THE AFLATOXINS

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Naturally occurring aflatoxins

Aflatoxins consist of a group of approximately 20 related fungal metabolites, although only aflatoxins B1, B2, G1 and G2 are normally found in foods. Aflatoxins B2 and G2 are the dihydro derivatives of the parent compounds. They are produced by at least three species of Aspergillus, A. flavus, A. parasiticus and A. nomius, and can occur in a wide range of important raw food commodities, including cereals, nuts, spices, figs and dried fruit.

Although the highest concentrations are formed in food crops grown and stored in the warmer areas of the world, the international trading of these important commodities ensures that aflatoxins are not only a problem for the producing nations but are also of concern for importing countries. Aflatoxins M1 and M2 are the hydroxylated metabolites of aflatoxins B1 and B2 and are produced when cows or other ruminants ingest feed contaminated with these mycotoxins. They are then excreted in the milk and may subsequently contaminate other dairy products such as cheese and yoghurt.

Chemical and physical properties

Some important physical and chemical properties of the aflatoxins are given in the table:

<table>
<thead>
<tr>
<th>Aflatoxin</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Melting Point</th>
<th>UV absorption max (ε), nm, methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>C_{17}H_{12}O_{6}</td>
<td>312</td>
<td>268-269</td>
<td>265 360-362</td>
</tr>
<tr>
<td>B2</td>
<td>C_{17}H_{14}O_{6}</td>
<td>314</td>
<td>286-289</td>
<td>12,400 21,800</td>
</tr>
</tbody>
</table>

Aflatoxin B1
Aflatoxins are crystalline substances, freely soluble in moderately polar solvents such as chloroform, methanol and dimethyl sulfoxide, and dissolve in water to the extent of 10-20 mg/litre. They fluoresce under UV radiation.

Crystalline aflatoxins are extremely stable in the absence of light and particularly UV radiation, even at temperatures in excess of 100 ºC. A solution prepared in chloroform or benzene is stable for years if kept cold and in the dark. The lactone ring makes them susceptible to alkaline hydrolysis, and processes involving ammonia or hypochlorite have been investigated as means for their removal from food commodities, although questions concerning the toxicity of the breakdown products have restricted the use of this means of eradicating aflatoxins from food and animal feeds. If alkaline treatment is mild, acidification will reverse the reaction to reform the original aflatoxin. In acid, aflatoxins B₁ and G₁ are converted to aflatoxins B₂a and G₂a by acid catalytic addition of water across the double bond of the furan ring. Oxidising reagents react and the molecules lose their fluorescence properties.

**Toxicity and importance**

Aflatoxins are both acutely and chronically toxic. Aflatoxin B₁ is one of the most potent hepato-carcinogens known, and hence the long-term chronic exposure to extremely low levels of aflatoxins in the diet is an important consideration for human health. In the temperate, developed areas of the world, acute poisoning in animals is rare and in man is extremely unlikely. The outbreak of so called 'Turkey-X disease', which caused the deaths of 100,000 turkeys and other poultry in the United Kingdom in 1960, was caused by extremely high concentrations of aflatoxins in imported groundnut meal. This alerted industry and governments to the potential devastating effects of mycotoxins, particularly of the aflatoxins.

Acute aflatoxin toxicity has been demonstrated in a wide range of mammals, fish, birds; rabbits, dogs and primates. Ducks, turkeys and trout are all highly susceptible. Age, sex and nutritional status all affect the degree of toxicity. Young animals are particularly susceptible and males more than females. For most species the LD₅₀ (lethal dose) is between 0.5- and 10mg/kg body weight. The liver is the principal target organ, although the site of the hepatic effect varies with species. Effects on the lungs, myocardium and kidneys have also been observed and aflatoxin can accumulate in the brain. Teratogenic effects following administration of high doses of aflatoxin have been reported in some species.
Acute poisoning of man by aflatoxins does occur occasionally in some areas of the world. Cases of human aflatoxicosis have been reported sporadically, mainly in Africa or Asia. The majority of reported cases involve consumption of contaminated cereals - most frequently maize, rice or cassava, or cereal products such as pasta or peanut meal. A classic case occurred in Malaysia in 1990 when approximately 40 persons were affected and 13 children died after eating noodles highly contaminated with aflatoxin and boric acid. High levels of aflatoxin were found on autopsy in liver, lung, kidney, heart, brain and spleen. Aflatoxin may not always be the primary cause of death in these acute cases. Autopsy brain (cerebrum) specimens from 18 kwashiorkor children and 19 other children who had died from a variety of other diseases in Nigeria showed aflatoxin present in 81% of the cases.

Aflatoxins have been implicated in sub-acute and chronic effects in humans. These effects include primary liver cancer, chronic hepatitis, jaundice, hepatomegaly and cirrhosis through repeated ingestion of low levels of aflatoxin. It is also considered that aflatoxins may play a role in a number of diseases, including Reye’s syndrome, kwashiorkor and hepatitis. Aflatoxins can also affect the immune system (Pier 1991).

Aflatoxin B1 is a potent mutagen causing chromosomal aberrations in a variety of plant, animal and human cells. The carcinogenicity and mutagenicity of aflatoxins B1, G1 and M1 are considered to arise as the result of the formation of a reactive epoxide at the 8, 9 position of the terminal furan ring and its subsequent covalent binding to nucleic acid, and the carcinogenicity of aflatoxin B1 has been studied in at least 12 different species. Although aflatoxins G1 and M1 have been tested less extensively, they appear to be toxicologically similar to aflatoxin B1. They are slightly less potent liver carcinogens but slightly more potent kidney carcinogens.

Products affected and natural occurrence

In most areas of the world, primary products such as cereals and nuts or animal products are screened routinely for aflatoxins, both by the producers and subsequently by importers or food manufacturers. Governments of many developed nations carry out surveillance on a regular basis to monitor the intake of aflatoxins by the human population so that action can be taken if this becomes necessary. Aflatoxins in peanuts moving in international trade have been found at levels of 1000 µg/kg or more, and products such as peanut butter may be contaminated by smaller amounts. Other nuts particularly prone to contamination are pistachios and brazils. Climate plays a crucial part in the conditions that encourage aflatoxin, so that the problem varies in severity from year to year. Drought leading to crop stress followed by rain is particularly unwelcome for cereals producers.

Sampling and analysis

Moulds and aflatoxins occur in an extremely heterogeneous fashion in food commodities. It is thus crucial that sampling is carried out in a way that ensures that the analytical sample is truly representative of the consignment. Failure to do this may invalidate the subsequent analysis. Analytical methods used are based on TLC, HPLC or ELISA. Extraction with aqueous acetonitrile or methanol, followed by clean-up of the extract solutions using immunoaffinity columns, provides sensitive and selective results for a wide range of foods
and animal feed. Detection by TLC or HPLC is based on their fluorescence under UV radiation, although aflatoxin B1 and aflatoxin G1 need derivatisation to enhance the fluorescence to a similar level to that of aflatoxin B2 and aflatoxin G2. Limits of detection below 1 µg/kg can be routinely achieved with analytical precision of ±30% for foods and below 0.05µg /kg for aflatoxin M1 in milk. On TLC plates, the four substances are distinguished on the basis of their colour, B standing for blue and G for green with subscripts relating to the relative chromatographic mobility. Aflatoxin B1 is usually but not exclusively found in the highest concentration.

**Stability and persistence**

Aflatoxins are quite stable in many foods and are fairly resistant to degradation. The effectiveness of some processes in reducing concentrations of aflatoxins in food can be affected by many factors, such as the presence of protein, pH, temperature and length of treatment. Commercial processing of raw commodities using cleaning regimes including the removal of broken particles, milling and sorting can reduce aflatoxin concentration considerably. Further information on decontamination will appear in the specialist fact sheet on this topic.

**Legislation and control**

The complete elimination of aflatoxins in human and animal food, while desirable, is extremely unlikely as they have the potential to arise in a wide range of agricultural products. Risk assessments have been carried out for aflatoxin. Because aflatoxin B1 is a genotoxic carcinogen, most agencies, including the Joint Expert Committee on Food Additives (JECFA) and the US Food and Drug Administration, have not set a total daily intake figure. In common with other dietary carcinogens, it is generally accepted that amounts in food should be reduced to the lowest levels that are technologically possible. Regulations have been set for human food and animal feed in many countries. The EC has established maximum permissible limits for aflatoxins in a range of commodities, including nuts, dried fruit and cereals, and for aflatoxin M1 in milk and dairy products. In the United Kingdom and elsewhere, regulation of aflatoxin B1 in animal feed has been effective in steadily reducing amounts of aflatoxin M1 in milk as shown by regular surveillance.