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Mycotoxin review – 2. *Fusarium*

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The genus *Fusarium* is surely known by everyone who looks at moulds associated with plants and soil, especially with its characteristic hyaline, multiseptate, slightly curved macroconidia. Nevertheless, it still seems a daunting task identifying isolates to species. The complexity of the genus is reflected in the number of ascomycete genera such as *Nectria*, *Calonectria* and *Gibberella*, which have *Fusarium* as the anamorph state. On the other hand many species of *Fusarium* have no known teleomorph. Colin Booth, President of the BMS in 1977, produced an invaluable monograph on the genus (Booth, 1971) and later, reviews by Nelson *et al.* (1983) and Gerlach & Nirenberg (1982) also help to make the genus more accessible.

Although *Fusarium* is especially important in plant pathology, several species also produce a diverse range of toxic metabolites which may contaminate foods and animal feeds of plant origin (Marasas *et al.*, 1984) but only a small number are judged to be important in the context of human health. The recently published account of a symposium of the American Chemical Society on mycotoxins and food safety (DeVries *et al.*, 2002) includes ten chapters on fusarial toxins including the fumonisins, the trichothecenes T-2 toxin and deoxynivalenol, and zearalenone which are reviewed below.

Fumonisin B_1

It is difficult to imagine a single toxin which can cause the death of horses by massive necrosis of brain tissue (equine encephalomalacia), of pigs by chronic accumulation of fluid in the lung (porcine pulmonary oedema), and that of rats by necrosis of the liver, and which may also be the aetiological agent of oesophageal carcinoma in humans! Such a toxin is fumonisin B_1 (Fig 1,A) produced by *Fusarium moniliforme* Sheldon (= *F. verticillioides* (Sacc.) Nirenberg), *F. proliferatum* (Matsushima) Nirenberg and related species of the Liseola group. Fumonisins characteristically develop in the field, principally in maize, and it would only be under exceptional circumstances of a total breakdown of storage conditions that the concentration would increase during storage. A number of fumonisins, of which fumonisin B_1 is the most important, were isolated and characterised as recently as 1988, although *F. moniliforme*, especially associated with maize, had been recognised as a toxigenic species at the beginning of the twentieth century (Butler, 1902). Since the isolation and characterisation of this intriguing molecule there has been a lot of interest, and a considerable literature reflected in a monograph on fumonisins in food (Jackson *et al.*, 1996).

Fumonisin B_1 is a potent competitive inhibitor of the enzyme which links sphinganine to a long chain fatty acid to form ceramide which is itself involved in the biosynthesis of a wide range of complex sphingolipids. The activity of fumonisin thus has two consequences: (1) blocking the biosynthesis of such important molecules as sphingomyelin, which may form 18-19% of the plasma membrane in the liver, neutral glycolipids, which may form 40% of the outer monolayer of myelinating cell membrane, and gangliosides, which are important membrane bound receptors for signalling between cells, and (2) accumulation of sphinganine which is itself an important signalling molecule.

Thus, because fumonisin is active in the arena of membrane biochemistry, it is perhaps understandable that its presence in food, or animal feed, may be associated with a wide range of disorders which all have in common the aspects of membrane integrity and signal recognition between cells. The Joint FAO/WHO Expert Committee on Food Additives (2002) has prepared an evaluation of certain mycotoxins in food including the fumonisins. It considered that the nephrotoxicity seen in some strains of rat was the most sensitive toxic effect of pure fumonisin B_1 which led to an estimate of an overall no observed effect level (NOEL) of 0.2 mg/kg of body weight per day. This, in turn, leads to an estimated provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg body weight

using a safety factor of 100. Most national estimates of intake of fumonisin B_1 from the human diet are below this figure, although there will be communities in some parts of the world, such as southern Africa, where it is exceeded.

Although the fumonisins are heat stable, some forms of food-processing do remove them from the human food chain. The separation and removal of screenings, before whole grain goes into storage, certainly removes a significant proportion of these mycotoxins. The aqueous washings from wet milling also remove some of the fumonisins from the grain. Nevertheless these compounds are widespread in maize from many parts of the world and further work is required to assess their role in human health.

Trichothecenes

The trichothecenes are a family of epoxysesquiterpene metabolites, derived from three molecules of mevalonate, which are conveniently considered in three classes on the basis of their chemical structures. These are type A, of which T-2 toxin is an example; type B, of which deoxynivalenol (DON) is an example; and macrocyclic trichothecenes, which are not produced by *Fusarium*, and will be dealt with in part 3 of these reviews. The epoxide ring in these molecules is remarkably stable but it is an essential feature for the expression of toxicity.

T-2 toxin

T-2 toxin (Fig 1,B) is one of the most acutely poisonous of the fusarial toxins and is produced by *Fusarium acuminatum* Ell. & Kellerm and *F. equiseti* (Corda) Sacc. Also, of particular importance, are *F. poae* (Peck) Wollenw. and *F. sporotrichioides* Sherb. which were posssibly associated with the devastating outbreaks of alimentary toxic aleukia (ATA) in the former Soviet Union during, and immediately after, world war two. A shortage of manpower meant that cereals could not be harvested and were allowed to overwinter in the field. An acute shortage of food in the following spring meant that these cereals were gathered and eaten, leading to the deaths of many thousands of people. *F. sporotrichioides* is a saprophytic species able to grow at temperatures as low as –2°C at the very high water activities (> 0.88) of these overwintered cereals. The illness associated with the consumption of these contaminated cereals included nausea, vomiting, necrotic lesions in the mouth and throat (making it difficult to eat), severe haemorrhagic diarrhoea and haemorrhaging in many of the body organs. T-2 toxin is also a potent immunosuppressant as well as causing irreversible damage to the bone marrow, leading to a characteristic reduction in white blood cells (aleukia). Indeed, although continued consumption of T-2 toxin will ultimately lead to death, many of the fatalities during these outbreaks of ATA had probably occurred due to pulmonary infections which their defective immune systems could not cope with.

It may be thought unlikely that outbreaks of ATA would happen again but recent evaluation of T-2 toxin in the European diet by JECFA (Joint FAO/WHO Expert Committee on Food Additives, 2002) suggested that there could be an incidence of contamination as high as 11% in European grain samples, albeit at very low concentrations. Using a lowest observed effect level (LOEL) of 0.029 mg/kg body weight per day for a sensitive biological test, and a safety factor of 500, the Committee derived a permitted maximum total daily intake of 60 ng/kg body weight which is about eight times the estimated mean exposure level in Europe.

One of the complexities of trying to estimate the risk from toxins such as T-2 is that, in naturally contaminated cereals, there may be related fusarial toxins of similar toxicity, such as HT-2 toxin and diacetoxyscirpenol, and the analytical challenge of determining the concentration of all these compounds is considerable. Nor is it known whether they act synergistically, or additively, so the large safety factor used by JECFA seems sensible at the present.

Deoxynivalenol (DON)

DON (Fig 1,C) is also known as vomitoxin because of its potent emetic properties and its action as a feed refusal factor. It is much more common than T-2 toxin in cereals such as wheat, barley, oats, rye and maize and is produced primarily by two important cereal pathogens, *Fusarium graminearum* Schwabe (and its teleomorph *Gibberella zeae* (Schw.) Petch) and *F. culmorum* (W. G. Smith) Sacc. which cause ear rot in maize and head blight in wheat. These two species have different optimum temperatures for growth (25°C and 21°C respectively) and this probably influences their geographical distribution. The *Fusarium* species used for the industrial manufacture of food quality mycoprotein was once considered to be a non-toxigenic strain of *F. graminearum*, but is now referred to the related but distinct species *F. venenatum* Nirenberg.

Although much less poisonous as an acute toxin than T-2 toxin, DON may still have a biological effect at

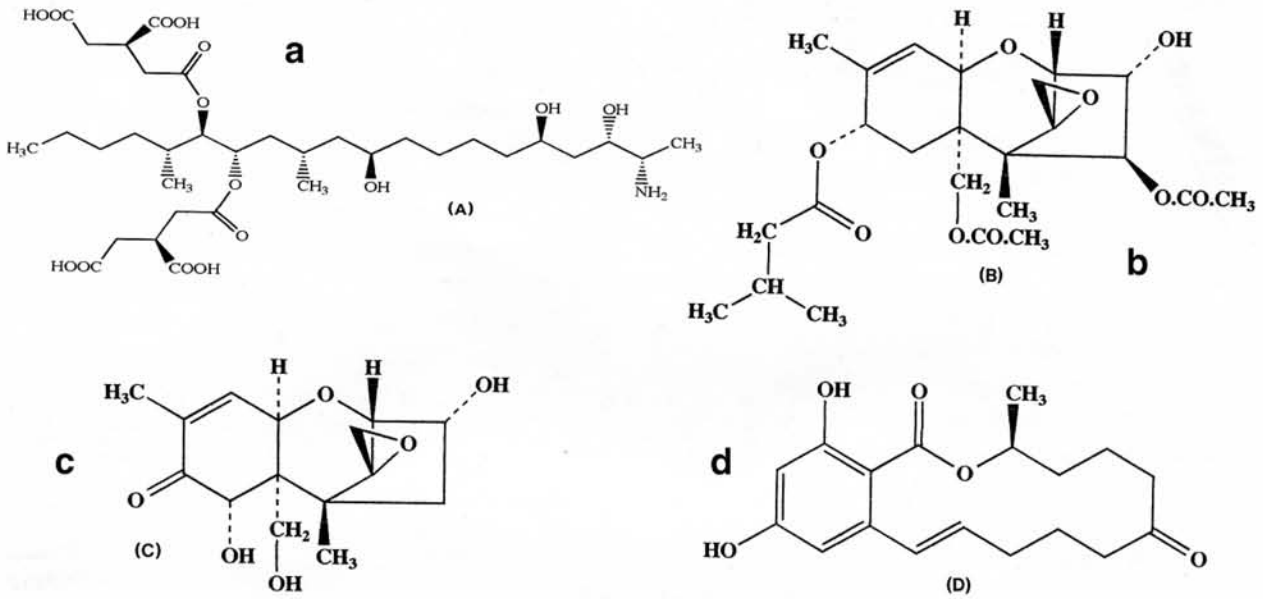

Fig 1 Structures of four fusarial mycotoxins (A) fumonisin B_1, (B) T-2 toxin, (C) deoxynivalenol (DON), (D) zearalenone.

very low concentrations. Thus, the single dose LD_{50} of DON for mice is about 70 mg/kg body weight, compared with about 5 mg/kg body weight for T-2 toxin, whereas DON shows immunosuppressant activity in mice at doses as low as 0.25 mg/kg body weight per day and the pig is even more sensitive to this activity. There have been instances in Asia of illness in humans, such as vomiting, nausea, dizziness and headaches, associated with the consumption of cereals contaminated with DON and possibly much lower concentrations of other trichothecenes.

The occurrence of DON in cereals is certainly widespread and surveys from South America, Canada, China and many countries of Europe have shown contamination levels in excess of 50% in oats, barley and wheat with mean concentrations as high as 9 mg/kg in barley. There are considerable annual variations in DON contamination which usually show a direct correlation with the incidence of head blight disease in the field caused by infection with *F. graminearum* and *F. culmorum*. In its turn head blight disease is most likely to occur if there is rain at the time of flowering (anthesis). This stage in the development of cereals is probably the most sensitive to infection and the spores of *Fusarium*, which are thin walled and easily wetted, are readily dispersed by rain splash.

The widespread occurrence of DON, and the possibility that low, persistent exposure to this mycotoxin may suppress resistance to bacterial infections such as *Listeria* and *Salmonella*, require a continued vigilance for a clearer understanding of its role in human disease. This should be linked to understanding how best to control the diseases of cereals caused by species of *Fusarium*.

Zearalenone

Unlike the previous mycotoxins zearalenone (Fig 1,D), which is a polyketide derived metabolite, is not acutely toxic with LD_{50} values measured in grams/kg body weight. It was, however, discovered as the cause of a reproductive disorder in pigs known as vulvovaginitis and as little as 0.1 mg/kg body weight can have an observable effect on the genital organs of a pig. Although it is especially associated with the pink ear rot of maize caused by *Fusarium graminearum*, like DON it is also produced by *F. culmorum* and may occur as a cocontaminant with DON in cereals. Because of its hormonal activity there is as much information about zearalenone and its derivatives in the patent literature on growth hormones, used in animal production, as there is in the literature on mycotoxins. Its use for increasing meat production in cattle is permitted in some countries, such as the USA, and banned in others, such as the countries of the European Community. Such differences in legislation in different parts of the world give rise to difficulties in trade between such countries.

Any compound with hormonal activity may be genotoxic and/or carcinogenic and there is some evidence that zearalenone may show both types of activity in some animal species. On the basis of the evidence available a report prepared for the Nordic

Council of Ministers (Eriksen & Alexander, 1998) accepted the temporary Total Daily Intake of 0.1 μg/kg body weight first proposed by Kuiper-Goodman *et al.* (1987).

Conclusion

The four *Fusarium* toxins described here show a very wide range of acute toxicity but they all have in common that they may show biological effects at low concentrations. Three of them are quite widespread as contaminants of animal feeds and, to a lesser extent, of foods for human consumption. A useful tabulation and full bibliography of the natural occurrence of fumonisin, DON and zearalenone, up to 1998, is provided by Pittet (1998). Despite the extensive studies on their toxicology it is still not certain what their significance is to human health; and even less understood is the possibility of synergistic interactions when two or more occur together. With the possibility of continued changes in agricultural practice, such as a decrease in the use of fungicides as a result of increasing demands for "organic" farming, it is important to continue to monitor the occurrence of these mycotoxins in cereals and cereal products.

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