Mycotoxins in Australian Maize: a Risk Assessment

Barry J Blaney
Department of Primary Industries & Fisheries, Locked Mail Bag No 4, Moorooka Q 4105

Introduction

This document provides a review of available information on mycotoxin occurrence in Australian maize, including published sources and unpublished data from industry testing programs. The extensive literature on the chemistry and toxicology of relevant mycotoxins is covered only briefly, with primary focus on Australian work. Risks to human health and that of pets and livestock from contamination are also discussed only in the Australian context. Emphasis is given to assessing the risks that Australian maize will exceed domestic standards and internationally accepted limits for mycotoxins, which could impact on marketing. This is a companion document to 'Mycotoxin contamination of Australian maize: a strategic risk management plan' (Blaney 2007) and 'Supply chain & export protocols for Australian maize' (Blaney and Cogswell 2007).

Aflatoxins

Chemistry and toxicity

Aflatoxins are a group of chemically similar compounds produced by *Aspergillus flavus* and *A. parasiticus.* They are highly oxygenated heterocyclic compounds characterised by dihydrodifurano moieties fused to a substituted coumarin. When extracted, separated on thin-layer-chromatography plates and viewed under ultraviolet light, two fluoresce blue (aflatoxins B1 & B2) and two green (G1& G2).

Aflatoxin B1 is one of the most potent liver carcinogens known (IARC 1993), and has been associated as a co-carcinogen with hepatitis B in the high incidence of liver cancer in parts of Africa and southern Asia (Groopman et al 1988, Peers et al 1987, Henry et al 2002). Hepatocellular cancer is the most common cancer in the world with nearly half a million new cases diagnosed each year. Aflatoxins can also cause acute poisoning if ingested by humans and other animals in high doses, such as occurred in Kenya in April 2004 when consumption of aflatoxin-contaminated maize affected 317 people, with 125 deaths (Lewis et al 2005).

Natural cases of human disease caused by aflatoxin have never been recorded in Australia, but chickens (Gardiner and Oldroyd 1965), turkeys (Hart 1965), ducks (Bryden et al 1980), dogs (Ketterer et al 1975), pigs (Ketterer et al 1982) and cattle (McKenzie et al 1981) have been poisoned in the past. The principal result of aflatoxicosis is severe liver damage (Blaney et al 1987a), but aflatoxin damages other organs and chronic exposure can damage the immune system, increasing susceptibility to infectious disease. Aflatoxin B1 is converted to aflatoxin M1 in the mammalian liver, and about 2-6% of aflatoxin B1 ingested by lactating animals is transmitted into milk as M1.

Tolerances to aflatoxins vary between livestock species and class, and the risk of milk contamination demands that dairy rations be additionally limited. On the basis of total aflatoxins (mg/kg) in the total diet, guidelines for maximum levels for various species are: ducklings, 0.005; turkeys, dogs, cats, 0.01; chickens, weaner pigs 0.02; dairy cows, dairy goats and dairy sheep 0.02; young cattle, goats and sheep 0.05; breeding pigs, cattle and sheep 0.05; horses, 0.05; grower and finisher pigs, 0.1; mature cattle, sheep and goats, 0.1. Lot-fed mature cattle, sheep and goats and finisher pigs can tolerate >0.1 for short periods.

Sources of contamination

Maize and peanuts grown in tropical conditions around the world often have serious aflatoxin contamination problems. Aflatoxins are best known in Australia as a problem in rain-fed (non-irrigated) peanuts grown in parts of the Burnett region in south-east Queensland (Graham 1982, Blaney 1985, Rachaputi et al 2002) although in Africa, southern Asia and parts of the United States the problem in maize is well recognised. *A. flavus* is able to grow in maize of lower moisture content (16% at 35°C; water activity ~0.8) and at higher

temperatures (12 – 43°C; optimum 30°C) than many other fungi found on field crops, and for this reason it was originally classified as a 'storage fungus'. In healthy peanuts and maize, plant defences prevent growth of *Aspergillus* spp., but when low available moisture and high temperatures affect kernel development, plant defences are lowered and these fungi can invade.

The sources of aflatoxin in the Australian livestock poisoning episodes referred to above were peanut meals and by-products, mouldy bread and other bakery waste, and grain sorghum and maize that were dried insufficiently and stored with high moisture contents. Aflatoxins are far less common in winter crops, although they have been detected in extremely mouldy wheat from temporary bunker stores in southern Qld (Blaney 1986), and on a single occasion at high concentrations in mouldy barley stored in a sealed silo in summer near Dubbo in NSW (Blaney unpublished data). However, significant concentrations of aflatoxins have never been detected in wheat and barley (or grain sorghum) without a history of storage problems. Cottonseed, other oilseeds and nuts like pistachios have minor contamination problems in Australia compared to elsewhere. Traces might on rare occasions be detected in mouldy hay and straw, but generally *A. flavus* and *A. parasiticus* are not competitive with other fungi on these high-cellulose materials, and they are also far less suitable substrates for aflatoxin production than grain and oilseeds. In summary, maize is the only grain crop where aflatoxins are known to cause serious pre-harvest contamination.

Occurrence in Australian maize

The main growing regions for maize in Queensland are the Darling Downs and Burnett in southern Qld and the Atherton Tableland and surrounding areas of northern Qld, with smaller amounts grown in central Qld. In New South Wales, most maize is grown in the Northern Rivers and North Coast, the Liverpool Plains, Central NSW and the Riverina. Some maize is also grown in the Murray Valley in Victoria and in Western Australia.

Southern Queensland

The first Australian survey for aflatoxin in maize was in 1978, when the Queensland Grain Growers Association (QGGA), aware of serious aflatoxin contamination of the peanut crop in the Burnett region, and problems in maize in the south-east of the USA (Lillihoj 1986), approached the Department of Primary Industries (DPI) to conduct surveys for aflatoxins in maize. The resultant survey showed a low level of contamination, and also validated the association of a Bright, Greenish-Yellow-Fluorescence (BGYF) of cracked grain under long-wave ultraviolet light, with aflatoxin contamination, as originally demonstrated in the USA (Fennell *et al*. 1973). In this initial survey, 800 maize samples grown in the Burnett region were screened under ultraviolet light for BGYF - of 140 samples judged most likely to be contaminated, aflatoxins were detected in 15 (11% positive), average 0.045 mg aflatoxins/kg, and from 70 samples chosen at random from the remainder, only 2 were positive (3%), average 0.007 mg aflatoxins/kg (Blaney 1981).

In the drought year of 1980, contamination followed a similar pattern - the QGGA conducted a survey of 768 samples from the Burnett and Darling Downs, from which 41 samples were selected as BGYF positive and 28 of these contained >0.02 mg aflatoxins B1/kg (J G Twyford, personal communication).

In a more recent survey in southern Qld in 1998 (mainly southern Darling Downs with some from the Burnett), 12 samples out of 73 were positive (average 0.01 mg aflatoxins/kg) (Maryam and Blaney unpublished). None of these surveys indicated alarming aflatoxin concentrations of maize at harvest in relation to the main target market of stockfeed, and maize is usually blended with other grains in compounded stockfeed, which also reduces the risk. However, the average concentration across all samples in some years might have exceeded the human food standard of 0.005 mg/kg, so monitoring by millers was warranted.

A series of serious droughts and high temperatures has increased the aflatoxin risk for rain-fed maize in southern Qld over the past decade, particularly in the Burnett. This was demonstrated by surveys conducted over the 2004, 2005 and 2006 seasons (Bricknell et al 2007). In the Burnett, 27 out of 168 crop samples assayed exceeded the milling standard of 0.005 mg total aflatoxins/kg, although another 33 samples were positive but <0.005 mg/kg. The central Burnett region, particularly the Coalstoun Lakes district, appears to present an unacceptable high risk for production of milling maize. On the Darling Downs, only 3 out of 146 samples exceeded milling grade (Bricknell et al 2007).

Northern Queensland

On the northern tablelands, the cooler and wetter climate is more suited to *Fusarium* species (see below) than *A.flavus*. BGYF screening is not effective in these circumstances. Two surveys for aflatoxins were reported prior to 2003. In 1982, 293 samples were all negative and in 1983, only 3 from 174 samples were positive (average 0.019 mg/kg) (Blaney *et al.* 1984b, 1986). This low frequency of contamination was mirrored by the minor aflatoxin contamination of peanuts grown there (Blaney 1985).

Of 41 samples tested from the 2004 and 2005 maize crops (Bricknell et al 2007), all met the standard for milling grade (0.005 mg/kg). Consequently, the risk of aflatoxin contamination can be considered low in traditional growing districts. On the other hand, maize production is expanding in some areas of northern Qld where the climate is hotter, drier and more conducive to contamination, so continued monitoring is warranted.

Central Queensland and Callide

Maize production is relatively recent in this region, and is dependant on rainfall and irrigation. No surveys were conducted in this region prior to 2004, but occasional samples tested as a result of diagnostic investigations by DPI did show some cause for concern (Blaney unpublished). One survey conducted during 2004 showed moderate to heavy contamination of a single large crop grown under partial irrigation (Bricknell et al 2007). Climatic modeling (Chauhan et al 2007) suggests that the high temperatures occurring over summer in this region might not prevent stress on the maize plant leading to aflatoxin contamination even with adequate irrigation, and planting time should be adjusted to avoid kernel formation during January/February (see below for discussion of control measures).

Northern rivers and northern tablelands NSW

No surveys for aflatoxins have been reported, but this region has moderate temperatures and more reliable rainfall than that in the inland grain growing regions of NSW, which suggests low risk of pre-harvest aflatoxin contamination. One out of 13 samples collected during 2004, 2005 and 2006 from this region assayed by Bricknell et al (2007) had a trace of aflatoxin (<0.002 mg/kg).

Liverpool Plains and central NSW

No surveys have been reported prior to those conducted recently. However, there have been indications (personal communication from industry representatives) that occasional cases of pre-harvest contamination had been detected, although much higher concentrations could be associated with flooding and storage problems, such as occurred in 2001 (Blaney et al 2007). Bricknell et al (2007) detected aflatoxins in 7 out of 74 samples collected from these regions in 2004, 2005 and 2006: only 4 samples exceeded the milling standard of 0.005 mg/kg, and all of these were from around Narromine in the central west.

Data provided to the author from another large processing company in NSW indicated contamination in only 3 from 108 crops harvested in 2005 at various sites in central NSW and the MIA, but more serious contamination in maize harvested around the Liverpool Plains and the MIA in 2006 which required substantial grading to meet requirements. From 96 crops, 32 would not have met the NACMA milling grade, and 17 fell into the Feed #2 grade before cleaning and processing. Some suspicion fell on the hybrids grown, but planting times, climatic conditions and insect damage are likely to have played a large part.

Riverina

It has been known for over 20 years that maize grown under irrigation in the MIA was not exempt from aflatoxin problems. Occasional contamination was generally ascribed to patches of maize on the fringes of fields suffering irregular water deficits and kernel stress cracks, and being exposed to increased insect attack (personal communications from milling industry representatives). BGYF screening and aflatoxin testing were used in the past by one large milling company to divert contaminated maize away from human food into livestock feed (personal communications). All 96 samples tested by that milling company from the 1996/97

Comments to: Barry Blaney, phone 040807 5691 email: Barry.Blaney@dpi.qld.gov.au

season until 2001/02 met the company standard of <0.005 mg/kg, although frequent detection of 0.001 – 0.003 mg/kg levels did cause some unease.

In 2002/03, irrigation restrictions and high temperatures increased crop stress, and 9 crop samples out of 25 received from contracted growers exceeded the limit of 0.005 mg/kg. Four of these samples exceeded 0.015 mg/kg, the present standard for 'prime' maize. This caused major concern and disruption, not only to growers who had to find stockfood markets, but also to the milling company and its clients.

Subsequent testing by Bricknell et al (2007) in this region in the 2004/05 and 2005/06 seasons showed that 68 out of 73 samples met the milling grade and the other 5 met the prime grade of 0.015 mg/kg. However, about 20% of crops harvested by a processing company in the MIA (Griffiths, Colleambally, Hay, etc) in 2006 had substantial aflatoxin levels (0.02-0.1 mg/kg).

Northern Victoria

The risks are probably similar to the Riverina, but insufficient data are available. Five samples tested in 2004/05 all met milling grade (Bricknell et al 2007).

South-western Western Australia

Insufficient data are available. Of two samples tested by Bricknell et al (2007), one contained low levels of aflatoxin, but both met milling grade (<0.005 mg/kg).

Risk factors for aflatoxin

The combination of drought and high ambient temperatures is now recognised as the primary environmental factor leading to aflatoxin contamination in the growing crop, with insect damage also contributing. Although aflatoxin research into factors predisposing maize to contamination has mostly been conducted in the USA (Lillehoj 1986), Australian observations support similar principles. The American research has shown that the critical period for aflatoxin production begins approximately twenty (20) days after anthesis and, if average temperatures exceed 27°C and approach 32°C, two conditions are met: firstly, the natural resistance of the maize plant to fungi in general is compromised; and secondly, the relatively heat-tolerant *A.flavus* has the advantage over other fungi present. At this stage, windblown fungal spores (*A. flavus* spores are highly resistant to desiccation) can enter through the silks. Physical damage to the ear from insects (especially boring insects) or birds also is a critical factor in aflatoxin contamination, since it exposes the endosperm to premature drying and *A. flavus* invasion. The specific problems associated with European corn borer and other insects in the USA are not relevant in Australia, and the impact of insects in Australian maize has not been quantified.

Climatic modeling (Chauhan et al 2007) has since shown that the high temperatures experienced in January/February in many growing regions (central Burnett in Qld, central highlands in Qld, central west NSW, MIA) present an inherently high risk for aflatoxin contamination in crops planted late so that kernel formation occurs in this hot period, and if irrigation is inadequate.

Aflatoxin contamination is often limited to ears in a small section of a field, for example if soil depth is shallower and/or irrigation across the field is uneven so that a few plants get less water than the rest. After harvest, most aflatoxin can be present in a tiny proportion of kernels (eg 0.1%) in a given batch of maize. However, once fungal growth has begun, it can continue until the moisture content of the grain reduces below 14%, so that delaying harvest can increase contamination.

Risk management

Risk management for aflatoxins in Australian maize has been reviewed by Bricknell et al (2007), and should involve both good agricultural practice (GAP) and quality systems based on Hazard Analysis and Critical Control Point (HACCP) (Codex 2003).

GAP for managing aflatoxin in growing maize involves selection of planting times to avoid extreme temperatures during the critical period of kernel formation, maintaining irrigation evenly across fields while monitoring any sections where soil holds less moisture, good nutrition, insect control, early harvest, minimising light-weight material at harvest, and drying (if necessary) to <14% moisture before storage. In regard to field monitoring, aerial photography can be used to detect patches of stressed and wilting plants due to lesser soil depth and water availability, as is done with peanuts in the Burnett region (Wright et al 2002).

Aflatoxin can be an even greater problem in stored maize. At moisture contents even slightly above 14%, temperature fluctuations will cause the smaller amount of 'available moisture' to migrate into pockets and if these pockets reach 16% with average temperatures around 35°C , the 'water activity' (aw) of maize reaches the minimum of 0.80 at which *A. flavus* can start to grow. Initially, the fungus will grow in the very small proportion of infected kernels, but this growth releases more moisture from the maize and the fungus will rapidly spread into adjacent sound kernels. This process is greatly accelerated by storage insects which damage kernels, transport fungal spores into the kernels and also release more moisture as they consume grain. Good agricultural practice for aflatoxin management includes: minimising damaged kernels before storage, either during harvest or gravity grading; using appropriate types of storage – shape of container and grain depth must not restrict air flows; managing night-day air flows as appropriate for ambient temperatures to avoid moisture condensation; and controlling insects with appropriate chemicals or modified atmospheres (Hocking 1991).

For millers and processors of maize, risk management includes selective sourcing of maize from lower risk crops, grading to remove damaged grain, and taking into account the effects of processing on aflatoxin contents. Dry milling produces large grits used for corn flakes, bran, germ, meal and flour. Studies have shown that only a small proportion remains in the large grits, with highest concentrations in the germ, bran and fines (Brekke et al 1975). Wet milling also concentrates aflatoxins into the germ, fibre and gluten fractions (Bennett and Anderson 1978). The implications need to be taken account of when deciding acceptable aflatoxin levels in raw ingredient, and also the impact of increased aflatoxin in fractions used for subsequent incorporation into human food and pet food. The relative frequency of contaminated batches entering a processing stream and the variability in aflatoxin contents within each batch also involve risk assessment and management decisions.

Regulations and trading standards

Aflatoxin B1 is currently regulated to 0.02 mg/kg in grain for stockfood under Queensland state regulations (Anon 2003). Standards for human food, however, are applied nationally; the *Food Standards Code* is called into force by the *Food Act 1981* in Queensland, and by similar legislation in other States and New Zealand. In the 1999 review of Standard A12, it was recommended that the 0.005 mg/kg standard for aflatoxin in foods other than peanuts, peanut products, tree nuts and tree nut products be removed, as it was "unnecessary and inconsistent with the draft Codex Standard". However, it remains important to the maize industry that Australian maize can be demonstrated to meet international standards, the most commonly applied standard being 0.005 mg total aflatoxins/kg, which is also the NACMA trading standard for 'milling' grade maize.

It is noted that the Feed #2 NACMA standard of 0.08 mg total aflatoxins/kg also specifies a limit of 0.02 mg aflatoxin B1/kg to harmonise with the Qld Stockfood Regulations (Anon 2003). The suitability of these NACMA grades for different livestock species depends obviously on degree of dilution in the total diet, period of feeding, and risk of residues in animal products like milk. A brief summary was complied by Kopinski and Blaney (2006).

Cyclopiazonic acid

Chemistry and toxicity

Cyclopiazonic acid (CPA) is a tetramic acid which has been associated with 'Kodua' millet poisoning of humans in India (Rao and Husain 1985), and might also contribute to the toxicity of mouldy maize containing aflatoxin to livestock (Cole 1986). It only seems to be toxic once it passes a certain threshold, fairly high

concentration, when it causes damage to various organs (liver, kidney, spleen). It has been found to be mutagenic in the Ames assay, but is not known to be carcinogenic or proven to have other chronically toxic effects.

Sources of contamination

CPA is another mycotoxin that is produced by *A. flavus* (but not *A. parasiticus*), and by several other *Aspergillus* and *Pencillium* species.

Occurrence

CPA has been detected in maize in other countries such as the USA (Gallagher et al 1978) and Indonesia (Widiastuti et al 1988). It is produced by Australian fungi (Blaney et al 1991), but has not yet been surveyed in Australian maize.

Risk factors & management

Processes to minimise aflatoxin contamination of maize will also minimise CPA contamination.

Regulation

CPA is not currently regulated internationally (EMAN 2004).

Ochratoxin A

Chemistry and toxicity

Ochratoxin A is a substituted coumarin derivative with an acidic carboxy group. As with the aflatoxins, it is also highly fluorescent under long-wave ultraviolet fluorescence. It is known to cause severe kidney damage and immunosuppression in several animal species as well as inducing DNA damage in rodents in the laboratory. To date there is no conclusive evidence that the toxic effects of ochratoxin A are the same in humans as in animals, but given its effects as a kidney toxin in most animals tested it would be reasonable to expect it is also a kidney toxin in humans. Additional animal evidence is sufficient for the International Association for Research into Cancer (IARC 1993) to classify it as a possible human carcinogen.

There have no proven cases of ochratoxin poisoning of livestock in Australia. High concentrations (70 mg/kg) were identified in mouldy bread thought to be the cause of poisoning of goats (Connole et al 1981), but ochratoxin A is rapidly converted to the non-toxic ochratoxin α in the rumen of ruminants, which makes this less likely, and aflatoxins were also detected in the bread. Ochratoxin (0.1 mg/kg) was present in combination with aflatoxins (10 mg/kg) in mouldy sorghum that affected pigs in southern Qld (Ketterer et al 1982), but probably played little part in the syndrome, as a subsequent experiment showed that these toxins are not synergistic in pigs (Tapia and Seawright 1985).

In toxicity studies, dogs and pigs were found to be the most sensitive species. Young beagle dogs dosed with ochratoxin A at 0.1 and 0.2 mg/kg bw/day for 14 days did not have reduced kidney function, but microscopic lesions in the kidney were detected. This level equates to about 1 mg/kg in feed. A similar result was obtained in female pigs fed 1 mg/kg feed for 2 years (see review by Kuiper-Goodman and Grant 2007). Australian broiler chickens (3 weeks of age) fed 1 mg/kg for 5 weeks were not adversely affected (Reichmann et al 1982). In regards to tolerances of pets and livestock, 1 mg/kg has been shown to produce minor kidney damage in long-term feeding studies with dogs and young pigs, so 0.1 mg/kg would appear to offer a generous safety margin for these species.

Sources of contamination

Ochratoxin A is produced by *Aspergillus ochraceus*, *A. carbonarius*, *A. niger* and *Penicillium verrucosum* (Pitt et al 2000). Ochratoxin contamination has been identified in grapes and grape products in Australia as a result of growth, mainly of *A. carbonarius* (A Hocking pers. comm.) and is known as a contaminant of coffee beans and figs elsewhere. Growth of *P. verrucosum* in storage can cause serious ochratoxin contamination of barley and other grains in Canada and Europe, but the fungus is not significant in Australian barley (J Pitt pers. comm..). Webley and Jackson (1998) reported a single instance of ochratoxin A contamination in wheat stored in cold wet conditions, but negative results otherwise in extensive screening.

Ochratoxin A, probably produced by *A. ochraceus* has been detected on several occasions in grain sorghum stored with high moisture contents (>16%) in central Queensland, usually in combination with aflatoxins (Ketterer et al 1982, Blaney unpublished data). However, it was not detected during screening of several hundred 'fair average quality' sorghum samples from that region in 1983, 1984 and various other years (Blaney, unpublished data). Connole et al (1981) reported ochratoxin production by *A. ochraceus* isolates in Qld.

Occurrence in Australian maize

Ochratoxin A has been detected only on rare occasions and in very low concentrations (0.001 – 0.004 mg/kg) in maize at harvest in Australia. These detections were in irrigated maize in the Murrumbidgee Irrigation Area (MIA) (data provided by milling company). The most likely species producing ochratoxin in Australian maize is *A. ochraceus*. However, members of the *A. niger* group have relatively recently been identified as ochratoxin producers and, since these do occur in Australian maize, might also contribute to ochratoxin contamination. However, surveys of maize produced in northern Qld (Blaney et al 1984b, 1986), southern Qld (Maryam & Blaney unpublished) and other Australian regions (Bricknell et al 2007) have all been negative.

Ochratoxin in maize is also uncommon in the USA, where high concentrations (1-7 mg/kg) have only been associated with maize that has undergone extensive mould growth in storage. A similar instance was observed in southern Queensland some years ago (Blaney, unpublished data). Even in parts of south-east Asia where aflatoxins are common in maize due to slow sun-drying and storage problems, ochratoxins appear to be only occasional contaminants (Widiastuti et al 1988). All indications are that ochratoxin does not present a significant risk to human health or livestock in Australia. However, current proposals in the EU to set limits of 0.005 mg/kg in raw grain and 0.003 mg/kg in derived products (see below) has potential to affect trade risks for exported maize.

Risk factors and management

While it can be concluded that *A. ochraceus* is far less common than *A. flavus* in maize, the occasional case of contamination is hard to predict, since little is known about factors controlling pre-harvest *A. ochraceus* infection. In laboratory cultures, *A. ochraceus* grows over a similar range of temperature and moisture as *A. flavus*, but there are apparently other factors limiting toxin production in field maize. These factors could include survival of spores on soils (relative resistance to desiccation), ability to invade the developing ear, and ability to compete with other fungi like *A. flavus*, *A. niger* and *Fusarium* species for damaged kernels. Similarly, little is known about factors that might promote ochratoxin production by *A. niger* in maize. However, some interaction has been shown between *A. niger* and *A.flavus* in regard to mycotoxin production. Until more is known about these factors, it is reasonable to assume that processes for managing aflatoxin in maize will also minimise the risk of ochratoxin contamination.

Regulation and standards

The EU has proposed a limit of 0.005 mg/kg in raw grain materials and 0.003 mg/kg for derived cereal products and grain for direct human consumption (EMAN 2004). The impetus for these stringent controls are results from recent surveys (SCOOP) across the EU showing widespread and extensive contamination of grains, grape products, beer, coffee, figs, olives and processed meats with ochratoxin A (Walker 2002). This sensitivity could influence international regulatory bodies, and eventually impact on Australian maize exports.

In contrast to the situation in Europe and Canada, the only identified potential sources of ochratoxin A in the Australian diet are maize and some grape products (both of which appear quite insignificant) and perhaps some imported products like coffee, olives and red wine. However, there appears very little justification for regular monitoring of Australian maize for ochratoxin A, unless there is a history of mould growth in storage.

Fumonisins

Chemistry and toxicity

Fumonisins are a group of chemically related polar compounds based on a hydroxylated hydrocarbon chain with methyl and amino (or acetyl) substituents. The most common and most toxic is called fumonisin B_1 (FB_1), with FB_2 and FB_3 usually accompanying FB_1 but in much lower concentrations. Fumonisins are particularly toxic to horses, where they cause liquefaction of the brain known as equine leucoencephalomalacia. The disease has been reported in many countries, including Australia (Shanks et al 1995.) Pigs can be affected with pulmonary oedema (Osweiler et al 1992), but no cases have been confirmed in Australia. Whether or not fumonisins have a role in human disease is still being investigated, but they have been associated with oesophageal cancer and diseases resulting from inhibition of sphingolipid biosynthesis (Riley et al 1996). The toxicity and carcinogenicity of AF B_1 is thought to be linked to its conversion to a highly reactive epoxide (McKean et al 2006).

Tolerances to fumonisins vary between livestock species and class (CFSAN/FDA 2001). On the basis of total fumonisins (mg/kg) in the total diet, suggested maximum levels for various species are: horses and rabbits, 1; dogs, cats, weaner pigs, 5; grower/finisher pigs, weaner cattle, 10; breeder pigs, cattle, sheep and poultry, 15; dairy ruminants, 15; non-breeding poultry and lot-fed ruminants, 30. Fumonisins are not considered to produce significant residues in milk of meat.

Sources of contamination

Fumonisins appear only to occur in maize, being produced by several *Fusarium sp.* that are associated with ear rot and stalk rot in maize worldwide. The most common species is *Fusarium verticillioides* (previously called *F. moniliforme*, Seifert et al *2003*) which appears to be the main source of fumonisins. It is also the most common in Australian maize (Watson et al 2006), but *F. proliferatum, F. subglutinans, F. thapsinum and F. nygamai* have also been isolated from ear-rotted maize in NSW, and are on record as capable of producing fumonisins.

F. verticillioides is common in maize from both northern Queensland (Blaney *et al*. 1986) and southern Queensland. The incidence of kernel-rot varies between seasons, for example it was severe on the southern Downs in 1985/86 (Williams *et al*. 1992a)

Occurrence in Australian maize

Queensland

In 1998, a survey for fumonisins was conducted in 73 maize samples representing grower deliveries to bulk grain depots in southern Qld (Maryam and Blaney, unpublished). Fumonisins were detected in all samples, averaging about 3 mg/kg (range 0.1 to 8.5 mg/kg). Fumonisins were also detected in a few samples of maize-based human foods purchased in supermarkets at up to 6 mg/kg – which is at odds with reports from the USA suggesting that processing of human foods usually results in a substantial reduction in fumonisin concentration.

Some concentrated samples of damaged kernels obtained during 1985/86 on the southern Downs were later found to contain up to 40 mg fumonisins/kg. Feeding trials with pigs in that year (Williams *et al*. 1992b) did not indicate high toxicity, but withdrawal of two pigs with pneumonia from the trial in hindsight raised suspicions of pulmonary oedema.

Surveys by Bricknell et al (2007) in 2004, 2005 and 2006 found 95% of samples from the Burnett and Darling Downs met the milling standard of 2 mg/kg for fumonisins and only 2 samples out of 314 exceeded the Prime standard of 5 mg/kg. In northern Qld, one sample out of 41 exceeded the Prime standard.

New South Wales - general

One survey for fumonisins in Australian maize was reported by Bryden et al (1995). Of 53 samples from 'human and animal food manufacturers and grain merchants', only 6 did not contain the toxin, and concentrations ranged from 1 to 40 mg/kg. At the University of Sydney, a couple of hundred samples of maize (most from NSW and some from Qld) were assayed for fumonisin using rapid test kits during 1995-97. This showed a moderate frequency of contamination at levels around 1 mg/kg (W Bryden personal communication).

Samples associated with leucoencephalomalacia in horses in NSW contained 164 mg/kg (Christley et al 1993, Shanks et al 1995).

Surveys by Bricknell et al (2007) in 2004, 2005 and 2006 found that 66 out of 79 samples from parts of NSW other than the MIA met the milling grade, 6 met prime grade (2- 5 mg/kg), 1 met feed #1 (5-10 mg/kg), 5 met feed #2 grade (10-40 mg/kg) and 1 sample exceeded 40 mg/kg. These results show a possible trend towards greater fumonisin contamination in NSW than in Qld.

NSW - Riverina

Of 96 samples tested by a large milling company based in the Riverina (MIA) from the 1996/97 season until 2001/02, 40 samples contained 1-2 mg/kg, and only 2 samples contained 2-5 mg/kg. In 2002/03, irrigation restrictions and high temperatures increased crop stress, and 7 crop samples out of 25 received from contracted growers exceeded the company standard of 2 mg/kg (all <5 mg/kg). This caused major concern and disruption, not only to growers who had to find stockfood markets, but also to the milling company and its clients. As part of the process of sourcing new markets for this rejected grain, 66 samples were tested for fumonisins: 32 contained <2 mg/kg; 5 contained 2-5 mg/kg; 21 contained 5-10; and 8 exceeded 10 mg/kg. Only two samples of screening exceeded 40 mg/kg (56 & 152 mg/kg) (O'Keeffe 2003, Blaney & Bricknell unpublished).

Surveys by Bricknell et al (2007) assayed 73 samples collected in 2004, 2005 and 2006 from this region with the following results: milling grade, 57; prime grade, 8; feed #1, 1; feed #2, 5; and 2 >40 mg/kg. These results are similar to those in other parts of NSW, and also to those obtained in the MIA in 2003.

Risk factors for fumonisins

F. verticillioides is systemic in the maize plant, but seems to grow rapidly and increase fumonisin concentrations only when plant defences are impaired. *F. verticillioides* requires a higher moisture content than *A.flavus* and is less heat tolerant. While drought stress is a significant factor in fumonisin contamination, the association with very high temperatures is not as strong as with aflatoxin. This might underlay the apparent trend towards more fumonisin contamination in NSW than in Qld. Irregular water availability (which can occur at the edges of irrigated fields) can produce sudden contraction and expansion of the pericarp, causing a 'starburst' pattern of fine cracks which appears to be associated with increased growth of *F. verticillioides* and production of fumonisins. Insect damage can also increase fumonisin contamination. Physical damage increases access to the endosperm, and stress might also reduce the activity of a beneficial maize fungus *Acremonium zeae* (Wicklow et al 2005). Different maize hybrids could vary in susceptibility to fumonisin (Butron et al 2006), but more research is needed in this area.

Risk management

Stress reduction in crops by good irrigation, correct fertilization, and insect control appear the keys to minimising the risk of excessive fumonisin contamination. However, hybrid susceptibility might also be playing a part, and maize breeders need to accommodate this in breeding objectives.

Because *Fusarium* species require a moisture content of 30-40% and relative humidity of ~95%, fumonisins are very unlikely to increase in maize post-harvest.

When serious fumonisin contamination does occur, it has been shown that a major proportion can be in the lightweight fraction, and removable by gravity grading.

Processing has been shown in the USA to cause substantial reductions in fumonisins. Dry milling produces large grits used for corn flakes, bran, germ, meal and flour. It has been shown that highest concentrations remain the bran and fines, less in germ and smallest grit fraction, but very little in the largest grits used for corn flakes (Katta et al 1997). Wet milling leaves little fumonisins in the corn starch, but concentrates it into the gluten, fibre and germ fractions (Bennett et al 1996). These principles need to be checked in Australian processing plants, but it seems likely that similar results will be obtained. The implications need to be taken account of when deciding acceptable fumonisin levels in raw ingredient, and also the impact of increased fumonisins in fractions used for subsequent incorporation into human food and pet food. The price of these products needs to reflect the cost of fumonisin contents, the associated risks to end-users, and the costs of minimisation (additional testing, premiums for raw ingredient, and reduced value of by-products).

Regulation and standards

In the USA, recommended limits for fumonisins range from 1 to 50 mg/kg in the complete feed of livestock and companion animals, with higher concentrations allowed in raw maize ingredient (US-FDA 2001). In maize for human food, 2 mg/kg is recommended in degermed dry milled maize products such as grits, 3 mg/kg for popcorn, and 4 mg/kg for some other maize products. Fumonisins are regulated only in a few countries ranging from 1 to 3 mg/kg. This includes a few members of the European Community, but the EC has not yet harmonised limits (EC 2005).

Fumonisins are not currently regulated in Australia. However, the NACMA trading standards are consistent with overseas proposals (CFSAN/FDA 2001), and human dietary exposure to fumonisins in Australia appears similar to that in North America. The results obtained in surveys above suggests a need for regular monitoring of maize intended for humans, for pet food and for horses. The risks for other livestock appear low and given that maize is rarely the main grain component of mixed diets in this country, testing might only be warranted when there is evidence of substantial pre-harvest damage (a known history of extensive *F. verticilioides* kernel rot, or shrunken, lightweight grain).

Zearalenone

Chemistry & toxicity

Zearalenone (ZEA) is a substituted resorcyclic acid lactone. It is non-steroidal estrogenic mycotoxin that has been implicated in some forms of infertility in pigs, cattle, sheep and possibly other animals. It has not been proven to affect human health (Anon 2004). Young pigs appear to be the most susceptible animal species. It has a very low acute toxicity, and it has been described as a myco-oestrogen rather than a mycotoxin.

In Australia, ZEA poisoning of pigs is not common, but cases have been recorded: in the highlands of central Victoria (apparently, as zearalenone was not assayed; Pullar and Lerew 1937); on the Atherton Tableland of far-north Queensland (Blaney et al 1984); and in the Northern Rivers district of New South Wales (unpublished data). The effects of ZEA are often mixed with those produced by trichothecenes (see below) as both are produced by certain *Fusarium* spp.

Young female pigs appear to be the most susceptible class of animal to zearalenone, where oestrogenic effects can be produced at levels of 0.2 mg/kg in the complete diet, although these effects are more consistent at 1 mg/kg (Williams et al 1988). Chickens and non-breeding cattle and pigs can tolerate much higher concentrations. Some pet food manufacturers have expressed concern about ZEA possibly affecting breeding

of cats and dogs, but the scientific panel of the EC (Anon 2004) recently expressed the opinion that there were no reliable data for the effects of ZEA on cats, dogs, rabbits and horses. Limited data suggests that humans might have a similar susceptibility to pigs, and ZEA has been patented as an oral contraceptive (Hagler et al 2001).

Sources of contamination

In maize, zearalenone is primarily produced by *F.graminearum*, a fungus responsible for causing ear and stalk rots. There are a few other *Fusarium* species that produce zearalenone, such as *F. culmorum*, *F. pseudograminearum*, *F. equiseti* and *F. crookwellense*, but these are less prevalent in maize. Zearalenone is also produced in pasture grasses by some *Fusarium* species, including *F. culmorum* and *F. crookwellense* (Lauren et al 1988).

Occurrence in Australian maize

The only place where zearalenone in significant concentrations can be regularly detected in Australian maize is on the tablelands of far north Qld, where the persistently wet climate favours ear rot by *F. graminearum.* Concentrations of around 1 mg/kg can be quite common (Blaney *et al*. 1984b, 1986) and storage of maize without drying can increase this sufficiently to affect pigs (Blaney *et al.* 1984a). The most contaminated kernels can be distinguished by a dark purplish colouration. In samples collected in the worst affected regions in 1983 and 1984, average concentrations of ZEA in samples with <0.25% purple kernels was 0.04 mg ZEA/kg, in samples with 0.25-1% purple kernels was 0.21 mg ZEA/kg, and in samples with >1% purple kernels was 0.61 mg ZEA/kg. Samples with 2% of purple kernels contained about 1 mg ZEA/kg. Continued breeding of maize hybrids for resistance to ear rot appears to have decreased ZEA concentrations over the last 20 years.

Another area where *F. graminearum* is relatively common is in the wet coastal districts of south-east Queensland and northern NSW. Zearalenone contamination in these zones is related to the presence of inoculum, but incidence is determined by timing of rainfall in relation to silking and the relative resistance of the maize hybrids planted. While surveys have not been reported, a few positive samples have been identified in diagnostic investigations into livestock disease, and it is probable that zearalenone is regularly present (even if only at low concentrations) in maize grown in these areas.

Such weather conditions are uncommon in other Australian maize-growing regions (Burgess et al 1981). However, this does not mean that ZEA will be completely absent. Monitoring in the MIA by a milling company regularly detected levels of 0.05 – 0.1 ZEA mg/kg (10 – 100% of samples) over the years 1996/97 to 2002/03 (T. Micklasavich, pers,comm.). Lowest levels in feed known to affect young pigs, the most sensitive species, are about 0.25 mg/kg. It is of note that concentrations did not increase in 2002/03 when ear rot was particularly severe – another indication that *F. verticillioides* rather than *F. graminearum* was dominant (see risk factors).

Surveys in 2004, 2005 and 2006 by Bricknell et al (2007) across all regions detected levels exceeding 0.1 mg ZEA/kg in only a few samples out of nearly 600, mainly from the Atherton Tableland.

Risk factors

Factors favouring *F. graminearum* infection are the key to prevalence of ZEA in maize. *F. graminearum* causes head blight of wheat, and rotating wheat and maize is a common cause of increased infection in both crops if climatic factors suit (Blaney et al 1987b; Southwell et al 2003). *F. graminearum* has also been isolated from stalk rot of sorghum rotated with wheat and maize (Blaney and Dodman 2002), and inoculum persists in pasture grasses rotated with maize in wet areas like the Atherton Tableland (Burgess et al 1981).

On the Atherton Tableland, fungal isolations from kernels with a purple discolouration found *F. graminearum* to be most common (63%), followed by *F. moniliforme* (*F. verticillioides*) (24%), *F. subglutinans* (8%) and *F. oxysporum* (10%). However, in damaged kernels without the purple discolouration, *F. verticillioides* (47%) was much more prevalent than *F. graminearum* (12%), whereas in visibly sound kernels *F. verticillioides* was

still isolated at 35% frequency compared to only 4% for *F. graminearum* and less for other *Fusarium* spp (Blaney et al 1986). This result supports the prevailing hypothesis that *F. verticillioides* can be a widespread systemic infection in symptomless maize which increases (and produces fumonisins) under conditions of stress and physical damage. *F. graminearum* on the other hand tends to invade the ear at silking and attack developing kernels usually at the ear tip, from whence it can grow down the centre of the cob and eventually into other kernels.

These different patterns of invasion reflect the different modes of survival among *Fusarium* spp. According to Burgess (1981), *F. verticillioides* survives as mycelium in plant residues near the soil surface and is well adapted to dispersal in the atmosphere in dry or wet weather as it forms macroconidia in sporodochia and chains of microconidia in dry powdery masses which are easily dispersed by wind and rain. *F. subglutinans* is also in this category. Consequently, spores will always be present on maize and invade whenever the opportunity presents, such as when insect damage occurs. On the other hand, *F. graminearum* invades by active dispersal of ascospores from perithecia produced on infected crop debris on the soil surface. Warm, humid conditions are required for perithecia formation and ascospore discharge, which needs to occur when maize is silking and most vulnerable to infection. The extent of growth of *F. graminearum* and production of zearalenone is then favoured by persistently moist conditions during maturation. This combination of weather conditions occurs on the Atherton Tableland (see Blaney et al 2006), and in the warm, summer-dominant heavy rainfall areas on the NSW north coast and parts of southern coastal Qld.

In other Australian regions where maize is grown, *F. graminearum* infection is far less prevalent because warm, wet weather at silking is not as common, and there is less opportunity to build up inoculum on crop residues on the soil. Watson et al (2006) investigated severe ear rot of maize that occurred in the MIA in 2002/03, and isolated the following species in decreasing frequency: *F. verticillioides, F. proliferatum, F. subglutinans, F. thapsinum, F. nygamai, F. graminearum, F. semitectum* and *F. equiseti*. Of these, *F. graminearum* and *F. equiseti* can produce ZEA. This does not mean that ZEA will not occur in these regions, but that it will rarely reach concentrations that are significant to human health or to livestock.

Risk management

In the main Australian maize production areas, zearalenone does not appear to warrant specific controls, but if necessary good agricultural practice would involve reduced stubble retention and avoiding maize-wheat rotation. Climatic patterns should be monitored, as unusually warm and moist weather during silking could increase the risk if there is crop debris on the soil. On the Atherton Tableland in far-north Queensland, effective management involves use of the hybrids specifically developed by DPI&F for disease resistance in that region, which feature a very long and tight husk cover. This breeding material could be adapted to hybrids for other areas if zearalenone problems become significant.

Regulations and standards

There are no international regulations for zearalenone in maize at this time. However, maize represents a small fraction of the Australian diet and intake of ZEA from the main food grains wheat and barley is negligible in Australia, so a limit of 0.2 mg/kg in raw maize would appear safe and achievable. Imported maize (and wheat and barley) products appear to have a much higher risk of zearalenone contamination than local grain, and some scrutiny of such imports is justified.

A limit of 0.2 mg/kg should also be safe for maize and maize products to be incorporated into pet foods, given that the maize would represent from 10 – 50% of the final product. Complete diets for young pigs should be limited to 0.1 mg/kg; while that for cattle and poultry should be limited to 0.5 mg/kg. Maize containing up to 2 mg/kg or more would be acceptable for stock food after suitable dilution in diets, but the potential co-occurrence of trichothecenes should be considered (see trichothecenes below).

The NACMA standard (NACMA 2003) for all maize grades currently contains a ‘nil’ tolerance for Fusarium (pink) fungal stained grain. This was set in regard to wheat affected by *F. graminearum* head blight and is achievable because of the low incidence of head blight in Australia. It might require some modification in future for maize, since such a stringent standard could unnecessarily restrict marketing of maize from wetter

coastal areas of Australia, and because maize with <0.2% pink/purple Fusarium-infected grain is unlikely to contain >0.1 mg ZEA/kg (Blaney et al 1984b).

Trichothecenes

Chemistry and toxicity

Trichothecenes are a large group of sesquiterpenes, that are broadly divided into Type A (T-2 toxin; HT-2 toxin, diacetoxyscipenol, etc), Type B (deoxynivalenol, nivalenol, etc) and macrocyclic trichthecenes (verrucarins, roridins). Acute exposure to trichothecenes induces anorexia at low doses and emetic effects at higher doses as well as causing problems with cell replication, irritation of the gastrointestinal tract and effects on the immune system. There is no current evidence that they are either carcinogens or mutagens. Type A trichothecenes including T-2 toxin produced by *F. sporotrichioides* and *F. poea* in millet have been associated with the human disease alimentary toxic aleukia (ATA) that was first reported in Russia in the 19th century (Ueno 1983). The characteristic signs of ATA are fever, necrotic angina, leukopenia, haemorrhaging, exhaustion of bone marrow and death. Cold, moist grain storage appears to factor these fungi. On the other hand, Type B trichothecenes tend to be produced by *Fusarium* species that favour warm to temperate climates, such as *F. graminearum*. Human food poisoning by deoxynivalenol (DON, also referred to as vomitoxin) featured abdominal pain, dizziness, headache, nausea, vomiting, diarrhoea and blood in the stool (Pieters 2002).

Poisoning of either humans or livestock by Type A trichothecenes has never been diagnosed in Australia. Although DON, nivalenol (NIV) and their acetyl derivatives are known to contaminate maize in some parts of Australia, DON and NIV are far more common in maize in wetter regions such as parts of New Zealand (Lauren et al 1991). DON has been responsible for widespread economic losses in North America, particularly in wheat (Schaafsma 2002). Human exposure to DON and NIV from wheat is rarely significant in Australia (Tobin 1988, Webley and Jackson 1998), and problems with livestock production (vomiting and reduced feed intake, particularly by pigs) are also uncommon (Moore et al 1984; Tobin 1988).

The tolerance of pigs to DON (Williams et al 1988) and NIV (Williams and Blaney 1994) in naturally contaminated grain in Australia have both been tested. Vomiting at high intakes (DON only) and persistent feed refusal (DON and NIV) were the only adverse effects noted, with the tolerance about 1 mg/kg: feed conversion was only affected when levels exceeded 8-9 mg DON/kg. Chickens are generally regarded as more tolerant than pigs, but offered a choice between diets containing 12 mg DON/kg and control diets, chickens strongly selected against the DON-containing diet, and given no choice, intakes were down and daily gain was reduced by 12% (Mannion and Blaney 1988). Daily gain also declined by 3 to 8% in chickens fed maize-base diets containing 3 to 6 mg NIV/kg, respectively (Kopinski et al 1991). Cattle also are considered fairly tolerant. However, severe feed refusal, depression and scouring was observed in calves fed triticale grown around Casino in northern NSW, that was subsequently found to contain 30 mg DON/kg (Blaney, unpublished data).

Sources of contamination

In Australian maize, the fungus primarily responsible for producing NIV and DON is *F. graminearum* (Burgess et al 1981, Pitt 2000). *F. culmorum* is another source of DON but appears more common on wheat than maize, and prefers cooler latitudes where less maize is grown in Australia. In comparison, *F. graminearum*, *F. culmorum* and *F. crookwellense* are all associated with DON and NIV contamination of maize in New Zealand (Lauren et al 1996).

Infection by *F. graminearum* is favoured by warm, wet conditions during flowering and persistently wet weather during maturation, which are common in Australia only on wetter localities (Kairi, Malanda, Herberton) on the tablelands of far-north Queensland and the north coast of NSW (Casino, Kyogle, Grafton). Research indicates that infection in far-north Queensland produces NIV while infection with the same species in northern New South Wales tends to produce DON (Blaney and Dodman 2002), and this might be related to different genotypes (Burgess pers. comm.).

Other *Fusarium* species like *F. equiseti*, *F. semitectum*, *F. acuminatum* that have been isolated from maize and maize soils (Wearing & Burgess 1978; Watson et al 2006) have potential to produce Type A trichothecenes, but their relatively low frequency in maize suggests that the risk of contamination is also low. Australian isolates of *F. compactum* and *F. acuminatum* subsp. *armeniacum* and *F. acuminatum* subsp. *acuminatum* tested by Wing et al (1993) produced type A trichothecenes, while several isolates of *F. equiseti* were of low toxicity. While widespread, these fungi are weak pathogens on maize, and are more likely to be associated with severe weather damage and wet, cool storage, both of which are uncommon in Australian maize areas. Minor contamination of maize with T-2 toxin and scirpenol has been reported in New Zealand maize (Lauren 1991).

None of this argument rules out the possibility of contamination of Australian maize, which of course can only be demonstrated by surveys, but it does point to the risk of significant contamination being very low and probably confined to cooler and wetter districts.

Occurrence in Australian maize

On the north Qld tablelands, NIV (and ZEA) can be detected in individual maize crops, but concentrations rarely exceed 1 mg/kg. DON has also been detected in maize in the region, but at very low frequency compared to NIV (Blaney, unpublished data). As with ZEA, monitoring of pink-purple discoloured kernels in grain is a useful screening test. In samples collected in the worst affected regions of the northern tablelands in 1983 and 1984, average concentrations of NIV in samples with <0.25% purple kernels was 0.13 mg/kg, in samples with 0.25-1% purple kernels was 0.66 mg NIV/kg, and in samples with >1% purple kernels was 1.21 mg ZEA/kg. The maximum detected was 2.5 mg NIV/kg in a sample with >2% of purple kernels (Blaney, unpublished data). Continued breeding of maize hybrids for resistance to ear rot in the 20 years since those surveys has probably reduced the extent of contamination.

The characteristic purple discolouration has been observed in isolated maize crops in the sunshine coast and gold coast hinterlands in Queensland, but not in the Darling Downs and Burnett regions in the limited mycotoxin surveys conducted.

Surveys for DON and NIV in maize have not been reported from NSW. Since DON has been detected in wheat and triticale with head blight from the Northern Rivers district (Tobin 1988), there is little doubt that it will be an occasional contaminant of maize in the same region.

Industry monitoring in the MIA from 1996/97 until 2003/04 detected DON (>0.5 mg/kg) in 3 out of 90 maize deliveries (0.6, 0.6, 0.7 mg/kg).

Risk factors & Management

Maize grown on the wetter parts of northern Qld tablelands (Malanda, Kairi, Herberton, Atherton) will continue to have a high risk of NIV contamination. Currently, this is controlled by resistant hybrids developed in the region by DPI&F. Other commercial hybrids developed elsewhere are regularly demonstrated to be extremely susceptible to *F. graminearum* ear and stalk rots in the region, and presumably to NIV and DON contamination. This potential for contamination should be taken into account if there are intentions in future to export maize from this region. Currently, most of the maize is used as feed for dairy and beef cattle, with some going to pigs and poultry. DON and NIV are more concentrated in visually damaged grain, and the most contaminated kernels can be distinguished by a dark purplish colouration.

The occurrence of head blight in wheat on the Liverpool Plains in 1999 and 2000 (Southwell 2003) also points to a moderate risk of DON and NIV contamination of maize grown there, but the extent of ear rot will be determined by the amount of crop residues present (maize and sorghum), heavy rainfall at silking, and persistent rainfall during maturation, which is also an unlikely scenario in most years. However, increased production of maize under pivot irrigation in the region could be increasing the risk.

Surveys for DON and NIV from other maize growing regions of NSW have not been reported, but one investigation in 2002/03 did detect *F. graminearum* at low frequency to other *Fusarium* species in maize from central NSW (Watson et al 2006). Low levels of DON and NIV should be expected as occasional contaminants of maize in wetter seasons throughout NSW and Victoria.

Processing has an effect on DON levels in products. Dry milling of DON-contaminated maize concentrated most DON into the germ meal fraction (Scott 1991). Wet milling of maize was studied in New Zealand by Lauren and Ringrose (1997), who found that most DON and NIV went into the concentrated steep liquor, since they have some water-solubility.

Overall, the relatively low frequency of contamination suggests that the issue of NIV/DON contamination of Australian maize is relatively minor, requiring only that monitoring for ear rots and purple grain be conducted in unusually wet seasons.

Regulation and standards

In 1998 and 1999, contamination of wheat with up to 2 mg DON/kg in the Netherlands prompted the conduct of a risk assessment, which suggested a limit for 0.2 mg DON/kg in wheat, based on the high consumption of wheat products by children (Pieters et al 2002). In the USA, 1 mg/kg is the most stringent advisory standard for DON in finished wheat products, with higher concentrations accepted for tolerant classes of livestock (Park and Troxell 2002). Health Canada has set a guideline of 2 mg DON/kg in uncleaned soft wheat for human consumption. Austria has guidelines for 0.5 for wheat and rye and 0.75 in durum wheat. Russia permits a maximum of 1 mg DON/kg (and 0.1 mg T-2 toxin/kg) in wheat, flour and bran.

On present information, it seems that Australia could set a limit of 1 mg/kg for both DON and NIV in raw maize and 0.5 mg/kg for maize products for human food without any substantial risk of maize rejection at market. The risk to human health in Australian from DON/NIV is much smaller than in Europe and North America, as maize is a minor food component while wheat has no significant contamination. Maize used in pet food and young pigs might be limited to 1 mg/kg, 2 mg/kg would be suitable for chickens and young cattle, and 5 mg/kg for adult pigs, cattle and other ruminants.

The NACMA standard (NACMA 2003) for all maize grades currently contains a ‘nil’ tolerance for Fusarium (pink) fungal stained grain. This was set in regard to wheat affected by *F. graminearum* head blight and is achievable because of the low incidence of head blight in Australia. It might require some modification in future for maize, since such a stringent standard could unnecessarily restrict marketing of maize from wetter coastal areas of Australia, and because maize with <0.2% pink/purple Fusarium-infected grain appears unlikely to contain >0.5 mg NIV/DON/kg. Indeed, regular testing of maize for DON and NIV does not appear to be warranted except in maize grown in these areas, and checking for pink/purple grain in the first instance will further reduce the risks.

References

Anon (2003) Agricultural Standards Regulations 1997, Queensland Government. www.legislation.qld.gov.au /LEGISLTN/CURRENT/A/AgrStandR97.pdf

Anon (2004) Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to zearalenone as undesirable substance in animal feed, *The EFSA Journal* **89,** 1-35. http://www.efsa.eu.int

Bennett GA, Anderson RA (1978) Distribution of aflatoxin and /or zearalenone in wet-milled corn products: a review, *Journal of Agriculture and Food Chemistry*, 26, 1055-60.

Bennett GA, Richard JL (1996) Influence of processing on *Fusarium* mycotoxins in contaminated grains, *Food Technology* **50**, 235- 245.

Blaney BJ (2007) *Mycotoxin contamination of Australian maize: a strategic risk management plan*, Final project report on DAQ0066 to the Grains Research & Development Corporation.

Blaney BJ (1981) Aflatoxin survey of maize from the 1978 crop in the South Burnett region of Queensland, *Queensland Journal of Agricultural and Animal Sciences* **38,** 7-12.

Blaney BJ (1985) Mycotoxins in crops grown in different climatic regions of Queensland, pp 97-108 in *Trichothecenes and Other Mycotoxins* (Ed. J Lacey) (John Wiley and Sons, Ltd: Chichester).

Blaney BJ (1986) Mycotoxins in water-damaged and mouldy wheat from temporary bulk stores in Queensland. *Australian Journal of Agricultural Research*, **37,** 561-5

Blaney BJ, Bloomfield RC, Moore CJ (1984a) Zearalenone intoxication of pigs, *Australian Veterinary Journal* **61,** 24-7.

Blaney BJ, Cogswell A (2007) *Supply chain & export protocols for managing mycotoxins in Australian maize*. The Maize Association of Australia. www.maizeaustralia.com.au

Blaney BJ, Connole MD, Hill MWM (1987) *Aflatoxicosis: Australian Standard Diagnostic Techniques for Animal Diseases*' No. 34 (CSIRO, Aust Agric Council).

Blaney BJ, Dodman RL (2002) Production of zearalenone, deoxynivalenol, nivalenol, and acetylated derivatives by Australian isolates of *Fusarium graminearum* and *F. pseudograminearum* in relation to source and culturing conditions, *Australian Journal of Agricultural Research* **53,** 1317-1326.

Blaney BJ, Kelly MA, Tyler AL, Connole MD (1989) Aflatoxin and cyclopiazonic acid production by Queensland isolates of *Aspergillus flavus* and *Aspergillus parasiticus*, *Australian Journal of Agricultural Research* **40,** 395-400.

Blaney BJ, Moore CJ, Tyler AL (1984b) Mycotoxins and fungal damage in maize harvested during 1982 in Far North Queensland, *Australian Journal of Agricultural Research* **35,** 463-71.

Blaney BJ, Moore CJ, Tyler AL (1987) The mycotoxins 4-deoxynivalenol, zearalenone, and aflatoxin in weather-damaged wheat harvested 1983-1985 in south-eastern Queensland, *Australian Journal of Agricultural Research,* **38**, 993-1000.

Blaney BJ, O'Keeffe K, Bricknell LK (2007) Managing mycotoxins in maize: case studies, *Australian Journal of Experimental Agriculture* (in press)

Blaney BJ, Ramsey MD, Tyler AL (1986) Mycotoxins and toxigenic fungi in insect-damaged maize harvested during 1983 in Far North Queensland, *Australian Journal of Agricultural Research* **37,** 235-44.

Blaney BJ, Williams KC (1991) Effective use in livestock feeds of mouldy and weather damaged grain containing mycotoxins – case histories and economic assessments pertaining to pig and poultry industries of Queensland. *Australian Journal of Agricultural Research* **42**, 993-1012.

Brekke OL, Peplinski AJ, Nelson GEN, Griffin EL (1975) Pilot-scale dry milling of corn containing aflatoxin, *Cereal Chemistry*, **52**, 205-11.

Bricknell LK, Blaney BJ, Ng JC (2007) Risk management for mycotoxin contamination of Australian maize, *Australian Journal of Experimental Agriculture* (in press)

Bryden WL (1991) Occurrence and biological effects of cyclopiazonic acid, in *Emerging Food Safety Problems Resulting from Microbiological Contamination* (K Mise, JL Richard eds) Toxic Microorganisms Panel of the UJNR, Tokyo.

Bryden WL, Lloyd AB, Cumming RB (1980) Aflatoxin contamination of Australian animal feeds and suspected cases of mycotoxicosis, *Australian Veterinary Journal* **56**, 176-180.

Bryden WL, Ravindran G, Salahifar H, Gill RJ, Burgess LW (1995) Fumonisin content of Australian maize, *Proceedings of the Nutrition Society of Australia* **19,** 46.

Burgess LW (1981) General ecology of the Fusaria, pp 225-135 in *Fusarium: Diseases, Biology and Taxonomy* (ed PE Nelson, TA Toussoun, RJ Cook), The Pennsylvania State University Press, University Park and London.

Burgess LW, Dodman RL, Pont W, Mayers P (1981) Fusarium diseases of wheat, maize and grain sorghum in eastern Australia. In 'Fusarium: Diseases, Biology and Taxonomy'.pp64-76 (Eds PE Nelson, TT Toussoun, RJ Cook) (The Pennsylvania State University Press: University Park, Pennsylvania).

Butron A, Santiago R, Mansilla P, Pintos-Varela C, Ordas A, Malver R (2006) Maize (*Zea mays* L.) genetic factors for preventing fumonisin contamination, *Journal of Agricultural and Food Chemistry* **54,**6113-6117.

Chauhan YS, Wright GC, Rachaputi NC (2007) Modelling climatic risks of aflatoxin contamination in maize, *Australian Journal of Experimental Agriculture* (in press).

Christley RM, Begg A.P, Hutchins DR, Hodgson DR and Bryden W (1993) Leukoencephalomalacia in horses, *AustralianVeterinary Journal* **70**, 225-6.

CFSAN/FDA (2001a) Background Paper in Support of Fumonisin Levels in Animal Feed. Center for Food Safety and Applied Nutrition, Food and Drug Administration. http://www.cfsan.fda.gov

CFSAN/FDA (2001b) Guidance for Industry - Fumonisin Levels in Human Foods and Animal Feeds Final Guidance. Center for Food Safety and Applied Nutrition, Food and Drug Administration. http://www.cfsan.fda.gov

Codex Alimentarius Commission (2003) Code of practice for the prevention and reduction of mycotoxin contamination of cereals, including annexes on ochratoxin A, zearalenone, fumonisins and trichothecenes, http://www.codexalimentarius.net /download/standards/406/CXC_051e.pdf

Cole RJ (1986) Etiology of turkey 'X' disease in retrospect: a case for the involvement of cyclopiazonic acid, *Mycotoxin research* **2**, 3-7.

Connole MD, Blaney BJ, McEwan T (1981) Mycotoxins in animal feeds and toxic fungi in Queensland 1971-80, *Australian Veterinary Journal* **57**, 314-8.

EMAN (European Mycotoxin Awareness Network) (2004) Cyclopiazonic acid http://www.lfra.co.uk/eman2/fsheet10.asp

EC (2005) Opinion of the scientific panel on contaminants in food chain on a request from the commission related to fumonisins as undesirable substances in animal feed, *The EFSA Journal* **235**, 1-32 http://www.efsa.eu.int

Fennell DI, Bothast RJ, Lillehoj EB, Peterson RE (1973) Bright greenish-yellow fluorescence and associated fungi in white corn naturally contaminated with aflatoxin, *Cereal Chemistry* **50**, 404-414.

Gallagher RT, Richard JL, Stahr HM, Cole RJ (1978) Cyclopiazonic acid production by aflatoxigenic and non-aflatoxigenic strains of *Aspergillus flavus*, *Mycopathologia* **66,** 839-42.

Gardiner MR, Oldroyd B (1965) Avian aflatoxicosis, *Australian Veterinary Journal* **41**, 272-276.

Graham J (1982) Aflatoxin in peanuts: occurrence and control. *Queensland Agricultural Journal* **108**, 119-122.

Groopman JD, Cain LG, Kensler TW (1988) Aflatoxin exposure in human populations: measurements and relation to cancer, *CRC Critical Reviews in Toxicology* **19**, 113-145

Hagler WM, Towers NR, Mirocha CJ, Eppley RM, Bryden WL (2001) Zearalenone: mycotoxin or mycoestrogen? pp321-331 in *Fusarium: Paul E. Nelson memorial symposium* (Eds BA Summerell, JF Leslie, D Backhouse, WL Bryden, LW Burgess) APS Press: The American Phytopathological Society, St Paul, Minnesota.

Hart L (1965) Avian aflatoxicosis, *Australian Veterinary Journal* **41**, 395-396.

Henry SH, Bosch FX, Bowers JC (2002) Aflatoxin, hepatitis and worldwide liver cancer risks, p229-233 in *Mycotoxins and Food Safety* (Eds DeVries JW, Trucksess MW, Jackson LS) Kluwer Academic/Plenum Publishers, New York.

Hocking AD (1991) Effects of fumigation and modified atmosphere storage on growth of fungi and production of mycotoxins in stored grains In *Fungi and Mycotoxins in Stored Products*, pp86-98 in ACIAR Proceedings No 36. (Eds. B.R. Champ, E. Highley, A.D. Hocking and J.I. Pitt) (The Griffin Press Ltd.: Netley, S.A.)

IARC (1993) Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins, Monograph 56, Lyon: International Agency for Research on Cancer.

Katta SK, Cagampang AE, Jackson LS, Bullerman LB (1999) Distribution of *Fusarium* molds and fumonisins in dry-milled corn fractions, *Cereal Chemistry* **74**, 858 -

Ketterer PJ, Blaney BJ, Moore CJ, McInnes IS, Cook PW (1982) Field cases of aflatoxicosis in pigs, *Australian Veterinary Journal* **59,** 113-7.

Ketterer PJ, Williams ES, Blaney BJ, Connole MD (1975) Canine aflatoxicosis, *Australian Veterinary Journal* **51,** 355-357

Kopinski J, Blaney B (2006) Managing mycotoxins in maize – options when feeding animals. *The Cob Magazine* Spring 2006 (available from the authors, or www.maizeaustralia.com.au)

Kopinski JS, Blaney BJ, Barram KM, Young R (1991) Responses of broilers offered diets containing the mycotoxin nivalenol. In *Recent Advances in Nutrition in Australia*, University of New England, Armidale. 7-10 April.

Kuiper-Goodman T, Grant DL (2007) Ochratoxin A (First draft) WHO Food Additive Series 28 http://www.inchem.org /documents/jecfa/jecmono/v28je19.htm

Lauren DR, Agnew MP, Smith WA, Sayer ST (1991) A survey of the natural occurrence of *Fusarium* mycotoxins in cereals grown in New Zealand in 1986-1989, *Food Additives and Contaminants* **8**, 599-605.

Lauren DR, diMenna ME, Greenhalgh R, Miller JD, Neish GA, Burgess LW (1988) Toxin-producing potential of some *Fusarium* species from as New Zealand pasture, *New Zealand Journal of Agricultural Research* **31**, 219-225.

Lauren DR, Jensen DJ, Smith WA, Dow BW, Sayer ST (1996) Mycotoxins in New Zealand maize: a study of some factors influencing contamination levels in grains, *New Zealand Journal of Crop and Horticultural Science* **24**, 13-20.

Lauren DR, Ringrose MA (1997) Determination of the fate of three *Fusarium* mycotoxins through wet milling of maize using an improved HPLC analytical technique, *Food Additives and Contaminants* **14**, 435-443.

Lewis L, Onsongo M, Njapau h, Schurz-Rogers H, Luber G, Kieszak S, Nyamongo j, backer L, Dahiye AM, Misore A, DeCock K, Rubin C (2005) Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya, *Environmental Health Perspectives* **113**, 1763-1767.

Lillehoj EB (1986) The aflatoxin-n-maize problem; the historical perspective pp13-32 in *Aflatoxin in Maize: a proceedings of a workshop*, El Batan, Mexico April 7-11, 1986.

McKean C, Tang L, Tang M, Billam M, Wang Z, Theodorakis CW, Kendall RJ, Wang JS (2006) Comparative acute and combinative toxicity of aflatoxin B1 and fumonisin B1 in animals and human cells, *Food and Chemical Toxicology* **44**, 868-876.

McKenzie RA, Blaney BJ, Connole MD, Fitzpatrick LA (1981), Acute aflatoxicosis in calves fed peanut hay, *Australian Veterinary Journal* **57,** 284-6.

Mannion PF, Blaney BJ (1988) Responses of meat chickens offered 4-deoxynivalenol and zearalenone-containing wheat, naturally infected with *Fusarium graminearum*. *Australian Journal of Agricultural Research* **39**, 533-40.

Moore CJ, Blaney BJ, Spencer RA, Dodman RL (1985), Rejection by pigs of mouldy grain containing deoxynivalenol, *Australian Veterinary Journal* **62,** 60-2.

NACMA (2003) Agricultural Commodity Standards Manual. A project of the National Agricultural Commodities Marketing Association (NACMA), 550 Wilberforce Rd, Wilberforce, NSW 2756.

O'Keeffe K (2003) Fusarium in the Riverina, *The Cob* **3** (5):11. www.maizeaustralia.com.au

Osweiler GD, Ross PF, Wilson TM, Nelson PE, Witte ST, Carson TL, Rice LG, Nelson HA (1992) Characterisation of an epizootic of pulmonary edema in swine associated with fumonisin in corn screenings, *Journal of Veterinary Diagnostic Investigations* **4**, 53-59.

Park DL, Troxell TC (2002) US Perspective on mycotoxin regulatory issues, p277-285 in *Mycotoxins and Food Safety* (Eds DeVries JW, Trucksess MW, Jackson LS) Kluwer Academic/Plenum Publishers, New York.

Peers F, Bosch X, Kaldor J, Linsell A, Plumen M (1987) Aflatoxin exposure, hepatitis B virus infection and liver cancer in Swaziland, *International Journal of Cancer* **39**, 545-553

Pieters MN, Freijer j, Baars B-J, Fiolet CM, van Klaveren J, Slob W (2002) Risk assessment of deoxynivalenol in food: concentration limits, exposure and effects, p235-248 in *Mycotoxins and Food Safety* (Eds DeVries JW, Trucksess MW, Jackson LS) Kluwer Academic/Plenum Publishers, New York.

Pitt JI (2000) Toxigenic fungi: which are important? *Medical Mycology* **38**, Supplement 1, 17-22.

Pitt JI, Basilico JC, Abarca ML, Lopez C (2000) Mycotoxins and toxigenic fungi, *Medical Mycology* **38**, Supplement 1, 41-46.

Pullar EM, Lerew WM (1937) Vulvovaginitis of swine, *Australian Veterinary Journal* **13**, 28-31.

Rachaputi N, Wright GC, Krosch S (2002) Management practices to minimise pre-harvest aflatoxin contamination in Australian peanuts, *Australian Journal of Experimental Agriculture* **42**, 595-605.

Rao LB, Husain A (1985) Presence of cyclopiazonic acid in kodo millet (Paspalum scrobiculatum) causing 'kodua poisoning' in man and its production by associated fungi, *Mycopathologia* **89**, 177-180.

Reichmann KG, Blaney BJ, Connor JK, Runge BM (1982) The significance of aflatoxin and ochratoxin in the diet of Australian chickens. *Australian Veterinary Journal* **58**, 211-2.

Riley RT, Wang E, Schroeder JJ, et al (1996) Evidence for disruption of sphingolipid metabolism as a contributing factor in the toxicity and carcinogenicity of fumonisins, *Natural Toxins* **4**, 3-15.

Schaafsma AW (2002) Economic changes imposed by mycotoxins in food grains: case study of deoxynivalenol in winter wheat, p271-276 in *Mycotoxins and Food Safety* (Eds DeVries JW, Trucksess MW, Jackson LS) Kluwer Academic/Plenum Publishers, New York.

Scott PM (1991) Possibilities of reduction or elimination of mycotoxins present in cereal grains, in *Cereal Grain Mycotoxins, Fungi and Quality in Drying and Storage*. J. Chelkowski, ed., Elsevier, Amsterdam.

Seifert KA, Aoki T, Baayen RP, Brayford D, Burgess L, Chulze S, Gamms W, Geiser D, de Gruyter J, Leslie JF, Logrieco A, Marasas WFO, Nirenberg HI, O'Donnell K, Rheeder J, Samuals GJ, Summerell BA, Thrane U, Waalwijk C (2003) The name *Fusarium moniliforme* should no longer be used, *Mycological Research News* 643-4.

Shanks G, Tabak P, Begg AP, Bryden WL (1995) An outbreak of acute leukoencephalomalacia associated with fumonisin intoxication in three horses, *Australian Equine Veterinarian* **13,** 17-8.

Southwell R, Moore K, Manning W, Hayman P (2003) An outbreak of *Fusarium* head blight of durum wheat on the Liverpool Plains in northern New South Wales in 1999, *Australasian Plant Pathology* **32**, 465-471

Tapia MO, Seawright AA (1985) Experimental combined aflatoxin B1 and ochratoxin A intoxication in pigs. *Australian Veterinary Journal* **62**, 33-7.

Tobin NF (1988) Presence of deoxynivalenol in Australian wheat and triticale – New South Wales Northern Rivers region 1983, *Australian Journal of Experimental Agriculture* **28**, 107-110.

Ueno Y (1983) Historical background of trichothecene problems, p1-6 in *Trichothecenes- chemical, biological and toxicological aspects* (ed Ueno) Elsevier, Tokyo.

USDA (2003) Aflatoxin Handbook. http://www.usda.gov/gipsa) (verified 23 March 2006)

US-FDA (United States food and Drug Administration) (2001) Guidance for Industry: Fumonisin levels in Human Foods and Animal Feeds, November 9, 2001.

Walker R (2002) Risk assessment of ochratoxin: current views of the European scientific community, the JECFA and the Codex committee on food additives and contaminants, p249-255 in *Mycotoxins and Food Safety* (Eds DeVries JW, Trucksess MW, Jackson LS) Kluwer Academic/Plenum Publishers, New York.

Watson A, Burgess LW, Summerell BA, O'Keeffe K (2006) Fusarium spp. associated with ear rot of maize in the Murrumbidgee irrigation area of New South Wales, p127-130 in *Water to Gold: Proceedings of the Maize Association of Australia 6th triennial conference*. Griffiths, NSW.

Wearing AH, Burgess LW (1978) Distribution and mode of survival of Fusarium roseum 'graminearum' Group 2 in maize soils of eastern Australia, *Transactions of the British Mycological Society* **70**, 480-486.

Webley DJ, Jackson KL (1998) Mycotoxins in cereals - a comparison between North America, Europe and Australia, pp 63-66 in *Proceedings of the Australian Postharvest Technical Conference 1998.*

Wicklow DT, Roth S, Deyrup ST, Gloer JB (2005) A protective endophyte of maize: Acremonium zeae antibiotics inhibitory to *Aspergillus flavus* and *Fusarium verticillioides*, *Mycological Research* **109**, 610-618.

Widiastuti R, Maryam R, Blaney BJ, Salfina, Stoltz DR (1988) Cyclopiazonic acid in combination with aflatoxins, zearalenone and ochratoxin A in Indonesian corn, *Mycopathologia* **104,** 153-6.

Williams KC, Blaney BJ (1994) Effect of the mycotoxins, nivalenol and zearalenone, in maize naturally infected with *Fusarium graminearum* on the performance of growing and pregnant pigs, *Australian Journal of Agricultural Research* **45**, 1265-79.

Williams KC, Blaney BJ, Magee MH (1988) Responses of pigs fed wheat naturally infected with *Fusarium graminearum* and containing the mycotoxins 4-deoxynivalenol and zearalenone, *Australian Journal of Agricultural Research* **39,** 1095-1105

Williams KC, Blaney BJ, Dodman RL, Palmer CL (1992a) Assessment for animal feed of maize kernels naturally-infected predominantly with *Fusarium moniliforme* and *Diplodia maydis*: I. Fungal isolations and changes in chemical composition, *Australian Journal of Agricultural Research* **3**, 773-82.

Williams KC, Blaney BJ, Young RA, Peters RT (1992b) Assessment for animal feed of maize kernels naturally-infected predominently with *Fusarium moniliforme* and *Diplodia maydis*: II. Nutritive value as assessed by feeding to rats and pigs, *Australian Journal of Agricultural Research* **43,** 783-94.

Wing N, Bryden WL, Lauren DR, Burgess LW (1993) Toxigenicity of *Fusarium* species and subspecies in section Gibbosum from different regions of Australia, *Mycological Research* **97**, 1441-1446.

Wright GC, Lamb DW, Medway J (2002) Application of aerial remote sensing technology for the identification of aflatoxin affected areas in peanut fields, in *Proceedings of 11th Australasian Remote Sensing Conference*, Brisbane, Qld, Sept, 2002.