confirmed using both rat hepatocytes and a pig kidney cell line LLC-PK_1 (Wang *et al.*, 1991; Yoo *et al.*, 1992). Merrill *et al.* (1993) also studied the role of FB1 in the inhibition of the biosynthesis of the sphingolipids in rat (Swiss 3T3) fibroblasts. The addition of FB1 led to a large accumulation of sphinganine and sphingosine by inhibiting the metabolism of sphinganine by the enzyme N-acyltransferase. In turn, the accumulation of the sphingoid bases stimulated a doubling of DNA synthesis. The same group of workers also argue that FB1 can be regarded as a tumour promoter because of the mitogenic properties of the accumulated sphingoid bases at cellular concentrations of 0.2-2.0 nmol/mg protein (Schroeder *et al.*, 1994).

Shephard *et al.* (1992) monitored the metabolic fate of ^{14}C-labelled FB1 in rats following either ip or oral administration. For the ip-administered toxin, 66, 32 and 1% of the radioactivity were recovered in the faeces, urine and liver, respectively, after 24 h of dosing. When administered orally, all the label occurred in the faeces. Similar results were obtained when FB1 was administered to vervet monkeys.

It appears that FB1 (and possibly other fumonisins) is rapidly excreted after ingestion in a largely unchanged form. Consequently, the pathological effects induced by FB1 in the rat must be caused either by extremely low concentrations of toxin retained by the rat, or by secondary reactions initiated by the presence of FB1.

THE BIOMONITORING OF MYCOTOXINS

Biomonitoring of aflatoxins

The introduction of methods for biomonitoring individual members of a population is a major development which will contribute significantly to the confirmation of the perceived linkage between mycotoxin exposure and human disease.

The development of biological markers (biomarkers) for the aflatoxins is based on knowledge of the metabolism and the critical macromolecular adduct formation of these toxins. Aflatoxin biomarkers may be used as a means of estimating the exposure of individuals to these toxins, and of predicting the risk of developing liver cancer and other diseases.

The role of aflatoxin biomarkers in humans is now discussed in terms of their role as markers of internal dose, biologically effective dose, early biological effect, and susceptibility.

Markers of internal dose

Markers in urine

Aflatoxin M_1 is a predominant metabolite in human urine. Its presence may be detected by thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA). Immunoaffinity columns have also been used to clean up the samples prior to quantification.

Zhu *et al.* (1987) analysed 252 urine samples over a three-day period in the Guangxi Region of China, to compare dietary exposure to the aflatoxins with urinary excretion of aflatoxin M_1. Between 1.2 and 2.2% of the dietary aflatoxin B_1 appeared in the urine as aflatoxin M_1, with a good correlation ($r = 0.65$) between the ingested and excreted toxins.

Markers in milk

The occurrence of aflatoxin M_1 in human breast milk is an indicator of both exposure of individual mothers to aflatoxin in food, and of the exposure of their infants to this toxin. However, a good correlation between levels of ingested aflatoxin B_1 and levels of aflatoxin M_1 in human breast milk has not been reported.

In a study in The Gambia, milk from five lactating mothers was collected once a day for four to five days and analysed for aflatoxins by a combination of immunoaffinity clean-up and HPLC quantification (Zarba *et al.*, 1992). The carry-over of aflatoxin, in the form of aflatoxin M_1, varied from 0.09 to 0.43%. Aflatoxin G_1 was also found in the milk of three of the five women.

The occurrence of aflatoxin M_1 in human breast milk was also studied in Zimbabwe and France (Wild *et al.*, 1987). In Zimbabwe, 11% of 54 samples of milk contained up to 50 pg/ml (approximately 0.05 ppb) aflatoxin M_1; none of the 42 samples collected in France was contaminated.

Markers in blood

Unmetabolized aflatoxin B_1 in human blood serum has been used as an indicator of recent exposure to aflatoxins in food. For example, aflatoxin B_1 has been detected in serum samples collected in Japan, Nigeria and Sudan (Hendrickse *et al.*, 1982; Tsuboi *et al.*, 1984; Denning *et al.*, 1988). The detection methods used were ELISA and HPLC; up to 3 ng/ml (approximately 3 ppb) B_1 were detected. In a recently reported study (Denning, personal communication) in The Philippines, blood samples were collected from 115 children with acute lower respiratory infection (ALRI). Aflatoxin B_1 was detected in 33% of the serum samples, with a mean value of 462 pg/ml (approximately 0.5 ppb). There was a strong association between those serum samples with no detectable aflatoxin and patient death, perhaps reflecting, in turn, an association between anorexia (and the presumed concomitant inability to metabolize aflatoxin) and poor patient outcome.

In a study carried out during the dry season in Songkla, Thailand, Denning *et al.* (1990) demonstrated that aflatoxins can cross the placental barrier in humans. In a group of 35 mothers, 17 of the cord sera samples contained the aflatoxins B_1, G_1 and Q_1 whereas only two maternal sera samples were contaminated.

Similarly, the aflatoxins M_1, M_2, B_1, B_2, G_1 and G_2 have been detected in cord sera from Ghana (34% of 188 samples), and aflatoxins M_1, M_2 and B_2 were present in cord sera collected in Nigeria (12% of 78 samples) (Lamplugh *et al.*, 1988).

Markers of biologically effective dose

Two biomarkers of biologically effective dose have been developed. The first is a urinary aflatoxin B_1-DNA adduct and the second is an adduct between aflatoxin B_1 and serum protein.

Aflatoxin B_1-DNA adduct in urine

The urinary excretion kinetics of specific metabolites after a single exposure to aflatoxin B_1 were studied in rats. Aflatoxin-N7-guanine (the primary B_1-DNA adduct; *see* Figure 17) accounted for 7.5% of the total detectable aflatoxins, and the aflatoxins P_1, Q_1, M_1 and B_1 accounted for 31.5, 3.0, 2.2 and 0.3% of total aflatoxins, respectively (Groopman *et al.*, 1992). During the 24 h after exposure, an excellent correlation ($r = 0.99$) existed between the oral dose of aflatoxin B_1 and the urinary aflatoxin-N7-guanine adduct. The other metabolites showed no such relationship.

Systematic evaluation in several human populations has validated the aflatoxin-guanine adduct as a measure of biologically effective dose. Studies performed in Kenya and China, for example, have confirmed the correlation between human exposure to aflatoxin B_1 and urinary aflatoxin-guanine adduct (Autrup *et al.*, 1987; Groopman *et al.*, 1992).

In the Murang'a district of Kenya, 12.6% of over 1000 urine samples contained the aflatoxin-guanine adduct. The levels of the adduct reflected regional differences, with the highest concentrations being found in samples from the Western Highlands and Central Province.

The Guanxgi Region is one of the areas of high liver cancer incidence in China. Dietary exposure to aflatoxin (mainly from corn) and corresponding levels of urinary aflatoxin metabolites were measured, over one week, in a group of 30 males and 12 females. A combination of immunoaffinity clean-up and HPLC was used to determine the levels of a range of metabolites. The most commonly detected compounds were aflatoxin-guanine adduct and the aflatoxins M_1, P_1 and B_1. However, acceptable correlation between exposure to aflatoxin and urinary metabolic excretion occurred only for the aflatoxin-guanine adduct ($r = 0.65$) and aflatoxin M_1 ($r = 0.55$).

Aflatoxin-albumin adduct in blood serum

Blood samples from the same subjects in the Guanxgi Region of China were also analysed for the aflatoxin-serum albumin adduct (*see* Figure 17) using a combination of immunoaffinity clean-up and competitive radio-immunoassay (RIA) (Gan *et al.*, 1988). A highly significant correlation of adduct level with aflatoxin exposure ($r = 0.69$, $P < 0.000001$) was observed. It was calculated that between 1.4 and 2.3% of ingested aflatoxin B_1 appeared as the serum albumin adduct.

The use of the aflatoxin-albumin adduct as a marker of the biologically effective dose offers two advantages over the measurement of the aflatoxin-DNA adduct. First, whereas the aflatoxin-DNA adduct reflects exposure to aflatoxin on the previous day, the level of aflatoxin-albumin adduct is a measure of chronic exposure to aflatoxin over the previous two to three months (Hall and Wild, 1994). Second, the collection of finger-prick samples of peripheral blood is far more convenient than the collection of urine.

The aflatoxin-albumin adduct has been measured in children and adults from a variety of African and other countries. In Africa, between 12 and 100% of the samples contained the adduct whereas in Thailand, levels and incidence were lower. Studies in France and Poland failed to identify any positive sera samples (Hall and Wild, 1994).

In The Gambia, a study of 30 pregnant women demonstrated a good correlation ($r = 0.52$, $P = 0.001$) between levels of the aflatoxin-albumin adduct in sera from maternal venous blood and the umbilical cord (Wild *et al.*, 1991).

Markers of early biological effect

Measures of mutation spectra

Studies in the field of molecular biology have led to a better understanding of the genetic alterations which occur during the progression from initiation to tumour formation, and to the development of sensitive tests for the diagnosis of tumours.

The p53 tumour suppressor gene is mutated in more than 50% of all human tumours (Hollstein *et al.*, 1991). The numbers and types of mutations in this gene (the mutation spectrum) are not equally distributed, but occur in specific hot-spots which vary with the aetiology of tumour formation. For example, studies on liver cancer tumours in areas with a high exposure to the aflatoxins (Qidong, China; Mozambique) showed a high frequency of G → T transversion at codon 249 of the p53 gene (Bressac *et al.*, 1991; Hsu *et al.*, 1991). To date, the same mutation has rarely been observed in North America, Europe or Japan where exposure to the aflatoxins is low and does not appear to be related to hepatitis B virus infection (Ozturk *et al.*, 1991).

In vitro studies using the human p53 gene have shown that codon 249 is the preferential site for the formation of aflatoxin-N7-guanine adducts (Pusieux *et al.*, 1991). Exposure of cultured human liver cells to aflatoxin B_1 has produced codon 249 mutations *in vitro* (Neal, personal communication).

Although the link between aflatoxin exposure and specific p53 gene mutations in human populations has still to be confirmed, the gene mutation spectrum has considerable potential as a marker for exposure to, and damage from, the aflatoxins. Currently, mutations detected in the p53 gene of epithelial cells shed into the urine are used to diagnose bladder cancer (Sidransky *et al.*, 1991). Another possibility is the examination of DNA from lymphocytes isolated from blood samples for evidence of mutation (Neal, personal communication).

Measures of immunosuppressive activity

Although the effect of aflatoxin on susceptibility to infection has been clearly demonstrated in a range of animal species (including chickens, turkeys, pigs, guinea pigs, calves, rabbits, rats, mice and cows), little or no data are avail-

able on the immunosuppressive effects of aflatoxins in human populations (Pier and McLoughlin, 1985; Denning, 1987). Current tests for immunosuppressive activity include (a) neutrophil monocyte activity and (b) lymphocyte transformation (Denning, personal communication).

Markers of susceptibility

Measures of genetic variation in metabolism

Susceptibility to a particular agent will depend on the ability of individuals to absorb, distribute and metabolize that agent, and on the nature of the metabolic process. The ability of individuals to repair damage inflicted by the agent will also contribute to the level of susceptibility.

Studies with human liver microsomes have shown that the cytochrome P450s involved in the activation (epoxidation) of aflatoxin B_1 varies with the level of exposure. The activation of high levels of aflatoxin B_1, for example, is performed by cytochrome P450 3A4 (CYP3A4), with the simultaneous production of aflatoxin Q_1 (Shimada and Guengerich, 1989). Conversely, low levels of aflatoxin B_1 (typical of human exposure) are activated by cytochrome CYP 1A2, with the simultaneous formation of aflatoxin M_1.

Since cortisol is also a substrate for CYP3A4 and is metabolized to 6β-hydroxycortisol, the ratio of these two steroids in the urine can be used as a marker for hepatic CYP3A4 activity (Joellenbeck *et al.*, 1992). The steroids may be determined either by ELISA or HPLC methods. A marker for CYP 1A2 activity has not been reported.

To date, biomarkers of cytochrome activity have not been applied to populations exposed to substantial levels of aflatoxins.

Although some workers have accorded the enzyme, glutathione S-transferase mu, a significant role in the detoxification (by conjugation to glutathione) of the aflatoxin epoxide metabolite, others have now demonstrated the importance of alpha class enzymes in the detoxification of aflatoxin in the mouse ((Liu *et al.*, 1991; Raney *et al.*, 1992; Neal, personal communication).

Although an *in vitro* assay (involving trans-stilbene as the substrate) and a polymerase chain reaction (PCR)-based genotyping assay are available for the monitoring of glutathione S-transferase mu, these procedures have not been used in populations exposed to significant levels of aflatoxins (Zhong *et al.*, 1991).

Biomonitoring of ochratoxin A

A variety of HPLC methods have been reported for the determination of OA in animal and human products.

Phillips *et al.* (1983) developed a method for estimating OA in chicken kidney and human plasma, and Bauer and Gareis (1987) reported a method for OA in blood.

One HPLC procedure for the determination of OA in kidney (and corn and barley) was subjected to an IUPAC collaborative study (Nesheim *et al.*, 1992). Briefly, extraction with chloroform:phosphoric acid was followed by liquid partitioning and solid phase extraction (SPE) (C18) clean-up. Reversed phase HPLC was then performed, using water:acetonitrile:acetic acid as the mobile phase, and fluorescence detection.

A combined SPE/C18 HPLC method for the determination of OA in pig serum has also been described by Takeda *et al.* (1991). The presence of OA was confirmed by temperature-controlled, pre-column methylation to partially derivatize the sample toxin and afford a specified methyl-OA:OA ratio. A detection limit of 0.1 ng/ml OA in pig serum was reported.

Immunoaffinity clean-up cartridges have also been used in conjunction with HPLC for the determination of OA in kidneys and pig liver ((Bisson *et al.*, 1994; Marley *et al.*, 1995). In the pig liver test, a 1:1 ratio of methanol:-phosphate buffered saline was used for extraction, followed by an immunoaffinity clean-up. The OA in the cleaned-up sample was quantified by reversed phase C18 HPLC in conjunction with fluorescence detection.

OA and its metabolites were determined using an ion-pair reversed phase HPLC analysis method (Fink-Gremmels *et al.*, 1993). This involved elution of a Spherisorb ODS 2 column (250 x 4.6 mm ID) with two buffered (pH 6.6) acetonitrile-based solvent systems containing tetrabutylammonium as the ion-pairing agent. Fluorescence detection (335 nm excitation; 450 nm emission) was employed.

Ion-pair reversed phase HPLC with fluorescence detection has also been used to detect OA in samples of blood and milk collected in Sweden (Breitholtz-Emanuelsson *et al.*, 1993). A Pasteur pipette packed with silica gel was used for sample clean-up. The reported limits of detection and quantification for OA were 10 and 40 ng/litre, respectively, for cow's and human milk; the limit of quantification in human blood was 60 ng/litre. OA was found in five (14%) of 36 samples of cow's milk and 23 (58%) of 40 samples of human milk. Blood samples were collected from 39 of the 40 human milk donors and analysed for OA. All samples were contaminated at a mean concentration of 167 ng/litre (range 90-940 ng/litre). The concentration of OA in human milk was $\leqslant$0.1 of that in human blood. (However, the narrow concentration range in this study means that this value should be treated with some caution.)

The application of an ELISA method to the analysis of OA in pig kidneys was reported by Clarke *et al.* (1994). The lowest reproducible OA detection limit in spiked pig kidney samples was 7.81 µg/kg, with an interassay co-efficient of variation of 8.9%. Rabbit antisera had a high degree of cross-reaction with OA (and ochratoxin C), but not with ochratoxin B and α- or 4-hydroxy-OA.

Biomonitoring of fumonisin B_1

Wang *et al.* (1992) concluded that the ratio of sphingosine to sphinganine could possibly be used as an early marker for exposure to the fumonisins. A method involving the reversed phase HPLC determination of sphingosine and sphinganine in urine is currently under development (Wild, personal communication).

Shephard *et al.* (1992) have developed a reversed phase HPLC method for the determination of FB1 in plasma and urine. A detection limit of 50 ng/ml was reported when a solid-phase, anion-exchange clean-up column, pre-column derivatization with ortho-phthaldialdehyde, and fluorescence detection, were employed.

The presence of FB1 (and FB2) in milk has been determined using immunoaffinity clean-up and reversed phase (C18) HPLC, with fluorescence detection (Scott *et al.*, 1994). The reported recoveries for both fumonisins

averaged 79–109%, and the detection limits were about 3–7 ng/ml. Aminopentol (AP1, the hydrolysis product of FB1), was determined in milk by a combination of C18 solid phase extraction clean-up and reversed phase HPLC. A transmission study using four cows dosed with pure FB1 either orally (1.0 and 5.0 mg FB1/kg body weight) or by iv injection (0.05 and 0.2 mg FB1/kg body weight) showed no detectable residues of FB1 or AP1 in the milk, with or without hydrolytic treatment with β-glucuronidase/sulfatase to liberate any conjugates.

Future research needs

Future activities within the mycotoxin arena should address the Control System illustrated in Figure 11 in order to facilitate the effective operation of the four 'control' sub-systems:

- identification of quality constraints
- prevention of contamination
- detoxification of contaminated feed
- identification and segregation of contaminated material.

The effective operation of these sub-systems requires the introduction of **process quality management**. This involves the formation of inter-disciplinary teams to address those key intra- and inter-commodity system interactions which determine the quality of the **process-commodity matrix** (*see* Figure 9). In order for these teams to operate successfully, the following general requirements should be met:

- a better understanding of the aetiology of mould and mycotoxin production in the field, including an evaluation of those moulds and mycotoxins considered to be, potentially, of future importance;
- a better understanding of the occurrence of mycotoxins in fruit and vegetables;
- a better understanding of the human metabolism of mycotoxins;
- a better understanding of the role of mycotoxins in the epidemiology of human disease;
- the development of measures to prevent contamination (including drying and storage) which can be utilized by resource-poor communities in climatically-challenged environments;
- the development of mould- and/or mycotoxin-resistant crop varieties;
- the identification of detoxification procedures which yield a 'safe' product;
- the introduction of harmonized sampling and analysis procedures.

It is essential that significant resources are directed towards obtaining a better understanding of the role of mycotoxins in the epidemiology of human disease. The establishment, for example, of a firm relationship between mycotoxin exposure and immunotoxicity would highlight the need to devote resources to the removal of mycotoxins from the human food chain, especially in those regions where high risk commodities constitute a major component of the staple diet.

In order to facilitate the attainment of these requirements, work should continue with the development and validation of the following:

- cost-effective sampling methods for a variety of mycotoxin/commodity combinations
- rapid, simple, low cost mycotoxin analysis methods which can be used in unsophisticated laboratories

- biomarkers for the detection of exposure of individuals to mycotoxins, and for the detection of immunotoxicity
- simple bioassays for the detection of toxic principles
- short-term *in vitro* tests (reflecting both acute and chronic *in vivo* responses) using systems which have been transfected with human cytochromes
- gene probes for toxigenic moulds.

Again, it is especially important that work should continue on the development of biomarkers for the detection of exposure of individuals to mycotoxins, and for the detection of immunotoxicity, as means of evaluating the nature of the relationship between mycotoxin exposure and immunotoxicity.

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