Mycologist, Volume **16**, *Part 3 August 2002.* ©*Cambridge University Press Printed in the United Kingdom.*
DOI: 10.1017/S0269915X02003014

Mycotoxin review – 1. *Aspergillus* and *Penicillium*

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Keywords: aflatoxin, ochratoxin, patulin

When a major wild bird food producer advised, in their catalogue for the year 2000, that they could not offer peanuts because of the high levels of aflatoxin, the phenomenon of mycotoxins had really come into the public domain. In 2001 the same company was able to advise that 'Although present in some peanut crops this season, aflatoxin is not currently creating any major peanut supply problems'. Mycotoxins, and specifically peanuts and the health of wild birds, were reviewed in earlier volumes of this journal (Isaac 1994 *a*, *b*) but the passage of time has not led to any reduction in the problems associated with the presence of mycotoxins in foods and animal feeds. Indeed, the improvement in analytical methods, and the implications of legislation in many countries have served to increase awareness of mycotoxins.

Aflatoxins

The production of aflatoxin B_1, the most toxic of this family of mould metabolites, is still only known from a small number of species of the genus *Aspergillus*: i.e. *A. flavus*, *A. parasiticus*, *A. nomius*, *A. ochraceoroseus*, *A. pseudotamarii* and a species with the perfect stage *Emericella venezuelensis*. *Aspergillus bombycis*, isolated from insect frass in silkworm rearing houses in Japan, was recently described as a new aflatoxigenic species by Peterson *et al.* (2001) and their paper is a useful source of references on the discovery of aflatoxigenic moulds. The biosynthetic pathway to this molecule from a decaketide precursor is complex, and involves a significant number of genes, so that it is perhaps not surprising that its production is limited to a small number of species. This raises the issue of the significance of aflatoxin production to the producing organisms and it has been suggested that they may have evolved as antifeedants providing the moulds with an advantage over their main competitors for plant seeds as a nutrient source, i.e. insects and small mammals. The acute and chronic toxicity of the aflatoxins, and their widespread occurrence in plant products, makes them significant in human and animal health.

The aflatoxins were first discovered in 1959/1960 because of their acute toxicity, being responsible for the deaths of many turkey poults in East Anglia, and young game birds are amongst the animals most sensitive to this acute form of poisoning. The birds had been fed on a pelleted feed containing groundnut meal which was shown to be the toxic constituent. The groundnut meal was infected with *Aspergillus flavus* and contaminated by a compound which fluoresced blue under ultra violet light. This compound, now known as aflatoxin B (*A. flavus* toxin with blue fluorescence), was shown to be a metabolite of the mould and within a few years its structure (Fig 1) was elucidated. Once appropriate analytical procedures had been developed it became apparent that the aflatoxins were and still are fairly common in a range of vegetable products of tropical and sub-tropical origin, especially peanuts and maize, and two important aspects of their toxicity were soon made clear.

1.) For some animal species, such as the rat, aflatoxin B_1 is one of the most carcinogenic mould metabolites known.
2.) There is a wide range of sensitivity to all aspects of the toxicity of aflatoxin B_1 amongst animal species and, indeed, between the sexes of the same species, the male generally being more sensitive than the female.

So what about humans? Are we as sensitive to the acute toxicity as the rabbit, cat or dog (LD_{50} values less than 1 mg/kg body weight) or as resistant as the hamster (LD_{50} 10.2 mg/kg body weight)? Are aflatoxins carcinogenic to humans? As far as acute toxicity is concerned there is no doubt that aflatoxin can kill and it is probable that we are somewhere between the two extremes. There are several reports of fatalities of humans arising from the consumption of aflatoxin contaminated food. The most serious occurred in India in 1974 when nearly 1000 people were ill and just over 100 died following the consumption of contaminated maize (Krishnamachari *et al.* 1975). It is clearly important to know whether aflatoxins are carcinogenic

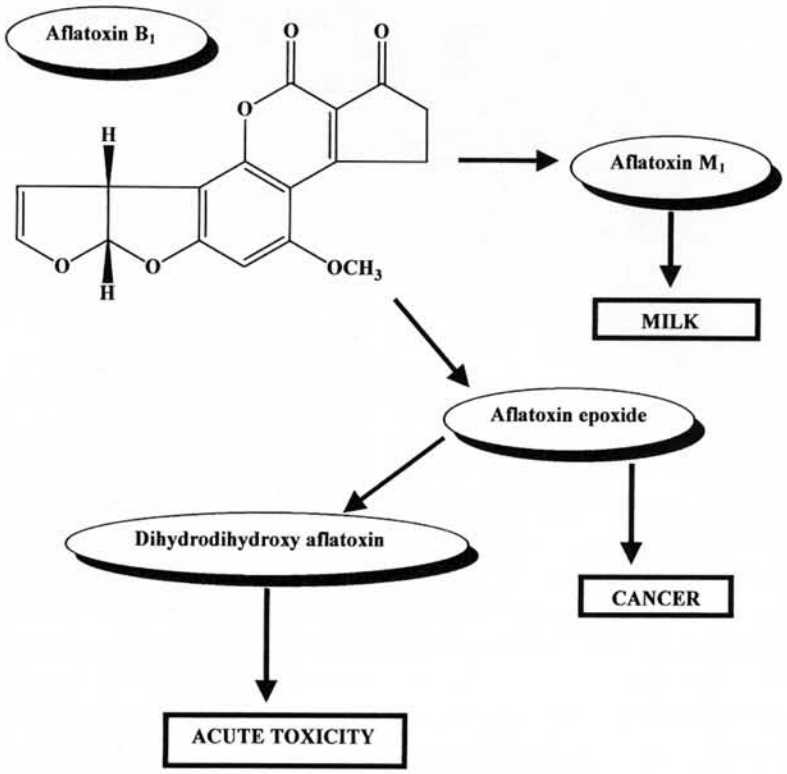

Fig 1 Those aspects of the metabolism of aflatoxin B_1 significant in its acute toxicity and carcinogenicity to animals.

to humans and, as long ago as 1977, epidemiological evidence seemed to show a strong correlation between the incidence of human liver cancer in Africa and aflatoxin in food. However, it was also suspected that hepatitis B infection could account for the high incidence of liver cancer in Africa. Once the molecular mechanisms by which aflatoxin acts as both an acute toxin and a liver carcinogen had been worked out it became clear that we have to assume that aflatoxin is potentially carcinogenic to humans. It also became clear that hepatitis B and aflatoxin can interact synergistically in the epidemiology of human liver cancer.

The toxicity of aflatoxin B_1 requires metabolism by the animal before it is expressed and this process occurs principally in the liver. It is probable that the mould metabolite itself is not toxic but has the appropriate physicochemical properties to be efficiently transported into liver cells where it is metabolised by several different enzymes (Fig 1). The first oxidative step leads to an epoxide, which is the potential carcinogen by reacting with guanine residues in DNA, but this may be further metabolised to a dihydroxy compound which is the acute toxin being very reactive with lysine residues in enzymes. The extent to which aflatoxin is metabolised and the balance between these two reactions will determine whether an animal species is especially sensitive to the carcinogenicity or acute toxicity. Other reactions may also occur which lead to products more readily secreted from the body and which may confer enhanced resistance to aflatoxin poisoning. One such product is aflatoxin M_1 secreted in the milk of several mammals including cows and humans. This compound is almost as toxic and carcinogenic as B_1 when it is consumed as a contaminant of milk and milk products. In the situation where a mother is consuming aflatoxin contaminated food, her baby will suffer from a complex interaction of the mother's poor diet, which undoubtedly influences the quality and quantity of her milk for the baby, and the milk also being contaminated with aflatoxin M_1.

On the assumption that aflatoxin B_1 is carcinogenic many countries have set a legislative limit to the acceptable levels in food for human consumption. The European Commision has set a limit of 2 µg/kg for a number of cereals and groundnuts and has recently extended the range of commodities to include spices for which a limit of 5µg/kg has been set. An especially stringent level of 0.05 µg/l has been set for aflatoxin M_1 in milk to protect the very young from long term exposure. The regulations are actually more complex than indicated here and some relaxation is allowed for some commodities, such as peanuts, if they are subjected to sorting, or other physical treatment, before use as a food ingredient. The most recent version of

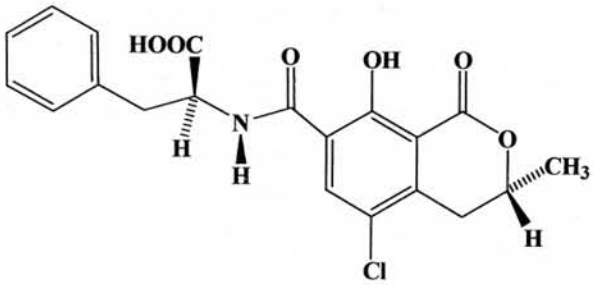

Fig 2 Structure of Ochratoxin A.

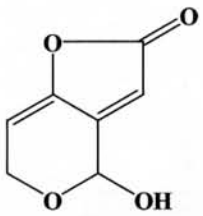

Fig 3 Structure of patulin.

these regulations was published in the *Official Journal of the European Communities* on 13th February 2002 as Commission Regulation (EC) No 257/2002. A consequence of these legislative limits in the E.C. is that problems arise with imports from other parts of the world and during 2001 these problems have involved peanuts from China and South Africa, pistachios from Iran, Brazil nuts from Brazil and hazel nuts from Turkey. The sampling and analytical procedures for enforcing this legislation are complex and demanding but clearly can be achieved. From an international perspective a worrying aspect of legislative limits is that different countries have set different limits for aflatoxins. Generally, producing exporting countries have less stringent legislation than importing countries.

The highest levels of aflatoxins are produced as a result of inadequate post harvest storage when high moisture content and warm temperatures facilitate rapid growth of moulds. Appropriate post harvest treatment to reduce moisture content and sound storage under dry cool conditions should control this source of aflatoxin contamination. However, aflatoxin producing species of *Aspergillus* have more complex interactions with plants such as maize and groundnuts. They can establish a benign endophytic relationship with the plant in the developing seed and, if the plant is stressed, for example by drought, some aflatoxin may be produced and it is then present in the crop, in the field, before harvest. Although concentrations are much lower than those associated with post harvest storage, they may be significant and higher than levels permitted in many countries. This field contamination of crops with aflatoxin is much more difficult to control than post harvest spoilage and is a major contribution to the problems encountered by the bird food producer in 2000.

Ochratoxin

Although aflatoxin B_1 is of major concern there are several other mycotoxins which are controlled by legislation in a number of individual countries and may be subjected to EC regulation in the near future (see below). Perhaps the most important is ochratoxin A (Fig 2) which is also quite widespread in occurrence. In temperate climates it is produced by *Penicillium verrucosum* and *P. nordicum* (Larsen *et al.* 2001), primarily on cereals such as barley, but in warmer parts of the world it is produced by several species of *Aspergillus*. The species from which it gets its name is *A. ochraceus* but it is also produced by *A. sulphureus*, *A. alliaceus*, *A. sclerotiorum*, *A. melleus* and several of the *niger* group such as *A. carbonarius*, *A. citricus* and *A. fonsecaeus*. It is one or more of these aspergilli which are probably responsible for the presence of ochratoxin in commodities such as cocoa, coffee, wine and vine products. Ochratoxin has a remarkably long residence time in the animal body and so can also occur in meat and meat products produced from animals which have been fed on ochratoxin-contaminated feed. Ochratoxin is predominantly a nephrotoxin and is implicated in the epidemiology of nephropathy in pigs and possibly Balkan endemic nephropathy in humans. However, the main reason for increased interest in ochratoxin A is the mounting evidence that it is also carcinogenic. During the year 2000 the E.C. discussed regulatory limits for this mycotoxin of 5 µg/kg in cereals and 3 µg/kg in cereal products. It is generally accepted that, in Europe, cereals will be the main source of ochratoxin in the diet but discussions have been taking place about imposing limits on other commodities such as wine and coffee. The original time table was to publish the agreed limits by October 2001 but this was deferred to 12th March 2002 when the following maximum levels were published in Commission Regulation (EC) No 472/2002:

Raw cereal grains (including raw rice and buckwheat) 5 µg/kg; all products derived from cereals (including processed cereal products and cereal grains intended for direct human consumption) 3 µg/kg; dried vine fruit (currants, raisins and sultanas) 10 µg/kg. Although ochratoxin may also occur in coffee, even after roasting, and in wine, beer, grape juice, cocoa and spices, there is no agreement over maximum permitted levels in these commodities.

Patulin

A third mycotoxin being considered by the EC is patulin (Fig 3), a relatively small molecule produced by a wide range of species of *Penicillium*, *Aspergillus* and other genera of moulds but, in the context of human exposure, *Penicillium expansum* is the most important. This mould is the agent of a common brown soft rot of apples although it can also be isolated from a number of other fruits (Pitt & Hocking, 1997). The production of patulin in apple tissue is confined primarily to the infected rotten tissue and a few mm beyond the edge of the rot. It is thus unlikely that whole fresh fruit will be a significant source of patulin in the diet although some varieties, such as the Bramley apple, have an open core where infection may occur and not be apparent on the outside of the fruit. However, a perfectly acceptable apple juice can be recovered from apples containing some rot and fresh apple juice can certainly be contaminated with patulin. The UK has an advisory limit of 50 µg/kg for patulin in apple juice and the EC is discussing whether this, or a lower level, should be set as a maximum permitted level. In 1993 a popular daily paper had a major headline 'Apple juice in cancer scare' arising from the report, by what was then MAFF, of samples from supermarket shelves with levels higher than the advisory limit. The Food Standards Agency continues to monitor retail apple juices as well as apple-based products, other fruit juices and tomato-based products and considers that, in the UK, there is no cause for concern with regard for public health in the present situation. It is worth noting the following points. Compared with many mycotoxins patulin is an unstable molecule but it is most stable in the weakly acidic conditions of apple juice. It is metabolised by actively fermenting yeasts and so does not occur in cider (see Moss & Long, 2002). It is excreted very rapidly from the body and there is no clear evidence that it is carcinogenic when consumed orally. Compared with aflatoxin B_1 and ochratoxin A it is a low priority, but its presence in apple juice is a useful indicator that the juice has been manufactured using rotten apples and clearly that is an unacceptable practice.

There are several metabolites of species of *Fusarium* which may have a more profound chronic effect than patulin. They include the trichothecenes, zearalenone and the fumonisins and these will form the subjects of a further review.

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