

AFLATOXIN

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Beneath those rugged elms, that yew-tree's shade,
Where heaves the turf in many a mouldering heap,
Each in his narrow cell for ever laid,
The rude forefathers of the hamlet sleep.

Elegy Written in a Country Churchyard
Thomas Gray 1716–1771.

In this famous poem Thomas Gray describes a hamlet's dead forefathers quietly mouldering under "heaves" of turf in a country churchyard. This represents a traditional view whereby moulds were principally associated with decay and disintegration of living matter following death.

However, in recent years scientific investigations have revealed the wider economic, toxicological and public health importance of certain mould species. For example, it is now known that in some circumstances mould-infected foods can be associated with serious toxicity, and sometimes death.

In this chapter the toxicity of a group of compounds called aflatoxins are described in order to illustrate the importance of the toxins produced by moulds.

Description, Occurrence, Uses

Description

Moulds are organisms belonging to the fungal kingdom. They are

either saprophytic, growing on dead organic matter, or more rarely parasitic, existing on other living organisms. They are capable of growing on many substances of importance to man (e.g. foodstuffs, wood, clothing), their growth often being highly dependent on the presence of appropriate conditions of humidity and temperature.

Some moulds are beneficial and economically important. These include the cultivated varieties used in cheese making which provide the distinctive aroma, taste and veining which makes these cheeses so attractive and delicious (e.g. Roquefort, Blue Vinney, Stilton). *Penicillium* is an example of another well-known mould made famous by Sir Alexander Fleming when he discovered the potential of penicillin, produced by the mould, as an antibacterial medicine.

However, many moulds are far from beneficial to man. They may damage stored food, clothing, leather, wood and other materials of economic importance. They may also cause extensive crop losses in the form of blights and rusts. Finally, they may pose health hazards by producing toxic substances called mycotoxins (from the Greek: *mukes*~mushroom, *toxikon*~toxic). The enormous public health and economic implications of mycotoxin contamination are illustrated by the fact that the Food and Agricultural Organisation of the United Nations estimates that up to 25% of the worlds food crops are affected by mycotoxins.

Examples of mycotoxins include the ergot alkaloids produced when the ergot fungus grows on rye (responsible for outbreaks of a disease called ergotism, or St Anthony's Fire), trichothecanes produced by *Fusarium* species (associated with alimentary toxic aleukia fatalities in the Second World War) and the aflatoxins.

Aflatoxins are a group of chemically related mycotoxins which are produced by particular species of moulds. Their name derives from the fungus *Aspergillus flavus* on which much of the early work with these substances was performed (i.e. the genus Aspergillus, the species **f**lavus and the suffix **t**oxin).

Subsequent research revealed that aflatoxins are produced by strains of *A. flavus* and strains of the related species *A. parasiticus*, *A. nominus* and *A. niger*. Furthermore, it was discovered that there are

a number of distinct, but structurally related aflatoxin compounds – the four most commonly seen being designated B1, B2, G1 and G2. The B designation of aflatoxins B1 and B2 resulted from the exhibition by these compounds of **B**lue fluorescence under the ultraviolet (UV)-light, whereas the G designation refers to yellow-**G**reen fluorescence under UV-light.

Aflatoxins M1 and M2, are hydroxylated derivatives of aflatoxin B1 and B2 that may be found in milk, milk products or meat (hence the designation M). They are formed by metabolism of B1 and B2 in the body of the animals, following absorption of contaminated feed. Aflatoxin B1 is the most frequent of these compounds present in contaminated food samples and aflatoxins B2, G1 and G2 are generally not reported in the absence of aflatoxin B1.

Thus, the aflatoxins form a family of highly oxygenated heterocyclic compounds with closely similar chemical structures, that are formed naturally by certain species of moulds.

Occurrence

Human exposure to aflatoxins occurs mainly through growth of the *Aspergillus* species *A. flavus* and *A. parasiticus*. Whether exposure is predominantly to aflatoxin B1, or to mixtures of various aflatoxins, depends upon the geographical distribution of the strains. *A. flavus*, which produces aflatoxins B1 and B2, occurs worldwide, while *A. parasiticus*, which produces aflatoxins B1, B2, G1 and G2, occurs principally in the Americas and in Africa.

Aflatoxins occur both in food crops in the field prior to harvest, and in improperly stored food where mould species have found an opportunity to grow. Fungal growth and aflatoxin contamination are a consequence of an interaction between the mould, the host organic material (i.e. crop, foodstuff) and the environment. The appropriate combination of these factors determines the degree of the colonisation of the substrate, and the type and amount of aflatoxin produced. Humidity, temperature and insect damage of the host substrate are major determining environmental factors in mould infestation and toxin production.

In addition, specific crop growth stages, poor fertility, high crop densities and weed competition have all been associated with increased mould growth and toxin production. For example, preharvest aflatoxin contamination of peanuts and corn is favoured by high temperatures, prolonged drought conditions and high insect activity; while postharvest production of aflatoxins on corn and peanuts is favoured by warm temperatures and high humidity.

Aflatoxins have been detected in milk, cheese, corn and other cereals, peanuts, cottonseed, nuts, figs and other foodstuffs. Milk and milk products, eggs and meat products are sometimes contaminated (generally with aflatoxins M1 and M2) because of the animals consumption of aflatoxin-contaminated feed.

Worldwide, corn contamination is probably of the greatest concern because of its widespread cultivation and its frequent use as the staple diet in many countries. However, due to local practices, customs or conditions, other foodstuffs may represent the greatest problem in certain localities.

One such area is West Africa where contamination of ground nuts (peanuts) is a significant problem. Ground nuts represent an important cash crop and foodstuff for rural farmers in West African countries such as The Gambia and Senegal. However, inappropriate storage conditions in the hot, humid climate can lead to contamination with aflatoxin. Indeed, black powdery moulds can often be seen growing on mounds of ground nuts stored in rural village huts.

Uses

Aflatoxins have no beneficial uses for man – their importance lies in their economic and medical significance in terms of spoilage of foodstuffs and toxicity to animals and man.

However, following the Gulf War in 1991, and the subsequent emergence of “Gulf War syndrome”, there has been increased concern regarding the use of biological agents as weapons of mass destruction and/or terrorism. Subsequent investigations have revealed that the Iraqi Government experimented with a variety of

biological agents including bacteria, viruses and mycotoxins. Thus, the sinister prospect has been raised of the possible future use of aflatoxins as a biological weapon.

Although there is no firm evidence that aflatoxin was used in the Gulf War, it has been reported that the Iraqis had produced 2200 litres of aflatoxin-containing material, and made seven aflatoxin-containing bombs. Although the properties of aflatoxin are not necessarily ideal as a direct acting agent against military personnel, it has been suggested that their use on foodstores and crops would result in contamination and subsequent economic and logistic disruption in the food supply.

In response to this information the US government added aflatoxins and certain other biological materials to a list of “select agents” covered under “The Antiterrorism and Effective Death Penalty Act of 1996”. This law requires the registration of facilities that work with these select agents, and imposes harsh penalties for noncompliance.

Although not a conventional “use”, it should be noted that aflatoxins have been incorporated into the medium of popular fiction. Graham Greene in his novel “The Human Factor” (1978) describes a character disposing of a suspected double agent by poisoning him with aflatoxin surreptitiously mixed in his whisky! The agent's subsequent death from liver failure is then conveniently ascribed to his propensity for heavy drinking.

Properties – Mechanisms of Biological Interaction

For aflatoxins the liver is the primary target organ for toxicity in all species studied. The precise manifestations of toxicity depend upon a number of factors, including dose and duration of exposure. However it is the potent ability of aflatoxins to induce liver cancer, and the significant economic and public health consequences that follow, that has stimulated much of the work on these compounds over the last 30 years.

Research work has followed a number of different lines of enquiry. Firstly, long term studies have been performed with

aflatoxins and mixtures of aflatoxins to characterise the ability of these compounds to induce cancer in a variety of animal species. Secondly, studies to investigate the mechanisms underlying the carcinogenic activity have been performed including genotoxicity, binding and metabolism studies. Thirdly, epidemiological studies have been performed in man to investigate the associations between diet, aflatoxin exposure, occurrence of hepatocellular carcinoma and other factors. These approaches are described in the following sections.

Carcinogenicity studies in experimental animals

Globally, primary hepatocellular carcinoma is among the most common forms of cancer in man. Incidence of the disease varies greatly in different areas of the world, suggesting involvement of environmental etiological factors, and much research has been devoted to the identification of such factors. Because many organic chemicals have been shown to have the capability of inducing primary hepatocellular carcinoma in animals, they have been extensively studied with respect to their possible significance as etiologic agents for primary hepatocellular carcinoma in man. Particular emphasis has been placed on aflatoxins because of their known widespread occurrence as food contaminants.

Mixtures of aflatoxins and aflatoxin B1 have been tested for carcinogenicity in several strains of mice and rats, in hamsters, fish, ducks, tree shrews and monkeys. Following oral administration, these compounds caused hepatocellular and/or cholangiocellular liver tumours, including carcinomas, in all species tested except mice. However, intraperitoneal administration of aflatoxin B1 to infant mice did induce high incidences of liver tumours. Additionally, in some species, the compounds produced tumours at other sites in the body. For example, tumours in the kidney and colon were also found in rats.

Aflatoxins B2, G1 and M1 have been tested separately in rats and induced liver tumours after oral or intraperitoneal

administration. However, these compounds appeared to be of lower hepatocarcinogenic potency than aflatoxin B1.

In conclusion, aflatoxin B1, mixtures of aflatoxins and other specific aflatoxins have all shown evidence of carcinogenic potential in animal species.

Mechanistic studies

In order to understand how and why the aflatoxins mediate their toxicity a number of experimental approaches have been taken. One approach has been to investigate the toxicity of these compounds to the genetic material within cells (e.g. mammalian DNA). Most data is available on aflatoxin B1, and this has consistently been shown to possess genotoxic potential in a variety of test systems. For example, in human and animal cells in culture it produces DNA damage, gene mutation and chromosomal anomalies; in insects and lower eukaryotes it induces gene mutations; and in bacteria it produced DNA damage and gene mutation. Other aflatoxins have not been so extensively investigated, but in a variety of studies B2, G1, G2, and M1 have all shown evidence of genotoxicity.

Another approach has been to examine how the aflatoxins are metabolised in the body. Studies using human liver material have shown that aflatoxin B1 is metabolised to a highly reactive chemical compound, called the 8,9-epoxide. Following its formation this compound binds very rapidly to protein, DNA and other important constituents of living cells, forming "adducts". Formation of these adducts disrupts the normal working processes of the cell, and in the case of DNA adducts, can ultimately lead to a loss of control over cellular growth and division. Humans metabolise aflatoxin B1 to the major aflatoxin B1-N7-guanine adduct at levels comparable to those in species which are susceptible to aflatoxin-induced hepatocarcinogenicity, such as the rat.

However, both humans and animals possess enzyme systems which are capable of reducing the damage to DNA and other cellular constituents caused by the 8,9-epoxide. For example, glutathione

S-transferase mediates the reaction (termed conjugation) of the 8,9-epoxide to the endogenous compound glutathione. This essentially neutralises its toxic potential. Animal species, such as the mouse, that are resistant to aflatoxin carcinogenesis have three to five times more glutathione S-transferase activity than susceptible species, such as the rat. Humans have less glutathione S-transferase activity for 8,9-epoxide conjugation than rats or mice, suggesting that humans are less capable of detoxifying this important metabolite.

There is considerable *in vitro* and *in vivo* evidence to support the view that humans possess the biochemical processes necessary for aflatoxin-induced carcinogenesis. Thus, presence of DNA and protein aflatoxin adducts, urinary excretion of aflatoxin B1-N7-guanine adducts and the ability of tissues to activate aflatoxin B1 have all been demonstrated for humans. In addition, studies have suggested that oncogenes are critical molecular targets for aflatoxin B1. A high frequency of mutations at a mutational “hotspot” has been found in p53 tumour suppressor genes in hepatocellular carcinomas from patients residing in areas considered to offer a high risk of exposure to aflatoxins, and where there is a high incidence of hepatocellular carcinoma.

In contrast, this mutational pattern is not found in hepatocellular carcinoma samples from moderate or low aflatoxin exposure countries or regions. Therefore, this hot-spot mutation is believed to be a molecular fingerprint linking the initial event of aflatoxin B1-DNA adduct formation with the ultimate development and progress of human hepatocellular carcinomas.

Human carcinogenicity data

Despite the strong supportive evidence for animal and mechanistic studies, there have been major difficulties in assessing the precise role of aflatoxin in the causation of liver cancer in humans.

Unlike laboratory conditions where exposure of laboratory animals can be accurately defined, exposure of humans to aflatoxins cannot generally be estimated with any great certainty. Exposure to aflatoxin in tropical areas of Africa and parts of Asia and Latin

America can begin very early in life, and episodically thereafter, thus making accurate assessments of exposure extremely problematic. Furthermore, the number of episodes, and the degree of exposure to aflatoxin, varies greatly by country and region, by agricultural and crop storage practices, by season and by other factors difficult to control in any scientific study.

Secondly, there is a high geographical correlation between exposure to aflatoxin, the hepatitis B virus and increased incidence of hepatocellular carcinoma. Prospective epidemiological studies have shown a high incidence of primary hepatocellular carcinoma among hepatitis B virus carriers in endemic areas. Clinical studies have also shown that most primary hepatocellular carcinoma patients are carriers of the hepatitis B surface antigen, and have chronic active hepatitis. Recently, hepatitis B virus sequences have been found to be integrated into the liver cell genome in some, but not all, patients with chronic hepatitis or primary hepatocellular carcinoma. This evidence has identified hepatitis B virus as a major etiological factor for primary hepatocellular carcinoma in certain populations, particularly in Taiwan and the People's Republic of China.

Some epidemiological studies have suggested that aflatoxin poses no detectable independent carcinogenic risk for man, and that it poses risks only in the presence of other risk factors such as hepatitis B infection. Such studies have indicated that the potency of aflatoxins in hepatitis B surface antigen-positive individuals is substantially higher than the potency in surface antigen negative individuals. Clearly, reduction in prevalence of hepatitis B infected individuals through vaccination of those at risk may therefore have an important impact on the risk of liver cancer in these populations. Further studies attempting to define the relationships between the aflatoxin exposure and hepatitis B infection factors are ongoing in Africa and the far East. Studies are also examining the role of hepatitis C virus infection in this complex set of potentially interdependent risk factors for the occurrence of primary hepatocellular carcinoma.

Despite these difficulties, aflatoxin B1 has been classified as a Group I carcinogen (i.e. it is considered that sufficient evidence exists to define aflatoxin B1 as carcinogenic to humans) in humans by IARC (International Agency for Research on Cancer) parameters. Furthermore, the Food and Agriculture Organisation of the United Nations and World Health Organisation Joint Expert Committee on Food Additives concluded in 1997 that they are considered to be human liver carcinogens. However, these expert bodies agree that exact mechanisms of aflatoxin hepatocarcinogenesis have not yet been fully elucidated, and some important points remain to be clarified.

It is to be hoped that better information will be generated as a result of on-going intervention projects, and agricultural development programmes, and by monitoring exposure to aflatoxin and the incidence of liver cancer in areas where hepatitis B virus vaccination is effectively reducing the prevalence of carriers of the viral surface antigen. In addition, initiatives must continue which reduce exposure through measures such as improved farming and storage practices, improved monitoring of foodstuffs and through enforcing food standards both within countries and across borders.

Toxicity Produced – Toxicity Profile

The adverse biological properties of aflatoxin seen in poisoning episodes in animals can be categorised in two general forms:

- **acute aflatoxicosis** which occurs following the ingestion of high doses of aflatoxins over a relatively short period of time. Specific acute episodes of disease may include haemorrhage, acute liver damage, oedema, alteration in digestion, absorption and/or metabolism of food, and possibly death
- **chronic aflatoxicosis** which occurs following the ingestion of low to moderate doses of aflatoxins over a prolonged period. The effects may be subclinical or difficult to recognise. Some of the more frequently described symptoms include impaired food conversion and slower rates of growth, with or without the

occurrence of an overt aflatoxin syndrome as seen with acute poisoning. Underlying these symptoms is a chronic poisoning of the liver leading ultimately to cirrhosis and/or liver cancer (see description of genotoxicity and carcinogenicity data above).

Laboratory investigations in a number of animal species have confirmed that aflatoxins can produce acute necrosis, cirrhosis and carcinoma of the liver. No animal species has been shown to be refractory to aflatoxin toxicity, however, a wide range of acute lethal doses have been observed, indicating different degrees of acute susceptibility. For most species the doses that killed 50% of the animals treated ranged from 0.5 to 10 mg/kg body weight. Species differ in their susceptibility to the acute and chronic effects, and toxicity can be influenced by dose, duration of exposure, age, health, nutritional status and environmental factors.

Further information relating to toxicity profiles are given below in relation to examples of toxic episodes published in the scientific literature.

Examples of Endemic Problems – Toxic Episodes

Examples of toxic episodes in animals

In 1960 more than 100,000 young turkeys on poultry farms in England died in the course of a few months from a mysterious new disease. In view of the lack of an explanation for the disease, it was named “Turkey X disease”. Soon, however, it was found that the problem was not limited to turkeys; ducklings and young pheasants were affected, and also showed heavy mortality.

Intensive investigation of the early outbreaks of the disease indicated that they were all associated with particular meals given to the birds. On feeding the meal to poults and ducklings, the symptoms of Turkey X disease were rapidly produced. The suspect feed was imported Brazilian peanut meal and initial speculation was that a fungal toxin might be involved.

Further investigations did in fact demonstrate that the meal was heavily contaminated with *Aspergillus flavus*, that this organism

was responsible for producing a toxin (aflatoxins were isolated and the chemical structures identified for the first time), and that the disease was the result of aflatoxin ingestion.

Examples of toxic episodes in man

Northwest India 1974

In the fall of 1974 an epidemic occurred in more than 150 villages in adjacent districts of two neighbouring states in a rural area of Northwest India. The disease was characterised by onset with high fever, rapidly progressive jaundice and ascites. According to one report of the outbreak, 397 persons were affected and 108 people died. One notable feature of the epidemic was that it was heralded by the appearance of similar symptoms in the village dogs.

Liver biopsy specimens from eight cases, and autopsy material from one human case and two dogs were studied. Characteristic features were centrizonal scarring, hepatic venous occlusion, ductular proliferation and cholestasis, focal syncytial giant-cell transformation of hepatocytes, and pericellular fibrosis.

Analysis of food samples revealed that the disease outbreak was probably due to the consumption of maize (corn) heavily infested with the fungus *Aspergillus flavus*. Unseasonable rains prior to harvest, chronic drought conditions, poor storage facilities and ignorance of dangers of consuming fungal contaminated food all seem to have contributed to the outbreak.

The levels of aflatoxin in food samples consumed during the outbreak ranged between 2.5 and 15.6 microgram/g. Anywhere between 2 and 6 mg of aflatoxin seems to have been consumed daily by the affected people for many weeks. In contrast, analysis of corn samples from the same areas the following year (1975) revealed very low levels of aflatoxin (i.e. less than 0.1 microgram/g), and this may have explained the absence of any reoccurrence of the outbreak in 1975. A ten-year follow-up of the epidemic found the survivors fully recovered with no ill effects from the experience.

Kenya 1981

Between March and June 1981, 20 patients (eight women and 12 men aged 2.5 to 45 years old) were admitted to three hospitals in the Machakos district of Kenya with severe jaundice. The patients reported that they had first exhibited symptoms of abdominal discomfort, anorexia, general malaise and low grade fever. After about seven days, jaundice and dark urine had appeared, and the patients had sought admission to hospital.

The patients came from rural areas of mixed woodland and bushed grassland about 150 km Southeast of Nairobi. The rainiest season is from March to May each year, when about 70% of the annual rainfall occurs. 1980 had been an extremely dry year with a poor harvest, but in 1981 the rains had come early, were heavy and prolonged. Maize is the major crop in the area, but some millet, sorghum, beans, cowpeas, pigeon peas and vegetables are also grown for home consumption.

Interestingly, the relatives and friends of one family told that many of the local doves had died, then the local dogs, and finally the people had become sick. The dogs were known to be consuming essentially the same diet as the local people.

On admission to hospital all patients were jaundiced, some with low grade fever, and extremely weak. Tachycardia and oedema (of the legs and to a lesser extent face and trunk) were seen. The liver was tender in all patients. Eight of the 20 patients improved with a return of appetite, disappearance of jaundice and discharge from hospital in six to 20 days. However, hepatic failure developed in the remaining 12 patients and they died between one and 12 days following admission.

An extensive investigation of the outbreak was performed. Aflatoxin levels in foods were measured and showed high levels of aflatoxin B1 and B2. For example, maize grains from the two homes where severe and fatal illness had occurred contained 12 mg/kg and 3.2 mg/kg of aflatoxin B1, while maize from unaffected homes had a maximum of 0.5 mg/kg aflatoxin B1. Liver samples were obtained from two patients at necropsy and these indicated

aflatoxin B1 levels of 39 and 89 $\mu\text{g}/\text{kg}$. Histologically the livers showed evidence of toxic hepatitis – marked centrilobular necrosis with minimal inflammatory reaction. Blood samples from the patient were also tested for possible viral infections and three were found to be positive for hepatitis B surface antigen.

The cumulative evidence suggests that aflatoxin poisoning was the cause of the acute liver disease in this incident. Contributing factors may have included the exceptionally prolonged and heavy rainy season that year which would have provided favourably moist conditions for the growth of aflatoxin producing moulds. Other factors could have been that the previous year's poor harvest had forced some individuals onto a protein deficient diet (this is known to potentiate aflatoxin poisoning in monkeys), and that the severity of the aflatoxin toxicity could have been worsened by the pre-existing liver damage due to hepatitis B viral infection in three of the subjects.

Preventative Measures

There are a variety of strategies which are aimed at minimising the animal and human exposure to aflatoxins. Firstly, reductions in exposure can be achieved through avoidance measures such as improved farming and proper storage practices and/or enforcing standards for food or feed within countries and across borders. Secondly, numerous strategies for the detoxification of aflatoxin contaminated foodstuffs have been proposed. However, it must be recognised that strategies aimed at reducing the risks posed by aflatoxins are dependent upon the resources available, and that this may be a particular constraint in poorer countries and those with a developing infrastructure.

Avoidance strategies

Good farming and storage practices are aimed at eliminating the conditions which encourage the growth of moulds in crops and stored food. For example, ripe crops should not be left in the field

too long, and cereal grains, rice and nuts should not be stored under damp, inadequately ventilated conditions.

However, since some degree of aflatoxin contamination is considered unavoidable, even where good manufacturing practices have been followed, many countries have introduced regulatory controls over the levels of these substances allowed in certain high risk foodstuffs.

In the UK, the Ministry of Agriculture, Fisheries and Food (MAFF) have been monitoring the levels of aflatoxins in foods for some years. The "Feeding Stuffs Regulations 1991" set maximum levels for aflatoxin B1 in animal feed, and thus restricts the amount of aflatoxin M1 carried over into milk and milk products. Regulations to limit the levels of aflatoxins in certain human foodstuffs (Aflatoxins in Nuts, Nut Products, Dried Figs and Dried Figs Products Regulations 1992) were introduced at the end of 1992. National limits for aflatoxin content of foodstuffs remain under surveillance, and international regulatory activities are co-ordinated at the regional and WHO level.

In the United States, the Food and Drug Administration (FDA) regulates the quality of food, including the levels of environmental contaminants. The FDA has established guidelines for the levels of aflatoxins permitted in human foodstuffs and animal feed. The maximum permitted level for human food is 20 parts per billion of total aflatoxins. Higher levels are permissible for feed destined for animal consumption.

Detoxification strategies

Because it is impossible to completely avoid some degree of aflatoxin contamination, a variety of strategies for their detoxification in foodstuffs have been proposed. These strategies have included physical methods of separation, thermal inactivation, irradiation, solvent extraction, adsorption from solution, microbial inactivation, chemical methods of inactivation and fermentation. Two of these strategies are described in more detail below.

A wide range of chemicals have been tested for the ability to degrade and inactivate aflatoxins. However, although a number of these chemicals can react to destroy aflatoxins effectively, most are impractical, too expensive or potentially unsafe because of the formation of toxic residues, or the perturbation of the nutrient content of the food. Two chemical approaches that have received considerable attention are ammoniation and reaction with sodium bisulphite.

Studies have shown that treatment of aflatoxin-contaminated corn with ammonia is an effective detoxification approach. Ammonia appears to produce hydrolysis of the lactone ring and chemical conversion of the parent compound to numerous products that exhibit greatly reduced toxicity. Similarly sodium bisulphite reacts with aflatoxins to form water soluble degradation products.

An alternative approach is to attempt to reduce the absorption of aflatoxins from contaminated feed in animals. This may be achieved by the addition of inorganic sorbent materials such as sodium calcium aluminosilicate (HSCAS) in the diet of animals. HSCAS tightly binds aflatoxins in the gastrointestinal tracts of animals, preventing their absorption into the body so that they are passed out unabsorbed in the faeces. This results in a major reduction in the body burden (i.e. exposure) of the animals to the mycotoxin.

Case History of Poisoning

Histories of probable poisoning with aflatoxins are described above in relation to the toxic episodes reported in Northwest India and Kenya. The case described below is of interest in that a full medical investigation and follow-up was performed which indicated that the patient remained well up to 14 years post-exposure. In addition, this case is extremely unusual in that a reasonably accurate estimate of exposure to aflatoxin can be made.

In a deliberate suicide attempt, a 25-year-old female American laboratory worker reported ingesting approximately 5.5 mg

aflatoxin B1 over a two-day period, and six months later, approximately 35 mg over a 14-day period. These amounts were consistent with those missing from the laboratories stocks, and can therefore be assumed to be accurate.

After the first episode she was reported have transient rash, nausea and headache, but there were no other ill effects – physical, radiological and laboratory examinations being normal except for sulphobromophthalein retentions of 9% and 7% at 45 minutes (sulphobromophthalein clearance is used as a measure of liver function – clearance in healthy individuals being essentially complete at 45 minutes). Following the second episode the only symptom reported was nausea. Liver biopsies after each episode were normal, and in a 14-year follow-up a physical examination and blood chemistry, including tests for liver function, were normal.

The authors of this report commented that additional factors, such as malnutrition and hepatitis virus, which were absent in this patient, may be necessary for aflatoxin carcinogenesis in humans. Alternatively, the latent period for liver tumour formation may be greater than the 14-year follow-up period, or the exposure levels of aflatoxin in this subject were insufficient to provoke more serious toxicity.

Concluding Remarks

Aflatoxins have only been recognised as a significant issue for human health in the past 35 years. During this time an enormous amount of information has been accumulated on the nature, occurrence, exposure and health effects of these mycotoxins. Clearly, further work is required to clarify their role in the occurrence of primary hepatocellular carcinoma, the molecular, biochemical and pathological mechanisms underlying their toxicity and optimal strategies for minimising both their health and economic impacts on the human populations they affect.

Suggested Further Reading

- Eaton DL and Groopman JD (1994). *The Toxicology of Aflatoxins*. Academic Press, New York.
- Greene G (1978). *The Human Factor*. The Bodley Head.
- International Agency for Research into Cancer (IARC) (1993). Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 56, pp. 245–395.
- Krishnamachari KA, Bhat RV, Nagaragen V and Tilak TB (1975). Hepatitis due to aflatoxin. An outbreak in West India. *Lancet* **i**, 1061–1063.
- Ngindu A, Johnson BK, Kenya PR, Ngira JA, Ocheng DM, Nandwa H, Omondi TN, Jansen AJ, Ngare W, Kaviti JN, Gatei D and Siongok TA (1982). Outbreak of acute hepatitis caused by aflatoxin poisoning in Kenya. *Lancet* **1**, 1346–1348.
- Willis RM, Mulvihill JJ and Hoofnagle JH (1980). Attempted suicide with purified aflatoxin. *Lancet* **1**, 1198–1199.
- Zilinskas R (1997). Iraq's biological weapons: the past as future? *JAMA* **278**, 419–424.