Toxigenic fungi and mycotoxins

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Growth of commonly occurring filamentous fungi in foods may result in production of toxins known as mycotoxins, which can cause a variety of ill effects in humans, from allergic responses to immunosuppression and cancer. The most important mycotoxins are aflatoxins, ochratoxin A, fumonisins, trichothecenes and zearalenone. Aflatoxins are potent carcinogens and, in association with hepatitis B virus, are responsible for many thousands of human deaths per annum, mostly in non-industrialised tropical countries. Ochratoxin A is a probable carcinogen, and may cause urinary tract cancer and kidney damage in people from northern and eastern Europe. Fumonisins appear to be the cause of oesophageal cancer in southern Africa, parts of China and elsewhere. Trichothecenes are highly immunosuppressive and zearalenone causes oestrogenic effects in animals and man. Currently available records and statistics do not reflect the major role played by mycotoxins in mortality attributable to food-borne micro-organisms.

Only in the last 30 years has it become clear that commonly occurring fungi growing in foods and feeds may produce toxins, known as mycotoxins. These toxins have caused major epidemics in man and animals during historical times. The most important epidemics have been: ergotism, which killed hundreds of thousands of people in Europe in the last millennium; alimentary toxic aleukia (ATA), which was responsible for the death of at least 100,000 Russian people between 1942 and 1948; stachybotryotoxicosis, which killed tens of thousands of horses in the USSR in the 1930s; and aflatoxicosis, which killed 100,000 young turkeys in the UK in 1960 and has caused death and disease in other animals, and probably in man as well.

Mycotoxins are secondary metabolites, i.e. they appear to have no role in the normal metabolism involving growth of the fungus. Many are bizarre molecules, with structures ranging from single heterocyclic rings with molecular weights of scarcely 50 Da, to groups of irregularly arranged 6 or 8 membered rings with total molecular weights greater than 500 Da. Such small molecules induce no response in the human immune system. A major potential danger of mycotoxins in the human diet, therefore, resides in our inability to detect them biologically.
Mycotoxins have four basic kinds of toxicity: acute, chronic, mutagenic and teratogenic. The most commonly described effect of acute mycotoxin poisoning is deterioration of liver or kidney function, which in extreme cases may lead to death. However, some mycotoxins act primarily by interfering with protein synthesis, and produce effects ranging from skin sensitivity or necrosis to extreme immunodeficiency. Others are neurotoxins, which in low doses may cause sustained trembling in animals, but at only slightly higher doses cause brain damage or death.

Long-term effects of low levels of mycotoxin ingestion are also varied. The prime chronic effect of many mycotoxins is the induction of cancer, especially of the liver. Some toxins affect DNA replication, and hence can produce mutagenic or teratogenic effects.

The symptoms of mycotoxicoses are almost as diverse as the chemical structures of the compounds themselves. Some compounds may elicit few symptoms until death results, while others may produce severe effects including skin necrosis, leucopenia and immunosuppression. Doses producing chronic disease are usually far below those responsible for acute effects, and so long-term effects such as cancer or tumour induction are undetected at the time of ingestion and, indeed, may remain so until disease is quite advanced.

Many of the toxigenic fungi are ubiquitous and, in some cases, apparently have a strong ecological link with human food supplies. The natural fungal flora existing in conjunction with food production is dominated by three genera: Aspergillus, Fusarium and Penicillium. Fusarium species are destructive pathogens on cereal crops and other commodities, and produce mycotoxins before, or immediately after, harvest. Certain species of Aspergillus and Penicillium are also plant pathogens or commensals, but these genera are more commonly associated with commodities and foods during drying and storage. The most significant toxigenic species and mycotoxins are described below.

Aflatoxins

Aflatoxins are both acutely and chronically toxic to animals, including man, causing acute liver damage, liver cirrhosis, induction of tumours and teratogenic effects. The four major naturally produced aflatoxins are known as aflatoxins B₁, B₂, G₁ and G₂. 'B' and 'G' refer to the blue and green fluorescent colours produced by these compounds under UV light on thin layer chromatography plates, while the subscript numbers 1 and 2 indicate major and minor compounds, respectively. When aflatoxin B₁ and B₂ are ingested by lactating cows, a proportion (about 1.5%) is hydroxylated and excreted in the milk as aflatoxins M₁ and
M\textsubscript{2}, compounds of lower toxicity than the parent molecules, but significant because of the widespread consumption of cows’ milk by infants. Because of their high toxicity, low limits for aflatoxins in foods and feeds have been set by many countries. Under recent agreements, 15 \textmu g/kg of total aflatoxins is likely to become the maximum level permitted in all food commodities in world trade.

Acute toxicity of aflatoxins to humans has been observed only rarely\textsuperscript{8}. In 1974, an outbreak of hepatitis that affected 400 Indian people, of whom 100 died, almost certainly resulted from aflatoxins\textsuperscript{9}. The outbreak was traced to maize heavily contaminated with \textit{A. flavus}, and containing up to 15 mg/kg of aflatoxins. Consumption of toxin by some of the affected adults was calculated to be 2–6 mg in a single day. It can be concluded that the acute lethal dose for adult humans is of the order of 10–20 mg.

Aflatoxin B\textsubscript{1} has been demonstrated in animal species to be the most potent liver carcinogen known. Human liver cancer has a high incidence in central Africa and parts of Southeast Asia, so a link with aflatoxins appears likely. Studies in several African countries and Thailand showed a correlation between the logarithm of aflatoxin intake and the occurrence of primary human liver cancer\textsuperscript{10}. However, studies in areas of the US where dietary aflatoxin is appreciable indicated that aflatoxins are unlikely to contribute significantly to the incidence of liver cancer in the US\textsuperscript{11}.

The resolution of this conflict is now apparent: hepatitis B virus is also a liver carcinogen. Aflatoxins and hepatitis B are co-carcinogens, and the probability of people developing cancer of the liver is much higher in areas where both aflatoxins and hepatitis B are prevalent\textsuperscript{12}. Considerable evidence exists that high aflatoxin intakes are causally related to high human liver cancer incidence\textsuperscript{13,14}. Aflatoxin B\textsubscript{1} is considered to be a class 1 human carcinogen\textsuperscript{15}.

Levels of aflatoxins in some tropical foods\textsuperscript{16} and blood samples\textsuperscript{17} are sometimes unacceptably high. Based on the data of Pitt and Hocking (\textsuperscript{16} and unpublished), it has been estimated that the number of deaths from liver cancer due to aflatoxin in Indonesia alone exceeds 20,000 per annum\textsuperscript{18}.

From the medical viewpoint, the recent discovery that aflatoxins appear to be immunosuppressive is also important. Other effects observed include an influence on protein energy metabolism, haemoglobin levels and effectiveness of vaccines\textsuperscript{17}. Increased susceptibility to disease among people likely to have low resistance due to nutritional and environmental factors can only add to the toll.

Aflatoxins are produced in nature only by \textit{Aspergillus flavus}, \textit{A. parasiticus} and a recently described species, \textit{A. nomius}. \textit{A. flavus} is ubiquitous. Since the discovery of aflatoxins, it has become the most widely
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reported food-borne fungus, reflecting its economic and medical importance and ease of recognition, as well as its universal occurrence. *A. parasiticus* is apparently less widely distributed\(^1\), but the extent of its occurrence may be obscured by the tendency for *A. flavus* and *A. parasiticus* to be reported only as *A. flavus*. *A. nomius* is not of practical importance.

*A. flavus* and *A. parasiticus* have a particular affinity for nuts and oilseeds. Peanuts, maize and cotton seed are the three most important crops affected. Early work assumed that invasion was primarily a function of inadequate drying or improper storage, and these factors are certainly important in the occurrence of aflatoxins in the humid tropics. However, in temperate zones, invasion of these crops by *A. flavus* before harvest is of prime importance. Invasion of peanuts occurs as a result of drought stress and related factors\(^2\). Preharvest invasion in maize is partly dependent on insect damage to cobs, but the fungus can also invade down the silks of the developing ears\(^3\). Most other nuts are also susceptible to invasion\(^4\).

Cereals are a common substrate for growth of *A. flavus* but, unlike the case of nuts and oilseeds, small grain cereal spoilage by *A. flavus* is almost always the result of poor handling. Aflatoxin levels in small grains are rarely significant\(^5\). Spices sometimes contain *A. flavus*\(^6\), and aflatoxin levels may be high.

In industrialised countries, stringent sorting and clean up procedures are used to reduce aflatoxins to low levels in foods with a perceived risk. For peanuts, where fungal growth is usually accompanied by discolouration of the kernel, this includes the use of sophisticated colour sorting equipment. Statistically based sampling, the drawing of large samples, homogenising before subsampling and standardised aflatoxin assays are used to ensure that susceptible crops and foods meet the stringent requirements of health laws in both exporting and importing countries. Non-industrialised countries are often less fortunate. Established patterns of local consumption, where substandard nuts and maize may be consumed without any form of sorting or inspection, mean that aflatoxin ingestion remains far too high in many countries, especially in rural areas.

**Ochratoxin A**

Ochratoxin A is an acute nephrotoxin, with oral LD\(_{50}\) values of 20 mg/kg in young rats and 3.6 mg/kg in day-old chicks. It is also lethal to mice, trout, dogs and pigs\(^7\). Necroses of the renal tubules and periportal liver cells have been the main pathological changes observed after fatal doses. Ochratoxin A has immunosuppressive, embryonic, and probably
carcinogenic effects. Ochratoxin A plays a major role in the aetiology of nephritis (kidney disease) in pigs in Scandinavia\textsuperscript{24}, and indeed in much of northern Europe. This a serious animal health problem.

Because ochratoxin A is fat soluble and not readily excreted, it accumulates in the depot fat of affected animals, and from there is ingested by humans eating pork. A second source is bread made from barley or wheat containing the toxin. Ochratoxin A has been found in human blood over wide areas of Europe, with levels up to 35 \( \mu \text{g/kg} \) reported\textsuperscript{25}, and in human milk at similar concentrations\textsuperscript{26}. Although clear evidence of human disease is still elusive, such levels indicate a widespread problem with ochratoxin A in Europe.

Ochratoxin A was originally described as a metabolite of \textit{A. ochraceus}\textsuperscript{27}, a species with natural habitats in drying or decaying vegetation, seeds, nuts and fruits. \textit{A. ochraceus} and closely related species are widely distributed in dried foods of various kinds\textsuperscript{22}. Nuts are also a major source. Although \textit{A. ochraceus} has been isolated from a wide range of cereals, records are rather infrequent\textsuperscript{22}. It may be an important source of ochratoxin A in green coffee beans, however.

Ochratoxin A was also reported to be produced by \textit{Penicillium viridicatum}\textsuperscript{28}, and this view prevailed for more than a decade. Eventually it became clear that isolates regarded as \textit{P. viridicatum} but producing ochratoxin were correctly classified in a separate species, \textit{P. verrucosum}\textsuperscript{29}. \textit{P. verrucosum} has been reported almost exclusively in grain from temperate zones. It is associated with northern European barley and wheat, and has also been isolated quite frequently from meat products in Germany and other European countries. It does not appear to be common elsewhere\textsuperscript{29}.

Occasionally, isolates of the common species \textit{Aspergillus niger} can produce ochratoxin A\textsuperscript{30}. However, the closely related \textit{A. carbonarius} is a more common producer\textsuperscript{31,32}, and a much more important source of ochratoxin A. These species are widespread in tropical foods\textsuperscript{33,34}, and survive sun drying. \textit{A. carbonarius} is an important source of ochratoxin A in dried vine fruits, wines and probably coffee. The impact on human health of ochratoxin A from this species has not yet been assessed.

**Fumonisins**

Fumonisins were discovered in the late 1980s\textsuperscript{35,36} as the result of many years of study of the disease known as equine leucoencephalomalacia (LEM). Fumonisins consist of a 20 carbon aliphatic chain with two ester linked hydrophilic side chains, resembling sphingosine, an essential phospholipid in cell membranes. The toxic action of fumonisins appears to result from competition with sphingosine in sphingolipid metabolism\textsuperscript{37}. 
Symptoms of fumonisin toxicity vary widely with animal type, dosage and toxigenic fungal isolate. The best defined disease, LEM, is characterised by liquefactive necrotic lesions in the white matter of the cerebral hemispheres of horses and other equine species. Marked neurotoxicity is evident, with aimless walking and loss of muscle control followed by death, which usually occurs about 2 weeks after toxin ingestion.

The effect of fumonisins on humans has not been fully established, but much evidence suggests a role in human oesophageal cancer. Maize is the major staple food in areas of the Transkei in southern Africa where oesophageal cancer is endemic, and the most striking difference between areas of low and high incidence was the much greater infection of maize by *F. moniliforme* in the high incidence areas\(^{38}\). A similar situation occurs in parts of China with an exceptional incidence of oesophageal cancer\(^{39}\). The International Agency for Research on Cancer\(^{15}\) found that fumonisin B\(_1\) was a possible human carcinogen, but was neither mutagenic nor genotoxic. It alters the capacity of cells to proliferate\(^{37,40}\).

The major producer of fumonisins are *Fusarium moniliforme* and closely related species, which are endemic in maize throughout the world. Maize is the only significant source of these compounds\(^{22}\).

**Trichothecene toxins: deoxynivalenol and nivalenol**

Deoxynivalenol (DON; also known as vomitoxin) and nivalenol are among the many trichothecene mycotoxins produced by *Fusarium* species\(^{41}\). DON causes vomiting and feed refusal in pigs at levels near 8 mg/kg of feed\(^ {42}\). It was responsible for a large-scale human toxicosis in India in 1988, and human toxicoses have also been reported from China, Japan and Korea\(^ {43}\). Symptoms in humans include anorexia, nausea, vomiting, headache, abdominal pain, diarrhoea, chills, giddiness and convulsions\(^ {44}\).

Along with other trichothecenes, deoxynivalenol and nivalenol cause a variety of immunological effects in laboratory animals, leading to increased susceptibility to all kinds of microbial diseases\(^ {45}\). These toxins do not appear to be carcinogenic, but may act synergistically with aflatoxins\(^ {15}\).

The major source of these toxins is *F. graminearum*, a species endemic in wheat and other cereals throughout the world\(^ {22}\).

**Zearalenone**

Zearalenone is an oestrogenic toxin, also produced by *F. graminearum* and closely related species. The effect of zearalenone in animals is a well-
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defined syndrome. Maize, barley and wheat grains infected with *F. graminearum* and containing zearalenone cause genital problems in domestic animals, especially pigs. Symptoms include hyperaemia and oedematous swelling of the vulva in prepubertal gilts, or, in more severe cases, prolapse of the vagina and rectum. Reproductive disorders in sows include infertility, fetal resorption or mummification, abortions, reduced litter size and small piglets. Male pigs are also affected: atrophy of testes, decreased libido and hypertrophy of the mammary glands are all well documented\(^46\). Zearalenone has been implicated in several incidents of precocious pubertal changes in children\(^47\).

**Conclusions**

Mycotoxins are much more wide-spread and of much more concern in human food supplies than was believed a decade ago. The documentation of excessive levels of aflatoxin in foods and blood samples from people in non-industrialised countries, along with the synergistic effects of hepatitis B, mean that these toxins are a significant cause of death in parts of Africa and Southeast Asia at least. The detection of ochratoxin A in a wider range of foods than was previously supposed, and in the blood of many people, has raised awareness that this toxin is widespread. The realisation that many mycotoxins, including aflatoxins, fumonisins and trichothecenes, are immunosuppressive has wide implications for the ability of human populations to resist disease. It is very likely that mycotoxins play a significant role in the perceived poorer health of many tropical people. Food-borne bacteria rightly are a major cause for concern to human health, but it is difficult to escape the conclusion that mycotoxins in foods are responsible for much higher numbers of human deaths than are food-borne bacteria.

**References**

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