Management of mycotoxins in Australian maize

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enTox- National Research Centre for Environmental Toxicology
Abstract

Mycotoxins are toxic products of secondary metabolism produced by a range of fungi. In 2001, the United Nations Food and Agriculture Organisation (FAO) estimated that at least 25% of agricultural crops worldwide are contaminated with mycotoxins. In 2003, the Australian maize harvest experienced a high level of mycotoxins contamination in crops from the Murrumbidgee Irrigation Area (MIA), causing serious financial pressure on growers.

The most well-known mycotoxins are the aflatoxins, a group of chemically similar compounds produced by species of *Aspergillus*. Aflatoxins are known to occur in maize in Australia and overseas. Another group of commonly occurring mycotoxins occurring almost ubiquitously in maize are the fumonisins, produced by species of *Fusarium*. Other mycotoxins of concern to human health that have been shown to occur in maize include Ochratoxin A, zearalenone and the trichothecenes, deoxynivalenol and nivalenol.

Until now, data regarding mycotoxin contamination of Australian-grown maize have been limited to specific geographic regions, single seasons or individual outbreaks. The survey of raw maize conducted as part of this project provides the most extensive information to date to assist in assessing the risk presented to human health from contaminated maize and to support the development of targeted extension materials for the industry.

The survey results indicate that, while mycotoxins are often present at low levels, Australian maize is generally of good quality with 85% of samples meeting the voluntary National Agricultural Commodities Marketing Association (NACMA) trading standards. Aflatoxins were detected in 25% of samples but results indicate that contamination is mainly a concern for companies supplying the human food and pet food markets that are aiming to meet the NACMA milling standard of 0.005 mg/kg.

The primary environmental factors influencing aflatoxin contamination are ambient temperature and available moisture, with aflatoxin contamination occurring at higher levels and more frequently in crops grown in regions relying on rainfall rather than irrigation. Crops produced in the peanut growing areas of the South Burnett were more likely to demonstrate contamination with both B and G aflatoxins than crops produced in other regions, leading to an assumption that *A. parasiticus* is the primary mycotoxin-producing species in that region.
Fumonisins were identified in 75.8% of all samples and across all regions, albeit at low levels. Higher levels of contamination occurred in crops grown in the Murrumbidgee Irrigation Area and New South Wales than in maize-growing regions in Queensland.

There was ample evidence suggesting that aflatoxins and fumonisins co-occur. Zearalenone appears to occur very infrequently and Ochratoxin A was not detected.

The risk to human health from exposure to aflatoxin B₁ (AB₁) and fumonisin B₁ (FB₁) was assessed using a Monte Carlo simulation. Results indicate that exposure to AB₁ through maize consumption is extremely low in Australia, with 95% of exposures calculated to be below 2.02 ng/kg BW AB₁/day in adults and below 3.57 ng/kg BW AB₁/day in children. These figures indicate that acute intoxication from aflatoxin contained in maize-based foods is extremely unlikely. In terms of chronic exposure and associated carcinogenicity, when the estimated exposure for both adults and children is compared with the no observable adverse effect level, adverse effects related solely to AB₁ contamination of maize-based food products also appear unlikely. Based on the data, less than 0.00025 cases of hepatocellular cancer (9x10⁻⁶ cases/100,000) are likely to occur annually in Australia as a result of maize-based foods contaminated with AB₁ (p<0.05).

The Australian adult population is exposed to significantly less FB₁ than the tolerable daily intake (TDI) (2.0µg fumonisins/kg BW/day) with the intake of 95% of people being less than 0.74 µg/kg BW/day. While the estimated risk of either chronic or acute health effects in adults is therefore low, the estimated exposure of children may be of concern. While the exposure of 95% of children to less than 1.75µg /kg BW /day falls below the TDI, there is little room for a safety factor to allow for raw product exceeding the NACMA standards or for high levels of contamination in imported foodstuffs. More research is required into the exposures of children to fumonisins and the potential effects of this exposure.

By integrating the findings of the survey with the published literature, the mycotoxin-related hazards inherent in the Australian maize production system were able to be identified. As a result, a methodology combining good agricultural practices and the hazard analysis critical control point framework aimed at managing the risk in the maize production industry was developed. The potential implications of climate change were also considered. A number of recommendations are proposed, including the introduction of a documented quality assurance scheme, the investigation of mycotoxin contamination in imported food products and an outline of issues requiring further research.
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Bricknell, L 2008, 'Aflatoxins in Australian maize: potential implications of climate change', in 10th International Federation of Environmental Health World Congress "Environmental health: a sustainable future, 20 years on" Brisbane [Appendix C]


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Contributions by others to the thesis

Professor Jack C. Ng, of EnTox, University of Queensland (Principal Supervisor), contributed significantly to the initial project concept, funding proposal and research design as well as providing guidance throughout the project and in thesis preparation.

Mr Barry Blaney (Associate Supervisor 2004-2009) of the Queensland Department of Primary Industries & Fisheries (Biosecurity) acted as manager of the overarching collaborative project, contributing significantly to the initial project concept, funding proposal, activity coordination and research design. He provided daily supervision to the candidate over the period 2004-2006 during sampling and laboratory analysis and also contributed guidance in selecting, modifying and refining laboratory analytical methods.

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<td>Aflatoxin B&lt;sub&gt;1&lt;/sub&gt;</td>
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<tr>
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<td>Aflatoxin B&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>Aflatoxins</td>
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<td>Aflatoxin G&lt;sub&gt;1&lt;/sub&gt;</td>
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<td>Aflatoxin G&lt;sub&gt;2&lt;/sub&gt;</td>
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<tr>
<td><strong>ANZFA</strong></td>
<td>Australia &amp; New Zealand Food Authority</td>
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<tr>
<td><strong>AOAC</strong></td>
<td>Association of Analytical Chemists</td>
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<tr>
<td><strong>BW</strong></td>
<td>Body weight</td>
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<tr>
<td><strong>CQ</strong></td>
<td>Central Queensland</td>
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<tr>
<td><strong>DON</strong></td>
<td>Deoxynivalenol</td>
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<tr>
<td><strong>DOWNS</strong></td>
<td>Darling Downs</td>
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<tr>
<td><strong>EFSA</strong></td>
<td>European Food Safety Authority</td>
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<tr>
<td><strong>EU</strong></td>
<td>European Union</td>
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<tr>
<td><strong>FAO</strong></td>
<td>Food and Agriculture Organisation</td>
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<td><strong>FB</strong>&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Fumonisin B&lt;sub&gt;1&lt;/sub&gt;</td>
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<td><strong>FSANZ</strong></td>
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<td><strong>FUM</strong></td>
<td>Fumonisins</td>
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<td><strong>GIPSA</strong></td>
<td>Grain Inspection, Packing and Stockyards Administration</td>
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<td><strong>GRDC</strong></td>
<td>Grains Research &amp; Development Corporation</td>
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<tr>
<td>Term</td>
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<tr>
<td>HACCP</td>
<td>Hazard Analysis Critical Control Point</td>
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<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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<td>JECFA</td>
<td>Joint Expert Committee on Food Additives</td>
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<tr>
<td>LOAEL</td>
<td>Lowest observable adverse effect level</td>
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<td>MIA</td>
<td>Murrumbidgee Irrigation Area</td>
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<td>MTDI</td>
<td>Maximum Tolerable Daily Intake</td>
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<td>NACMA</td>
<td>National Agricultural Commodities Marketing Association</td>
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<td>Nivalenol</td>
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<tr>
<td>NOAEL</td>
<td>No observed adverse effect level</td>
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<td>NSW</td>
<td>New South Wales</td>
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<td>OTA</td>
<td>OTA</td>
</tr>
<tr>
<td>QLD</td>
<td>Queensland</td>
</tr>
<tr>
<td>ROP</td>
<td>Reverse osmosis purified</td>
</tr>
<tr>
<td>SAX</td>
<td>Selective Anion Exchange</td>
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<tr>
<td>SPE</td>
<td>Solid Phase extraction</td>
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<tr>
<td>TDI</td>
<td>Tolerable daily intake</td>
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<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
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<tr>
<td>VIC</td>
<td>Victoria</td>
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<td>WA</td>
<td>Western Australia</td>
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<td>WHO</td>
<td>World Health Organisation</td>
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<td>WTO</td>
<td>World Trade Organisation</td>
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<td>ZER</td>
<td>Zeralenone</td>
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Chapter 1  Introduction

1.1 Background

Mycotoxins are toxic products of secondary metabolism produced by a range of fungi on a wide variety of substrates, several of which are known or suspected to be toxic to humans and animals. The toxic effects of these compounds have been known for centuries; in the Middle Ages, when rye bread was a dietary staple, the biblical *staff of life* became known as the *sceptre of death* as a result of the outbreaks of hallucinations, manic depression, gangrene, abortion, decreased fertility and painful convulsive death (Atkins & Norman 1998). These symptoms were caused by ergot, a mycotoxin produced in grain colonised by *Claviceps purpureum*.

The United Nations Food and Agriculture Organisation (FAO) has estimated that at least 25% of agricultural crops worldwide are contaminated with mycotoxins (FAO 2001). Recent research has linked increased mycotoxin contamination with climate change (Magan, Medina & Aldred 2011; Marroquín-Cardona et al. 2014; Van Der Fels-Klerx et al. 2012), meaning that this figure may very well have increased in intervening years. A leading expert in the field has described mycotoxins in food as the most important chronic food safety risk factor behind foodborne pathogens (Reddy et al. 2010), presenting a greater risk than anthropogenic contaminants, pesticides and food additives (Kuiper-Goodman 2004). In Australia, past investigations, as well as data collected by millers and manufacturers, have identified aflatoxins, fumonisins, ochratoxin A, trichothecenes (including nivalenol and deoxynivalenol) and zearalenone in maize (Blaney 1981, 1999, 2001; Blaney, Moore & Tyler 1984; Blaney, Ramsey & Tyler 1986; Connole, Blaney & McEwan 1981). Potentially harmful outbreaks of mycotoxin contamination are known to occur sporadically, and their occurrence is difficult to predict. While there have been many models postulated to predict the growth of mycotoxin-producing fungal species (Garcia et al. 2009), there is poor correlation between fungal growth and mycotoxin production (Garcia et al. 2009). There is therefore no widely recognised primary model that can be used to predict the occurrence of contamination with any real accuracy (Garcia et al. 2009).

In 2003, the Australian maize harvest experienced a high level of contamination in crops from the Murrumbidgee Irrigation Area (MIA), causing serious financial pressure on growers (Blaney, O'Keeffe & Bricknell 2008). To date, no comprehensive investigation of mycotoxin contamination of Australian-produced maize has been conducted and little is known about seasonal variations, the extent or the magnitude of contamination.
The ability to demonstrate that maize is free of contaminants is critical to the competitive performance of Australia’s grains value chain. Increasing media attention to residues in food makes it likely that all consumers and customers for Australian maize will shortly be demanding evidence of freedom from such contaminants. A consistent and coordinated approach to detect contaminated maize and ensure that it is diverted away from human food and sensitive markets is an obvious solution but this is difficult with production and marketing spread among many different groups and regions. Some sections of the industry regularly test incoming maize for mycotoxins while other sectors are unaware of the potential problem, leaving the industry vulnerable to incidents of contamination. To date, little data has been collected with respect to mycotoxins in maize based food commodities in Australia, and the risk to human health has not been adequately estimated. In addition, both aflatoxins and fumonisins in maize were identified by the World Health Organisation as priority contaminants requiring further study through total diet studies and risk assessment (WHO 2002).

When setting standards for contaminants in food, both the Food Standards Australia New Zealand Act 1991 (s.8(2)) and the WTO Sanitary and Phytosanitary Agreement require standard relating to food contaminants to be based on and assessment of risk, using the best available scientific evidence (García-Cela et al. 2012). In its most basic form, risk assessment is the process of identifying and measuring the likelihood of potential adverse effects of an activity to occur. In the last few decades it has become obvious that it is no longer sufficient to describe situations as “safe” or “unsafe” (Langley 2005) and that some form of quantification of risk is necessary.

No matter what model of risk assessment is used, there are a number of key elements. Firstly, a hazard must exist. Secondly, there must be some uncertainty in the likelihood of the hazard causing harm and the potential outcome. Thirdly, there must be at least one possible adverse outcome, a target and a time frame. Finally there must be some importance attached to the risk by people potentially affected by it (Thomas & Hrudey 1997). Elements of a risk assessment typically include the following:

- Identification of a hazard
- An assessment of the hazard
- An assessment of exposure to the hazard
- Risk characterisation (often including quantification where possible)

Once the risk has been characterised, methods for minimising or controlling the risk can be proposed.
1.2 Research question

The research described in this thesis was conducted as part of a project that addressed several strategies from the Grains Research Development Corporation’s five year plan—specifically, to develop quality management strategies, systems and processes that assist growers and the industry to assess and manage maize quality and enhance market opportunities. It particularly addressed the issue of increasing grower awareness and knowledge of mycotoxin contamination and the development of new skills and innovative agricultural management practices. As the doctoral research comprised only part of this broad aim, although the data collected informed most of the broader outcomes, it was important to clearly define the research question relevant to the doctoral studies.

What level of risk does the presence of mycotoxins in the Australian maize crop present to the health of the Australian population? How can this risk be minimised and managed?

1.3 Objectives

- Identify the hazard by determining which mycotoxins contaminate the Australian maize crop
- Assess the hazard by
  - determining the extent and nature of contamination
  - identifying agricultural practices and environmental conditions conducive to contamination in the Australian context
- Assess the exposure of the Australian population through the consumption of maize based foods
- Characterise the attributable risk to the Australian population
- Propose methods for the minimisation and management of this risk.

1.4 Thesis structure

The objectives described above have been formulated in accordance with the elements of risk assessment process. While this thesis follows the traditional scientific format in most respects, the discussion has been framed to address these elements in order to provide a clear reflection of the progress toward resolution of the research question.

1.5 Scope

Mycotoxins occurrence is strongly variable, relating to a wide range of dynamic factors. Results are limited to the seasons surveyed and should be interpreted as a snapshot only. Reported levels of
contamination are thus not intended to definitively represent levels in past or future seasons, although
the assumption is made that the three seasons of the survey are generally representative of the situation
in the Australian industry for the purposes of formulating recommendations.

Although many of the factors identified are relevant to maize production generally, the study has been
conducted in the Australian context and is not generalisable to maize producing regions in international
locations. Recommendations are made from this perspective and should not be considered applicable to
other nations.
Chapter 2  Literature review

2.1  Introduction

Mycotoxins are a relatively large and chemically diverse group of natural toxins produced as secondary metabolites by a wide range of fungi (CAST 2003; Sabater-Vilar 2003; Whitlow Jnr & Hagler Jnr 2003). They can be found in most regions of the world, making them significant environmental pollutants (CAST 2003; Sabater-Vilar 2003). As secondary metabolites, these compounds are produced by special, differentiated cells but are not required for growth or development (Sabater-Vilar 2003; Sengbusch 2003). Their primary purpose is not known, although some are known to exert an antibiotic effect on competitor microbes, indicating a survival benefit to production of the toxin (Hell 1997). Mycotoxins are often released into the medium in which the fungus is growing (CAST 2003) and can cause significant problems for both food and feed industries.

Significant research into mycotoxins and their effects began in the 1960s, following a number of deaths in turkeys fed contaminated peanut meal. The birds suffered a collection of symptoms that became known as Turkey X Disease, later identified as aflatoxicosis (Blount 1961; CAST 2003; DeVries, Trucksess & Jackson 2002). Prior to this, the only known mycotoxicoses were mushroom poisoning and ergotism, caused by mouldy grain (CAST 2003). Research continued, with aflatoxins, fumonisins, tricothecenes, patulin and other mycotoxins being found in food and feed crops in all parts of the world (DeVries, Trucksess & Jackson. 2002). It became clear that these secondary metabolites could be important players in diseases of animals and humans (CAST 2003; Wu, Groopman & Pestka 2014) and increasing public concern about food safety has ensured that appropriate management of mycotoxin contamination has become a priority (Munkvold & Muntzen 2004).

2.2  Mycotoxins

A practical definition of a mycotoxin is “a fungal metabolite that causes an undesirable effect when animals or humans are exposed” (Whitlow Jnr & Hagler Jnr 2003). Mycotoxins that have been found to occur in maize include aflatoxins, citrinin, cyclopiazonic acid (CPA), fumonisins, OTA, penicillic acid, tricothecenes (including nivalenol and deoxinivalenol) and zearalenone (Abbas et al. 2006; Ayalew 2010; Blaney, Moore & Tyler 1984; Blaney, Ramsey & Tyler 1986; Bryden et al. 1995; CAST 2003; González et al. 1999; Hooker & Schaafsma 2005; Scudamore & Patel 2000). Of these, aflatoxins, fumonisins, zearalenone, trichothecenes and ochratoxin A are of concern, because of their risk to human health as food contaminants (CAST 2003; Kuiper-Goodman 2004; Pitt & Tomaska 2001, 2002;
Whitlow Jnr & Hagler Jnr 2003). Table 2-1 illustrates the major mycotoxins known to occur in maize, the fungi associated with each mycotoxin and some of the known related health effects.

### Table 2-1 Mycotoxins, fungi and related health effects

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Fungi associated</th>
<th>Symptoms/toxicology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin</td>
<td><em>Aspergillus flavus,</em> <em>A. parasiticus</em></td>
<td>liver necrosis, liver tumours, reduced growth, depressed immune response, carcinogen</td>
</tr>
<tr>
<td>Fumonisin</td>
<td><em>Fusarium moniliforme,</em> <em>F. proliferatum</em></td>
<td>equine leukoencephalomalacia, porcine pulmonary oedema</td>
</tr>
<tr>
<td>Deoxynivalenol/ Nivalenol</td>
<td><em>F. graminearum</em></td>
<td>feed refusal, reduced weight gain, diarrhoea, vomiting</td>
</tr>
<tr>
<td>OTA</td>
<td><em>Penicillium verrucosum,</em> <em>A. ochraceus</em></td>
<td>porcine nephropathy; various symptoms in poultry</td>
</tr>
</tbody>
</table>

(Koenning & Payne 1999)

While the usual route of exposure to mycotoxins is via ingestion as food contaminants (CAST 2003; Whitlow Jnr & Hagler Jnr 2003), indirect exposure may also occur in instances where toxic residues persist in milk, eggs or edible tissues (CAST 2003). Tolerable daily intakes per kilogram of body weight (BW) per day for the major mycotoxins in maize are listed in Table 2-2.

### Table 2-2 Tolerable daily intakes (TDI) of major mycotoxins

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>TDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B₁</td>
<td>As low as reasonably achievable (ALARA principle)</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>0.005 μg/kg BW/day</td>
</tr>
<tr>
<td>Fumonisin B₁</td>
<td>2.0 μg/kg BW/day</td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>1.0 μg/kg BW/day</td>
</tr>
<tr>
<td>Zearalenone (temporary TDI)</td>
<td>0.2 μg/kg BW/day</td>
</tr>
</tbody>
</table>

(Sabater-Vilar 2003)

In the first French Total Diet study, the primary route of exposure to mycotoxins, particularly ochratoxin A, zearalenone and deoxynivalenol, was through cereals and cereal products, including maize (Leblanc et al. 2005). Maize and maize products also potentially represent sources of exposure in Zambia, Kenya and other sub-Saharan African countries (Ediage et al. 2011; Kankolongo, Hell & Nawa 2009; Muthomi et al. 2009; Muture & Ogana 2005); Europe (Oroian et al. 2009; Pietri, Zanetti & Bertuzzi 2009) and Brazil (Moreno et al. 2009). The mean Australian maize consumption per capita has been estimated to be 5.9 g/day (ANZFA 1996), predominantly in the form of moist maize kernels.
on the cob, canned products or processed products such as corn flakes and cornflour (Pitt & Tomaska 2001). When sweet corn consumption is removed from this intake, for the purposes of dietary risk assessment ANZFA (2001) estimates the consumption of processed maize products by Australians to be 3.48 g/day. This includes products in which maize flour is an ingredient (including corn flour, corn meal, custard powder, breakfast foods, tortilla, taco shells, pasta). Although other corn products exist, these products are considered to represent the major processed corn products available on the market (ANZFA 2001). To date, there has been no thorough assessment of exposure to any mycotoxin from the consumption of Australian maize.

Mycotoxin production and fungal growth is related to a variety of environmental conditions, including weather extremes, plant stress, excess hydration of stored grain (Oroian et al. 2009; Whitlow Jnr & Hagler Jnr 2003). Fungi on crops produce mycotoxins in the field, during handling and in storage (Oroian et al. 2009; Whitlow Jnr & Hagler Jnr 2003). In the field, environmental conditions such as heat, low water availability and insect damage lead to plant stress, leading to a predisposition for mycotoxin contamination (Whitlow Jnr & Hagler Jnr 2003). Unfortunately, optimum conditions for mycotoxin formation are difficult to reproduce in the laboratory (Whitlow Jnr & Hagler Jnr 2003), leading to uncertainty regarding the specifics of production.

An indication of a potential hazard can be gathered from the identification of toxigenic fungi from a food or commodity, but conclusions can only be drawn from an assay identifying specific mycotoxins (Sabater-Vilar 2003). A number of factors cause this difficulty. Firstly, the presence of a toxigenic fungus does not necessarily mean a specific mycotoxin is also present (Fink-Gremmels 1999; Sabater-Vilar 2003). Secondly, it is not simply a case of matching a mycotoxin to a specific fungus or even fungal genus. As previously stated, certain fungi are known to produce more than one mycotoxin (CAST 2003; Fink-Gremmels 1999; Sabater-Vilar 2003; Whitlow Jnr & Hagler Jnr 2003) and certain mycotoxins are known to be produced by more than one genera of fungi (CAST 2003; Fink-Gremmels 1999; Sabater-Vilar 2003). Thirdly, mycotoxins may persist in a substrate after the fungi are no longer present (Fink-Gremmels 1999; Sabater-Vilar 2003).

2.2.1 Aflatoxins

Aflatoxins are produced by fungi of the genera Aspergillus, specifically *A. flavus*, *A. parasiticus*, *A. nomius* and *A. pseudotamrri* (CAST 2003). The primary species of interest are *A. flavus* and *A. parasiticus* (Anderson, Nehring & Wichser 1975; Diener & Davis 1987; Hell 1997; Pitt & Tomaska...
2001). *A. flavus* is predominant in maize (Diener & Davis 1987; Whitlow Jnr & Hagler Jnr 2003) with *A. parasiticus* being more common in peanuts than corn (Whitlow Jnr & Hagler Jnr 2003).

There are four major aflatoxins, designated AB₁, AB₂, AG₁ and AG₂ (CAST 2003; Pitt & Tomaska 2001). The B tag indicates blue coloured fluorescence, while G is indicative of green fluorescence. The subscript indicates the relative chromatographic mobility (CAST 2003; Pitt & Tomaska 2001). The chemical structures of these four aflatoxins are shown in Figures 2-1 to 2-4.

![Figure 2-1 Chemical structure of AB₁](image1)
![Figure 2-2 Chemical structure of AB₂](image2)

Two other aflatoxins have also caused concern because of their potential impact on human health. Aflatoxins M₁ and M₂ are metabolites of aflatoxins B₁ and B₂ found in the milk of lactating animals and are significant as direct contaminants of food (CAST 2003; Pitt & Tomaska 2001). Given that they are not related to maize, the M aflatoxins will not be discussed further.

*Aspergillus sp.* use a large number of enzymes for their development, allowing them to occur on a wide variety of substrates (Hell 1997). Aflatoxins are widely known for their presence in peanuts and peanut products, but they are also known to occur in Australian maize crops (Bennett et al. 1978).

*Aspergillus sp.* are known to favour the heat and drought stress associated with warmer climates (Whitlow Jnr & Hagler Jnr 2003) and outbreaks of acute toxicosis have occurred from infected commodities in regions such as Africa (Hell 1997; Pitt & Tomaska 2001) and India (Bommakanti &
Waliyar 2000). One of the largest and most recent of these occurred in Kenya in 2004 as a result of consumption of contaminated maize leading to 317 cases of acute aflatoxicosis and 125 deaths (Daniel et al. 2011; Lewis et al. 2005).

LD₅₀ values for AB₁ range between 0.5 and 10 mg/kg BW, depending on the species, age and nutritional status of the animal under investigation (Watson 1998). Data from a single toxic event in humans in Kenya in 1982 indicates that acute exposure to 38 µg/kg BW of aflatoxin B₁ can result in significant health effects, with up to 60% of cases being fatal (Lampel, Al-Khaldi & Cahill 2012). In a widespread event in northwest India in 1974, an estimated 55 µg/kg BW exposure resulted in a 27% fatality rate (Lampel, Al-Khaldi & Cahill 2012). Both exposures occurred over an undetermined number of days.

In a rare case of deliberate self-poisoning, a laboratory worker ingested 12 µg /kg BW of AB₁ per day over a 2-day period and 6 months later, 11 µg /kg BW per day over a 14-day period. Mild health effects were noted, including transient rash, nausea and headache. No long-term effects occurred, with a physical examination and tests for liver function returning normal results in a follow up investigation fourteen years later (Lampel, Al-Khaldi & Cahill 2012). A crude NOAEL of 10 µg /kg BW per day over periods up to 14 days could be assumed for acute effects on the basis of this instance.

Aflatoxins are considered potent carcinogens, mutagens and teratogens (Pitt & Tomaska 2001; Wu, Groopman & Pestka 2014), primarily affecting the liver in humans (Hell 1997; Pitt & Tomaska 2001). They have been found to be carcinogenic and teratogenic in animals (Bommakanti & Waliyar 2000; CAST 2003; Hell 1997; IARC 1993a) and are also implicated in impairment of protein formation, blood coagulation, weight gain and immunogenensis (Hell 1997). Preliminary evidence suggests that there may also be an interaction between chronic aflatoxin exposure and malnutrition, immunosuppression, impaired growth, and diseases such as malaria and HIV/AIDS (WHO 2005).

The International Agency for Research into Cancer (IARC) has classified aflatoxin B₁ as a Class 1 carcinogen. Owing to its status as a naturally occurring genotoxin, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) have not set a value for tolerable daily or weekly intake for this mycotoxin, recommending that its level in food should be as low as reasonably achievable (ALARA) . The no observed adverse effect level (NOAEL) of AB₁ has been estimated at 0.75 µg/kg BW AB₁/day (Weidenbörner 2001). If the IARC classifications are accepted, any aflatoxin contamination in maize destined for human consumption can be clearly identified as a hazard to human health.
Dietary aflatoxin exposure is considered to be an important risk factor in the development of hepatocellular cancer in some regions of the world (Sudakin 2003; Wu, Groopman & Pestka 2014). It is proposed that the risk of liver cancer is influenced by a number of factors, especially carriage of the hepatitis B virus (Henry, Bosch & Bowers 2002). The potency of aflatoxins in serologically positive individuals has been shown to be higher by an approximate factor of 30 (Henry, Bosch & Bowers 2002) although this was not indicated in two studies conducted in China and reviewed by IARC when aflatoxins were re-evaluated in 1993 (Table 2-3).

Table 2-3 Human aflatoxin toxicity studies reviewed by IARC

<table>
<thead>
<tr>
<th>Location</th>
<th>Type of study</th>
<th>Population/ cohort</th>
<th>Parameter/ exposure</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Netherlands</td>
<td>Cohort</td>
<td>Oilpress workers</td>
<td>Exposure to contaminated dust</td>
<td>Increased mortality from cancer</td>
</tr>
<tr>
<td>China</td>
<td>Cohort</td>
<td>Villagers</td>
<td>Exposure to contaminated food products</td>
<td>Excess mortality from liver cancer; AB\textsubscript{1} effects are independent of Hepatitis B infection</td>
</tr>
<tr>
<td>Denmark</td>
<td>Cohort</td>
<td>Workers</td>
<td>Exposure to contaminated feed products</td>
<td>Excess hepatocellular carcinoma</td>
</tr>
<tr>
<td>China</td>
<td>Cohort</td>
<td>Unspecified</td>
<td>Aflatoxin metabolites in urine</td>
<td>Elevated risk of hepatocellular carcinoma; AB\textsubscript{1} effects are independent of Hepatitis B infection</td>
</tr>
<tr>
<td>Philippines</td>
<td>Case control</td>
<td>Hospital patients</td>
<td>Heavy vs. light exposure to aflatoxins</td>
<td>Significantly increased risk of hepatocellular carcinoma in those with heavy exposure</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>Case control</td>
<td>Hospital patients</td>
<td>Heavy vs. light exposure to aflatoxins</td>
<td>No association</td>
</tr>
<tr>
<td>Thailand</td>
<td>Case control</td>
<td>Hospital patients</td>
<td>Heavy vs. light exposure to aflatoxins</td>
<td>No association</td>
</tr>
<tr>
<td>Thailand</td>
<td>Case control</td>
<td>Unspecified</td>
<td>AB\textsubscript{1}-albumin adducts in sera</td>
<td>No association with hepatocellular carcinoma</td>
</tr>
<tr>
<td>Thailand</td>
<td>Case control</td>
<td>Unspecified</td>
<td>AB\textsubscript{1}-albumin adducts in sera</td>
<td>No association with cholangiocarcinoma</td>
</tr>
<tr>
<td>Swaziland</td>
<td>Correlation</td>
<td>Unspecified</td>
<td>Aflatoxin intake/ Hepatitis B infection status</td>
<td>Strong association with hepatocellular carcinoma; stronger association between hepatocellular carcinoma and AB\textsubscript{1} than Hepatitis B infection</td>
</tr>
</tbody>
</table>

(IARC 1993a)

Pitt and Tomaska (2001) estimated the risk to Australians of acquiring liver cancer from aflatoxin exposure using data from the NHMRC (1992) and ANZFA (1996, 1998) and from unpublished ANZFA public health analyses. From these sources, the median aflatoxin contamination of peanuts was estimated to be 4.0µg/kg. Peanut consumption was estimated to vary between 2.9-13.1 g/day, giving an estimated aflatoxin intake of between 11.6 and 54 ng/60 kg adult/day. This estimate closely compares
to an estimate proposed by JECFA for a European population (Pitt & Tomaska 2001). A major problem with this estimate is that it ignores the potential for aflatoxin exposure from other sources.

Both the 19th and 20th Australian Total Diet Surveys analysed a range of products for aflatoxins but the toxins were only identified in a single sample of peanuts (FSANZ 2001). The only foods potentially containing maize to any extent were cornflakes and infant cereal, meaning that the survey provides little information about the contribution of contaminated maize to exposure in Australian consumers. A comprehensive study of breakfast and infant cereals in Canada identified that half of each type of sample contained detectable levels of aflatoxin, although predominantly at low levels (Tam et al. 2006), but there are many other food products on the market that also contain maize at significant levels and have the potential to contribute to aflatoxin exposure in Australians. The 20th iteration of the Survey sampled Breads, biscuits, rice, oats, processed wheat bran, breakfast cereals (including infant cereal), instant coffee, peanut butter, almonds and milk chocolate for aflatoxins but, again, maize based foods were not represented to a sufficient extent to determine their potential contribution (FSANZ 2003). The subsequent 23rd Australian Total Diet Survey (FSANZ 2011) reports that aflatoxins were again not identified in any of the foods sampled, which included almonds, baked beans in tomato sauce, mixed grain breakfast cereals, single grain breakfast cereals, mixed infant cereal, rolled oats, white rice, peanut butter, meat pie, meat and savoury sauce (non-tomato). While a more extensive range of products were analysed than that conducted previously, this survey still fails to include commonly consumed products with high maize content such as snack foods, and thus it remains difficult to determine a comprehensive estimate of dietary aflatoxin exposure in Australians and provides no indication of any potential contribution of maize based foods.

Lubulwa & Davis (1994) used data later published by Pitt & Hocking (1996) to estimate a daily intake of aflatoxins for the Indonesian population, a figure calculated at 14 ng/day.

In 2002, the World Health Organisation identified aflatoxins in maize in the core list of priority contaminants of concern and recommended that they become a focus for future total diet surveys worldwide. Since then, the French total diet survey has been designed to include foods subject to mycotoxin contamination (Leblanc et al. 2005; Sirot et al. 2009).

### 2.2.2 Fumonisins

Fumonisins are known to occur worldwide, predominantly in maize crops, at levels of mg/kg (Ariño et al. 2007; Feng et al. 2011; IARC 2002; Shephard et al. 1996; Wei et al. 2013; Yoshizawa, Yamashita & Luo 1994). Despite the lack of human data, evidence of toxicity on animals has led IARC to classify...
FB₁, the toxin produced most predominantly, as being possibly carcinogenic to humans (Group 2B) (IARC 2002).

The toxicity of fumonisins is thought to result from the blockage of sphingolipid biosynthesis (Diaz & Boermans 1994; Whitlow Jnr & Hagler Jnr 2003). Fumonisins are similar in structure to sphingosine, a component of sphingolipids, which are in high concentrations in myelin and some nerve tissues (Whitlow Jnr & Hagler Jnr 2003). The chemical structure of the most toxicologically important fumonisin, FB₁, is illustrated in Figure 2-5. Fumonisins are known to exert acute toxic effects, with horses and pigs being particularly susceptible to acute fumonisin toxicosis (CAST 2003). In other model animals, FB₁ has a low acute toxicity (JECFA 2003; Pitt & Tomaska 2001). Fumonisins are poorly absorbed in animal species, are rapidly excreted and not metabolised in animal systems (IARC 2002).

FB₁ is hepatotoxic and nephrotoxic in all animal species tested (IARC 2002), including rats and mice (CAST 2003; IARC 2002) but little clear evidence is available regarding their carcinogenic effects in humans. It is suspected that fumonisins have a role in causing human oesophageal cancer, based on investigations carried out in Chile; the Transkei, South Africa; and Linxian County in China (Bryden et al. 1994; CAST 2003; Gelderblom et al. 1988; Pitt & Tomaska 2001; Wu, Groopman & Pestka 2014; Yoshizawa, Yamashita & Luo 1994) It has also been shown in vitro that fumonisins are a cause of neural tube defects (NTD) in mouse embryos and it has been proposed that fumonisin exposure is a contributing factor to such conditions in humans (Marasas 2004). An investigation of fumonisin contamination of maize flour used in the preparation of indigenous food products in Central America was conducted and compared with the incidence of NTD (Marasas 2004). Concentrations of the toxins was variable; in 1995, 66% of samples contained over 10 mg/kg fumonisins, while in 2000-2002 data from follow-up studies found only 6-8% of samples were contaminated to a level >3.7 mg/kg (Marasas 2004). Areas consuming high proportions of indigenous food products were found to have significantly...
higher incidences of NTD than the USA and similar results were to be found in other regions of the world where maize products formed a staple part of the diet, including the Transkei in South Africa and China (Marasas et al, 2004). A significant relationship between the consumption of tortillas and NTDs has also been proposed in the Texas-Mexico border region (Marases et al. 2004 citing unpublished data by Missmer et al.).

Horses are recognised as the most sensitive species to the acute toxic effects of fumonisins and research has established an approximate NOAEL in horses of 0.2 mg FB₁/kg BW/day (JECFA 2003). JECFA used this value in 2003 when establishing a group TDI of 2 µg fumonisins/kg BW/day addressing both acute and chronic effects (JECFA 2003).

In 2001, Pitt & Tomaska reported that, in a small survey of Australian maize for fumonisins, 49% of samples contained fumonisins at concentrations between 1-5 mg/kg, and a further 12% at concentrations greater than 5 mg/kg. In the 23rd Australian Total Diet Survey, a range of foods, including baked beans, breakfast cereals (mixed grains, single grain and infant cereal), meat pie and frozen sweet corn kernels were analysed for FB₁ and FB₂ but were not detected (FSANZ 2011). As with aflatoxins, however, this did not include a comprehensive range of maize based foods and, given fumonisin contamination predominantly occurs in maize (Gareis et al. 2003; IARC 2002; WHO 2002), this cannot be seen to be conclusive evidence of the absence of these mycotoxins in the Australian diet.

In 2003, the European Commission published a report of the findings of an in depth survey into the occurrence of *Fusarium* toxins in food and an assessment of dietary intake by the population of EU member states. In this report, exposure to fumonisins in the EU population was well below the TDI of 2 µg/kg BW per day, with the average adult intake being between 0.8-13.2% of the TDI (Gareis et al. 2003). Higher intakes occurred among young children, although the average was still just 22.3% of the TDI (Gareis et al. 2003).

Maize was found to dominate as one of the main contributors to the daily intake of fumonisins (Gareis et al. 2003). Of the maize samples, 66% were positive for FB₁, with corn derived products returning positive proportions from 9% in sweet corn to 100% in polenta samples (Gareis et al. 2003).

A European survey of dietary intake concluded that the primary source of fumonisin intake was cereals, with maize and wheat being the major contributors (Gareis et al. 2003). This survey compared a TDI of 2 µg/kg BW with estimated intakes in European member states and concluded that the intakes for all population groups was well within these limits (Gareis et al. 2003). The report did, however, qualify
their findings by identifying problems with representative sampling procedures and inaccurate consumption data (Gareis et al. 2003). The Second International Workshop on Total Diet Studies in 2002 identified fumonisins in maize as a priority contaminant of concern and recommended that they become a focus for future total diet surveys worldwide (WHO 2002).

Based on a maize consumption rate of 5.9 g/day (ANZFA 1996), and an assumed mean concentration of 1 mg/kg fumonisins in maize consumed, Pitt & Tomaska (2001) concluded that the risk of a death occurring in Australia as a result of fumonisin consumption is very low. The authors did qualify their conclusion with the remark that this was yet to be validated by sampling and analysis of Australian produce.

Using figures published in the Australian Exposure Assessment Handbook (enHealth 2012b), the average adult Australian body weight can be estimated to be 74 kg and the average child’s (under 8y) body weight, 15 kg. Based on the 5.9 g/day maize intake and 1 mg/kg average fumonisin contamination used by Pitt & Tomaska (2001), the average daily intake of fumonisins for Australian adults can be calculated to be 0.08 μg/kg BW, with the average intake for Australian children under the age of eight years being 0.39 μg/kg BW. Both estimates are well below the 2 μg/kg BW TDI recommended in the EU report, indicating that the risk of adverse health effects from exposure to fumonisins in Australia is indeed very low, although the arbitrary nature of the average fumonisin concentration proposed by Pitt and Tomaska (2001) means that this estimate is not in any way robust.

_Fusarium verticillioides_ (syn., _moniliforme_) and _F. proliferatum_, closely related fungal species ubiquitous in corn, are the main fungal species producing high fumonisin yields throughout the world (Bolger et al. 2001; Bryden, Salahifar & Burgess 1995; CAST 2003; Gelderblom et al. 1988; Munkvold & Muntzen 2004; Pitt & Tomaska 2001; Whitlow Jnr & Hagler Jnr 2003), although _F. proliferatum_ is not common in Australia (CAST 2003). These fungi are rarely found in other grains, making maize the primary source of concern for intake of fumonisins by humans (Pitt & Tomaska 2001). Three fumonisin toxins, designated FB1, FB2 and FB3, have been identified in naturally contaminated maize (Cawood et al. 1991; Whitlow Jnr & Hagler Jnr 2003).

Fusarium mycotoxins are known to survive processing and tend to concentrate in feed products (Bennett & Richard 1996). Fumonisins have also been found to survive the brewing process (Bennett & Richard 1996). Fumonisins are not degraded during the fermentation process, but do not appear to occur in the final distilled product, tending to remain in the waste products (Bothast et al. 1992). Despite this, fumonisins have been found in food products (Bennett & Richard 1996; CAST 2003; Pitt
& Tomaska 2001) and toxic events caused by fumonisins tend to arise from heavily infected corn (Bennett & Richard 1996)

2.2.3 Zearalenone

Zearalenone (ZER) is primarily produced by *Fusarium graminearum*, a fungus responsible for causing ear and stalk rots in maize (Whitlow Jnr & Hagler Jnr 2003). ZER has been shown to be produced on corn in Australia, Europe, North America, the Philippines, Thailand, and Indonesia (Eriksen, Pennington & Schlatter 2000). It is a non-steroidal estrogenic mycotoxin that has been implicated in numerous mycotoxicoses in farm animals, especially in pigs (Eriksen, Pennington & Schlatter 2000). The chemical structure of ZER is illustrated in Figure 2-6.

No evidence of any assessment of exposure to ZER in the Australian population is to be found in the literature. In the European Commission report, it was concluded that exposure to ZER in the EU population was within TDI of 0.2 μg/kg BW per day, with the daily average of the population being only 13.4% of the TDI. Higher intakes occurred among young children, although the average intake was still well below the TDI (Gareis et al. 2003).

Maize was found to be one of the main contributors to the daily intake, with the highest ZER intake from maize being 64.3 ng/kg BW per day in British males (Gareis et al. 2003). Correspondingly, the highest maize intake occurred in males 6-64 years of age, 12.4 g/day; with the lowest being in infants 6-12 months of age at 3.3 g/day.

Maize and maize products had the highest level of contamination in the survey, with 79% of raw maize samples returning positive results for ZER (Gareis et al. 2003). Of maize derived products and maize-based products, 51% and 53%, respectively, returned positive assays for ZER (Gareis et al. 2003).
ZER production tends to occur in heavily and moderately damaged kernels (Bennett et al. 1988), with dry cleaning, screening, curing or milling failing to significantly reduce ZER concentrations (Bennett & Richard 1996). The brewing process fails to remove 51% of ZER from an initially contaminated raw product (Bennett & Richard 1996). All fractions following milling have been found to be contaminated (Bennett & Richard 1996). The highest concentrations tend to occur in the hull and high fat fractions (Bennett & Richard 1996), which are usually sold for animal feed rather than for human consumption (Bennett & Anderson 1978). Despite this, 20% of ZER in contaminated maize has been found to occur in prime product mix, consisting of grits, low fat meal and flour (Bennett & Richard 1996).

### 2.2.4 Ochratoxin A

Ochratoxin A (OTA) is a metabolite of *Aspergillus ochraceus, A. carbonarius, A. niger, A. alliaceus* and *Penicillium verrucosum* (Bayman et al. 2002; Frohlich, Marquardt & Ominski 1991; Pitt & Tomaska 2002). The chemical structure of OTA is illustrated in Figure 2-7.

![Figure 2-7 Chemical structure of OTA.](image)

Of the variety of fungi known to produce OTA, *A. ochraceus* is one that is often found at very low levels in food (Pfohl-Leszkowicz & Manderville 2011; Pitt & Tomaska 2002). Early work indicated that only a small percentage of *A. ochraceus* isolates were toxigenic (Pitt & Tomaska 2002). More recent work investigating the fungus in coffee beans has shown a much higher proportion of isolates to be capable of producing OTA (Pitt & Tomaska 2002) although other research has indicated that field isolates are often less likely to produce the toxin (Bayman et al. 2002). *P. verrusocum* is virtually unknown in Australian cereal crops, despite extensive searching (Pitt & Tomaska 2002) and is thus unlikely to be a significant source of OTA in the Australian diet.

OTA is known to cause renal toxicity, nephropathy and immunosuppression in several animal species as well as inducing DNA damage in rodents *in vivo* and in rodent cells *in vitro* (JECFA 2001). The target organ for carcinogenicity in all model species is the kidney, with lesions resulting from both
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Lisa K. Bricknell
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acute and chronic exposure (Pitt & Tomaska 2002). Although no adequate data are available on the
genetic and related effects of OTA in humans, IARC considers there to be sufficient evidence in
experimental animals for the carcinogenicity of OTA and have classified it as possibly carcinogenic to
humans (Group 2B) (IARC 1993b).

Acute toxicity varies according to species, from LD50 0.2 mg/kg BW in dogs to about 50 mg/kg BW in
mice (Pitt & Tomaska 2002). Ruminants appear to degrade the toxin in the foregrastomach (Sorrenti et al.
2013) and thus have been shown to exhibit few effects at any “reasonable dose” (Pitt & Tomaska 2002).
Kidney degeneration has been observed in rats after long term oral exposure (Pitt & Tomaska 2002).

OTA has been shown to be both embryotoxicto, reprotoxic and teratogenic in laboratory and farm
animals (Malir et al. 2013). Table 2-4 presents LOAELs and NOAELS for nephrotoxicity and
carcinogenicity in animal species described by Walker (2002), demonstrating that pigs are clearly the
most sensitive of the species tested. Consequently, JECFA used data from a two year study of pigs to
establish a provisional Tolerable Weekly Intake (TWI) of 112 ng OTA/kg BW (equivalent to 16 ng
OTA/kg BW/ day) (JECFA 1991; Walker 2002). This figure was rounded down to 100 ng OTA/kg
BW/week in 1996 (JECFA 1996) and this revised value still stands, following re-evaluations in 2001

Table 2-4 LOAELs and NOAELs for nephrotoxicity and carcinogenicity of ochratoxin.

<table>
<thead>
<tr>
<th>Species</th>
<th>Effect</th>
<th>Duration of study</th>
<th>LOAEL (μg/kg BW/day)</th>
<th>NOAEL (μg/kg BW/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Kidney tumours</td>
<td>2 years</td>
<td>4,400</td>
<td>130</td>
</tr>
<tr>
<td>Rat</td>
<td>Karyomegaly of cells of proximal tubule</td>
<td>90 days</td>
<td>15</td>
<td>not demonstrated</td>
</tr>
<tr>
<td>Pig</td>
<td>Kidney tumours</td>
<td>2 years</td>
<td>70</td>
<td>21</td>
</tr>
<tr>
<td>Pig</td>
<td>Impaired renal function</td>
<td>90 days</td>
<td>8</td>
<td>not demonstrated</td>
</tr>
<tr>
<td>Pig</td>
<td>Progressive nephropathy</td>
<td>2 years</td>
<td>40</td>
<td>8</td>
</tr>
</tbody>
</table>

(Walker 2002)

The European Food Safety Authority (EFSA) also used nephrotoxicity in pigs as the endpoint criterion
to recommend a provisional TWI of 120 ng OTA/kg BW/week (equivalent to 17 ng OTA/kg BW/day)
(EFSA 2006). The use of this endpoint, however, is contested in the literature by a number of
authorities, who recommend that, on the basis of OTA’s carcinogenic properties and uncertainty in its
status as a genotoxin and mutagen, a precautionary approach should be taken and the provisional values
be much lower (Kuiper-Goodman 1996; Kuiper-Goodman et al. 2009; Miraglia & Brera 2002; Walker
The European Union has suggested that exposures should be lower than 5 ng OTA/kg BW/day, based on an opinion published by the EU Scientific Committee on Food (Miraglia & Brera 2002). Likewise, a Nordic expert group evaluated OTA in 1991, basing their assessment on carcinogenic properties and recommended a TDI of 5 ng OTA/kg BW/day (Kuiper-Goodman et al. 2009; Walker 2002). Despite recent research indicating that OTA is indeed mutagenic (and thus genotoxic) in the target tissue of male rat kidney (Hibi et al. 2011; Kuroda et al. 2014; Pfohl-Leszkowicz & Manderville 2011), the status of OTA as a genotoxin remains controversial (Haighton et al. 2012; Pfohl-Leszkowicz & Manderville 2011). Health Canada has chosen to follow the precautionary approach of other nations to regulate OTA as a non-threshold carcinogen (Haighton et al. 2012; Kuiper-Goodman et al. 2009; Pfohl-Leszkowicz & Manderville 2011), proposing an even more stringent TDI of 4 ng/kg BW/day (Pfohl-Leszkowicz & Manderville 2011). It remains to be seen if these new findings will be considered by JECFA and other authorities in a revision of the exposure standards for OTA.

There has been much written in the literature about the possible role of OTA in the etiology of Balkan Endemic Nephrology (Lin et al. 1998; Vrabcheva et al. 2004). The symptoms of this kidney disease appear to be similar to those of OTA toxicosis in animals (Pitt & Tomaska 2002). To date, however, there is no conclusive evidence to suggest that OTA is the cause of the condition, and it is more likely that other toxins or contributing factors are involved (Lin et al. 1998; Pitt & Tomaska 2002).

Human exposure to OTA can be detected in blood and breast milk (Breitholtz et al. 1991; Miraglia & Brera 2002). In Canada, a mean exposure level was estimated at 1.6 ng/kg BW/day using serology. This estimate was confirmed using food consumption data; based on average intake of cereals and pig meat, a mean daily intake was estimated to be 1.5 ng/kg BW/day (Pitt & Tomaska 2002, citing Kuiper-Goodman et al 1993). A similar study was conducted in Europe in 1995 to determine the dietary intake of OTA in the European population. Thirteen countries participated by reporting data on the occurrence of OTA in food products, consumption of these food products, and on the occurrence of OTA in human blood and milk. Consumption patterns indicated a mean daily intake of 1.8 ng/kg BW/day, whereas an intake of 0.2-2.4 ng/kg BW/day was estimated using serology results (Jørgensen & Bilde 1996).

In Europe, OTA produced by P. verrucosum is present in bread and flour based foods and also in the meat of animals grown with cereals as a major dietary component (Pitt & Tomaska 2002). As a result, serology indicates most Europeans have a considerable concentration of OTA in their blood (Pitt & Tomaska 2002). OTA has been shown in numerous studies to be a common contaminant of human
blood, breast milk and the human kidney in Europe, the USA, Canada and elsewhere in the world (Bauer & Gareis 1987; Frohlich, Marquardt & Omsinski 1991; Hadlok 1989; Hadlok & Wagner 1993; Hald 1989; Petzinger & Ziegler 2000; Solti et al. 1997; Ueno et al. 1998). In the UK, an average intake of OTA in the range 0.26-3.54 ng/kg BW/day based on food analysis was reported by Gilbert, Brereton and MacDonald (2001). Results from plasma serology were not significantly correlated, however the results of urine analysis did show a significant association (Gilbert, Brereton & MacDonald 2001).

OTA exposure from the total diet was investigated in Japan, with results indicating exposure to be generally low and resulting from consumption of a variety of foods and beverages (Sugita-Konishi 2007). The authors did recommend, however, that monitoring systems and standards be implemented to ensure the risk remained low (Sugita-Konishi 2007).

The European exposure model assumes that the major sources of OTA are cereals, wine, dried vine fruits and coffee (Pitt & Tomaska 2002). While there is evidence that OTA occurs in Australian grapes, there is no evidence to date of OTA occurring in cereals intended for human consumption in Australia (Pitt & Tomaska 2002), thus removing a significant source of intake from the Australian diet. Pitt & Tomaska (2002) assumed that the Australian diet parallels that of Europe but subtracted the contribution of cereals to intake. This resulted in an estimate of mean daily intake of 17 ng/day, the equivalent of 0.3 ng/kg BW/day in a 60 kg person. A flaw in this calculation is that it is known that OTA bioaccumulates in the meat of animals fed grain (Pitt & Tomaska 2002; Zurovac et al. 1996). Given that meat is an important part of the Australian diet, and the majority of Australian maize is used as animal feed, meat may be a significant source of OTA. While there is no evidence that the toxin occurs in Australian cereals intended for human consumption, the evidence does not extend to cereals used for animal feeds. The authors qualify their findings by asserting that the identification of OTA in commodities previously ignored, such as dried fruit and wine, is becoming an issue of interest to regulators and industry (Pitt & Tomaska 2002). The 23rd Australian Total Diet survey, conducted on 2008, examined a range of foods for OTA, including a limited range of meats. No evidence of contamination was detected in any food. As with both aflatoxins and fumonisins, the limited nature of this survey makes it difficult to conclusively state that OTA exposure is unknown in Australia but it appears likely that exposure is relatively low when compared with European populations. Reliable data on OTA prevalence and concentration in Australian commodities is necessary before a true assessment of risk can be made.
2.2.5 Tricothecenes

The tricothecenes are a group of nearly 150 related compounds produced by several fungal genera (CAST 2003). The most important genera when referring to maize is again *Fusarium*. There are two subdivisions of tricothecenes; type A including T2-toxin, HT2 toxin and neosolaniol and type B, including deoxynivalenol (DON) and nivalenol (NIV) (Mélotte 2004).

Tricothecenes known to contaminate maize include DON, also referred to as vomitoxin, and NIV (Blaney & Dodman 2002; Blaney, Moore & Tyler 1984; Blaney, Ramsey & Tyler 1986; CAST 2003; JECFA 2012). The chemical structures of these toxins are illustrated in Figures 2-8 and 2-9.

![Figure 2-8 Chemical structure of DON](image)

![Figure 2-9 Chemical structure of NIV](image)

DON and NIV have been found to occur primarily in heavily or moderately damaged kernels (Bennett et al. 1988). DON is a very stable compound, known to survive processing (Bennett & Richard 1996; Pieters et al. 2002) and is known to be present in finished food products in the US and elsewhere (Bondy & Petska 1991).

DON has been implicated in incidents of mycotoxicoses in both humans and farm animals (Canady et al. 2001). Acute exposure to DON induces anorexia at low doses and emetic effects at higher doses (Pieters et al. 2002). Other tricothecenes induce similar symptoms (Pieters et al. 2002). Other effects include the inhibition of DNA and RNA synthesis; protein synthesis at the ribosomal level; irritation of the gastrointestinal tract; alteration of a variety of blood parameters and effects on the immune system (Pieters et al. 2002). Table 2-5 presents examples of toxicity data from animal studies.

Research by Bondy & Petska (1991) indicates that DON may be an etiologic factor in human IgA nephropathy and other forms of mucosal immune dysfunction. DON is known to stimulate early differentiation of IgA-secreting cells in the Peyer’s Patch instead of at effector sites in the mucosa (Bondy & Petska 2000). This leads to symptoms analogous to those that occur in human IgA nephropathy, a kidney disorder caused by deposits of IgA inside the glomeruli (NKUDIC 2003). Such symptoms include increased IgA-secreting plasma cells in the systemic compartment, increased serum
IgA production and mesangial IgA deposition (Bondy & Petska 1991). To date there is no evidence that DON is a carcinogen or mutagen (Pieters et al. 2002).

Table 2-5 Summary of animal toxicity studies for DON

<table>
<thead>
<tr>
<th>Species</th>
<th>Study</th>
<th>Effect</th>
<th>Parameter</th>
<th>Dose (mg/kg body wt/ day)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Subacute</td>
<td>Reduced food uptake</td>
<td>-</td>
<td>0.03-0.7</td>
<td>(Eriksen &amp; Alexander 1998)</td>
</tr>
<tr>
<td>Pig</td>
<td>Subacute</td>
<td>Reduced food uptake</td>
<td>-</td>
<td>0.6-1.2</td>
<td>(Rotter et al. 1994)</td>
</tr>
<tr>
<td>Mouse</td>
<td>Acute</td>
<td>Mortality</td>
<td>LD₅₀ oral</td>
<td>46-78</td>
<td>(Eriksen &amp; Alexander 1998)</td>
</tr>
<tr>
<td>Pig</td>
<td>Acute</td>
<td>Vomiting</td>
<td>-</td>
<td>0.05-0.2</td>
<td>(Eriksen &amp; Alexander 1998)</td>
</tr>
<tr>
<td>Mouse</td>
<td>5 weeks</td>
<td>Decreased α1/ α2 ratio</td>
<td>NOAEL</td>
<td>0.25</td>
<td>(Eriksen &amp; Alexander 1998)</td>
</tr>
<tr>
<td>Pig</td>
<td>6 weeks</td>
<td>Reduced growth, reduced food uptake, stomach corrugation</td>
<td>LOAEL</td>
<td>≤0.15</td>
<td>(Rotter et al. 1994)</td>
</tr>
<tr>
<td>Mouse</td>
<td>Immunotoxicity</td>
<td>Increased susceptibility to infections</td>
<td>NOAEL</td>
<td>0.25</td>
<td>(Tryphonas, Iverson &amp; So 1986)</td>
</tr>
<tr>
<td>Mouse</td>
<td>Teratogenicity</td>
<td>Foetal skeleton abnormalities</td>
<td>NOAEL</td>
<td>0.5</td>
<td>(Khera, Arnold &amp; Whalen 1984)</td>
</tr>
<tr>
<td>Mouse</td>
<td>Reproductive toxicity</td>
<td>Mortality of pups</td>
<td>NOAEL</td>
<td>0.375</td>
<td>(Khera, Arnold &amp; Whalen 1984)</td>
</tr>
<tr>
<td>Pig</td>
<td>Reproductive toxicity</td>
<td>Reduced growth (maternal toxicity)</td>
<td>LOAEL</td>
<td>0.03-0.07</td>
<td>(Eriksen &amp; Alexander 1998)</td>
</tr>
</tbody>
</table>

At their 72nd meeting in 2010, JECFA reviewed the provisional tolerable daily intake (TDI) for DON, with consideration given to the contribution that acetylated derivatives make to overall exposure (JECFA 2012). It was concluded that, as the acetylated derivatives (particularly 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol) are converted to DON in vivo, the toxicity of these derivatives should be considered to be equal to that of DON (JECFA 2012). Consequently, the provisional maximum TDI was revised to become a group value of 1 μg/kg BW for DON and its acetylated derivatives (JECFA 2012).

In 2002, Pitt & Tomaska attempted to ascertain exposure to tricothecenes in the Australian population, but found little information related to DON and NIV in Australian commodities. Available data suggested only very low levels of contamination in grain products (Pitt & Tomaska 2001) and the authors subsequently concluded that dietary exposure was likely to be very low. The authors limited their assessment to wheat products, although it is known that both DON and NIV occur in Australian maize (Blaney 2004; Blaney, Ramsey & Tyler 1986).

Fusaric acid is known to occur naturally with DON (Diener & Davis 1987; Whitlow Jnr & Hagler Jnr 2003). Smith & MacDonald (1991) suggested that this combination of mycotoxins may result in more severe symptoms than would occur with either alone.
2.3 Sampling and Analysis

2.3.1 Sampling

Sampling is perhaps the single most important step in accurately identifying and quantifying mycotoxins. Up to 90% of error in a single analysis can be attributed to initial sampling procedure. Obtaining a representative sample is difficult, but crucial (Whitlow Jnr & Hagler Jnr 2003; Wrigley 1999). The main problem in obtaining a representative sample is that mycotoxin contamination in any sample is non-homogeneous (Pohland & Yess 1992). Fungal colonies are rarely, if ever, uniformly distributed throughout a load, having a tendency to form in “hot spots” (Whitlow Jnr & Hagler Jnr 2003). As a result, the entire sample must be finely milled, mixed and subsampled, which leads to the second largest source of error in analysis (Whitlow Jnr & Hagler Jnr 2003). It has been estimated that the variability in results is the sum of the variability inherent in the sampling, subsampling and analysis, and tends to be greater at lower concentrations (Pohland & Yess 1992)

2.3.2 Traditional analytical methods

Extraction

Organic solvents most commonly used for extraction of mycotoxins are chloroform and acetone. Acetonitrile and methanol are also used, usually mixed with a given ratio of a more polar solvent (water, dilute acid, aqueous solution of salts) to assist in the breaking of weak electrostatic bonds which bind some mycotoxins to other substrate molecules (Karunyavanij 2002). The ground sample is either shaken with the extraction solvent for up to 45 minutes or blended at high speed for about 3 minutes (Karunyavanij 2002)

Mycotoxins are such a diverse group of chemical compounds that it is difficult to find a simple procedure which specifically removes non-mycotoxin "interfering" compounds whilst leaving the mycotoxins in the extract. For this reason it is difficult to find a good method for screening a wide-range of mycotoxins simultaneously. It is possible, however, to devise procedures which remove interfering non-mycotoxin compounds from the extract of a particular commodity and leave a particular mycotoxin or group of mycotoxins in the extract. Common clean-up techniques include defatting, solid phase extraction using commercially available columns and liquid-liquid partition. These clean-up procedures are those employed in officially approved methods for aflatoxin analysis, and they are also used in analytical methods for many other mycotoxins.
After clean-up, the extract is prepared for detection and/or quantification of the mycotoxins under consideration. At this stage, the extract is often dissolved in a large volume of non-aqueous solvent, such as chloroform and then evaporated to near dryness. Care must be taken at this stage as some mycotoxins can break-down if the dry extract is overheated.

A more recent technology used to produce clean extracts with high recoveries is the immunoaffinity column. These columns are specific to single or groups of mycotoxins and require little preparation of the sample prior to cleanup. The major benefit of immunoaffinity columns is that they are very specific to the analyte of interest. This means that contamination with co-eluants is highly unlikely, making the resulting extract highly suitable for HPLC analysis. The major drawback is the expense associated with analysing large numbers of samples.

**Detection and quantification**

2.3.2.1 Thin layer chromatography (TLC)

TLC is most commonly used for aflatoxins and is also used for OTA and ZER. Fumonisins are more problematic because they do not fluoresce and the developed TLC plate must be pre-treated before viewing under ultra violet light. TLC is becoming less popular with the greater availability of HPLC which can provide a more precise quantification, although TLC is still often used for “qualitative” assays, for screening purposes (Semple et al. 2002) and in developing countries (Anklam & Stroka 2004).

TLC for mycotoxins almost exclusively uses silica gel (Karunyavanij 2002). Other stationary phases, such as polyamide and formamide-impregnated diatomaceous earth, have been trialled, but have not had the same success (Karunyavanij 2002).

In many cases, one-dimensional TLC is not adequate to separate aflatoxins from interfering constituents. It is also not adequate when concentrations lower than 1 µg/kg are to be detected. The simplest technique for improving separation is multiple developments with the same or different solvents in a single dimension. An extension of this technique is two dimensional TLC (Karunyavanij 2002). This method is particularly useful when assaying for aflatoxins, ZER and OTA, as all three mycotoxins can be identified in a single chromatogram.
2.3.2.1.2 **High performance liquid chromatography (HPLC)**

Reversed phase HPLC is the most commonly used method for identification and quantification in mycotoxin analysis (Kos & Krska 2003b). Stationary phases for mycotoxin analysis usually include C\textsubscript{18} material with mobile phases being mixtures of water, methanol or acetonitrile (Kos & Krska 2003a, 2003b; Krska 1998; Visconti & Pascale 1998). A pre-column or guard column is employed to prevent heavy contamination blocking or carrying over to the main separation column (Kos & Krska 2003b). A number of different detectors are used in HPLC; for mycotoxin analysis, fluorescence detection is most commonly used. For compounds that do not fluoresce naturally, or fluoresce weakly, a derivatising agent is added to the sample.

To quantify aflatoxins by HPLC using fluorescence detection, pre-or post-column derivatisation is usually performed for low-level detection, because aflatoxins are rather weak emitter of fluorescent light. Br\textsubscript{2}, I\textsubscript{2} (for B and G aflatoxins) or trifluoro-acetic acid (TFA for aflatoxin M\textsubscript{1}) can be used as derivatising agents. The emitted light is detected at 435 nm after excitation at 365 nm (Kos & Krska 2003a).

Fumonisins require pre-column derivatisation with o-phtaldialdehyde (OPA) and mercaptoethanol and can then be detected with detection limits of 50 μg/kg or better (Kos & Krska 2003b; Visconti & Pascale 1998).

Direct fluorescence detection of ZER is possible at 465 nm (after excitation at 270 nm). The limit of detection (LOD) for samples containing ZER analysed with RP-HPLC-FLD has been reported to be 3-6 μg/kg (Kos & Krska 2003b). Fluorescence detection of OTA in cereals is possible with a limit of detection of 10 μg/kg sample (Kos & Krska 2003b).

2.3.2.1.3 **Mass spectrometry**

Mass spectrometry is the newest method used in quantifying mycotoxins from a range of substrates. It is usually used in tandem with HPLC (HPLC-MS), with the analyte of interest being separated on the HPLC column and then identified according to its molecular composition by mass spectrometry either singularly or by employing a number of mass spectrometers in sequence (HPLC-MS-MS). The detection method is very specific, removing the problem of co-eluents and has the particular advantage of being able to detect multiple mycotoxins in the same sample extract. HPLC-MS is sensitive, selective and accurate, with HPLC-MS-MS providing the highest degree of confidence in quantification (Songsermsakul & Razzazi-Fazeli 2008). One recently published paper describes an
HPLC-MS-MS method for quantification of twenty-five different mycotoxins in a variety of grain products, including maize (Ediage et al. 2011), while a more recent study reported the detection of over three hundred mycotoxins in a single run (Abia et al. 2013).

2.3.3 Rapid assessment techniques

*Enzyme linked immunological assay (ELISA)*

Immunological assays for mycotoxins have been developed primarily to screen out negative samples and identify positive samples for quantitative analysis (Pohland & Yess 1992). While these kits are relatively fast, user friendly and allow the processing of large numbers of samples, it is essential that they be used with proper understanding of their limitations and under quality controlled laboratory conditions.

*Bright green-yellow fluorescence (BGYF) and near-infra red spectrometry (NIR)*

BGYF is caused by an enzyme in the plant that oxidises kojic acid produced concurrently with aflatoxin by *A. flavus* (Anderson, Nehring & Wichser 1975). Early work showed a strong correlation between BGYF and aflatoxin (Anderson, Nehring & Wichser 1975) and BGYF in cracked maize kernels has been used for many years as a rapid indicator of aflatoxin contamination. It is important to recognise, however, that all BGYF is not aflatoxin, it may be a completely harmless, unrelated compound (Anderson, Nehring & Wichser 1975).

Attempts have been made to establish aflatoxin levels by numbers of BGYF particles or by the weight and by area of BGYF particles in whole kernel samples. Although a relationship was shown to exist between numbers of BGYF particles and kernels and aflatoxin levels, the correlation was not high enough to encourage use of the numbers as an indication of aflatoxin content (Karunyavanij 2002). This method of detection is now not recommended except as a way of identifying batches for chemical analysis (Karunyavanij 2002).

Despite this, BGYF continues to be assessed for its usefulness in detecting and quantifying mycotoxin contamination, particularly as a screening and sorting technique. Near-Infrared Spectrometry (NIR) has been used experimentally in the United States to rapidly analyse single kernels of corn for the presence of attributes such as BGYF, aflatoxin and fumonisin mycotoxins and *Fusarium sp.* Data from the correlation of NIR spectra to aflatoxin and fumonisin content and to the presence of *Fusarium sp.* were analysed and showed potential for NIR instruments to detect these parameters (Dowell 2000). Berardo *et al* (2005) used NIR to detect mycotoxigenic fungi and mycotoxins in naturally contaminated maize,
A recent paper reported that over 57% of the aflatoxin contamination in a sample was found to occur in kernels exhibiting both visible damage and BGYF, with 35% of kernels occurring in damaged kernels with no BGYF (Pearson, Wicklow & Brabec 2010). These particles were removed using a commercial high speed dual wavelength sorter operating using both visible and near-infrared spectra. A single pass removed an average of 46% of the aflatoxin contamination with a second pass resulted in an overall 88% reduction in aflatoxin content in white maize, only rejecting 13% of the maize sample in the process (Pearson, Wicklow & Brabec 2010). It was considered that two passes were required for the operation to be effective, and that this was more than likely related to the substantial portion of contamination that occurs in kernels with only minor discolorations. These results are similar to those reported in an earlier study on naturally contaminated yellow corn, where aflatoxin contamination was reduced by an average of 81% (Pearson, Wicklow & Pasikatan 2004).

These same studies evaluated the potential for Bright Orange Fluorescence (BOF) as a method for identifying kernels contaminated with fumonisins. In the sample analysed, over 33% of fumonisin contamination was found to occur in kernels exhibiting BOF alone and almost all fumonisin occurring in kernels that either exhibit BOF or visible discoloration (Pearson, Wicklow & Brabec 2010). Dual wavelength sorting using wavelengths of 500nm and 1200nm proved successful in reducing the fumonisin contamination in contaminated white corn by 57%, with only a 4-9% rejection rate (Pearson, Wicklow & Brabec 2010). A similar study in yellow corn using wavelengths of 750 nm and 1200 nm demonstrated a reduction in contamination by 85% (Pearson, Wicklow & Pasikatan 2004). These results indicate that this modern technology may potentially be used in the near future commercially to reduce mycotoxin contamination at harvest.

**Mini-columns**

A miniature column, called a mini-column is used in many rapid assay methods to remove interfering compounds and to qualitatively detect aflatoxin down to a few µg/kg (Karunyavanij 2002). The use of these small chromatographic columns was introduced for the detection of aflatoxins in peanuts in 1968 and many different mini-columns are now available (Karunyavanij 2002). This method is now routinely used in the peanut industry for rapid identification and semi-quantification of aflatoxins. The procedure can be completed within 15 minutes and has a limit of detection of less than 10 µg/kg. The final extract from this procedure can also be used for TLC (Karunyavanij 2002).
2.4 Environmental factors conducive to mycotoxin contamination

The conditions required for the production of mycotoxins are complex and involve a combination of conditions favourable to fungal infection and growth and those conducive to mycotoxin formation. While some conditions are conducive to a number of fungi and their associated mycotoxins, others relate to a single fungal species. Fungi on crops are known to produce mycotoxins in the field, during handling and in storage (Whitlow Jnr & Hagler Jnr 2003).

Fungal spores remain dormant in soil from crop to crop and from year to year, present in layers of infected trash material. Increasing adherence to no-till cultivation, aimed at preserving topsoil, has led to an increase in soil contamination with fungal spores (Codex Alimentarius Commission 2003). Wheat and maize share a susceptibility to some *Fusarium sp.*, particularly *F. graminearum* (CAST 2003; Codex Alimentarius Commission 2003). Rotating these two crops potentially increases the availability of inoculum and subsequent ZER, NIV and/or DON contamination in these crops, particularly if there is rainfall during anthesis and persistently moist conditions during maturation.

Although aflatoxin was originally believed to be predominantly a storage problem, it is now believed that much of the aflatoxin problem in maize originates in the field (Bennett & Anderson 1978; Blaney 1981; Whitlow Jnr & Hagler Jnr 2003). The combination of drought and high ambient temperatures has long been understood to be the primary environmental factor leading to aflatoxin contamination (Abbas et al. 2002; Bruns 2003). Stress conditions at the time of pollination can lead to pre-harvest contamination, when spores as inoculum are plentiful (Whitlow Jnr & Hagler Jnr 2003). Aflatoxin contamination is enhanced by insect damage pre-and post harvest (Kankolongo, Hell & Nawa 2009; Whitlow Jnr & Hagler Jnr 2003). There appears to be a 6-8 week window in which the kernel is susceptible to fungal attack, occurring from approximately after flowering until the kernel dries to 18-20% moisture content (Anderson, Nehring & Wichser 1975). Aflatoxin contamination in maize is increased if maize is planted in the same field consecutively, or in a crop rotation that incorporates other crops that support the growth of *A. flavus* (Hell 1997). The critical period for aflatoxin production begins approximately twenty (20) days after anthesis (Bruns 2003). Drought stress is also a significant factor in fumonisin production (Munkvold & Desjardins 1997), probably through impairing plant defence mechanisms.

There are two routes by which toxigenic fungi can penetrate the maize kernel; firstly through infection of the silk channels and secondly through damage to the kernel caused by insects or machinery. An
important pest in the southern United States, causing significant yield losses as well as aflatoxin contamination, is the Southwestern corn borer (*Diatraea grandiosella* Dyar) (Williams et al. 2005).

*Fusarium sp.* are generally considered field fungi, but have been known to occur in storage (Whitlow Jnr & Hagler Jnr 2003). In the field, *Fusarium spp.* are associated with ear rot and stalk rot in maize (Whitlow Jnr & Hagler Jnr 2003). Fungi of this genus tend to grow at higher available moisture (*A_w*) and lower temperatures than those of *Aspergillus* (Whitlow Jnr & Hagler Jnr 2003) and, in maize, *F. verticillioides* is more often associated with a “cool, wet growing season with warm conditions at silking and wet conditions late in the growing season” (Herrera et al. 2010; Whitlow Jnr & Hagler Jnr 2003).

Fumonisins have been found in high concentrations in maize grown in the high rainfall areas of Zambia (Kankolongo, Hell & Nawa 2009), Spain (Herrera et al. 2010), China (Sun et al. 2011), Italy (Mazzoni et al. 2011). A combination of rainfall and high relative humidity on the first two days of silking have been strongly correlated to *F. verticillioides* infection, leading to a potentially higher fumonisin contamination (Martínez et al. 2010) of the ear at harvest. Higher concentrations of fumonisins have also been associated with prolonged duration in the field after maturation and prior to harvest (Blandino, Reyneri & Vanara 2009). In an Italian study, it was found that fumonisin accumulation was inversely related to the free water (*A_w*) and kernel moisture content, with hybrids that lose greater amounts of moisture suffering increased levels of contamination (Battilani et al. 2009). This would be consistent with a combination of the moist conditions during silking favouring infection identified by Martinez et al. (2010) with the drying effect of prolonged field exposure reported by Blandina et al. (2009).

Like other members of the *Fusarium* genus, *F. graminearum* is associated with a cool, wet growing season (Eriksen, Pennington & Schlatter 2000; Kankolongo, Hell & Nawa 2009; Whitlow Jnr & Hagler Jnr 2003) with warm conditions at silking (Whitlow Jnr & Hagler Jnr 2003)., making the climate of the maize growing areas on the Atherton Tableland area in North Queensland particularly conducive to the development of infection (Blaney, Moore & Tyler 1984).

Mycotoxin production in storage is governed by moisture content and temperature. *Fusarium* species grow best at 30-40% moisture, which is normal in the developing maize ear. *Aspergillus* species are most competitive at lower moisture contents of 18 – 30%, and so pre-harvest invasion is associated with premature drying of maize kernels. Unless maize is harvested with abnormally high moisture contents, *Fusarium* mycotoxins are not produced in storage. On the other hand, moisture migration and
accumulation in harvested maize can easily provide pockets of maize with >18% moisture, favouring rapid growth of *Aspergillus* species and aflatoxin production at temperatures above 30ºC. Acceptable moisture content decreases as ambient temperature increases (FAO 2001).

### 2.5 Mycotoxin contamination of maize in Australia

Little research has been done to determine the severity and extent of mycotoxin contamination in Australian maize crops. Aflatoxins were first reported in Australian maize in 1981 (Blaney 1981; Connole, Blaney & McEwan 1981) as a result of a decade’s testing of animal feed for a range of mycotoxins and a survey of the 1978 South Burnett crop. Further research was conducted on damaged maize harvested during 1982 and 1983 in far north Queensland (Blaney, Moore & Tyler 1984; Blaney, Ramsey & Tyler 1986). In these studies, a range of mycotoxins were identified, including significant concentrations of ZER, with low levels of aflatoxins and OTA.

ZER contamination of maize-based human foods was first reported in 1995, with 35% of a small range of maize-based foods containing ZER at levels of 10-68 µg/kg (mean of positive samples 23µg/kg) (Bryden, Salahifar & Burgess 1995).

Fumonisin contamination of Australian maize was first reported by Bryden et al. in 1995. A total of 47 out of 53 maize samples were found to be positive for fumonisins, with most contaminated kernels appearing “normal and unmoulded” (Bryden et al. 1995). Natural contamination of fumonisins is likely to be widespread in Australian maize (Bryden et al. 1995), borne out by a major Australian incident in the Murrumbidgee Irrigation Area during the 2003 season. These Australian isolates of *F. verticilliodes* were found to have high toxigenicity when compared with strains in other parts of the world (Bryden et al. 1995).

### 2.6 Risk assessment

Intuitive risk assessment is fundamental to human survival. Every time we undertake an activity with an intrinsic risk, we analyse the harm that may occur, the severity of that harm and the likelihood of it occurring in the context of what we propose to do. Extending this assessment process to become a tool in decision-making is a natural progression and a variety of methods are now used routinely in engineering, occupational health & safety, finance, ecology and environmental health.

When conducting a risk assessment, the quality of the information is a crucial element in producing a reliable outcome. An effective risk assessment requires good quality information to minimise uncertainty and a high level of knowledge and expertise on the part of the risk assessor. In particular,
outcomes are dependent on the amount and quality of information available in terms of the toxicology and epidemiology of the hazard and data with respect to exposure. Such information is in many cases sketchy or difficult to come by and, as a result, outcomes may not always be definitive (enHealth 2012b; Langley 2005). One approach to risk assessment is that proposed by the Australian enHealth Council (2012b) and reproduced in Figure 2-10.

![Figure 2-10 enHealth risk assessment framework](image)

**2.7 Risk Management**

**2.7.1 Environmental measures**

The most important factors for controlling fungal growth in stored grain are moisture and temperature with drying being a principle means of aflatoxin control (Sauer & Tuite 1986). Pre-harvest control includes proper irrigation to prevent plant stress and pesticide application to prevent insect damage (Whitlow Jnr & Hagler Jnr 2003). Post harvest, temperature control of stored grain is critical to prevent fungal growth (Sauer & Tuite 1986).
The use of fungicides in the field has been proposed for mycotoxin control, however the associated stress reaction in the plant may in fact increase the likelihood of mycotoxin contamination (Whitlow Jnr & Hagler Jnr 2003). This is supported by (Duncan et al. 1984), who write that fungicides have little efficacy in controlling pre-harvest contamination in maize.

After maturity, maize is usually left to dry in the field to prevent damage during harvest. Depending on the date of sowing and the period of time it takes for the hybrid to mature, the potential for exposure to inoculum, climatic conditions conducive to fungal growth and mycotoxin formation and insect attack varies. A recent in-field study in the US indicated that late season planting of a late maturity maize hybrid resulted in a significant risk of mycotoxin contamination when compared with earlier planting and medium maturity hybrids (Blandino, Reyneri & Vanara 2009).

2.7.2 Breeding

Over many years, there has been research aimed at breeding maize hybrids resistant to fungal infection. Selection of a hybrid that is resistant to infection, adapted for local conditions and suitable for the proposed end-use is a key decision. It has been known for many years that hybrids with long cobs with tight husk cover are more resistant to insect attack than other hybrids and experience less aflatoxin contamination (Bruns 2003). Battliani et al (2009) proposed the selection of hybrids for their capacity to lose water slowly during ripening rather than for their international market class, thus minimising the potential for aflatoxin and fumonisin contamination. Other varieties are more tolerant to drought and thus experience less stress in dry conditions. In the United States there has been some success in identifying inbred genotypes for aflatoxin resistance, although the majority of these lack traits that make them suitable for commercial purposes (Betrán & Isakeit 2004; Betrán, Isakeit & Odvody 2002). Early maturing hybrids common in the Midwestern corn belt of the USA were trialled in Mississippi to avoid the high temperatures commonly occurring in the grain filling stage in that state, however, these early maturing varieties had looser husks that made cobs susceptible to insect attack and subsequent aflatoxin contamination and the trial was not successful (Betrán & Isakeit 2004).

New techniques in genetic engineering are aimed at improving resistance to toxigenic fungi and their toxins. Three basic strategies being investigated by genetic researchers are: reducing infection by the toxigenic fungi; inserting genes capable of degrading the resultant toxin/s; interfering with the biosynthetic pathway to reduce mycotoxin accumulation (Munkvold 2003). The first commercially available transgenic variety is Bt (Bacillus thuringiensis) corn which has been shown to suffer less corn borer damage, less F. verticillioides infection and lower fumonisin contamination than non-transgenic
corn (Hammond et al. 2004; Munkvold & Muntzen 2004; Williams et al. 2005). Bt genes have not been shown to directly affect the fungi or the resultant mycotoxins; their primary role is in providing resistance to insect attack (Munkvold & Muntzen 2004). In some trials, Bt corn also showed lower concentrations of other mycotoxins, although this result was not consistent (Munkvold & Muntzen 2004).

2.7.3 Processing

Drying of the grain is the most important and commonly used method for preventing contamination. Should kernels be insufficiently dried in-field, mechanical drying is often employed to achieve the low moisture levels proven to prevent mould growth and subsequent mycotoxin contamination during storage (Blandino, Reyneri & Vanara 2009). The traditional method use is to use hot air to dry the kernels, although this has proven to be extremely energy intensive (Blandino, Reyneri & Vanara 2009). A 2009 study by Blandino et al compared the hot air method with infrared and combined infrared-hot air methods. The study found that combining the two drying methods resulted in significant improvement in energy consumption and more rapid drying, making it a more effective method than either hot air drying or infrared alone.

A number of processes have been shown to reduce the mycotoxin concentrations in contaminated maize, although there appears to be no successful method of complete decontamination. The milling process appears to be effective in reducing the amount of aflatoxin in products for human consumption. During wet milling, aflatoxins tend to concentrate in components destined for animal feed rather than human food products (Bennett & Anderson 1978). The final starch product was found to be free of aflatoxins (Bennett & Anderson 1978). Crude oil extracted in the wet milling process has been found to contain aflatoxins, but the alkali refining process is apparently effective in removing the toxins (Bennett & Anderson 1978).

Dry milling also appears to be effective to some extent in decreasing both aflatoxin and fumonisin contamination. Pietri et al (2009) found that aflatoxin could be reduced by 8-11% of AB$_1$ and 11-14% of FB$_1$ in a “normally” contaminated lot (3.6 µg/kg AB$_1$ and 5379 µg/kg FB$_1$) by applying a cleaning step in the milling process. This cleaning step involved passing unprocessed maize kernels through a winnower, a dry de-stoner and an intensive scourer coupled with an aspirator prior to the conventional degerming process (Pietri, Zanetti & Bertuzzi 2009). A highly contaminated lot (91.1 µg/kg AB$_1$ and 8841 µg/kg FB$_1$) showed a reduction in contamination of 57-59% of AB$_1$ and 34-38% of FB$_1$, probably due to physical damage to highly contaminated kernels during transport and their subsequent disposal.
The conventional dry milling process is quite effective in reducing aflatoxin contamination in particular, with a significant percentage being contained in the germ (Pietri, Zanetti & Bertuzzi 2009), which is traditionally used for animal feed. This does, however, present potential risks to human health if the feed is used for dairy cattle.

Dehulling has been shown to reduce aflatoxin levels by as much as 92% (Siwela et al. 2005). If this is the case, the method may prove to be particularly useful in rendering highly contaminated loads suitable for use as animal feed in resistant species. This result was not replicated by Pietri et al (2009), however, who found that 18-47% of aflatoxin contamination occurred in the germ of the kernel, rather than the outer layers.

Alkaline cooking of maize with lime, known as nixmatalization, is a traditional method of food processing in South America. The process softens the pericarp and allows the endosperm to absorb water which is then more easily ground. Nixmatalization increases protein content and nutrient availability; improves flavour and aroma; and has been shown to inactivate some mycotoxins. Unfortunately, under certain conditions, it is believed that the inactivated mycotoxins can regenerate later, either in products such as dough or the digestive tract (Méndez-Albores et al. 2004). Aguiano-Ruvalcaba et al (2005) found that nixmatalization effectively inactivated aflatoxin in maize and that acidic treatment prevents its regeneration in dough. This effect is not supported by Mendez-Albores et al (2004), whose results indicate that acidification of aflatoxins, such as occurs in digestion, leads to a regeneration of the inactivated aflatoxin molecule.

*Lactobacillus* fermentation has been shown to bind to AB1 (Haskard et al. 2001; Peltonen et al. 2001; Peltonen et al. 2000) and has been shown to significantly reduce AB1 in maize meal (Mokoena, Chelule & Gqaleni 2006).

ZER concentrations and purple coloured kernels indicating *F. graminarum* infection are strongly correlated (Blaney, Ramsey & Tyler 1986). It is thought that removing such kernels during harvest would remove a significant proportion of tricothecenes and ZER (Bennett et al. 1988).

Some mycotoxins can be destroyed through ammoniation of grain. Allameh et al (2005) studied productivity in broiler poultry concluded that replacing aflatoxin infected maize with ammoniated grain can significantly suppress aflatoxicosis, leading to improvements in production parameters. Méndez-Albores et al (2004) consider this to be a both effective and cost-efficient method of reducing
aflatoxin content of animal feed, although this is not a practical method for treating affected products already in storage according to Whitlow Jnr and Hagler Jnr (2003).

2.7.4 Regulation

Food regulation largely revolves around three concerns; adulteration, marketing controls and public health. The type of regulatory instrument used to regulate these concerns varies, with offences for undesirable behaviour, prescriptive provisions, codes and standards all being used at one time or another throughout history and with varying success.

The regulation of food began in England with bread in mediaeval times, largely involving price controls of a staple that was often in short supply (Reynolds 2011). Another issue was the adulteration of bread with alum, added to whiten bread, (Reynolds 2004) noted by historians in the 19th century and eventually regulated under the *Adulteration of Food & Drink Act of 1860* (Reynolds 2004).

Food regulation in Australia also started with bread (Reynolds 2011), reflecting the importance of this staple food. The *Adulteration of Bread Act 1838* broadened into the *Adulteration of Food Prevention Act 1979*. This statute addressed both public health issues in the form of “ingredients injurious to health” and commercial interests relating to fraudulent practices to increase the weight or bulk of food (Reynolds 2011). The first decade of the 20th Century saw the introduction of specified standards for certain foods in Victoria and NSW, with failing to comply constituting an offence under the so-called “Pure Food” Acts (Reynolds 2011). Other States passed similar laws during this decade (Reynolds 2011), incorporating a variety of provisions in addition to food standards. These included general hygiene requirements and the licensing/registration of dairies and premises selling food for on-site consumption (Reynolds 2004).

Historically, there has always been opposition to food regulation (Reynolds 2004). The first food laws were not strongly enforced (Reynolds 2011), probably due to the relative importance attached within society to public health in general. Some might argue that this has not significantly changed in the intervening years, with economic issues continuing to compete with public health concerns.

*Food standards*

The most commonly used method to manage mycotoxin related risk in human food commodities is to instigate standards for maximum permitted levels. Approximately ninety-nine countries are known to regulate for mycotoxins, primarily aflatoxins, in foods and animal feeds (FAO 2004). Table 2-6
compares regulatory standards, guidelines and recommendations currently in place for the major mycotoxins in maize and maize products.

Table 2-6 Regulatory limits, guidelines and recommendations for mycotoxins in maize for human consumption and maize-based food products

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Australia/NZ</th>
<th>Japan</th>
<th>EU</th>
<th>USA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxins</td>
<td>None</td>
<td>B1 5μg/kg</td>
<td>B1 2μg/kg</td>
<td>20 μg/kg (action limit)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total 10μg/kg</td>
<td>Total 4μg/kg</td>
<td></td>
</tr>
<tr>
<td>Fumonisins</td>
<td>None</td>
<td>None</td>
<td>Various, see Table 2-8</td>
<td>Various, see Table 2-8</td>
</tr>
<tr>
<td>ZER</td>
<td>None</td>
<td>None</td>
<td>No standard or recommendation</td>
<td>None</td>
</tr>
<tr>
<td>OTA</td>
<td>None</td>
<td>None</td>
<td>3μg/kg</td>
<td>None</td>
</tr>
<tr>
<td>DON</td>
<td>None</td>
<td>None</td>
<td>No standard or recommendation</td>
<td>None</td>
</tr>
<tr>
<td>NIV</td>
<td>None</td>
<td>None</td>
<td>No standard or recommendation</td>
<td>None</td>
</tr>
</tbody>
</table>

(CAST 2003; Pietri, Zanetti & Bertuzzi 2009)

**Codex Alimentarius**

No standards currently exist in the international standard Codex Alimentarius, because it is believed that elimination of mycotoxin contamination is not feasible (Codex Alimentarius Commission 2003). Instead, Codex supports the ALARA principle, and has formulated a code of practice for the prevention and reduction of mycotoxin contamination in cereals for human consumption (Codex Alimentarius Commission 2003). Despite this recommendation, many countries throughout the world have still chosen to instigate standards for mycotoxins in food products.

**Australia & New Zealand**

In Australia, the only mycotoxin currently regulated is AB1, and only in peanuts. Previously, a second standard existed for aflatoxins in all other food products, but this standard was removed in an overhaul of the Australian and New Zealand Food Standards Code. In the 1999 review of Standard A12, it was recommended that the 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” (ANZFA 1999). Standards were specifically not recommended for other mycotoxins reviewed (including OTA and Fusarium toxins such as fumonisins, DON and ZER) because the need for further monitoring in Australian and New Zealand produce made establishing such standards immature. No standards have since been established.
Standard A12 of the *Food Standards Code* indicates that mycotoxins (other than aflatoxins in peanuts and peanut products and phomopsins in lupins) are not considered “contaminants” under the provisions of the Code and are thus not included in the general requirement requiring unspecified contaminants to be absent from all food products.

**National Agricultural Commodities Marketing Association**

The National Agricultural Commodities Marketing Association (NACMA) introduced the recommendations shown in Table 2-7 in 2004 (National Agricultural Commodities Marketing Association 2004). These are not regulatory standards but guidelines to assist industry in determining quality and are applied throughout the maize industry. Milling grade and Prime maize is of higher standard and returns greater profits than maize falling into other categories and so it is in the grower’s best interest to limit mycotoxin contamination as much as possible.

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Milling</th>
<th>Prime</th>
<th>Feed 1</th>
<th>Feed 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total aflatoxins (µg/kg)</td>
<td>5</td>
<td>15</td>
<td>20</td>
<td>80 (not more than 20 µg/kg AB₁)</td>
</tr>
<tr>
<td>Total fumonisins (mg/kg)</td>
<td>&lt;2</td>
<td>5</td>
<td>10</td>
<td>40</td>
</tr>
</tbody>
</table>

**European Union**

Until recently, there was no unified standard for maize products in the European Union (EU), although there were a variety of standards for animal feed (CAST 2003). Standards for EU member countries varied, most only implementing standards for aflatoxins, although France included standards for ZER and OTA and the Netherlands, somewhat optimistically, prohibited cereal based foods containing any level of any mycotoxin (CAST 2003).

Harmonised minimum admissible standards have been formulated gradually in the EU since 1998, with a ruling in 2001 that standards for aflatoxin were to be in force in all member states by 5 April 2002 (Byrne 2001). Currently, the EU enforces standards for aflatoxins, fumonisins, OTA, ZER, DON and patulin (European Commission 2006). Member states retain the right to keep national regulations for mycotoxin/commodity combinations that are not covered by the harmonised EU regulations (CAST 2003). EU standards relevant to maize and maize based food products are presented in Table 2-8.

Chemical decontamination of products is forbidden as well as blending contaminated products with good quality products to achieve compliance with the maximum permissible level (Byrne 2001; Visconti 1998). Clear labelling is required for products intended for sorting or other physical treatment to lower aflatoxin contamination prior to human consumption (Visconti 1998).
Table 2-8 Summary of EU standards or mycotoxins in selected maize and maize-based food products

<table>
<thead>
<tr>
<th>Intended use</th>
<th>Aflatoxin AB₁</th>
<th>Fumonisins (FB₁+FB₂)</th>
<th>OTA</th>
<th>DON</th>
<th>ZER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processed cereal-based foods and baby foods for infants and young children</td>
<td>0.1 µg/kg</td>
<td>200 µg/kg</td>
<td>0.5µg/kg</td>
<td>200µg/kg</td>
<td>-</td>
</tr>
<tr>
<td>Dietary foods for special medical purposes intended specifically for infants</td>
<td>0.1 µg/kg</td>
<td>-</td>
<td>0.5µg/kg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Maize based foods for direct consumption excluding foods with specified limits</td>
<td>2.0 µg/kg (Total 4.0 µg/kg)</td>
<td>1000 µg/kg</td>
<td>3.0µg/kg</td>
<td>750µg/kg</td>
<td>75µg/kg</td>
</tr>
<tr>
<td>Maize-based breakfast cereals and maize-based snacks</td>
<td>2.0 µg/kg (Total 4.0 µg/kg)</td>
<td>800 µg/kg</td>
<td>3.0µg/kg</td>
<td>-</td>
<td>100µg/kg</td>
</tr>
<tr>
<td>Unprocessed maize, except that intended to be processed by wet milling</td>
<td>-</td>
<td>4000 µg/kg</td>
<td>5.0µg/kg</td>
<td>1750µg/kg</td>
<td>320µg/kg</td>
</tr>
<tr>
<td>Milling fractions of maize and other maize milling products with particle size &gt; 500 µm not used for direct human consumption</td>
<td>-</td>
<td>1400 µg/kg</td>
<td>-</td>
<td>750µg/kg</td>
<td>-</td>
</tr>
<tr>
<td>Milling fractions of maize and other maize milling products with particle size ≤ 500 µm not used for direct human consumption</td>
<td>-</td>
<td>2000 µg/kg</td>
<td>-</td>
<td>1250µg/kg</td>
<td>-</td>
</tr>
</tbody>
</table>

(European Commission 2006)

United States

The US Food and Drug Administration (FDA) has established action levels for aflatoxins in human food products (Table 2-9). These action levels are used as a guide by field staff to determine when enforcement action should be taken, but are not binding on the public, the government or the industry (CAST 2003). Mixing uncontaminated maize with maize exceeding the action level of aflatoxin to achieve an acceptable concentration is considered adulteration and is prohibited (CAST 2003).

Table 2-9 U.S. Guidance levels for fumonisins in maize products

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Total Fumonisins (FB₁+FB₂+FB₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degermed dry milled corn product</td>
<td>2 mg/kg</td>
</tr>
<tr>
<td>Whole/partly degermed dry milled corn product</td>
<td>4 mg/kg</td>
</tr>
<tr>
<td>Dry milled corn bran</td>
<td>4 mg/kg</td>
</tr>
<tr>
<td>Cleaned corn intended for popcorn</td>
<td>3 mg/kg</td>
</tr>
<tr>
<td>Cleaned corn for masa production</td>
<td>4 mg/kg</td>
</tr>
</tbody>
</table>

(CAST 2003; FDA 2007)
The FDA has issued a guidance document aimed at the food industry, outlining levels of fumonisins in maize and maize products that are considered adequate to protect human health (Table 2-9). It is considered practical to meet these maximum levels by good agricultural practice. (USFDA 2001) In addition to action and guidance levels, the FDA encourages good agricultural practice in accordance with the ALARA principle advocated by Codex (CAST 2003; USFDA 2000, 2001).

2.8 The maize industry in Australia

2.8.1 Industry Profile

The maize industry in Australia is a specialised, market-driven industry producing about 300-500 kt annually from about 70-80 000 ha (Grains Council of Australia 2005). The total volume of production in Australia in 2010-11 was 357 kt from a cultivated area of 62, 000 ha (Australian Bureau of Statistics 2013b) and estimated at 454.5kt from approximately 70, 000 ha in 2011-2012 (Australian Bureau of Statistics 2013a). The historical peak in production to date was in 2001-02 at 454 kt (Australian Bureau of Statistics 2013b). The area under cultivation for maize has steadily declined since the early 1920s, while production increased notably from the early 1980s (Australian Bureau of Statistics 2013b).

Major maize-growing areas in Australia include the Murrumbidgee Irrigation Area (MIA) in NSW and the Darling Downs and Burnett regions of Queensland, together contributing roughly equally and comprising an estimated 94% of the 2011 crop (Australian Bureau of Statistics 2013a). Small quantities of maize are also grown in the Atherton and Northern Tablelands in North Queensland, Central Queensland around Emerald, northern and central New South Wales, southern Western Australia, Victoria and South Australia.

It is difficult to accurately estimate the number of maize growers in Australia at any one time. The industry is small, compared with those of wheat and rice, and is rarely quantified in ABS datasets—maize usually being included in the catchall category of coarse grains. Maize is also a rotation crop in Australia, used to provide a grower with an additional source of income during the period after the harvest of their major annual crop. A Grains Council of Australia report in 2005 estimated that sixty-five growers produce maize in Australia (Grains Council of Australia 2005). This is in stark contrast to a report by the Australian Bureau of Statistics (2012), which reported that 800 agricultural businesses contributed to the crop of 2011-2012.

Most Australian grown maize is used domestically, for stock feed, pet food and human consumption, although a small amount is exported, predominantly to Japan, South Korea, Taiwan, Malaysia, New
Zealand, Sri Lanka and the Pacific Islands. Some maize is ensilaged for on-farm stock feed, with the remainder grown as hard grain, sweet corn and popcorn.

An industry consultation seminar involving leading members of the Australian maize industry was held in Brisbane in August 2006, hosted by EnTox, the National Research Institute for Environmental Toxicology and organised by the Queensland Department of Primary Industries and Fisheries (now the Department of Agriculture, Fisheries and Forestry, since the election of the Newman Government in 2012). Discussions identified the key economic issues to be:

- Water availability, despite the relatively higher water use efficiency of modern maize production systems
- Variable market demand
- Freight
- Costs of production
- High input costs, meaning growers operate on tight margins
- Research into ongoing improvements in efficiency of production

Future expansion is dependent on development of new market opportunities. These may take the form of new export markets or new value added product development. Ethanol production may offer increased demand for maize in the near future. Export opportunities may exist in supplying niche markets if Australia remains GMO free; however traits such as improved insect/disease resistance and improved nutrition in genetically modified crops may open up new markets, so the co-existence of “natural” and genetically modified hybrids needs to be fully investigated by the industry.

2.8.2 Industry stakeholders

The Australian maize industry is comprised of a network of stakeholders from a variety of sectors. In the maize supply chain, stakeholders can, in most cases, be assigned to one of eight categories, namely: seed companies, producers, accumulators, processors, end users, analysts, extension/research agencies or representative agencies. Table 2-10 illustrates the range of stakeholders, their maize-related interests and their position in the network.
Table 2-10 Stakeholder interests in the maize industry

<table>
<thead>
<tr>
<th>Industry Sector</th>
<th>Maize-related interests</th>
<th>Deal with…</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Seed companies</strong></td>
<td>Breeding improved varieties for human consumption, stock feed &amp; silage</td>
<td>Growers, Industry reps, Extension/Researchers</td>
</tr>
<tr>
<td><strong>Producers</strong></td>
<td>Producing optimum yield for best price</td>
<td>Seed companies, Accumulators, End-users, Industry reps</td>
</tr>
<tr>
<td><strong>Accumulators</strong></td>
<td>Client-based commodity marketing-livestock &amp; human consumption markets</td>
<td>Producers, Processors, End-users</td>
</tr>
<tr>
<td><strong>Bulk handlers</strong></td>
<td>Client-based commodity marketing-livestock &amp; human consumption markets</td>
<td>Producers, Processors, End-users</td>
</tr>
<tr>
<td></td>
<td>Grain handling &amp; storage</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Drying grain</td>
<td></td>
</tr>
<tr>
<td><strong>Exporters</strong></td>
<td>Grain export, storage</td>
<td>Growers, End users (international)</td>
</tr>
<tr>
<td><strong>Processors</strong></td>
<td>Purchasing</td>
<td>Growers, Bulk handlers, Manufacturers</td>
</tr>
<tr>
<td><strong>Millers</strong></td>
<td>Sale of milled product for human consumption &amp; by-products for stock feed</td>
<td>Growers, Bulk handlers, Manufacturers</td>
</tr>
<tr>
<td></td>
<td>Analysis for quality control</td>
<td></td>
</tr>
<tr>
<td><strong>Manufacturers</strong></td>
<td>Food products for human consumption (eg) popcorn, grits, snack foods, baking premixes, starch</td>
<td>Growers, Accumulators, Processors</td>
</tr>
<tr>
<td></td>
<td>Pet food</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stock feed</td>
<td></td>
</tr>
<tr>
<td><strong>End users</strong></td>
<td>Maize/ maize silage purchased as feed</td>
<td>Growers, Bulk handlers, Feedlots</td>
</tr>
<tr>
<td><strong>Commodity producers (dairy, beef)</strong></td>
<td>Maize/ maize silage purchased as feed</td>
<td>Growers, Bulk handlers, Feedlots</td>
</tr>
<tr>
<td><strong>Feedlots</strong></td>
<td>Purchasing stock feed &amp; silage</td>
<td>Accumulators, Commodity producers</td>
</tr>
<tr>
<td><strong>Analysts</strong></td>
<td>Laboratory analysis for compliance</td>
<td></td>
</tr>
<tr>
<td><strong>Industry representative bodies</strong></td>
<td>Supporting industry interests</td>
<td></td>
</tr>
<tr>
<td><strong>Extension/ research agencies</strong></td>
<td>Researching new alternatives, providing advice</td>
<td></td>
</tr>
</tbody>
</table>

Mycotoxins impact upon almost every sector in this network, from higher premiums paid for milling quality maize for growers, through potential negative human and animal health effects from mycotoxins in manufactured commodities. Exporters of both raw maize and manufactured products.
must comply with the standards of the nation to which they are exporting. AB₁ and AB₂ contaminating maize fed to dairy cattle are known to be metabolised into the derivative aflatoxins M₁ and M₂, also known to be carcinogenic, although to a lesser degree than their naturally occurring parents.

2.9 Future directions

Australia’s hot, dry climate is particularly conducive to the development of mycotoxins in maize. Many of the factors leading to contamination are known, but few have been thoroughly investigated in the Australian context. It is clear that there is a need for information about mycotoxins in local maize and maize products to enable an accurate estimation of the risk mycotoxins present to the Australian population.

With increasing attention on toxic contaminants internationally and the difficulty in decontamination, it is in the maize industry’s benefit to manage mycotoxin contamination at the production stage; rather than rely on regulatory standards that apply to the end product. In the absence of Australian standards and in keeping with the ALARA principle promoted by Codex, support for good agricultural management practices is necessary. While it is accepted that it is not possible to eliminate mycotoxin contamination (Blaney 2004), it is possible, through good agronomic practice, to minimise contamination and limit the negative effects to industry through effective management of occasional outbreaks. An integrated research programme aimed at educating growers in good practice; developing a model to predict, prevent and manage contamination events; implementing methods to detect and rapidly assess contamination; investigating routes to effectively utilise contaminated maize in animal feed; and promoting the development and use of resistant hybrids would be of significant benefit to the industry and, in turn, Australian consumers.
Chapter 3  Materials and Methods

3.1 Methodology

The research used a mixed methods methodology. The US National Institutes for Health, in their guide to Best Practices for Mixed Methods Research in the Health Sciences, describes the mixed methods research as *a research approach or methodology*

- focusing on research questions that call for real-life contextual understandings, multi-level perspectives, and cultural influences;
- employing rigorous quantitative research assessing magnitude and frequency of constructs and rigorous qualitative research exploring the meaning and understanding of constructs;
- utilizing multiple methods (e.g., intervention trials and in-depth interviews);
- intentionally integrating or combining these methods to draw on the strengths of each; and
- framing the investigation within philosophical and theoretical positions.

(Cresswell et al. 2011)

The project was applied in nature, focusing on the real life issue of mycotoxin contamination in Australian-grown maize and the associated risk to consumers. The problem-solving approach required an understanding of both the quantitative nature of mycotoxin contamination and qualitative aspects relating to the structure of the maize industry, current agricultural methods, stakeholder opinions and needs.

To meet the needs of the first part of the research question, quantitative and probabilistic research was required to determine the risk to the Australian community from exposure to maize contaminated with mycotoxins. A cross sectional design of samples of Australian grown maize from all maize growing areas were laboratory analysed for mycotoxin contamination, comprised the majority of the project. The risk assessment phase of the project then used the data collected through the quantitative laboratory analysis to compute custom distributions of contamination. These were employed in a probabilistic analysis to calculate risks quantitatively.

In order to address the second part of the research question, an understanding of the structure and needs of the maize industry was necessary. Qualitative data were collected using a focus group approach. The data were thematically analysed and used to inform the development of a targeted extension tool aimed at proactively reducing the risk of contamination during production.
3.2 Materials

3.2.1 Reagents

During analysis, unless otherwise stated, only reagents of analytical grade and solvents of HPLC grade were used. All solvents and other reagents were sourced from Selby Biolab, Unit 5, 138 Tennyson Memorial Avenue, Tennyson, QLD 4105.

Solvents

Acetone- HPLC Grade

Acetonitrile- HPLC grade- LabScan, 24 Rama 1 Rd, Patumwan, Bankok, Thailand

Chloroform- Analytical Reagent- Biolab (Aust) Ltd, Clayton, VIC

Dichloromethane- HPLC grade- LabScan

Ethyl Acetate- Chromatography grade- EM Science/ Merck, Darmstad, Germany

Methanol- HPLC grade- LabScan

Toluene- Analytical Reagent- LabScan

n-Hexane- HPLC grade- LabScan

Other reagents

2-Mercaptoethanol- 2-hydroxyethylmercaptan; β- mercaptoethanol 14.3 moles/L- Sigma-Aldrich

Disodium tetraborate- Univar/ Ajax chemicals

Formic Acid- 98-100% reagent grade- Scharlau, Barcelona, Spain

Hydrochloric acid- 32N- Univar/ Ajax chemicals

Iodine- solid- Univar/ Ajax chemicals

o- Phthalaldehyde- phtaldialdehyde min 99% HPLC grade- Sigma, St Louis, MO USA

o-Phosphoric acid- Univar/ Ajax chemicals

Potassium chloride- analytical reagent- Univar/ Ajax Chemicals

Sodium dihydrogen phosphate- analytical reagent- Univar/ Ajax chemicals
Sodium hydroxide (monobasic) pellets- analytical reagent- Univer/ Ajax Chemicals
Sodium sulphate (anhydrous) granular- AnalAR/ Merck Pty Ltd, Kilsyth, Victoria
Sulphuric acid- Univar/ Ajax chemicals
Trifluoroacetic acid- Sigma-Aldrich

### 3.2.2 Equipment

#### Glassware

Glass syringe (25 µL, 10 µL)
Conical flask (250 mL, 100 mL, 50 mL)
Separation funnel (250 mL)
Filter funnel, small
Graduated cylinder (1 L, 500 mL, 100 mL, 50 mL)
Borosilicate test tubes
Schott bottle (2 L, 1 L, 500 mL)

#### Liquid transfer

Eppendorf research pipette (5 mL, 1 mL, 100 µL, 50 µL)
Eppendorf multipette plus
Eppendorf tips (5 mL, 1 mL, 100 µL, 50 µL)
Eppendorf combitips (25 mL, 5 mL) plus adapter

#### Milling

Coarse corn mill (grinder), antique.
Hammer mill, manufacturer unknown.
Romer Mill, Romer Labs, Union MO, USA
High Performance Liquid Chromatography

High Performance Liquid Chromatograph- Shimadzu LC-10AD, comprised of:

- Shimadzu FCV-10A1 pump
- Shimadzu DGU-14A vacuum degasser
- Shimadzu SIL-10AD auto injector
- Shimadzu SCL-10A controller
- Shimadzu RF-10AxL fluorescence detector
- Shimadzu CTO-10A oven

Varian Pursuit C18 5μ column- PN 3000- 250 mm x 4.6 mm SN 9648815

Phenomenex AJ0.4287 C18 Guard column 4mmLx 3 mm D

Other equipment

VacElut SPS24, Varian Inc, Victoria, Australia

Pierce Reacti-Therm 18800 heating module with 18780 Reacti-Vap evaporating unit

3.2.3 Consumables

BondElut LRC Strong Anion Exchange (SAX) solid phase extraction (SPE) columns- 500 mg 10 mL- Varian Inc

BondElut LRC C18 solid phase extraction (SPE) columns- 500 mg 10 mL- Varian Inc

Filter paper, glass fibre- Grade 393 125 mm- Filtech, Armidale NSW

Filter paper, phase separation- 25 (special analysis) 110 mm- Advantec, Japan

Centrifuge tubes (50 mL, 15 mL)- Quantum Scientific

Pasteur pipettes, glass

Transfer pipettes, 2 mL

Thin layer chromatography plates- aluminium 20 cm x 20 cm silica gel 60 1.05553.0001- Merck, Germany
3.2.4 Standards

Aflatoxin B1- Sigma-Aldrich
Aflatoxin B2- Sigma-Aldrich
Aflatoxin G1- Sigma-Aldrich
Aflatoxin G2- Sigma-Aldrich
Fumonisin B1- Sigma-Aldrich
OTA- Sigma-Aldrich
Zearalenone-Sigma-Aldrich

3.3 Methods

3.3.1 Sampling

Maize samples were requested from the 2004-2006 maize growing seasons and were provided by a variety of industry sectors, including processors, growers, seed companies, bulk handlers, agricultural extension staff and government research stations.

2004

Samples of raw maize produced during 2004 were requested from members of the steering committee. No sample size was specifically requested.

2005-2006

The sampling protocol for the 2005 and 2006 seasons was more structured. A request for samples was circulated to growers and other industry members via the Maize Association of Australia newsletter, *The Cob (Vol 4 No2)* in Autumn 2005. A follow up reminder was included in the Spring edition of the same year. Samples were requested to be 5kg and “representative” of the load. Woven polyethylene bags were provided, labelled with the project name and with space for the name of the provider, their location and contact details to be written on the exterior. Providers were also given a sample form, on which to record a range of details about the sample, including dates of sowing and harvest, application of fertilisers, rainfall and irrigation.
Food products

A limited pilot survey of a range of commercially available maize-based food products was conducted. During sampling, care was taken to select examples from different batches, different brands (where available) and from different seasons. Each sample was assumed to be representative of the respective batch and was analysed in triplicate using the methods of analysis described later in this chapter.

3.3.2 Physical properties and rapid assessment methods

Damaged kernels

The proportion of damaged kernels was estimated by visual separation of broken, cracked and dead kernels in a 10 g sub-sample. These portions were weighed and percentages calculated.

Bulk density

Maize was poured through a funnel, allowing the grain to fall freely from a height of 30 cm into a 500 mL cylinder. The mass of the cylinder was then calculated using a fore-runner balance. This method proved unsuccessful for rapid prediction of contamination.

Discoloured kernels as an indicator of F. graminearum

The entire sample was spread in a single layer and visually inspected for pink/purple discoloured kernels.

Aflatoxins by ultraviolet exposure

The entire sample was coarsely cracked through a corn mill and examined under long wave ultra-violet light for bright greenish yellow fluorescence (BGFY) indicating potential aflatoxin contamination. Fluorescing particles were counted and recorded.

3.3.3 Milling

The entire raw maize sample was milled through a Romer Mill, set on the second-finest setting, with approximately 20% of the sample being collected for analysis. Prior to collecting the analytical portion the mill was “rinsed”, with the first 30 seconds of milled maize being discarded. After each batch of samples the mill was dismantled and cleaned with a paintbrush.
3.3.4 Detection and quantification

*Aflatoxins, OTA & ZER by TLC*

Milled sample (25 g) was disrupted ultrasonically for 60 seconds in 100 mL extraction solvent of acetonitrile: 4% aq KCl: 2N HCl acid (60:40:2). The extract was filtered through glass fibre filter paper and 50 ml extract diluted with distilled H₂O (50 mL) in a 250 mL separation funnel. The extract was defatted by shaking with n-hexane (50 mL) and allowed to partition. The lower, aqueous, partition was drawn off, and returned to the separation funnel. The top portion was discarded. Dichloromethane (50 mL) was added to the separation funnel, shaken briskly for approximately 60 seconds and allowed to partition. The lower, organic, partition was filtered in stages through phase separation filter paper containing sodium sulphate. This process was repeated with the top partition; the filtrates were combined and taken to near dryness over a steam bath under a stream of air.

The residue was quantitatively transferred to a test tube using dichloromethane and taken to absolute dryness in a small beaker of water over a steam bath under an airstream.

Extracts were stored at `17°C for no longer than seven days prior to screening by thin layer chromatography. Recoveries using this method were consistent at 80-90%. Results presented in this study were corrected for recoveries.

![Figure 3-1 TLC plate](image)

Each 20 cm x 20 cm TLC plate was cut into four 10 cm x 10 cm plates (see Figure 3-1). Extracts were reconstituted in 200 µl chloroform and 7.5 µl of reconstituted extract spotted on TLC plate at point A.

Different concentrations of combined aflatoxin: OTA standard or ZER standard were spotted at points B and C for comparison and quantification. A typical plate using standards is presented in Figure 3-2.
Plates were developed in the first dimension using chloroform: acetone (9:1). Plates were removed and allowed to dry before developing in the second dimension using toluene: ethyl acetate: 98% formic acid (50:40:10). Plates were then allowed to dry completely before viewing under long wave UV light.

Aflatoxins were confirmed by spotting plates with H₂SO₄ and observing for colour change from blue and green to yellow. The limit of detection for this method was 1 µg/kg.

**Aflatoxins by HPLC**

Extraction and clean up of milled samples was conducted using an adaptation of the method described by Sorbolev & Dorner (2002) and described below.

Milled sample (15 g) was weighed 50 mL disposable centrifuge tubes with methanol: water (75:25) solution (30 mL) and disrupted ultrasonically for 3 minutes at 35ºC. The tubes were centrifuged at 5000 rpm for 10 minutes.

A 2 mL aliquot of supernatant was diluted 1:1 with acetonitrile. This was eluted through 1 g aluminium oxide packed into a glass pasteur pipette and a 2 mL aliquot of the eluate was collected and taken to dryness. The dried extract was stored at -17ºC for no more than 7 days prior to quantification.
Quantification was performed according to the Association of Analytical Chemists (AOAC) method 994.08A (F) & (G), as described above, using a mobile phase of acetonitrile (17%), methanol (17%) and ROP water (76%) and pre-column derivatisation with trifluoroacetic acid (TFA). A typical trace from contaminated grain is illustrated in Figure 3-3.

Each run of samples analysed included at least one spiked sample and a known duplicate for quality control. Recoveries using this method were consistently high, with AB$_1$ averaging between 83%-99%; AB$_2$ 87%-98%; AG$_1$ 77%-89% and AG$_2$ 86%-98%. Results presented in this study were corrected for recoveries.

**FB$_1$ by HPLC**

FB$_1$ was extracted using an adaptation of the official method of the Association of Official Analytical Chemists (AOAC) 995.15, first described by Shephard (1998). Milled sample (8 g) was weighed into 50 mL disposable centrifuge tubes with 75% aqueous methanol (40 mL) and disrupted ultrasonically for 15 minutes at 35°C. The tubes were centrifuged at 5000 rpm for 10 minutes.

SAX SPE columns were conditioned with 5 mL 100% methanol and 5 mL 75% methanol before 10 mL of the sample extract was loaded. The columns were washed with 75% aqueous methanol (5 mL) and methanol (5 mL) before eluting with methanol: acetic acid (99:1, 10 mL) into 15 mL disposable centrifuge tubes under gravity. The extract was reduced to dryness using reacti-therm block
under a stream of nitrogen and stored below 4°C for no longer than 7 days before quantification by HPLC.

A mobile phase of 77% methanol and 23% 0.1M sodium di-hydrogen phosphate (pH adjusted to 3.35 with orthophosphoric acid) was used with a flow rate of 0.9 mL/minute. The HPLC apparatus was equilibrated for at least 45 minutes prior to use.

Dried extracts were reconstituted with 50% aqueous acetonitrile (500 µL) and vortexed for 10 seconds. Reconstituted extracts were loaded onto HPLC autosampler for precolumn derivitisation with OPA derivatising agent (80mg o-phthaldialdehyde dissolved in 2 mL methanol and diluted with 0.1M disodium tetraborate and 100 µl 2-mercaptoethanol). FB₁ was detected by fluorescence at Ex 335 nm; Em 440 nm and peaks appeared between 9-11 minutes. Standard curves were linear to 25 mg/kg, with samples contaminated to higher levels requiring dilution prior to quantification. Signal to noise ratio was excellent, with the limit of detection for FB₁ was 0.01 mg/kg. A typical trace from contaminated grain is illustrated in Figure 3-4.

Recoveries using this method were consistent at 65-75%. Each run of samples analysed included at least one spiked sample and a known duplicate for quality control. Results presented in this study were corrected for recoveries.
3.3.5 Statistical analysis

Statistical analysis was performed using Microsoft Excel and Statistical Packages for the Social Sciences (SPSS) software version 19. Prior to analysis, data were cleaned by removing cases of contamination that fell more than two standard deviations from the mean to eliminate the impact of obvious statistical outliers according to the two standard deviation rule (Abbas, Shier & Cartwright 2007; Ratcliff 1993).

Samples in which mycotoxins were not detected were assigned values at half the limit of detection. In the case of calculations using total aflatoxin concentration the value assigned was calculated as the sum of half the limit of detection for each individual aflatoxin. For samples from most Regions this is equivalent to 0.23µg/kg, the sum of half the limits of detection for AB1 and AB2. In the Burnett Region, where the G aflatoxins were almost universally detected, the value assigned was 0.45 µg/kg, taking into account the likely presence of these toxins produced by *A. parasiticus*.

The natural variation and heterogeneous occurrence of mycotoxin distribution significantly limited the use of conventional statistical tests. To confirm that this expected situation existed in the survey, distributions of mycotoxin concentration were analysed by region using the Kolmogorov-Smirnov test. It was found that each region in which contamination was detected demonstrated a unique distribution in both 2005 and 2006 seasons, in most cases due to the shape of the distribution as well as to the location of the mean. The Kolmogorov-Smirnov test is sensitive to variations in both shape and location of the distribution so, to eliminate the effect of location and allow shapes to be compared, the distributions were centred. This was accomplished by subtracting its location (in this case, the mean) from each of the variable's values. This confirmation of the heterogeneity of occurrence and variation in distribution presented significant challenges in the choice of appropriate statistical tests.

It would be usual, when statistically comparing aflatoxin concentrations between Regions, to use an analysis of variance (ANOVA) analysis. This test, however, assumes that values are distributed normally and, as evidenced above, it is clear that this is not the case in this study. Given the non-Gaussian nature of the aflatoxin distribution, non-parametric tests were required to appropriately analyse the available data.

The usual alternative to the ANOVA test in cases where the distribution of data are non-Gaussian and the independent variables are categorical is the Wilcoxon-Mann-Whitney test (also known as the Mann-Whitney U test). This test, however, gives flawed and inaccurate results when the samples are
drawn from populations with substantially different distributions as proven above. Consequently another test was sought to accurately determine regional differences.

Given that the most valuable information from an industry perspective is whether maize is suitable for milling (<5 µg/kg total aflatoxin) or stock feed (<20 µg/kg AB1), data were categorised according to these parameters. Samples could then be analysed as either complying with these standards or not, by Region.

In most cases, a Chi-squared analysis would be appropriate for this purpose. As the data were explored, however, it became obvious that there would be a number of cells in the contingency tables with counts below five, which is known to render the Chi-squared statistic inaccurate. As a result, Fisher’s Exact test (FET) was chosen to analyse the data in a number of 2x2 tables.

The Goodman-Kruskal Gamma test was used to determine relationships between variables. This correlation coefficient is preferred over more commonly-used nonparametric measures of correlation, such as Spearman’s rho and Kendall’s tau-b, when the data contains many tied observations, as occurs in this dataset (Siegel & Castellan 1988).

3.3.6 Risk assessment

Probabilistic risk assessment using the Monte Carlo method was performed using @Risk Industrial Edition 5.0 (Palisade Corporation, Ithaca, NY, USA) and Microsoft Excel software. Monte Carlo simulations substitute values randomly drawn from probability distributions in place of variables within a risk equation. Thousands of iterations of the equation are calculated to build a probability model that is then used to quantitatively estimate the probability of a result occurring.

Probability-based assessments such as Monte Carlo simulations are becoming increasingly common in toxicology, as the use of distributions allows the element of uncertainty to be included in calculations, making the resulting model more representative than it would be had point estimates been used (Arcella & Leclerq 2004). Other benefits of Monte Carlo analysis include the ability to determine not only a probably outcome but how likely that outcome is, making it particularly useful in the assessment of risk, which is probabilistic by definition. The Monte Carlo method also allows the researcher to identify which variables are most likely to cause variation in the final result and to model interdependent relationships between the input variables.

Despite this, Monte Carlo methods have not been used extensively to date in Australia for environmental health risk assessment (enHealth 2012b). One particular weakness of the Monte Carlo
method is the tendency to be used without a sufficient number of data points (Arcella & Leclerq 2004), although the size of the survey means that this is not the case here. To ensure the validity of the assessment discussed below, the guidelines set out by enHealth (2012b) for the use of Monte Carlo methods in assessing risks from exposure to environmental health hazards have been followed as far as practicable.

The exposure model, used in the Monte Carlo analysis for both AB1 and FB1, is shown below. A combination of point estimates and probability distributions was used.

\[ E = c \times g \times m \]

Where:

- \( E \) = exposure (per kg BW / day)
- \( c \) = concentration of mycotoxin in maize per gram
- \( g \) = quantity of maize consumed per day, excluding as sweet corn (g/day)
- \( m \) = body weight (kg)

Laboratory results for maize contamination were evaluated by the program against a range of distributions for goodness of fit and custom distribution models for AB1 and FB1 were computed for variable \( c \).

A point estimate for body weight \( m \), was set as 74 kg for the average adult Australian and 15 kg for the average child under 8 years of age, as recommended by EnHealth in the draft Australian Exposure Assessment Handbook (enHealth 2003). This value varies from the 60kg used by the WHO and the 70kg (13.2kg for a 2 year old child) proposed by the International Commission on Radiological Protection (ICRP 1975), which are also widely used. These values were considered more accurate for the Australian population, given the increase in average body mass in recent years (Byard & Bellis 2008), and data accurate enough for a distribution model was not available.

A distribution for daily maize consumption was based on the ANZFA (2001) estimate of processed maize products by Australians at 3.48 g/day. This includes products in which maize flour is an ingredient (including corn flour, corn meal, custard powder, breakfast foods, tortilla, taco shells, pasta). This value was used as the mean and a lognormal distribution calculated in @RISK. The lognormal distribution is considered an appropriate distribution for food consumption models (enHealth 2012b) and a distribution was used rather than a point estimate based on the ANZFA estimated mean.
consumption because it was considered necessary to reflect the uncertainty inherent in maize consumption across the population.

The Monte Carlo simulation was run for a total of 10 000 iterations.

3.3.7 Climate data

Data relating to climate was provided by the Australian Bureau of Meteorology and CSIRO. The specific variables used were a count of the days reporting maximum temperatures over 30°C and average daily precipitation. Data were selected from the nearest available weather station to the sample site, identified using the station coordinates and Google Earth.

3.4 Industry consultation

To ensure industry stakeholders were informed of major developments in the project, two papers and a poster were presented at the Maize Association of Australia biennial conference in Griffith, 2006. Regular updates were provided through the medium of The Cob, the official newsletter produced by the Maize Association of Australia and distributed to all registered maize producers. In addition, deidentified consultation data were provided by the then Department of Primary Industries & Fisheries (now the Department of Agriculture, Fisheries and Forestry) following a formal industry consultation seminar that was held in Brisbane in August 2006. No ethics approval was deemed necessary for the use of this information.

3.5 Limitations of methodology

The most significant limitation in the methodology lies in the sampling regime. As discussed in Chapter 2, the majority of error in any mycotoxin survey is caused by sampling because mycotoxins are never distributed homogeneously throughout a crop. While in an ideal world, sampling would have followed a strict pattern according to the protocols established by the US Grain Inspectors, Packers and Stockyards Administration (GIPSA), the geographic area and volume of maize requiring sampling made it necessary to request samples be provided by interested individuals or organisations rather than the project team collecting samples personally. In an attempt to minimise the inevitable variation in methodology, sample providers were requested to ensure their samples were “representative” and a 5 kg sample was requested rather than the smaller, combined samples recommended by GIPSA. While this has undoubtedly introduced undesirable variation in sampling technique, the large number and volume of samples are anticipated to reduce the impact of heterogeneous, within-crop distribution.
Related to this was the small number of samples collected for the food product survey. Maize is a very common element in a very wide range of foods–it is therefore impossible to satisfactorily sample all. Consequently, the results have not been used to assess the risk of exposure but have been used as a pilot study to indicate the potential for contaminated products to enter the market.

In the analysis phase, the inexperience of the analyst introduced potential inconsistencies in recoveries and errors in interpretation of the results. To combat this, methods were practised under the supervision of experienced chemists before results were recorded. Spiked and repeated samples were included in every batch to ensure recoveries remained consistent.

In early stages of analysis, the method used to extract fumonisins produced consistently low recoveries in spiked samples. The principle source of the problem proved to be an ambiguity in the published AOAC method. Prior to loading on the SAX SPE column, the method states that the extract “should have apparent pH ca 5.8. If necessary, adjust pH to 5.8–6.5 with 1M NaOH (only 2–3 drops should be required)”. Using Merck pH test strips, the apparent pH of extracts was usually well above this range; adjusting the pH with NaOH as recommended by the method resulted in serious negative effects on recovery and it was eventually concluded that pH adjustment was unnecessary.

Another source of low recovery was insufficient equilibration of the SPE columns. In instances where the initial methanol (5 mL) and 75% aq. methanol (5 mL) volumes were washed through the columns under vacuum at rates greater than 2 mL/min, recoveries were significantly reduced even when the flow rate of sample extracts and eluant was consistent at <2 mL/min. Once this rate was standardized and slowed, recoveries improved and became consistent. Samples extracted during this initial phase were re-extracted to ensure the process used throughout the project was consistent.

The formula used in the Monte-Carlo analysis uses constants for body weight in Australian adults and children. At the time the analysis was carried out, the best available information came from the enHealth draft Australian Exposure Assessment Handbook, released in 2003, which recommended 74 kg for Australian adults and 15 kg for children under the age of 10 years. Since then, new recommendations have been released in the Australian Exposure Factor Guide, providing more specific figures for age ranges in children and revised, and increased, body weights for Australian adults (enHealth 2012a). There is now also a recommended lifetime average body weight figure for Australian adults (enHealth 2012a), which would provide a better option for calculating the risk of chronic exposure.
The new figures do not vary substantially from those used in the calculations reported here. The recommended average Australian body weight for adults has been revised upward from 74 kg to 78 kg, with a lifetime average of 70 kg (enHealth 2012a). The recommended body weight of children varies from 11 kg for 1-2 years to 15 kg for 2-4 years to 24 kg for 4-8 years (enHealth 2012a).

These adjustments are unlikely to significantly affect the risk calculations. Using the recommended lifetime body weight of 70kg would increase the risk slightly over the lifespan, while the recommended adult body weight would reduce the calculated risk in adults by a similar proportion. For children, it would be fair to say that the figures reported most accurately reflect the risk to a 2-4 year old child. This is a fair point, given that the rapid development of children in this age range puts them at increased risk of negative health effects resulting from toxic exposures.
Chapter 4 Results

4.1 Sampling

Sampling represents the major maize growing areas of Australia i.e. the South Burnett, the Darling Downs and the Murrumbidgee Irrigation Area. Regions producing lesser quantities, including the Tablelands area of North Queensland and northern and mid-NSW are also appropriately represented. Regions from which samples were collected are illustrated in Figure 4-1.

![Sampling regions](image)

Sample distribution by Region and year of harvest is presented in Table 4-1. The samples from Central Queensland in 2004 were provided by a single grower from a test crop. The crop did not prove feasible and so samples were not available for subsequent seasons. Cyclone Larry devastated the North Queensland maize crop of 2006 and so no samples were provided from that region in that year. Only a very small number of samples were provided by single growers in Western Astralia and northern Victoria in 2005. Neither grower submitted samples for analysis in 2006.
Table 4-1 Sample distribution by region and year of harvest

<table>
<thead>
<tr>
<th>Region</th>
<th>Samples (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2004</td>
</tr>
<tr>
<td>Burnett</td>
<td>50</td>
</tr>
<tr>
<td>Darling Downs (DOWNS)</td>
<td>45</td>
</tr>
<tr>
<td>Central Queensland (CQ)</td>
<td>50</td>
</tr>
<tr>
<td>Northern and mid-NSW (NSW)</td>
<td>13</td>
</tr>
<tr>
<td>North Queensland (NQ)</td>
<td>25</td>
</tr>
<tr>
<td>Murrumbidgee Irrigation Area (MIA)</td>
<td>7</td>
</tr>
<tr>
<td>Victoria (VIC)</td>
<td>-</td>
</tr>
<tr>
<td>Western Australia (WA)</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>190</td>
</tr>
</tbody>
</table>

Given the maize industry in Australia is small, with an estimated sixty-five growers and samples were provided from in excess of 70 different locations over the three seasons of the project, is it assumed that most maize growers in Australia provided samples. A substantial number of samples were also provided by three different seed companies, mostly from locations in the MIA and Darling Downs.

4.2 Pilot study (2004)

Given the less stringent nature of the sampling procedure and smaller sizes of samples collected from the 2004 harvest, results for this year have been treated as a pilot study by separate analysis and have not been compared with those from the 2005 and 2006 seasons. No information was available with respect to what proportion of the entire crop these samples represent, so results should be interpreted with caution.

4.2.1 Contamination

Compliance with the NACMA standards is presented below (Tables 4-2 and 4-3). Clearly, CQ experienced significantly higher aflatoxin contamination during 2004, with over 50% of samples exceeding the NACMA standard for all purposes and 100% of samples testing positive for aflatoxin contamination. The variance of aflatoxin contamination of the CQ samples was calculated to be 1950.39 (SD 44.05), with a minimum concentration of 2.7 µg/kg and a maximum of 240.0 µg/kg. All of the CQ samples originated from different loads from the same crop, planted in the Emerald vicinity. Interestingly, when the loads were combined and further samples were analysed, the aflatoxin contamination averaged at 5 µg/kg. This demonstrates the heterogeneous nature of aflatoxin...
contamination documented in the literature and the potential to dilute contamination by mixing contaminated lots with uncontaminated grain.

Table 4-2 Compliance with NACMA standard for aflatoxins by Region of samples from 2004 harvest

<table>
<thead>
<tr>
<th>Region</th>
<th>Milling (&lt;5 µg/kg)</th>
<th>Prime (&lt;15 µg/kg)</th>
<th>Feed #1 (&lt;20 µg/kg)</th>
<th>Feed #2 (&lt;80 µg/kg with &lt;20 µg/kg AB₁)</th>
<th>Exceeds Std (&gt;80 µg/kg total or &lt;20 µg/kg AB₁)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NQ</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CQ</td>
<td>16%</td>
<td>30%</td>
<td>-</td>
<td>-</td>
<td>54%</td>
</tr>
<tr>
<td>Burnett</td>
<td>96%</td>
<td>4%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Downs</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NSW</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MIA</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Fumonisin contamination is subject to much less variation between Regions, with the majority of maize tested being of good quality. The apparent greater proportion of samples in the MIA experiencing higher fumonisin contamination is an artefact resulting from the significantly smaller number of samples received from that Region in 2004. Only two samples from the MIA returned results greater than 5 mg/kg, insufficient to draw conclusions about the actual incidence of contamination in that Region during 2004.

Table 4-3 Compliance with NACMA standard for fumonisins by Region of samples from 2004 harvest

<table>
<thead>
<tr>
<th>Region</th>
<th>Milling (&lt;2 mg/kg)</th>
<th>Prime (&lt;5 mg/kg)</th>
<th>Feed #1 (&lt;10 mg/kg)</th>
<th>Feed #2 (&lt;40 mg/kg)</th>
<th>Exceeds Std (≥40 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NQ</td>
<td>92%</td>
<td>4%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CQ</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Burnett</td>
<td>98%</td>
<td>2%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Downs</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NSW</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MIA</td>
<td>85.71%</td>
<td>-</td>
<td>-</td>
<td>14.29%</td>
<td>-</td>
</tr>
</tbody>
</table>

4.2.2 Physical characteristics

General visual assessment proved unreliable when predicting aflatoxin contamination. All samples from the Central Queensland region in 2004 were clean, dry, unmoleded and appeared to be of good quality; yet 94% of these samples were contaminated with aflatoxin, ranging from concentrations of 1.3 µg/kg AB₁ to an estimated 240 µg/kg AB₁.
Identification of pink kernels associated with F. graminearum infection was extremely rare and limited to samples originating from the North Queensland region. This rarity of occurrence made statistical analysis unworkable.

There appeared to be no significant correlations between the bulk density of maize and the concentration of fumonisin (G=0.29 p<0.05) or total aflatoxins (G=0.09 p<0.05) when results from 112 samples taken from all regions during 2004 were compared.

The correlation coefficient for total aflatoxins and the number of particles showing BGYF (glows) is not significant (p<0.05) and, at G=0.368, not a strong correlation. A \( \chi^2 \) analysis indicates the relationship between the presence of BGYF and the presence of aflatoxin to be statistically significant (p<0.01).

**4.3 Aflatoxins**

**4.3.1 Descriptive analysis**

Only samples from 2005-2006 seasons are considered here; these samples were collected in accordance with the same guidelines, providing some measure of uniformity. Compliance with NACMA standards is presented in Table 4-4.

<table>
<thead>
<tr>
<th>Region</th>
<th>Milling (&lt;5 µg/kg)</th>
<th>Prime (&lt;15 µg/kg)</th>
<th>Feed #1 (&lt;20 µg/kg)</th>
<th>Feed #2 (&lt;80 µg/kg with &lt;20 µg/kg AB₁)</th>
<th>Exceeds Std (&gt;80 µg/kg total or &lt;20 µg/kg AB₁)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NQ</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Burnett</td>
<td>72.00%</td>
<td>14.67%</td>
<td>6.67%</td>
<td>2.00%</td>
<td>4.67%</td>
</tr>
<tr>
<td>Downs</td>
<td>94.00%</td>
<td>6.00%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NSW</td>
<td>90.91%</td>
<td>3.03%</td>
<td>1.52%</td>
<td>1.52%</td>
<td>3.03%</td>
</tr>
<tr>
<td>MIA</td>
<td>89.47%</td>
<td>8.77%</td>
<td>-</td>
<td>-</td>
<td>1.75%</td>
</tr>
<tr>
<td>VIC</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WA</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

By identifying samples from hybrids considered suitable for milling purposes and those suitable for stock feed, it was calculated that 14% of samples potentially suitable for milling purposes were contaminated with aflatoxin at levels greater than 5 µg/kg total aflatoxin and 10% of samples from feed hybrids were contaminated at levels exceeding the accepted 20 µg/kg AB₁. Several hybrids are...
considered suitable for both purposes and a number of samples were from experimental hybrids with no information about intended end use. Where a hybrid was considered suitable for both purposes it was included in the milling category. Hybrids with no information about intended end use were excluded from these calculations.

The histogram of AB$_1$ concentrations for the study is presented in Figure 4-2. Visual assessment of this histogram clearly indicates that AB$_1$ concentrations are not distributed normally. Kolmogorov-Smirnov goodness-of-fit analysis confirms this (p=0.000) and also proves that the distribution is significantly different from exponential distribution (p=0.000).

Examination of the descriptive statistics presented in Table 4-5, particularly the medians, indicates clearly that in all regions at least half the samples returned results for total aflatoxin contamination that were below the limit of detection. The means and their associated confidence intervals indicate that samples from the Burnett appear to suffer substantially greater total aflatoxin contamination than those from other Regions. This is will be explored in more detail in forthcoming sections.
Table 4-5 Descriptive statistics for AB₁ concentrations by Region 2005-2006

<table>
<thead>
<tr>
<th>Region</th>
<th>n</th>
<th>Min</th>
<th>Max (µg/kg)</th>
<th>Mean (µg/kg)</th>
<th>Median (µg/kg)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>NQ</td>
<td>16</td>
<td>ND</td>
<td>2.70</td>
<td>0.3844 (CI₉₅% 0.0553-0.7134)</td>
<td>0.23</td>
<td>0.61750</td>
</tr>
<tr>
<td>Burnett</td>
<td>149</td>
<td>ND</td>
<td>157.7</td>
<td>7.7042 (CI₉₅% 4.7284-10.6799)</td>
<td>0.45</td>
<td>18.38141</td>
</tr>
<tr>
<td>Downs</td>
<td>107</td>
<td>ND</td>
<td>17.00</td>
<td>0.7826 (CI₉₅% 0.3214-1.2438)</td>
<td>0.23</td>
<td>2.40643</td>
</tr>
<tr>
<td>NSW</td>
<td>67</td>
<td>ND</td>
<td>120.00</td>
<td>4.3740 (CI₉₅% 0.0348-8.7132)</td>
<td>0.23</td>
<td>17.78944</td>
</tr>
<tr>
<td>MIA</td>
<td>65</td>
<td>ND</td>
<td>40.00</td>
<td>1.9092 (CI₉₅% 0.5706-3.2479)</td>
<td>0.23</td>
<td>5.40245</td>
</tr>
<tr>
<td>VIC</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WA</td>
<td>2</td>
<td>ND</td>
<td>4.00</td>
<td>2.1150 (CI₉₅% -21.8362-26.0662)</td>
<td>2.23</td>
<td>2.66579</td>
</tr>
<tr>
<td>Total</td>
<td>426</td>
<td>ND</td>
<td>157.7</td>
<td>4.2198 (CI₉₅% 2.8953-5.5443)</td>
<td>0.23</td>
<td>1.90786</td>
</tr>
</tbody>
</table>

Regional distributions

As is illustrated in Table 4-6, differences in the shape of each distribution from region to region were significant, even when the central tendencies of distributions were similar.

Table 4-6 Kolmogorov-Smirnov Z statistics for AB₁ concentrations by Region

<table>
<thead>
<tr>
<th>Regions compared</th>
<th>AB₁ (exact p, 2 tailed)</th>
<th>AB₁ centred (exact p, 2 tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2005</td>
<td>2006</td>
</tr>
<tr>
<td>Burnett vs Downs</td>
<td>1.68 (0.000)</td>
<td>2.853 (0.000)</td>
</tr>
<tr>
<td>Burnett vs MIA</td>
<td>1.489 (0.001)</td>
<td>1.078 (0.084)</td>
</tr>
<tr>
<td>Burnett vs NSW</td>
<td>1.179 (0.019)</td>
<td>2.368 (0.000)</td>
</tr>
<tr>
<td>Downs vs NSW</td>
<td>0.182 (1.000)</td>
<td>0.236 (0.149)</td>
</tr>
<tr>
<td>NSW vs MIA</td>
<td>0.170 (1.000)</td>
<td>1.139 (0.004)</td>
</tr>
</tbody>
</table>

4.3.2 Regional variation

There were no significant differences between Regions when the number of samples contaminated at levels >5 µg/kg were compared (p>0.05, FET). This was not true in 2006, however, when samples from the Burnett region were significantly more likely to produce samples considered unacceptable for milling purposes than all other Regions providing samples; specifically the Downs (p=0.000, FET),
NSW (p=0.000, FET) and the MIA (p=0.013, FET). Samples originating from the MIA were significantly more likely to be contaminated at levels >5 µg/kg than the Downs (p=0.026, FET).

When considering samples contaminated at levels >20 µg/kg AB1, a similar pattern emerged. No significant difference is evident between Regions in 2005 (p>0.05, FET). In 2006, samples from the Burnett regions were significantly more likely to be considered unacceptable for stock feed than those from the Downs (p=0.005, FET), NSW (p=0.040, FET) and the MIA (p=0.041, FET).

4.3.3 Seasonal variation between Regions

In the Burnett region, samples produced in the 2006 season were significantly more likely to be contaminated at levels >5 µg/kg than those from 2005 (p=0.004, FET). No other region demonstrated this variation between seasons.

4.3.4 Variations by locality within the Burnett region

The results described above indicate that the Burnett Region is potentially problematic with respect to aflatoxin contamination. An examination of the data implied that this problem was restricted to certain geographical localities in the Region, so significant differences between localities were investigated. These localities are illustrated in Figure 4-3.

![Sampling locations within Burnett region](image)

In 2005, significantly more samples originating from the Wooroolin area in the central part of the region were likely to be unsuitable for milling purposes than areas of the region (p=0.009, FET)
situated around Kingaroy. This was not the case in 2006. Samples from the Coalstoun Lakes area in the north of the region are more likely to produce samples >5 µg/kg aflatoxin in both 2005 (p=0.042, FET) and 2006 (p=0.030, FET). When considering samples exceeding NACMA standards for aflatoxin (>80 µg/kg total or >20 µg/kg AB1), there was no significant difference between localities in either 2005 (p=471, FET) or in 2006 (p=0.861, FET).

4.3.5 Occurrence of Aflatoxin G1 and G2

While both *A. flavus* and *A. parasiticus* are known to produce aflatoxins in maize, aflatoxins G1 and G2 are only produced by *A. parasiticus*. When the occurrence of the four aflatoxins was investigated, it was found that aflatoxin G1 and G2 were significantly more likely to occur in maize from the Burnett Region than other regions (p=0.043, FET). No significant relationship to season was noted, nor any variation between localities within the Burnett.

4.4 Fumonisins

4.4.1 Descriptive analysis

Although the occurrence of fumonisin contamination was high, with 75.8% of all samples containing detectable levels, the concentration of contamination was generally low with most samples meeting the NACMA requirements for milling purposes (Table 4-7).

<table>
<thead>
<tr>
<th>Region</th>
<th>Milling (&lt;2 mg/kg)</th>
<th>Prime (&lt;5 mg/kg)</th>
<th>Feed #1 (&lt;10 mg/kg)</th>
<th>Feed #2 (&lt;40 mg/kg)</th>
<th>Exceeds Std (≥40 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NQ</td>
<td>87.5%</td>
<td>12.5%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Burnett</td>
<td>95.33%</td>
<td>3.33%</td>
<td>1.33%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Downs</td>
<td>93.00%</td>
<td>7.00%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NSW</td>
<td>81.82%</td>
<td>7.58%</td>
<td>1.52%</td>
<td>7.58%</td>
<td>1.52%</td>
</tr>
<tr>
<td>MIA</td>
<td>82.46%</td>
<td>14.04%</td>
<td>-</td>
<td>3.51%</td>
<td>-</td>
</tr>
<tr>
<td>VIC</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WA</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Visual assessment of Figure 4-4 clearly indicates that FB1 concentrations are not distributed normally. Kolmogorov-Smirnov goodness-of-fit analysis confirms this assessment (p=0.000) and also proves that the distribution is significantly different from exponential distribution (p=0.000).
Figure 4-4 Histogram of FB1 concentrations for all Regions 2005-2006

Table 4-8 Descriptive statistics for FB1 concentrations (mg/kg) by Region 2005-2006

<table>
<thead>
<tr>
<th>Region</th>
<th>n</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>NQ</td>
<td>16</td>
<td>ND</td>
<td>2.9</td>
<td>0.59</td>
<td>0.418</td>
<td>0.844</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(CI95% 0.146-1.045)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burnett</td>
<td>118</td>
<td>ND</td>
<td>12.9</td>
<td>0.45</td>
<td>ND</td>
<td>1.506</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(CI95% 0.185-0.73368)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Downs</td>
<td>101</td>
<td>ND</td>
<td>4.2</td>
<td>0.70</td>
<td>0.350</td>
<td>0.827</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(CI95% 0.532-0.858)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSW</td>
<td>62</td>
<td>ND</td>
<td>10.5</td>
<td>1.02</td>
<td>0.250</td>
<td>2.137</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(CI95% 0.481-1.566)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIA</td>
<td>62</td>
<td>ND</td>
<td>13.0</td>
<td>1.25</td>
<td>0.315</td>
<td>2.424</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(CI95% 0.632-1.863)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIC</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WA</td>
<td>2</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>366</td>
<td>ND</td>
<td>13.0</td>
<td>0.75</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(CI95% 0.579-0.922)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Samples where FB$_1$ was not detected (ND) were assigned a value of half the limit of detection, i.e. 0.005 ppm. Samples with concentrations more than two standard deviations from the mean were removed as outliers. This amounted to a total of 8 samples, ranging from 17.1 mg/kg to 80.6 mg/kg, all originating from the MIA and NSW.

### 4.4.2 Regional distributions

Distributions of FB$_1$ concentrations were analysed by region using the Kolmogorov-Smirnov test, displayed in Table 4-9. Once again, significant differences in distributions between Regions were noted although, unlike the situation with aflatoxin, the Burnett, MIA and Downs Regions exhibited significantly similar distributions in 2006. The same similarity did not occur in 2005.

#### Table 4-9 Kolmogorov-Smirnov Z statistics for FB$_1$ concentrations by Region

<table>
<thead>
<tr>
<th>Regions compared</th>
<th>FB$_1$ (exact $p$, 2 tailed)</th>
<th>FB$_1$ centred (exact $p$, 2 tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2005</td>
<td>2006</td>
</tr>
<tr>
<td>Burnett vs Downs</td>
<td>3.103 (0.000)</td>
<td>0.917 (0.370)</td>
</tr>
<tr>
<td>Burnett vs MIA</td>
<td>2.435 (0.000)</td>
<td>0.634 (0.816)</td>
</tr>
<tr>
<td>Burnett vs NSW</td>
<td>1.95 (0.001)</td>
<td>1.254 (0.086)</td>
</tr>
<tr>
<td>Downs vs NSW</td>
<td>1.021 (0.249)</td>
<td>1.862 (0.002)</td>
</tr>
<tr>
<td>Downs vs MIA</td>
<td>0.985 (0.287)</td>
<td>1.072 (0.200)</td>
</tr>
<tr>
<td>NSW vs MIA</td>
<td>0.398 (0.997)</td>
<td>1.517 (0.020)</td>
</tr>
<tr>
<td>NQ vs Burnett</td>
<td>1.863 (0.002)</td>
<td>2.277 (0.000)</td>
</tr>
<tr>
<td>NQ vs Downs</td>
<td>0.919 (0.368)</td>
<td>1.429 (0.034)</td>
</tr>
<tr>
<td>NQ vs NSW</td>
<td>0.884 (0.425)</td>
<td>1.945 (0.001)</td>
</tr>
<tr>
<td>NQ vs MIA</td>
<td>0.726 (0.667)</td>
<td>2.075 (0.000)</td>
</tr>
</tbody>
</table>

### 4.4.3 Regional variation

Once again, Fisher’s Exact Test was used to examine relationships between the Region of origin and samples meeting the NACMA standards for milling (<2 mg/kg) and stock feed #1 (< 10 mg/kg). The Feed #1 standard was chosen because there were no instances of contamination at levels exceeding the Feed #2 standard included in the analysis. Isolated cases contaminated at such high levels were removed as part of the data cleaning process as random outliers.

NSW and the MIA were significantly more likely to produce maize unsuitable for milling during 2005. NSW had significantly more instances of contamination at levels >2 mg/kg than the Burnett (p=0.000, FET), Downs (p=0.000, FET) and NQ (p=0.035, FET), while the MIA produced significantly more...
samples in this range than the Burnett (p=0.000, FET) and the Downs (p=0.022, FET). The Burnett region demonstrated significantly less instances of contamination at levels <2 mg/kg than all other Regions; Downs (p=0.023, FET), NQ (p=0.027, FET), NSW (p=0.000, FET) and the MIA (p=0.000, FET).

In 2006, there was no significant difference between Region of origin and contamination at levels >2 mg/kg or >10 mg/kg.

In 2005, fumonisin contamination at levels >10 mg/kg occurred significantly more often in NSW than the Burnett (p=0.000, FET), Downs (p=0.002, FET) and NQ (p=0.027, FET). A similar pattern was evident in the MIA region, with samples being more likely to exceed the 10 mg/kg standard than either the Downs (p=0.013, FET) or the Burnett (p=0.001, FET). There was no significant difference between the NSW and MIA regions.

4.4.4 Seasonal variation between regions

The Burnett region produced significantly more cases of contamination >2 mg/kg in 2006 than in 2005 (p=0.005, FET), while NSW produced more cases in 2005 than in 2006 (p=0.000, FET). There were no significant differences between seasons for either the Downs or MIA regions.

At contamination >10 mg/kg, NSW again experienced significantly higher levels of contamination in 2005 than in 2006 (p=0.001, FET). Although there were similar proportions of samples >2 mg/kg in the MIA over these two seasons, a significantly greater proportion were >10 mg/kg in 2005 than in 2006 (p=0.022, FET). No other Regions were significantly different.

4.5 Ochratoxin A

OTA was not detected in any sample during the survey.

4.6 Zearalenone

ZER was detected in only a very small number of samples, all from the North Queensland region and all at low levels.

4.7 Aflatoxin and fumonisin co-contamination

There is ample evidence that aflatoxin and fumonisin contamination can co-occur. More than 25% of samples from the 2005 and 2006 seasons tested positive for both mycotoxins. Statistical analysis (χ²)
did not indicate any significant relationship between the two variables (p=0.564). Of those samples that were classified as unsuitable for sale as stock feed according to the NACMA standard, there were none that exceeded the standard for both mycotoxins. Of the twenty-nine (29) samples that exceeded the NACMA standard for aflatoxin, only two (2) demonstrated high fumonisin concentrations, and these samples were still below the 40 mg/kg limit for fumonisins. Only three (3) samples were classed as exceeding the standard or fumonisin, all of which were below the 5 µg/kg milling standard for aflatoxin.

4.8 Relationships between climate and mycotoxin contamination

Survey results for AB1 and FB1 concentrations were correlated with climate data for the kernel development period in each maize growing region.

4.8.1 Aflatoxins

Over the 2005-2006 seasons, concentrations of aflatoxin contamination proved to be negatively correlated with rainfall during kernel development (p=0.005). In 2006, the Burnett area of Queensland was significantly more likely to produce maize unsuitable for milling purposes than any other maize-producing region in Australia (p<0.05), which corresponded with lower daily rainfall averages over the kernel development period (p<0.01). Regions using irrigation reported significantly lower levels of aflatoxin contamination (p<0.01) as did areas with higher rainfall (p<0.01). A correlation between aflatoxin concentration and temperature was not evident, probably because all maize growing areas reported temperatures well over 30°C during the relevant kernel development periods; however a significant negative correlation was identified between average rainfall and the number of days over 30°C (p=0.002). It is reasonable to suggest that a combination of the increased water availability and associated lower temperature acted to reduce contamination.

4.8.2 Fumonisins

FB1 contamination was found to be negatively correlated with mean daily precipitation during the kernel development period (p=0.000). Significant FB1 concentrations were also correlated with the number of days experiencing maximum temperatures over 30°C during the kernel development period (p=0.004).
4.9 Preliminary survey of maize based food products

A preliminary survey of common, commercially available maize-based food products was conducted. Food samples included cornflakes, corn chips, polenta (corn meal), puffed corn and high fibre white bread, containing Hi-maize®. In most cases these products were manufactured in Australia from Australian product, however there were two exceptions- one brand of polenta was manufactured in Italy (1.28 mg/kg FB₁) and the puffed corn product was manufactured in the USA from maize produced in that country. Aflatoxin was not detected in any food product. Fumonisin contamination was more common, with all products apart from the bread returning at least some positive results, probably due to the low maize content. Results of the survey are presented in Table 4-10.

Table 4-10 Results of limited maize-based food products survey for AB₁ and FB₁ contamination

<table>
<thead>
<tr>
<th>Product</th>
<th>n</th>
<th>Aflatoxin (µg/kg)</th>
<th>Fumonisin (mg/kg)</th>
<th>Aflatoxin (n)</th>
<th>Fumonisin (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornflakes</td>
<td>5</td>
<td>ND</td>
<td>0.20</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Corn chips</td>
<td>4</td>
<td>ND</td>
<td>0.26</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Polenta</td>
<td>4</td>
<td>ND</td>
<td>0.51¹</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Puffed corn</td>
<td>4</td>
<td>ND</td>
<td>2.60</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Bread</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

¹Although all four polenta samples returned positive results for fumonisins, three were manufactured from Australian grown maize and concentrations were low, reducing the mean. The imported brand was contaminated with 1.28 mg/kg FB₁.

4.10 Risk assessment

An assessment of the risks to Australian consumers was conducted using the enHealth model described in Chapter 2.6. Community consultation, leading to issue identification, occurred prior to the commencement of the research and acted as the catalyst for the project’s inception. Results of the research were presented in a number of forums throughout the project. Additionally, key members of the industry were involved in a formal industry consultation seminar was held in Brisbane in August 2006, to present work to date and identify industry concerns and informational needs.

4.10.1 Hazard assessment

Hazard identification

Given the small nature of the pilot food survey and the low levels of contamination, an exposure model based on manufactured foods was not able to be performed using this data. The survey of raw maize
provides the most extensive information available on mycotoxin contamination of Australian-grown maize. Until now, data regarding contamination has been limited to specific geographic regions, single seasons or individual outbreaks. While there are certainly limitations and uncertainties regarding the data, the survey data provides the most complete dataset available. Mycotoxin contamination is difficult to reduce or eliminate once it has occurred. Results from hybrids not intended for human consumption and from samples that would be rejected by manufacturers for existing contamination were excluded from the dataset and an assumption was made that the balance of the maize crop would be included in the food chain.

**Toxicity**

This topic has been covered in detail in Chapter 2.2, and thus only a cursory examination of the toxicity of aflatoxin AB₁ and FB₁ relating to applicable exposure standards will be given here.

On the basis of a deliberate suicide attempt described by the USFDA (1992), a crude NOAEL of 10 µg /kg BW AB₁ per day over periods up to 14 days is assumed for acute effects. Owing to its status as a naturally occurring genotoxin, JECFA have not set a value for tolerable daily or weekly intake for this mycotoxin and thus any aflatoxin contamination in maize destined for human consumption is considered a hazard to human health.

Contamination of milling grade maize with fumonisins is of concern only if consumption has the potential to cause exposure to exceed the TDI of 2 µg /kg BW/day (JECFA 2003). This value was based on the NOAEL for renal toxicity in rodents observed in both short and long term studies (JECFA 2003).

### 4.10.2 Exposure assessment

**Model**

Using distribution models of available data, the risk of mycotoxin contamination in Australian maize to the Australian population was estimated with a probabilistic risk analysis utilising Monte Carlo simulation as described in section 3.3.6. The exposure model, shown again below, was used for both AB₁ and FB₁. A combination of point estimates and probability distributions was used in the model.

\[ E = c \times g \times m \]

Where:

\[ E = \text{exposure (per kg BW / day)} \]
\(c=\) concentration of mycotoxin in maize per gram

\(g=\) quantity of maize consumed per day, excluding as sweet corn (g/day)

\(m=\) body weight (kg)

Exposure analysis was conducted using the @Risk Industrial edition 5.0 computer software package. Survey results were evaluated by the program against a range of distributions for goodness of fit and a custom distribution model was computed for variable \(c\) (Figures 4-5 and 4-6).

As described in Section 3.3.6, body weight \(m\), was set as a constant 74 kg for the average adult Australian and 15 kg for the average child under 8 years of age, as recommended by EnHealth in the draft Australian Exposure Assessment Handbook (enHealth 2003).
The computed distribution for daily maize consumption is illustrated in Figure 4-7, and was based on the ANZFA (2001) estimate of processed maize products by Australians at 3.48 g/day.

![Figure 4-7 Modelled daily maize consumption in Australia](image)

**Aflatoxins exposure**

Results indicate that exposure to aflatoxin B₁ through maize consumption is extremely low in Australia, with a mean exposure of 0.54 ng/kg BW AB₁/day and a 95th percentile of 2.02 ng/kg BW AB₁/day for adults. For children, who have lower body weights, mean exposure is estimated to be 0.95 ng/kg BW AB₁/day and a 95th percentile of 3.57 ng/kg BW AB₁/day. It should be noted that the distribution of contaminated maize was based on the entire dataset, truncated at the 5 µg/kg point to account for the NACMA standard for milling maize. When only samples from hybrids used for milling were used to calculate the distribution, for adults the mean exposure drops to 0.19 ng/kg BW AB₁/day, with 95% of cases less than 0.74 ng/kg BW AB₁/day. For children, the mean exposure is 0.96 ng/kg BW AB₁/day, with 95% of cases less than 3.76 ng/kg BW AB₁/day.

These figures represent a scenario in which all aflatoxin contained in the raw maize survives processing and is present in the final manufactured product. In truth, significant portions of the maize kernel, particularly the hull and the germ, are in many cases diverted from the end product. Research of dry-milled corn suggests that aflatoxin tends to be concentrated in the hull and germ of the maize kernel (Brekke et al. 1975; Scudamore & Patel 2000), which would mean that much of the aflatoxin in raw maize is excluded from manufactured food products. Under some conditions, processing tends to reduce aflatoxin concentration and toxicity (Scudamore & Patel 2000). While the fate of aflatoxins during processing has been extensively researched, the literature illustrates varied results depending on the nature and type of processing, temperatures and matrices. Despite this, it is not unreasonable to
assume that actual aflatoxin exposure from Australian-grown maize is somewhat less than estimated here.

**Fumonisins exposure**

Again, results of the Monte Carlo simulation indicate that exposure to FB$_1$ through maize consumption is extremely low in Australia, with a mean exposure of 0.20 µg/kg BW FB$_1$/day and a 95$^{\text{th}}$ percentile of 0.76 µg/kg BW FB$_1$/day for adults. For children, mean exposure is estimated at 0.98 µg/kg BW FB$_1$/day with a 95$^{\text{th}}$ percentile of 3.73 µg/kg BW FB$_1$/day.

It should be noted that the distribution of contaminated maize was based on the entire dataset, truncated at the 2 mg/kg point to account for the NACMA standard for milling maize. When only samples from hybrids used for milling were used to calculate the distribution, the mean exposure drops to 0.18 µg/kg BW/day, once again with 95% of cases less than 0.74 µg/kg BW/day. For children, the mean exposure reduces to 0.72 µg/kg BW/day, with a 95$^{\text{th}}$ percentile of 1.75 µg/kg BW/day.

The same caveat with regard to fumonisins present in the hull and germ applies here. Fumonisins have been found to occur in higher concentrations in corn germ and corn bran (Scudamore & Patel 2000; Vanara, Reyneri & Blandino 2009). Fumonisins are also subject to degradation through some processing techniques, although to a lesser extent than aflatoxins (Scudamore et al. 2008). It is quite reasonable to assume that the actual exposure from maize-based food products is somewhat less than these estimates.

### 4.10.3 Risk characterisation

**Aflatoxins**

By comparing the values obtained through the simulation for aflatoxin exposure from Australian maize, with the 10 µg/kg BW AB$_1$/day NOAEL previously estimated, it is clear that the potential for an acute exposure event related to Australian maize is highly unlikely in both adults and children.

In terms of chronic exposure, when the estimated exposure for both adults and children is compared with the 0.75 µg/kg BW AB$_1$/day NOAEL proposed by Weidenbörner (2001), adverse effects related solely to AB$_1$ contamination of maize-based food products appear unlikely. The ALARA principle, however, indicates that there is no exposure to aflatoxins that should be considered to have no effect. As a result, it is appropriate to determine the risk of developing hepatocellular carcinoma from exposure to AB$_1$ from contaminated maize.
Liver cancer incidence in a population attributable to aflatoxins can be derived by combining estimates of aflatoxin potency in terms of the risk per unit dose and estimates of aflatoxin intake, or the dose per individual (Herman 2008). The potency of AB₁ in the general population has been calculated to be 0.01 cases per year/100 000 people per ng AB₁/kg BW per day, with a range of 0.002-0.03 cases per year/100 000 people per ng AB₁/kg BW per day (Herman 2008). In individuals who are serologically positive for HBV, the potency is approximately 30 times greater, at 0.3 cases per year/100 000 people per ng AB₁/kg BW per day, with a range of 0.05-0.5 cases per year/100 000 people per ng AB₁/kg BW per day (Herman 2008). The proportion of the Australian population who are serologically positive for HBV is estimated to be 0.67% (0.47-0.87%) (O'Sullivan et al. 2004). Taking this into account, the formula for calculating potency of AB₁ in the Australian population is:

\[
\text{Potency}_{\text{AUST/100,000}} = 0.01 \times 99.33\% + 0.3 \times 0.67\%
\]

This calculates an estimate of 0.012 cases per year/100 000 people per ng AB₁/kg BW per day, with a range of 0.002-0.038 cases per year/100 000 people per ng AB₁/kg BW per day.

The risk of developing hepatocellular cancer as a result of maize consumption in the Australian population is calculated by multiplying this potency with the exposure estimated previously. Using the distribution from the model and assuming HBV prevalence in the Australian population to be the mean of 0.67%, the risk is estimated to be less than 0.000009 cases /100 000 annually (p<0.05). Even in the worst case scenario, the risk remains extremely low, at less than 0.000010 cases /100 000 annually (p<0.05).

At the time of writing, Australia’s population was estimated at 22,798,394, with an estimated population increase of one person every 1.52 minutes (Australian Bureau of Statistics 2012). For ease, this can be rounded to 23,000,000. Taking this into account, even in the worst case scenario, less than 0.00025 cases of hepatocellular cancer occur annually in Australia as a result of maize-based foods contaminated with AB₁. This risk is extremely low, particularly when compared with the rate of cancers caused by alcohol consumption in Australia; estimated to be 5,663 cases annually, of which an estimated 180 are hepatocellular cancer (Cancer Council of Australia 2011).
4.11 Industry consultation

4.11.1 Sector specific issues and concerns

Industry representatives from all sectors were asked about specific issues they were concerned about with respect to mycotoxins in maize. **Seed companies** indicated they required information about physiological traits occurring in seed grain that can be monitored to minimise mycotoxin risks and mycotoxin-related information that can be included in the agronomic advice they provide to growers. They also expressed interest in whether genetically modified Bt maize used overseas is mycotoxin resistant; and whether there was a breeding solution to mycotoxin contamination of maize in the field.

**Growers** specified a need for information about signs and symptoms of mycotoxin affected grain to be alert for during production and advised that the problem was not well known amongst growers. Educating agronomists and consultants about the problem was identified as an appropriate target for extension programmes.

**Grain merchants** and **bulk handlers** identified a number of issues. In general terms, these ranged from concerns relating to pre-receipt sampling for analysis, through storage conditions to occupational health and safety risks to workers; as well as a need for clear information about mycotoxin hazards throughout the supply chain. These stakeholders were firstly concerned about mycotoxin contamination inherited from the grower and ways to sample and rapidly assess grain in the field, at the farm gate or immediately prior to accepting a delivery. Secondly, they were interested in the success of grading and other cleanup methods in the reduction of mycotoxin contamination. Thirdly, they requested clear storage protocols, particularly relating to the acceptability of the oft-quoted 14% moisture content for stored grain, and advice as to appropriate uses for graded, screened or rejected grain. Finally, they expressed a need for a set of industry-wide sampling and testing protocols for mycotoxin contamination in maize. This sector was also concerned about the potential for human health effects relating to mycotoxins and grain infected by fungi.

**Processors** indicated that their primary concern was having confidence that the product arriving at the factory door meets the standard required for the end use. Relates to this was a request for clarification of sampling methodology, including how to get a representative sample and correct sample size. In addition, processors asked how mycotoxin contamination can be minimised throughout the supply chain, including quality control during manufacture.
**End users** included representatives of the food, pet food and stock feed sectors. Many issues raised by these representatives reflected those raised by other sectors- including the need for a mycotoxin management protocol throughout the supply chain; quality assurance; advice as to uses for grain that is currently rejected; and the need for a standard sampling and analysis protocol, including rapid and cost-efficient tests.

Additionally, the **manufacturing sector** expressed a need for information about:

- existing standards for mycotoxins in food and feed products
- how the manufacturing process affects mycotoxin levels
- whether aflatoxin is an indicator of other mycotoxin levels
- what mycotoxins to test for
- the risk to human health
- the need to test for mycotoxins and conjugated toxins in animal organs used for wet products such as pet foods

Representatives described a general lack of knowledge and understanding of the mycotoxin issue in the manufacturing industry and requested information be disseminated.

Other concerns expressed by this group included economic issues, such as agreed payment levels for varying levels of contamination, such as that practised in the peanut industry; and the potential use of binders in stock feed and the associated costs. It was suggested that a form of accreditation be put into place in conjunction with a common management protocol throughout the supply chain; but questions arose as to responsibility for such a programme and whether the associated costs might increase costs excessively, making way for cheap imports from overseas. Inclusion of a standard mycotoxin management clause in the vendor declaration was suggested.

Representatives from **analytical laboratories** agreed that there was a need to develop a protocol for agreed sampling and testing methods. Connected to this was a need for growers and other sectors of the industry submitting samples to understand the requirements for accurate testing and the limitations of analysis.

**Extension and regulation agencies** agreed with the need for a protocol to minimise mycotoxin contamination throughout the supply chain, and also raised the question of the need for an export protocol. It was also suggested that it would be of greater benefit to offer premiums for high quality
maize grown in accordance with risk management principles as opposed to instigating penalties on contaminated grain.

Researchers identified the effect of binding agents on nutritional value and remediation methods for contaminated grain as areas in need of future research.

4.11.2 Common concerns

There were several issues that were raised by a number of industry sectors, indicating these should be addressed as a matter of priority.

- Dissemination of general information about mycotoxins in maize to growers, agronomists, consultants, bulk handlers, manufacturers and processors;
- Development of a management protocol to minimise mycotoxin contamination of maize, addressing the entire supply chain, including export, and using a risk management approach;
- Development of a sampling and analysis protocol;
- Development of recommendations for appropriate uses for contaminated maize.
Chapter 5  Discussion

5.1 Risks inherent in the Australian maize industry

Based on the results of the maize survey, the general quality of Australian grown maize with respect to mycotoxin contamination is excellent. Almost the entire harvest can be utilised for human/pet food or stock feed. Only a very small quantity each year exceeds trading standards and natural dilution of this contaminated grain reduces contamination even further.

The distribution of mycotoxin contamination that does occur is very dynamic. Outbreaks of aflatoxin contamination are particularly difficult to predict and, while fumonisins are almost endemic at low concentrations in many regions, outbreaks of highly contaminated grain do occur. These outbreaks are very dependent on environmental conditions, as demonstrated both in the survey results and the literature. Occasionally environmental conditions can cause a significant outbreak, causing problems in the maize supply chain, as occurred in 2003 (Blaney, O'Keefe & Bricknell 2008).

The absence of a statistically significant relationship between aflatoxin and fumonisin occurrence suggests that contamination occurs independently; the presence of contamination with one group of related mycotoxins does not appear to predispose the kernel to contamination with the other. Given that contamination depends on a wide variety of factors, co-occurrence would be more likely to be related to kernel damage, climatic conditions, plant stress and presence of inoculum. The evidence indicating that high levels of contamination with both mycotoxins is rare and is potentially related to the difference in climatic conditions favoured by the different fungal agents; while a kernel may be infected by both fungi, the climate will determine which species will more successfully produce toxin.

5.1.1 Presence of inoculum and cross-infection

Fungal infection usually occurs prior to harvest, but can also occur from dormant fungal spores present in grain dust residues in storage silos, which can be transported through grain by insects or rodents. Contamination of grain with soil-borne spores combined with damage to kernels may also make mycotoxin formation more likely during storage. While good agricultural practices (GAP) can reduce the availability of inoculum, it is impossible to eliminate it altogether, making this a significant hazard in maize production.

Clearly there is no shortage of fungal inoculum in maize growing regions of Australia; the wide occurrence of both aflatoxins and fumonisins demonstrate the presence of *A. flavus, A. parasiticus* and
F. verticillioides in most maize growing regions. Fungal spores remain dormant in soil from crop to crop and from year to year, present in layers of infected crop residues (Thomas et al. 2007). Increasing adherence to no-till cultivation, aimed at preserving topsoil, can thus increase soil contamination with fungal spores.

Rotating crops that share susceptibility to specific fungi increases the availability of inoculum in shared fields. The literature reports that wheat and maize share a susceptibility to some Fusarium spp., particularly F. graminearum (Vogelgsang et al. 2011). Rotating these two crops is therefore likely to increase the availability of inoculum and subsequent ZER, NIV and/or DON contamination in these crops (Vogelgsang et al. 2011). This is particularly likely to occur if there is rainfall during anthesis and persistently moist conditions during maturation (Blaney 2001). Such conditions rarely occur in the main grain production regions of Australia, although they did occur in 1999-2001 at a few localities on the Liverpool Plains of NSW (Southwell et al. 2003). The low occurrence of F. graminearum mycotoxins is possibly indicative of the rarity of this confluence of conditions during the sampling seasons.

While the conditions supporting F. graminearum cross contamination were, fortunately, rare, the same cannot be said of the conditions supporting aflatoxin contamination related to A. parasiticus cross contamination. In the Burnett Region, the two major crops are maize and peanuts and it is common practice amongst growers to rotate the two crops. As both are summer crops, the land is allowed to lie fallow for approximately seven months of the year (Maraseni 2007). The common occurrence of both B and G aflatoxins only in samples from the Burnett Region reflect this practice. As discussed in Chapter 2, G aflatoxins are produced only by A. parasiticus, and this fungi is the predominant cause of aflatoxin contamination in peanuts (Blaney et al. 1989).

5.1.2 Climate

Australia’s climate poses specific challenges in terms of mycotoxin contamination. Climate data provided by the Bureau of Meteorology shows that many maize growing areas of Australia, including the Murrumbidgee Irrigation Area (MIA), central west of NSW and Central Queensland can experience high temperatures and low precipitation during the maize growing season (Bureau of Meterology 2004). Maize crops in these areas are irrigated but aflatoxin problems still occur occasionally in parts of crops. This could be due to uneven irrigation or soil that is shallow in spots due to field levelling for flood irrigation. The risk would appear to increase if crops are planted in December, when the developing ear can be exposed to very high January/February temperatures, often exceeding 35°C.
Although less often subject to such high temperatures, crops in the Central Burnett, South Burnett and Darling Downs in Queensland are often rain-fed (Robertson et al. 2003). The data show more frequent aflatoxin contamination in these areas, particularly in the central and upper Burnett. As previously discussed, aflatoxin contamination of grain produced in these areas appears more common than in the other maize growing regions studied.

The clear variation in aflatoxin contamination between the northern and southern zones of the Burnett Region is interesting. This is most likely explained by climatic conditions. While the degree of drought in the Coalstoun Lakes is no greater than the southern area of the Burnett around Kingaroy, 100 years’ worth of collected data indicates that temperatures are consistently and appreciably warmer during the reproductive period (Chauhan, Wright & Rachaputi 2008). These higher levels of aflatoxin contamination in the Coalstoun Lakes area were accurately predicted by computer modelling conducted by Chauhan et al (2006).

Risk modelling by Chauhan et al (2006) using data collected as part of this survey also shows that in some regions during summer, even full irrigation may not provide sufficient water to the growing ear to combat the extreme evaporation rates from high temperature and dry winds. This modelling accurately predicts the aflatoxin contamination in maize samples collected from the Central Queensland region in 2004. Although the analysis reflects only a single crop from a single year, the fact that the computer model predicts the high risk of contamination of maize produced in this region indicates that it is likely to be a recurring problem. The crop that was sampled was produced under irrigation, however the high rate of contamination would indicate that climatic conditions (including temperature, humidity and evaporation) make irrigation ineffective.

The conditions in north-eastern NSW and the southern Darling Downs in south-east Qld are more moderate in terms of temperature and rainfall (Bureau of Meterology 2004), and aflatoxin contamination appears to be only a rare problem. Less data exist for fumonisins in these areas but samples collected in this project show no more contamination than in other regions. As the climate becomes cooler and moister, for example in proximity to the QLD-NSW border ranges, conditions become more conducive for growth of the mould that produces ZER, NIV and DON, \textit{F. graminearum}, but even so, significant contamination during the survey period was very rare.

As previously noted, parts of the north Queensland tablelands feature a cool, persistently wet climate during maize silking and maturation, and ZER and NIV contamination can occur, although instances such as these were rare during the seasons surveyed. Genetic variations and distribution of \textit{F.}
*graminearum* isolates mean that while both areas experience ZER contamination, NIV tends to occur in northern Queensland and DON in southern Queensland (Blaney, O'Keeffe & Bricknell 2008). In this region, aflatoxin was found to occur only rarely, although further study is warranted as maize production is extending into the hot, wet lowlands of this region.

**Implications of climate change on Australian maize**

Climate change is expected to have significant impacts upon Australia. Our continent is predicted to experience general increases in ambient temperature and reduced average rainfall (Australian Greenhouse Office 2006). These conditions clearly not only favour aflatoxin contamination but also induce plant stress, making the plant more susceptible to fungal infection.

Extreme climate events such as heat waves and drought are also tipped to occur more frequently (Australian Greenhouse Office 2006). This would indicate that episodes such as the 2001 and 2003 outbreaks of severe aflatoxin contamination experienced in Australia will also occur more frequently.

Climate change may make growing dryland maize in some Australian regions unprofitable and farmers may turn to more drought-resistant crops. The dryland maize-growing regions in South East Queensland are expected to grow warmer, with more hot days. A decline in annual rainfall is expected to occur, coupled with higher evaporative demand (Australian Greenhouse Office 2006). This may reduce the availability of Australian grown grain, causing an increase in imported maize to meet demand. Alternatively, if farmers continue to produce maize, irrigation will be required in greater volumes to meet the need for milling grade maize. Given the current water shortage and projected reductions in annual precipitation, this is unlikely to be a sustainable choice. Even in maize-producing areas customarily using irrigation, reduced water allocations may lead to maize being considered an unviable crop. By 2030, it is predicted that NSW will suffer increased water stress, with little change in rainfall but higher evaporative demand (Australian Greenhouse Office 2006).

Maize considered unsuitable for milling purposes would in many cases remain suitable for stock feed. A significant increase in the amount of maize available for this purpose would have the potential to reduce prices. Another use of contaminated maize is as a source of material for the production of biofuel, although the limited size of Australia’s maize industry would be unlikely to make this a profitable exercise.
Potential consequences for Australian consumers

If maize continues to be farmed but mycotoxin levels increase, harvests found to be unacceptable for milling purposes have a high probability of being sold for stock feed. Reduced rainfall may lead to lack of pasture and contaminated maize may be utilised for supplementary feed for dairy cattle, presenting obvious risk of the contamination of milk with aflatoxin M₁.

5.1.3 Insect damage

The general good quality of the samples provided for analysis indicates that Australian maize does not seem to experience the amount of insect damage reported in parts of North America. The vast majority of grain found to be contaminated with mycotoxins to detectable levels during the survey period was clean, unmoulded and undamaged. As reported in Chapter 4, there was no relationship between the percentage of damaged kernels and mycotoxin contamination. Despite these findings, the literature clearly identifies that maize does experience problems with a number of different species (Blaney, Ramsey & Tyler 1986; DAFF 2010b; Hardwick 2006; Murray & Miles 2003). The predominant insect pest in Australian pre-harvest maize is the ear worm, *Helicoverpa armigera* (Hübner) (Murray & Miles 2003). Eggs of this species are common on maize during silking and the larvae develop in the cob, leaving the kernels susceptible to fungal invasion (Murray & Miles 2003).

Another pest known to affect Australian maize is common armyworm, *Mythimna convecta* Walker (Lepidoptera: Noctuidae) (Hardwick 2006). During the seasons surveyed, the majority of mycotoxins occurred in undamaged grain, suggesting that contamination appears to be more related to climate than to insect attack. Supporting this hypothesis, one study in northern Queensland did not indicate increased ZER in maize infected with *F. graminearum* as a result of severe insect damage (*Spodoptera* sp.) (Blaney, Ramsey & Tyler 1986) but more investigation is certainly warranted.

Insects also play a role in rendering stored maize susceptible to fungal invasion. There are five major insect pests of stored cereal grain in Australia; moths (Angoumois, Tropical warehouse and Indian moths), weevils (*Sitophilus* spp.), the lesser grain borer (*Rhyzopertha dominica*), flour beetles (*Tribolium castaneum*), the saw-toothed grain beetle (*Oryzaephilus surinamensis*) and flat grain beetles (*Cryptolestes* spp.) (DAFF 2010b). Moths and the sawtooth grain beetle multiply rapidly at temperatures between 30-35°C and humidities between 75-80% (DAFF 2010b). Controlling temperature and humidity with aeration not only reduces mould growth, and thus mycotoxin production, but also insect populations.
5.1.4 Mechanical damage

Mycotoxin production during the actual harvest operation is unlikely, unless the process is interrupted and prolonged by rain, but mechanical harvesters can cause damage to kernels and leave them more vulnerable to fungal invasion. Mechanical damage is more likely to occur when grain is insufficiently dried before harvest, an uncommon situation in Australia, where it is more common to allow grain to dry to storage conditions before harvest. However, over-drying maize can lead to the kernel becoming brittle and susceptible to damage (Munkvold 2003).

5.1.5 Storage conditions

As with pre-harvest contamination, the factors conducive to fungal growth during storage are primarily related to the amount of inoculum present, temperature, relative humidity, moisture content and insect activity. Data provided by the Bureau of Meteorology indicates that the climate in major Australian grain production regions causes elevated temperatures (>30°C) in storage to be routinely experienced (Bureau of Meterology 2004), making the moisture content of stored grain critical. Even if the moisture content is in the range of 14-15%, at 30°C moisture migration and accumulation due to temperature differentials at the grain surface can easily provide pockets of maize with 16-18% moisture, favouring rapid growth of Aspergillus species and aflatoxin and ochratoxin production (Sanchis & Magan 2004). F. verticillioides requires a minimum moisture content of 18% and relative humidity of ~95%, and this fumonisins are unlikely to increase in maize postharvest. Conversely, maize stored (and maintained) at 10 -20°C is very unlikely to support significant aflatoxin production (Shapira 2004).

Another hazard is unexpected precipitation or high humidity during harvest, leading to high moisture conditions in storage or grain that has been insufficiently dried in the field prior to harvest and subsequent storage. While fumonisin, ZER, DON and NIV are predominantly pre-harvest problems in Australia, aflatoxin can be both a pre-harvest and post-harvest problem (Blaney, O'Keeffe & Bricknell 2008).

This was demonstrated by a case of contaminated grain exported to Japan in 2005 that was rejected by the Japanese authorities for aflatoxin contamination at levels exceeding the acceptable 0.005 mg/kg. The incident was investigated as part of this research project and described by Blaney (2008). The maize had been grown under irrigation during particularly hot and dry conditions and harvested during cool and showery weather. This resulted in maize that was borderline in terms of acceptable moisture content. In response to visible problems with quality, the grower graded the harvest to remove the majority of damaged kernels (Blaney, Bricknell & O'Keefe 2006). During the investigation, the grower
provided samples of both graded and ungraded grain from the same harvest to the author for analysis. Results indicated contamination levels of 0.002 mg aflatoxins/kg and 0.005 mg aflatoxins/kg in ungraded grain, clearly indicating the presence of inoculum in the load prior to shipment (Blaney, Bricknell & O'Keefe 2006). The increase in concentrations to unacceptable levels were probably the result of the grain being stored in unaerated containers and shipped across the Equator at temperatures up to 50°C before being deposited on the Japanese docks in midwinter (Blaney, Bricknell & O'Keefe 2006). Sufficient moisture had migrated from the kernels and condensed to allow some kernels to sprout—ideal conditions for the growth of A. flavus and subsequent production of aflatoxins (Blaney, Bricknell & O'Keefe 2006). Clearly both in-field and in-storage hazards need to be considered in the Australian maize production context.

5.2 The risk to human health

Fumonisins

When compared to the TDI of 2 µg fumonisins/kg BW/day, it is clear that the Australian adult population is exposed to significantly less than the tolerable daily dose and is, for all intents and purposes, safe from both acute and chronic toxic effects on the basis of current knowledge.

While the estimated adult exposure is extremely low and appears to pose little risk, the estimated exposure of children may be of concern. The standard child’s body weight at 15 kg, is one fifth that of the average adult and yet the amount of maize-based food products consumed has been assumed to be similar based on their consumption of corn-based breakfast cereal and snack food such as corn chips. This results in the exposure of children being substantially higher than adults. Children are always considered to be more susceptible to any kind of toxic exposure based on their smaller body mass and rapidly developing organs and immune systems. While the exposure of the majority of children at less than 1.75 µg fumonisins/kg BW/day still falls below the TDI (p<0.05), there is little room for a safety factor to allow for raw product exceeding the NACMA standards or for high levels of contamination in imported foodstuffs.

One other result of concern is the relatively high level of fumonisin contamination in the puffed corn food product. This product is manufactured in the USA from American maize and, according to the label, “contains corn germ”. With higher concentrations of fumonisin known to occur in the germ of the kernel, perhaps the higher levels are not surprising. The results described in Table 4.9 are supported
by a study of puffed (extruded) maize in Italy, where all samples were highly contaminated with both FB1 and FB2 at concentrations up to 6.1 mg/kg FB1 and 0.4 mg/kg FB2 (Doko & Visconti 1994a).

While Australia has no food standard or recommendation for fumonisins in any food product, the same is not true of the USA. The USFDA has a range of guidance levels for fumonisins in raw maize intended for human consumption. The highest of these, 4 mg/kg, is applied to dry milled corn bran, whole/partly degemmed dry milled corn product and clean corn for masa production, while a level of 3 mg/kg is applied to cleaned corn intended for popcorn, the closest analogy to the puffed corn product tested. The process of manufacturing puffed corn involves subjecting whole kernels to a steam and pressure treatment. There is no level particularly applicable for corn intended for puffing, however it is of concern that the processed product is contaminated to an amount comparable to the guidance level for raw maize used for similar human consumption purposes. A 100 g serve of this product each day would be enough to exceed the TDI for fumonisins. While the average serve is 14 g according to the label, consumption of the product as a snack rather than as a breakfast cereal could mean that in reality, serves are significantly larger.

5.2.1 Implications

In Australia, the only mycotoxin currently regulated is aflatoxin B1, and only in peanuts. Until 1999, a specific standard existed for aflatoxins in all other food products but this standard was removed as part of an overhaul of the Australian and New Zealand Food Standards Code. Standard A12 of the Food Standards Code also does not include mycotoxins in the general requirement requiring unspecified contaminants to be absent from all food products, as they are not classified as “contaminants” under the provisions of the Code.

In the 1999 review of Standard A12, it was recommended that the specific 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” (ANZFA 1999). Codex Alimentarius recommends that “contaminant levels in foods shall be as low as reasonably achievable” and that “maximum levels shall only be set for those foods in which the contaminant may be found in amounts that are significant for the total exposure of the consumer”. The position of ANZFA at the time was that the 19th Australian Total Diet Survey Survey (1998) had failed to detect aflatoxin in foods other than peanuts and thus, it appears, did not believe that the contaminant could occur in other foods at concentrations to significantly impact upon the consumer.
This failure to detect aflatoxin in Australian foods is not indicative of the contamination of maize-based foods at the time because the Authority did not choose to sample and analyse a range of maize-based foods for aflatoxin contamination. Both the 20th and 23rd Australian Total Diet Surveys returned similar results, based on the sampling and analysis of similar foods. A sample of maize-based foods manufactured from domestically-produced grain analysed after the introduction of the NACMA Standards in 2004 would probably return a similar result owing to the current practice of Australian manufacturers to test incoming loads of raw maize for a range of mycotoxins and reject those not meeting these voluntary standard for milling grade maize (Table 2-7). The survey of a range of foods containing significant proportions of maize carried out as part of our study appears to support this assumption, with no domestically-produced foods containing detectable levels of aflatoxin.

5.2.2 Imported products

While the application of the NACMA trading standards appears to protect the consumer from significant dietary exposure through food products based on domestically-produced maize, the same cannot be said for imported commodities. Of the foods tested as part of this study, two products tested positive for mycotoxins at significant levels (Italian polenta and puffed corn). The puffed corn product, imported from the USA, contained FB₁ at concentrations up to 4 mg/kg. It is worth noting that this concentration is significantly above the US Advisory Standard for fumonisin in food products. Likewise, the polenta imported from Italy was contaminated to a level of 1.28 mg FB₁/ kg, exceeding the EU Standard for maize based foods for human consumption (1 mg FB₁+FB₂/kg). This example serves to illustrate the vulnerability of the Australian market to unscrupulous dealers seeking to take advantage of Australia’s lack of regulation to offload product unsuitable for sale in home markets.

The potential use of contaminated maize for supplementary feed for dairy cattle, presents the risk of the contamination of milk with aflatoxin M₁. This is of particular concern should climate change result in large quantities of contaminated grain being diverted to use as stock feed. Australia has no standard for aflatoxin in milk or milk products. Additionally, milk powder also carries the potential for contamination with aflatoxin M₁ and is permitted for import from all areas certified as free from foot and mouth disease provided an import permit is granted (Anonymous). Once again, even if dairy feed were to be regulated in Australia, the lack of a food standard would leave Australia potentially vulnerable to import of contaminated product.
5.3 Managing the risks in Australian maize production

The data demonstrates that, despite mycotoxin contamination occurring at generally low levels, occasionally environmental conditions can cause a significant outbreak, causing problems in the maize supply chain, as occurred in 2003. The potential for these outbreaks to increase in frequency due to changes in climate in maize growing regions is also very real. This warrants the promotion of an industry wide risk management process that encourages growers to produce grain under optimum conditions.

Some factors increasing risk of contamination, such as weather variables, are not entirely controllable, although there are a number of good agricultural practices (GAP) that will assist in reducing contamination (Codex Alimentarius Commission 2003). Other factors such as insect pressure and storage conditions can be controlled. One framework for risk management is the Hazard Analysis Critical Control Point (HACCP) system. The *Code of Practice for the Prevention and Reduction of Mycotoxins in Cereals* identifies mycotoxin related hazards at each stage of cereal production in line with GAP and HACCP principles (Codex Alimentarius Commission 2003). A similar framework is used below, describing controls relating to hazards common across all maize growing areas as well as those specific to different Australian regions.

5.3.1 Pre-planting controls

Pre-planting planning should include attention to several critical steps in minimising mycotoxin contamination, including reducing available inoculum and selecting an appropriate hybrid. No-till cultivation methods can increase soil contamination with inoculum, because fungal spores can remain dormant in layers of infected crop trash (Thomas et al. 2007). No tillage and low tillage farming methods have increased in importance amongst agriculturalists in recent decades, aiming to reduce erosion, improve soil structure, increase water availability and increase yield (Knowler & Bradshaw 2007; Silburn, Freebairn & Rattray 2007; Thomas et al. 2007) requiring a trade-off between these outcomes and mycotoxin control if inoculum and resulting contamination is to be minimised.

As discussed earlier, rotation of crops that share a common susceptibility to mycotoxin producing fungi, such as wheat and maize with *F. graminearum* and peanuts and maize with *A. parasiticum*, increases the availability of inoculum.

Selection of a hybrid adapted for local conditions and suitable for the proposed end-use is a key decision. For example, the Queensland Department of Agriculture, Fisheries and Forestry has had a...
long-term breeding program in North Queensland to develop hybrids tolerant to *Fusarium* spp. infection (DAFF 2011) and, in this region, selection of appropriate hybrids may prove to be the most effective way to minimise ZER and NIV contamination. While no hybrids are currently available specifically for aflatoxin and fumonisin resistance, hybrids with increased resistance to insect attack and increased drought tolerance could be less susceptible. It has been known for many years that hybrids with long cobs with tight husk cover are more resistant to insect attack than other hybrids and experience less aflatoxin contamination (Bruns 2003). Other varieties are more tolerant to drought and thus experience less stress in dry conditions. In the United States there has been some success in identifying inbred genotypes for aflatoxin resistance, although the majority of these lack traits that make them suitable for commercial purposes (Betrán & Isakeit 2004; Betrán, Isakeit & Odvody 2002). Early maturing hybrids common in the Midwestern corn belt of the USA were trialled in Mississippi to avoid the high temperatures commonly occurring in the grain filling stage in that state, however, these early maturing varieties had looser husks that made cobs susceptible to insect attack and subsequent aflatoxin contamination and the trial was not successful (Betrán, Isakeit & Odvody 2002).

New techniques in genetic engineering are aimed at improving resistance to toxigenic fungi and their toxins. The first commercially available transgenic variety is Bt (*Bacillus thuringiensis*) corn which has proven partly resistant to aflatoxin contamination through resistance to certain boring insects (Hammond et al. 2004; Munkvold & Muntzen 2004; Williams et al. 2005). The Australian maize industry’s voluntary genetically modified organism (GMO) -free policy means that genetically engineered hybrids are not currently available to Australian producers and, given that early maturing hybrids have proven ineffective in climatic conditions similar to Australia’s in the USA, GAPs will remain the primary strategies to minimise aflatoxin contamination in the near future.

Timing planting dates to avoid high temperatures and/or drought stress during the period of kernel development and maturation could be an important precaution in the prevention of both aflatoxin and fumonisin contamination. The Queensland Department of Agriculture, Fisheries and Forestry is using computer modelling to assist growers to schedule planting and harvesting dates by predicting potential aflatoxin contamination in maize based on existing and historical climatic conditions (Chauhan, Wright & Rachaputi 2008). When sufficient irrigation is not available and long term climate predictions indicate below average rainfall, maize may not be an appropriate crop and producers should consider alternatives.
5.3.2 Growing & harvest

When the results of the survey are correlated with climate data, it appears that the major concern during the growing period is plant stress. As discussed earlier, the defences of a stressed plant can be compromised, making it more susceptible to infection. Additionally, high levels of stress can produce damaged kernels. Given that plant stress also affects yield, it is clearly in the grower’s interest to manage the problem effectively. The most commonly utilised controls are Good Agricultural Practices—particularly managing soil moisture and nutrient levels. Recommended approaches for specific regions are provided by local agricultural extension staff.

Infestations of the predominant insect pest in Australian maize production, *Helicoverpa armigera* (Hübner) are becoming difficult to treat with conventional pesticides as the species becomes resistant to commonly used chemicals (Scholz, Monsour & Zalucki 1998). Control of insect pests should be approached using Integrated Pest Management (IPM) programs which are available from local agricultural advisors.

Should conditions of rain or high humidity be forecast or expected to occur around harvest, early harvest should be considered. The most critical factor during harvest is accurate determination of moisture content, and ensuring that the entire crop meets desired moisture targets. Removal of trash and weeds is also very important, as admixture will compromise air flows in storage.

5.3.3 Storage, transport and export

As previously described, aflatoxins are the mycotoxin of most concern during storage. The hazards associated with mycotoxin production during transport and export in and from Australia are effectively the same as those occurring in stored grain. Maize should be sound and as free as possible of lightweight grain, cracked grain and contaminants. Only food grade containers that are clean and free of grain residues and dust should be used because such deposits can be heavily contaminated with fungal spores (Blaney, O'Keeffe & Bricknell 2008). Once these prior conditions are met, the primary reason for fungal growth and mycotoxin production during transport is moisture migration and accumulation within sealed containers (Blaney, O'Keeffe & Bricknell 2008).

The moisture content of the individual maize kernel is an important variable. Moisture content is measured as water activity ($A_w$) and represents that ratio of the vapour pressure of water in a material (in this case, a maize kernel) to the vapour pressure of water at the same temperature. The minimum $A_w$ is 0.78 for *A. flavus* growth and 0.83 for aflatoxin production (Hill et al. 1985). Avoiding aflatoxin
production in storage involves ensuring that the water activity of the maize is kept below 0.70, which corresponds to 14% moisture at 30°C (DAFF 2010a). While this is somewhat below the minimum $A_w$ stated above, acceptable moisture content for maize decreases as ambient temperature increases and ambient temperatures exceeding 30°C are common in maize growing areas of Australia. At 40°C, the water activity ($A_w$) of maize with 14% moisture rises to 0.75 and at 50°C to 0.8, so maize that might be subject to such temperatures during storage or transport should be dried to 12 – 13% moisture to reduce the risk as much as possible.

Aeration of stored grain assists in reducing both relative humidity and storage temperatures. Good aeration is essential when ambient temperatures are high, but is only effective when the external air has a relative humidity <80% and temperature of <20°C (Shapira 2004). For this reason aeration is usually best carried out at night.

During export, shipping containers are often held at tropical summer temperatures for several weeks, which can cause condensation to form on the grain and, in extreme conditions, allow the maize to germinate. The risks can be minimised by ensuring containers are placed on lower decks to avoid temperature fluctuations and including moisture absorbing materials in containers during transport. Commercial products are available for this purpose, based on silica gel or diatomaceous earth.

The most effective and widely accepted method of control of insect invasion during storage is prevention, through using airtight storage, hygiene, aeration, controlled atmosphere and drying. Market restrictions and grain-specific chemical registrations limit other pest control options. Carbaryl can be used a protective treatment for grain to be used on-farm or in feed grain but residues are not accepted in grain intended for human consumption. Phosphine fumigation is accepted in cereals by all markets; dichlorvos and other residual pesticides are only acceptable to non-restricted markets. With pest species becoming resistant to commonly used organophosphate chemicals, alternative chemical registrations for use in grain are expected in the future (Bullen, Burrill & Hughes 2007).

One method used widely throughout the industry to reduce contamination levels prior to storage or sale is gravity grading. Despite the lack of a statistically significant relationship between bulk density and mycotoxin contamination, \textit{ad hoc} analysis demonstrated that more than 90% of fumonisin can occur in the lightweight fraction and is thus removable by gravity grading. Likewise, grading of the grain destined for Japan in 2005 reduced aflatoxin contamination from an original 0.005 mg/kg to 0.002 mg/kg. The use of gravity grading is supported by research conducted by Johansson et al. (2006) and Munkvold & Desjardins (1997) although the latter qualify that the method is not completely...
effective. An explanation for the failure to identify a significant relationship between bulk density and aflatoxin contamination in this study is that the number of seriously damaged (and thus lightweight) samples available for analysis was very small and it is possible that these samples were excluded from the database as outliers.

5.3.4 A risk-based management system for Australian conditions

Mycotoxins cannot be easily eliminated from grain once contamination has occurred. It can be difficult to predict when contamination will occur and when it does, mycotoxins can be distributed extremely irregularly, both in maize growing in the field and in stored maize. If not detected before reaching the end-use, the costs can be very high in terms of rejected product, trade embargos and product recalls.

There are two approaches to deal with this problem. Firstly, it can be assumed that contamination is beyond control and perform multiple mycotoxin tests on each load of maize at harvest, each load sold from storage, and in each batch of final product. Alternatively, a quality control system can be applied at all stages of production, transport and storage, to minimise contamination, and limit mycotoxin tests to the occasional confirmatory assay.

A quality control system incorporates many of the specific measures already in place in most well-run maize growing, processing, transport, storage and marketing operations, particularly with respect to moisture control and storage. A formal quality control system includes appropriate documentation assuring that maize has been subject to appropriate care throughout its history. Although most stakeholders try to maintain a good quality product, without documentation there is no way to assure a purchaser that good practice has been followed and that the risk of contamination is therefore low.

The Food and Agriculture Organisation of the United Nations has published a manual on the application of the HACCP system in mycotoxin prevention and control (FAO 2001), but the case studies and examples in that document relevant to maize are for conditions in South East Asia rather than Australia. The risk factors for maize grown under Australian conditions are in many cases different to those described in these examples. Environmental parameters are critical in mycotoxin production and Australian conditions also significantly vary from those in the major maize growing centres of the USA and Canada.

In the northern states of the US and in Canada, maize is often harvested at higher moisture contents. In the lower ambient temperatures of these northern latitudes this does not present a significant problem (Abbas et al. 2002), but in Australia this would lead to a high risk of aflatoxin contamination occurring
during storage owing to much higher ambient temperatures in storage. In South East Asia, high relative humidity means maize is harvested at high moisture content and dried post-harvest prior to storage (FAO 2001). The major Australian maize growing areas are more subject to low relative humidities, making pre-harvest drying the normal procedure. Despite the recommendation by Codex that HACCP be used in production to prevent mycotoxin contamination, this has not yet been implemented in the Australian maize production industry. HACCP in general is not widely used in the Australian grain industry generally, despite the “Graincare” project of the 2000s.

In response to the identified hazard of mycotoxins in Australian maize and the lack of a suitable management tool adapted to Australian conditions, a guide book was developed for Australian maize producers (Bricknell & Blaney 2007)[Appendix A]. The Guide applies the principles of GAP in the Codex Alimentarius Code of practice for minimising mycotoxins in cereals and combines them with HACCP principles of quality control. The guide acknowledges the fact that the grower has the best understanding of their own process/production line. Consequently, a specific detailed plan has not been prescribed. Instead, a process was designed to assist operators to develop their own plan, using examples specific to Australian conditions and the maize industry. An example of hazards identified in a fictional Australian maize producing operation is provided in Table 5-1. In the guidebook, once hazards in their operation have been identified, the grower is guided through the process of identifying appropriate control measures. These control measures are then designated to be either GAPs or HACCP critical controls. Examples of GAPs are given in Table 5-2. For those controls considered critical, the grower is directed through the process of defining critical limits; and developing a monitoring programme for critical control points. An example of the resultant HACCP plan is shown in Table 5-3.
### Table 5-1 Mycotoxin-related hazards in the maize supply chain

<table>
<thead>
<tr>
<th>Step</th>
<th>Hazard</th>
</tr>
</thead>
</table>
| Purchase seed grain   | Hybrid unsuitable for local conditions  
Hybrid unsuitable for planned market  
Hybrid unsuitable for expected planting window  
Hybrid susceptible to local diseases (eg. hybrid susceptible to *F. graminearum* selected for planting on the Atherton Tableland) |
| Soil preparation      | Soil contaminated with excessive *F. graminearum* inoculum from previous wheat crop  
Soil contaminated with excessive *A. flavus* inoculum from trash of previous crop or previous peanut crop  
Soil of uneven depth or moisture holding capacity due to field levelling over different soil types or rocky outcrops. |
| Planting              | Planting time may expose developing kernels to high temperatures & low precipitation during kernel development |
| Pre-harvest/ Growing  | Low soil moisture leading to plant stress during kernel development  
Insufficient soil nutrients leading to plant stress during kernel development  
Insect attack leading to damaged kernels  
Damage to ears during mechanical cultivation or from birds |
| Harvest               | Damage to kernels from harvester  
Kernels insufficiently dried and susceptible to damage  
Rainfall or high humidity around harvest risks high moisture |
| Storage               | Moisture content of kernels excessive  
Insect attack, allowing fungi to penetrate kernel  
Insufficient aeration, allowing moisture migration and fungal growth  
Storage container contaminated with old grain residues containing high concentrations of fungal spores |
Table 5-2 Good Agricultural Practices to minimise mycotoxin contamination in maize

<table>
<thead>
<tr>
<th>Step in process</th>
<th>Hazard</th>
<th>Good Agricultural Practice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purchase seed grain</td>
<td>Hybrid unsuitable for local conditions</td>
<td>Select seed in accordance with advice from reputable seed dealer</td>
</tr>
<tr>
<td></td>
<td>Hybrid unsuitable for planned market</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hybrid unsuitable for expected planting window</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hybrid susceptible to local diseases</td>
<td></td>
</tr>
<tr>
<td>Soil preparation</td>
<td>Soil contaminated with excessive <em>F. graminearum</em> inoculum from previous wheat crop</td>
<td>Avoid rotating wheat and maize crops in susceptible areas</td>
</tr>
<tr>
<td></td>
<td>Soil contaminated with excessive <em>A. flavus</em> inoculum from trash of previous crops</td>
<td>Plough trash into soil</td>
</tr>
<tr>
<td></td>
<td>Soil of uneven depth or moisture holding capacity due to field levelling over different soil types or rocky outcrops</td>
<td>Prepare maps of fields showing shallow areas, that can be monitored for stress using infra-red photography and harvested separately</td>
</tr>
<tr>
<td>Planting</td>
<td>Planting time could expose developing kernels to high temperatures &amp; low precipitation during kernel development</td>
<td>Avoid planting times which will lead to the period of anthesis and the following 20 days occurring in periods of very hot weather.</td>
</tr>
<tr>
<td>Harvest</td>
<td>Rainfall or high humidity around harvest</td>
<td>Check weather reports and harvest earlier if necessary</td>
</tr>
<tr>
<td></td>
<td>Damage to kernels from harvester</td>
<td>Dry maize in field to 14% moisture before harvest</td>
</tr>
<tr>
<td>Storage</td>
<td>Storage container contaminated old grain residues containing high concentrations of fungal spores</td>
<td>Decontaminate container before storage</td>
</tr>
</tbody>
</table>
Table 5-3 Example of a possible HACCP plan for minimising mycotoxin contamination in maize

<table>
<thead>
<tr>
<th>Step/ CCP</th>
<th>Hazard Analysis</th>
<th>Control</th>
<th>Critical Limit</th>
<th>Monitoring</th>
<th>Frequency</th>
<th>Corrective action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-harvest/ Growing</td>
<td>Low soil moisture leading to plant stress during kernel development</td>
<td>Available soil moisture</td>
<td>Lower limit of critical $A_w$ (check with local agronomist for an exact value)</td>
<td>Measure soil moisture and record</td>
<td>Weekly</td>
<td>Irrigate; record amounts</td>
</tr>
<tr>
<td></td>
<td>Insufficient soil nutrients leading to plant stress during kernel development</td>
<td>Available soil nutrients</td>
<td>Soil N, P &amp; K as recommended for hybrid by local agronomists</td>
<td>Fertiliser applied (appropriate for soil type and hybrid); amounts and type recorded</td>
<td>As recommended for hybrid</td>
<td>Add fertilizer; record amount</td>
</tr>
<tr>
<td></td>
<td>Insect attack leading to damaged kernels</td>
<td>Integrated pest management (IPM) plan</td>
<td>Insect population within acceptable limits as determined by control program</td>
<td>Inspect for insects and record results</td>
<td>Weekly</td>
<td>Apply pesticide in accordance with IPM</td>
</tr>
<tr>
<td>Storage</td>
<td>Moisture content of kernels excessive</td>
<td>Kernel moisture content at point of storage</td>
<td>Moisture content $\leq 14%$</td>
<td>Measure and record grain moisture</td>
<td>Immediately prior to storage</td>
<td>Dry mechanically</td>
</tr>
<tr>
<td></td>
<td>Insect attack, allowing fungi to penetrate kernel</td>
<td>IPM plan</td>
<td>No evidence of insect or rodent infestation using inspection protocols specified in IPM plan</td>
<td>Inspect for pests and record results</td>
<td>Weekly</td>
<td>Control pests in accordance with IPM</td>
</tr>
<tr>
<td></td>
<td>High ambient humidity and temperature</td>
<td>Aeration</td>
<td>Temperature of air intake $&lt;20^\circ C^1$</td>
<td>Measure and record humidity, ambient temperature and airflow</td>
<td>Daily during storage</td>
<td>Adjust aeration-time of day or airflow</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Humidity of air intake $&lt;80%^1$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Shapira (2004)
5.3.5 Verification

In order to verify the success of any risk management plan, periodic testing of the final product is essential. In the Australian context, this is generally carried out at the point of sale using the NACMA standards to determine whether a load is rejected or accepted. Testing at the point of sale requires rapid methods of analysis, a range of which are evaluated below. In terms of verifying a mycotoxin management plan, a grower may wish to have confirmation that their processes are successful before submitting their grain for sale. Such instances would not rely upon rapid methods, in which case submitting a range of appropriately collected sample for analysis at a NATA accredited laboratory would be the most reliable option.

Rapid assessment techniques

**ELISA**

ELISA testing is widely used in industry as a rapid method for assessing the compliance of purchased maize for compliance with NACMA trading standards. Dilution is often required to quantify contaminations at levels above 20 µg aflatoxin/kg. Kits are now available that are capable of analysing total aflatoxins at higher concentrations and in some instances can detect a range of mycotoxins. For relatively fast results and to determine samples in need of more precise analysis, such kits would be useful, particularly in an industrial setting.

**BGYF**

Results would indicate that the number of glowing particles observed under ultraviolet light is an unreliable method of predicting the level of aflatoxin contamination. In previous years this method was used quite widely to rapidly assess the quality of grain at the point of purchase. While BGYF is not an accurate method for determining the extent of contamination, the significant correlation observed between the presence or absence of aflatoxin makes it a potentially useful qualitative method for identifying lots requiring quantitative analysis. BGYF should not be relied upon as a method for verification of a mycotoxin management plan.

**Bulk density**

Survey results indicate the bulk density is not a reliable method for identifying potentially contaminated maize although, as discussed earlier, the literature and anecdotal evidence indicates that an initial screening to remove lightweight particles is effective in reducing overall contamination to a
substantial degree (Blaney, Bricknell & O'Keefe 2006; Johansson et al. 2000; Munkvold & Desjardins 1997). Bulk density is not recommended, therefore, as a method for verification but remains useful as a method of reducing the overall contamination of a lot prior to sale.

**Visual inspection**

As discussed earlier, an initial screening of a lot to remove damaged and lightweight particles can substantially reduce overall contamination, but visual assessment cannot be relied upon to determine contamination, or lack of it. Maize that appeared to be of good quality to the naked eye was often found to be contaminated at substantial levels with both aflatoxin and fumonisin. Visual inspection should not be used as a method for verification of a mycotoxin management plan.

**5.3.6 Managing the risks in maize-based foods**

While the NACMA standards (Table 2-7) appear to protect the Australian community from exposure to mycotoxins in maize based food, there is a clear vulnerability to exposure from imported products due to the lack of food standards for food products other than peanuts and tree nuts. In this, Australia and New Zealand follow the example of Codex Alimentarius, which recommends no other regulatory limits for aflatoxins and no limits for fumonisins. The primary safeguard for the purposes of public health is contained in section 9 of the Importation of Food Act (Comm) 1992, which makes it an offence to import food that does not comply with applicable standards or is known to be a risk to public health. While it could be argued that there is clear evidence that products contaminated with mycotoxins are a risk to public health, a strong defence could be mounted on the basis that Australia has no legal standard to protect consumers and mycotoxin contamination must therefore not be considered a public health risk. A prosecution under this section would therefore be unlikely to succeed.

Despite the recommendations of Codex, a significant number of countries have chosen to instigate standards specifically for maize or cereal based products, while others have instigated additional standards for products for which no specific standards has been set. Examples of countries that have implemented such general standards include India and the USA where a general maximum limit for total aflatoxins for foods of 30 µg/kg and 20 µg/kg, respectively, are in place (Kubo 2012). It should be noted that figure quoted for the USA is an action limit only, “based on the unavoidability of the poisonous or deleterious substances and [does] not represent permissible levels of contamination where it is avoidable” (USFDA 2000). In South Africa, a general maximum limit for total aflatoxins is set at 10 µg/kg and additionally a general maximum limit for AB₁ is set at 5 µg/kg for all foodstuffs (Kubo
This conservative standard recognises that maize forms a substantial part of the African diet (Wagacha & Muthomi 2008) and African maize has been shown in published surveys to experience significant issues with contamination (Reddy et al. 2010; Wagacha & Muthomi 2008). As a consequence, the lower limit reflects the ALARA principle appropriate for carcinogenic contaminants. Japan maintains one of the strictest controls with total aflatoxin content required to be below 10 µg/kg in all foodstuffs (Kubo 2012).

Like many other countries on the international market, Australia has no standard for fumonisins in any product. As discussed in Chapter 2, the USA has set advisory standards ranging from 2 mg/kg in degermed dry milled corn product to 4 mg/kg in partly degermed corn product and cleaned corn intended for masa production. In Regulation No. 1881/2006, the European Commission stated that “monitoring control results of the recent harvests indicate that maize and maize products can be very highly contaminated by fumonisins and it is appropriate that measures are taken to avoid such unacceptably highly contaminated maize and maize products can enter the food chain”. On the basis of IARC’s designation of fumonisins as Class 2b carcinogens, the EU determined that the threshold approach is appropriate and established maximum limits for fumonisins in cereal and cereal based foods products (European Commission 2006), which would include the maize-based products discussed here (see Table 2-8). A number of other countries which refer to the EU legislation for food standards, including Turkey, Bosnia and Herzegovina, Norway and Switzerland, have established similar maximum limits for fumonisins (Kubo 2012).

Both aflatoxins and fumonisins are considered confirmed or potential carcinogens and thus the ALARA principle should apply with respect to exposure. The current unregulated system is not conforming to this principle, with foods other than peanut products and tree nuts potentially containing any concentration of the toxins. Clearly, the assumption that the only exposure to aflatoxins is through nut products is not able to be substantiated, given that Australian manufacturers are prepared to accept maize meeting the NACMA milling standards (<5 µg/kg aflatoxin) for manufacturing purposes and imported products are not regulated at all. A study of a variety of food products in Pakistan commonly eaten by infants and young children found 21% to have levels above 0.1µg aflatoxin/kg, exceeding the EU standard for such products (Mushtaq et al. 2012). Italian corn products routinely return positive results for fumonisin contamination- 100% of polenta samples tested in one survey demonstrated contamination of up to 3730 µg/kg (Doko & Visconti 1994b). These levels are significantly in excess of the current EU standard of 1000µg/kg. Five out of six brands of puffed corn in the same study...
contained levels of between 2220 \( \mu g/kg \) and 6100 \( \mu g/kg \) FB1, again significantly in excess of the current applicable EU standard. Had any of these products been exported to Australia, there would be no control over their sale and consumption, despite them now being ineligible for sale in the EU.

It is acknowledged that Australia’s position is in accordance with Codex Alimentarius. Despite this, the unavoidable presence of aflatoxins and fumonisins in the maize production chain and their acknowledged toxicity coupled with the potential for mycotoxin levels to increase as a result of climate change suggests that it may be time for Australia to also consider introducing some method of risk management for aflatoxins and fumonisins in foods currently without an established Codex limit.

As described, most countries have taken the path of setting general standards. Setting a food standard for a toxin is a significant task, well beyond the scope of this thesis, involving not only a survey of the toxicological data and other regulatory standards internationally but also a comprehensive program of public and industry consultation. Additionally, the findings of the research presented here suggest that the main reason for implementing such standards would be to protect the Australian community from exposure through imported products. This is more clearly defined in the case of fumonisins, owing to their almost exclusive occurrence in maize based foods- the application of the NACMA standards by the major food manufacturing companies appears to be managing the problem well on a local scale. Consequently, a general standard for all foods sold in Australia would potentially be overkill. In a HACCP system, a Critical Control Point should be identified at any point in the process where a risk may be introduced or increased. Clearly this is the case with imported foods, given that the risks are being minimised at earlier points on the chain by the application of the NACMA standards. Consequently, it would be considered prudent to introduce a Critical Control Point at the point where imported products enter the Australian market.

Under the World Trade Organisation Sanitary and Phytosanitary Agreement, WTO members have the right to take measures for the protection of human health from risks arising from toxins in imported foods, provided that they are applied only to the extent necessary to protect health, are based on scientific principles and are not maintained without sufficient scientific evidence. Under this Agreement, member countries can set their own rules but these rules must not be used to protect domestic producers from international competition, in accordance with Article 20 of the WTO General Agreement on Tariffs and Trade. As such, Australia would be entitled to instigate some form of quality control, provided it was supported by sound, risk-based information indicating that mycotoxin contamination in Australian maize

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contamination in imported products is more likely to occur owing to Australia’s more stringent quality control procedures during primary production and manufacture.

The presence of relatively high concentrations of fumonisins in the imported corn products reported in Chapter 4 clearly demonstrates that the risk to Australian consumers from imported product exists, providing a platform for a measure under the Sanitary and Phytosanitary Agreement. Both the imported puffed corn product from the USA, containing 2600µg fumonisin /kg and the imported polenta from Italy, containing 1280µg fumonisin/ kg would be unable to be sold in their countries of manufacture but are legally able to be sold in Australia. Given that the results of this research support the findings in the literature that mycotoxin contamination of maize based food products is common in countries that routinely export to Australian markets, often unavoidable, and, in the case of fumonisins, ubiquitous, adherence to the ALARA principle would indicate that a more rigorous approach to the management of mycotoxin contaminated food products should be considered by Australian authorities, particularly with respect to imports. There are a number of emerging metrics that may be used to achieve this aim that may be of more use than the traditional standard (García-Cela et al. 2012). The choice of metric is beyond the scope of this thesis, which addresses the management of mycotoxins in Australian maize, but is worthy of further research and consideration if the risk to consumers is to be managed effectively.

5.4 Recommendations

- Encourage the use of mycotoxin risk management plans in industry through the instigation of incentives or premiums attached to “mycotoxin-free” maize (or maize meeting relevant NACMA standards), similar to that used by the peanut industry;
- Conduct a more detailed survey of fumonisin and other mycotoxin contamination in imported maize based foods;
- Incorporate maize-based foods more specifically into a future Australian Total Diet Survey with an emphasis on fumonisins and other mycotoxins;
- Maintain general surveillance of mycotoxins in the Australia maize crop.
Chapter 6  Conclusion and future research

The initial aim of this research was to investigate the extent of mycotoxin contamination in Australian grown maize, assess the associated risk to human health and propose methods for managing that risk. Until this project commenced, there had been no extensive study of the occurrence of mycotoxins in the Australian maize crop and little consolidated evidence relating to the risk to human health presented by mycotoxin contaminated maize in the Australian diet. There was also no coordinated approach to managing the risk in the maize industry.

The results reported here indicate that, while mycotoxins are often present at low levels, in general Australian maize is of good quality. Certain regions appear to present higher risks for contamination, such as the Burnett Region (particularly the Coalstoun Lakes district) with respect to aflatoxin and NSW and the MIA with respect to fumonisins. Aflatoxins are the mycotoxins of greatest concern, primarily to manufacturers of human food products and pet food.

Despite this positive finding, with the worldwide move toward total quality control and risk management, it is to the maize industry’s benefit to manage mycotoxin contamination during production and on into manufacture and sale, rather than rely on industry and/or regulatory standards that apply to the end product. While it is not possible to eliminate mycotoxin contamination, it is possible to minimise contamination by using effective risk management strategies, including quality control during primary production, the application of trading standards to maize used for food manufacture and animal feed and the effective regulation of imported food products.

The risk to human health from exposure to AB$_1$ and FB$_1$ was assessed using a Monte Carlo simulation. Results indicate that exposure to aflatoxin B$_1$ through maize consumption is extremely low in Australia, with 95% of exposures calculated to be below 2.02 ng/kg BW AB$_1$/day in adults and below 3.57 ng/kg BW AB$_1$/day in children. These figures indicate that acute intoxication from aflatoxin contained in maize-based foods is extremely unlikely. In terms of chronic exposure and associated carcinogenicity, when the estimated exposure for both adults and children is compared with the no observable adverse effect level, adverse effects related solely to AB$_1$ contamination of maize-based food products also appear unlikely. Based on the data, less than 0.00025 cases of hepatocellular cancer (9x10$^{-6}$ cases/100,000) are likely to occur annually in Australia as a result of maize-based foods contaminated with AB$_1$ (p<0.05).
The Australian adult population is exposed to significantly less FB1 than the tolerable daily intake (TDI) (2.0 µg fumonisins/kg BW/day) with the intake of 95% of people being less than 0.74 µg/kg FB1 BW /day. While the estimated risk of either chronic or acute health effects in adults is therefore low, the estimated exposure of children may be of concern. While the exposure of 95% of children to less than 1.75 µg FB1/kg BW /day falls below the TDI, there is little room for a safety factor to allow for raw product exceeding the NACMA standards or for high levels of contamination in imported foodstuffs.

The research raised the issue of mycotoxin contamination in some imported foodstuffs. While the observance of voluntary trading standards by Australian manufacturers appears to be protecting consumers, imported products are subject to no such standards. The enforcement of strict standards overseas leaves the Australian consumer vulnerable to unscrupulous dealers seeking to offload product unacceptable for sale in home markets. The research was limited to the management of mycotoxin contamination in Australian maize, making the issue of risks associated with imported products beyond its scope. As a result, it is recommended that further research be conducted to determine the need for additional control measures to manage potential risks related to contaminated maize-based food products and the form such measures might take.

There remains scope for significant research in the field of mycotoxin management. In terms of minimising contamination during primary production, the success of a HACCP based system such as that described here must be evaluated. Part of such a process, as identified, is a system of validation. The use of NIR spectroscopy as a means for rapid assessment of mycotoxin contamination is currently in its infancy but appears promising in theory. Although the samples collected through this project were sent for NIR analysis, the survey was of a preliminary nature and has not been presented here. Many samples were available at the low end of contamination scale, and the small number of highly contaminated samples made it difficult to develop a robust model. Long term collection of samples to include a significant number of highly contaminated examples would be worth pursuing. This technology presents significant advantage to industry by providing a means of rapid, non-destructive analysis that can be performed on site at grain reception terminals.

A thorough analysis of maize based foods, both domestically produced and imported, would be a beneficial project to determine if the results of the preliminary study are representative of the market. Additionally, a total diet survey of food products for adult, child and infant consumption would enable
a more accurate determination of exposure to mycotoxins from multiple sources. More research is also required into the exposures of children to fumonisins and the potential effects of this exposure.

A survey investigating aflatoxin M₁ levels in milk both from dairy cattle fed feed containing maize and available generally in the marketplace would provide updated information on the occurrence of this mycotoxin in a staple food. There exists a paucity of current local research in this area and, given recent drought conditions, lack of pasture and the anecdotal use of peanut meal for supplementary feed, there may well be a resultant risk of aflatoxin contamination in milk and milk products. An extension of such a project could include a survey of aflatoxin M₁ contamination in powdered milk and baby formula, both brands produced domestically and imported.

This research has shown that managing mycotoxins in maize is a complex problem, requiring the implementation of control measures at all stages of production, processing and marketing—from pre-planting through food manufacture and sale. Such a complex problem requires a cooperative, multi-disciplinary response, involving industry, regulators and researchers. The significance granted to such a response should be in proportion to the importance of mycotoxins in food; a health issue that has been described as being greater than synthetic food contaminants, plant toxins, food additives or pesticide residues; the most important chronic food safety risk factor in the world today (Kuiper-Goodman 2004; Reddy et al. 2010).
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Appendices


Appendix A

Mycotoxins in Australian maize production: how to reduce the risk
Foreword

This Guide has been prepared as part of a joint project between the National Research Centre for Environmental Toxicology (EnTox), University of Queensland; University of Sydney; Queensland Department of Primary Industries & Fisheries; NSW Department of Primary Industries; and the Grains Research & Development Corporation. The project was supported by representatives of millers, growers, seed companies, bulk handlers and stockfeed manufacturers in collaboration with research and extension professionals and has been endorsed by the Maize Association of Australia.

The project was undertaken in response to an identified need to better manage mycotoxin contamination in Australian maize.

For more information, contact the Maize Association of Australia or the Department of Primary Industries in your state.
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Introduction

Over the last twenty years, occasional instances of increased mycotoxin contamination in Australian maize have been recorded. Despite only affecting a small percentage of Australian maize, these incidents have highlighted the need for an industry-wide management system to ensure Australian maize meets the standards of all domestic users and export markets.

What are mycotoxins?

Mycotoxins are toxic chemicals produced naturally by certain fungi. The term “mycotoxin” comes from the Greek “mykes”, meaning fungus, and the Latin word “toxicum”, meaning poison. Many mycotoxins have been identified, occurring on a wide variety of substrates. Some mycotoxins are produced by a number of different fungi; while some species of fungi can produce more than one mycotoxin. A good example is the chemically similar group of mycotoxins called aflatoxins, which are formed by both *Aspergillus flavus* and *Aspergillus parasiticus*.

Mycotoxins that have been found in maize include aflatoxins, fumonisins, ochratoxins, trichothecenes (including nivalenol and deoxynivalenol) and zearalenone; and these are of concern because of the risk they pose to human health as food contaminants. Several different mycotoxins can occur in a single batch of maize, for example aflatoxins and fumonisins can co-occur in maize affected by very high temperatures, while zearalenone and trichothecenes can co-occur in maize grown in cool, persistently wet climates.

The presence of a given fungus does not mean that the mycotoxin(s) associated with that fungus are also present. There are many factors, especially environmental conditions and agricultural practices, involved in the production of mycotoxins. Environmental conditions differ throughout Australia’s maize growing regions, making the type of mycotoxin problem different depending upon the region concerned. While climatic conditions cannot be altered, there are Good Agricultural Practices (GAP) that, when applied, can minimise mycotoxin contamination.

Managing mycotoxin contamination

Mycotoxins are common environmental pollutants which cannot be easily eliminated from grain once contamination has occurred. It can be difficult to predict when contamination will occur and when it does, mycotoxins can be distributed extremely irregularly, both in maize growing in the field and in stored maize. If not detected before reaching the end-use, the costs can be very high in terms of rejected product, trade embargos and product recalls. There are two approaches to deal with this problem. Firstly, we can assume that contamination is beyond our control and perform multiple mycotoxin tests on each load of maize at harvest, each load sold from storage, and in each batch of final product. Alternatively, we can apply a quality control system at all stages of production, transport and storage, to minimise contamination, and limit mycotoxin tests to the occasional confirmatory assay.

Sole reliance on extensive testing of the final product creates waste both in terms of wasted money and wasted grain, should a load be rejected for all potential purposes. Mycotoxins occur unevenly throughout a load and so accurate sampling for mycotoxin analysis is extensive, time consuming and requires substantial quantities of grain. Chemical analysis is complex, requiring trained analysts, costly consumables and significant time to complete each assay. Additionally, a significant number of chemically diverse mycotoxins occur in maize, with a specific chemical assay required for each one. These factors result in considerable expense for the operator.

Conversely, a quality control system incorporates many of the specific measures already in place in most well-run maize growing, processing, transport, storage and marketing operations, particularly with respect to moisture control and storage. Controlling moisture, for example, is significantly easier and less costly than monitoring for mycotoxins in the end product.

Why use a documented quality control system?

A formal quality control system includes appropriate documentation assuring that maize has been subject to appropriate care throughout its history. Although most stakeholders try to maintain a good quality
product, without documentation there is no way to assure a purchaser that good practice has been followed and that the risk of contamination is therefore low. While vendors can guarantee purchasers that grain has been handled safely whilst in their possession, there are no assurances on what has happened further up the chain. With a documented system, buyers can readily check that all protocols aimed at minimising the risk of mycotoxin contamination have been followed.

Overseas markets are becoming increasingly discriminating in today's primary industries. The push toward quality control overseas is occurring rapidly and in order to compete successfully in international markets, Australian primary production is finding it necessary to embrace quality control locally. Quality control has been successfully practised in many other sectors of Australian primary production, and the experience is that product marketed as being produced in compliance with an accredited quality control system demands significantly higher prices than product without the “tick of approval”.

Risk management planning

In this guidebook, we apply the principles in the Codex Alimentarius Code of Practice for minimising mycotoxins in cereals of Good Agricultural Practice (GAP) and combine them with HACCP (Hazard Analysis Critical Control Point) principles of quality control. The guide acknowledges the fact that the grower has the best understanding of their own process/production line. Consequently, we have not prescribed a specific detailed plan, but instead a process to assist operators to develop their own plan, using examples specific to Australian conditions and the maize industry.

Mycotoxins of concern in Australian maize

Aflatoxins

Aflatoxins are a group of chemically similar compounds produced by *Aspergillus flavus* and *A. parasiticus*. Four different aflatoxins (B1, B2, G1 and G2) are produced by *A. parasiticus* but only two (B1 & B2) are produced by *A. flavus*. When analysed and viewed under ultraviolet light, two fluoresce with a blue colour (B1 & B2) and two with a green colour (G1 & G2). There are another two aflatoxins that occur in milk (M1 & M2) as a result of cows metabolising aflatoxins B1 and B2, which are important when considering aflatoxin contamination of maize intended for feeding dairy cows.

Aflatoxins are one of the most potent liver carcinogens known, and have been associated as a co-carcinogen with hepatitis B in the high incidence of liver cancer in parts of south-east Asia. They can also cause acute affects if ingested by humans or animals in high doses, such as occurred in Kenya during 2004 when consumption of aflatoxin contaminated maize led to more than eighty deaths in a single incident. No natural cases of human disease caused by aflatoxin have ever been recorded in Australia, although livestock have occasionally been poisoned in the past. It is clearly critical that management systems are in place to ensure exposure to aflatoxin is minimised, and that Australian maize can be demonstrated to meet international standards.

What conditions make aflatoxins a problem?

Aflatoxins are best known in Australia as a problem in rain-fed peanuts grown in parts of south-east Queensland; although in Africa, southern Asia and parts of the United States the problem in maize is well recognised. In Australian maize, aflatoxins are more often produced by *A. flavus*, although *A. parasiticus* is not uncommon. *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 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 thrive.

The combination of drought and high ambient temperatures is now recognised as the primary environmental factor leading to aflatoxin contamination in the growing crop. Although aflatoxin research in maize has mostly been conducted in the USA, Australian investigations support similar principles. The critical period for aflatoxin production begins approximately twenty (20) days after anthesis and, if average day/night temperatures exceed 27ºC, two conditions are met. Firstly, the natural resistance of the maize plant to fungi in general is compromised; and secondly, the relatively heat-tolerant *Aspergillus 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. Aflatoxin contamination can be limited to a tiny proportion of kernels in a given batch of maize. 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.

Good agricultural practice (GAP) for managing aflatoxin in growing maize involves selection of planting times to minimise exposure to extreme temperatures during the critical period of kernel formation, maintaining irrigation evenly across fields, good nutrition, insect control, early harvest, minimising light-weight material at harvest, and drying (if necessary) to <14% moisture before storage.

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 35ºC, 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 eventually the fungus will rapidly spread into adjacent sound kernels. This process is accelerated by storage insects. 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 temperature using aeration- adjusting night-day air flows as appropriate for ambient external temperatures to avoid moisture condensation; and controlling insects with appropriate chemicals.

[Figure 1 Cob infected with *A. flavus* (Source: Integrated Crop Management, Iowa State University)]

**Ochratoxin A**

A number of fungi are known to produce ochratoxin A, including *Aspergillus ochraceus*, *A. carbonarius*, *A. niger* and *Penicillium verrucosum*. Of these, the most likely species producing ochratoxin A 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, could also contribute to
ochratoxin contamination. Ochratoxin A is known to cause 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) to classify it as a possible human carcinogen.

What conditions make ochratoxin A a problem?

Ochratoxin A has been detected only occasionally 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); surveys of maize produced in other regions have so far 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 and consequential heating. A similar case was observed in southern Queensland some years ago, but all indications are that ochratoxin does not present a serious risk to Australian maize quality. Aspergillus ochraceus is less common than A. flavus in maize, and less is known about factors controlling 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, a negative interaction has been shown between A. niger and A. flavus, which might affect 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.

Fumonisins

Fumonisins are another group of chemically related mycotoxins, the most common and most toxic called fumonisin B₁ (FB₁), with FB₂ and FB₃ common in lower concentrations. Fumonisins are particularly toxic to horses, where they cause liquefaction of the brain known as Equine Leucoencephalomalacia (ELEM). Pigs can also be affected with pulmonary oedema. 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.

Many Fusarium sp. are associated with ear rot and stalk rot in maize. The most common species in Australian maize is Fusarium verticillioides (previously called F. moniliforme) which is presumed to be the main source of fumonisins. However, F. proliferatum, F. subglutinans, F. thapsinum and F. nygamai have also been isolated from ear-rotted maize, and are on record as capable of producing fumonisins.

What conditions make fumonisins a problem?

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 Aspergillus 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. 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 (see photo).

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. Different maize hybrids could vary in susceptibility to fumonisins, but more research is needed in this area. When serious fumonisin
contamination does occur, it has been shown that the majority can occur in the lightweight fraction, and be removable by gravity grading. Because *Fusarium* species require a moisture content of 30-40% and relative humidity of ~95%, fumonisins are unlikely to increase in maize post-harvest.

**What conditions make zearalenone a problem?**

*F. graminearum* is associated with persistently cool, humid conditions during silking (flowering), conditions uncommon in the main Australian maize-growing regions. Exceptions are parts of the Atherton Tableland area in North Queensland and wet coastal areas like the Northern Rivers district of 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.

In the main Australian maize production areas, zearalenone does not appear to warrant specific controls, but if necessary this could involve reduced stubble retention and avoiding maize-wheat rotation. 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.

**Trichothecenes**

Trichothecenes are a group of over 150 structurally related toxins. Those known to contaminate maize in Australia include deoxynivalenol (DON, also referred to as vomitoxin), nivalenol and their acetyl derivatives. DON is far more common in maize in wet, cooler parts of North America and Europe than in Australia and has been responsible for widespread economic losses in North America. DON and nivalenol are more common in heavily or moderately damaged grain. They are known to survive processing and to be present in finished food products.

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. To date there is no evidence that DON is a carcinogen or mutagen.

What conditions make trichothecenes a problem?
In Australian maize, the fungus primarily responsible for producing these toxins is *F. graminearum*, but *F. culmorum* and other *Fusarium* species might also be involved. Research indicates that infection in North Queensland in the Atherton Tableland area produces nivalenol while infection with the same species in mid New South Wales tends to produce DON. This appears to be related to genetic variation in the fungal species rather than to differences in environmental conditions. Other maize producing regions in Australia appear unaffected. The primary similarity between the regions is their cooler climate and high humidity when compared with other maize producing areas.

**Figure 3 Cobs infected with *F. graminearum*. (Source: Integrated Crop Management, Iowa State University)**

**Mycotoxin-related hazards in Australian maize production**
Fungi on crops can produce mycotoxins in the field, during handling and in storage. The conditions required for the production of mycotoxins are complex and involve a combination of conditions favourable to fungal infection and growth and those conducive to mycotoxin formation and not all mycotoxins require the same conditions. Australian maize is grown in a range of climates which affects fungal growth and mycotoxin production.

Codex Alimentarius, in its Code of Practice for the Prevention and Reduction of Mycotoxins in Cereals, identifies mycotoxin related hazards at each stage of cereal production, in line with GAP and HACCP principles. A similar framework is used below, highlighting generic hazards as well as those specific to different Australian regions.

**Pre-planting**
Planning prior to planting or entering into a contract should include attention to several critical steps in minimising mycotoxin contamination. The first step lies in reducing exposure to infection though reducing the available fungal inoculum. Fungal spores remain dormant in soil from crop to crop and from year to year, present in layers of infected crop residues. Increasing adherence to no-till cultivation aimed at preserving topsoil, can increase soil contamination with fungal spores, requiring a trade off between mycotoxin control and soil conservation.

Rotating crops that share susceptibility to specific fungi increases the availability of inoculum in shared fields. Wheat and maize share a susceptibility to some *Fusarium* sp., particularly *F. graminearum*. Rotating these two crops increases the availability of inoculum and subsequent zearalenone, NIV and/or DON contamination in these crops, particularly if there is rainfall during anthesis and persistently moist conditions during maturation. Such conditions rarely occur in the
main grain production regions of Australia, although they did occur in 1999-2001 at a few localities on the Liverpool Plains of NSW.

While GAP can reduce the availability of inoculum, it is impossible to eliminate it altogether. Selection of a hybrid adapted for local conditions and suitable for the proposed end-use is a key decision. For example, the Queensland Department of Primary Industries and Fisheries has had a long breeding program in North Queensland to develop hybrids resistant to *Fusarium* sp. infection, and in this region selection of resistant hybrids may prove to be the most effective way to minimise zearalenone and NIV contamination. While no hybrids are currently available specifically for aflatoxin and fumonisin resistance, hybrids with increased resistance to insect attack and increased drought tolerance could be less susceptible.

### Planting

Timing planting dates to minimise exposure to high temperatures and/or drought stress during the period of kernel development and maturation could be an important precaution in the prevention of both aflatoxin and fumonisin contamination. The Queensland Department of Primary Industries & Fisheries is using computer modelling to assist growers to schedule planting and harvesting dates by predicting potential aflatoxin contamination in maize based on existing and historical climatic conditions.

### Pre-harvest/ growing

Australia’s climate poses specific challenges in terms of mycotoxin control. Many maize growing areas of Australia, including the Murrumbidgee Irrigation Area (MIA), central west of NSW and Central Queensland experience extremely high temperatures and low precipitation during the summer months. Crops in these areas are generally irrigated, but aflatoxin problems still occur occasionally in parts of crops if irrigation is uneven or if soil is shallow in spots due to field levelling for flood irrigation. The risk increases if crops are planted in December, when the developing ear can be exposed to very high January/February temperatures (maximum 35°C - 45°C).

Although less often subject to such high temperatures, crops in the Central Burnett, South Burnett and Darling Downs in Queensland are often rain-fed and have regularly suffered stress over the last 10 seasons. Surveys indicate more frequent aflatoxin contamination in these areas, particularly in the central Burnett. When irrigation is not available and long term climate predictions indicate below average rainfall, maize might not be an appropriate crop and producers should consider alternatives.

The conditions in north-eastern NSW and the southern Darling Downs in south-east Qld are more moderate in terms of temperature and rainfall, and aflatoxin contamination is rarely a problem. Less data exist for fumonisins in these areas but recent surveys show no more contamination than in other regions. As the climate becomes cooler and moister, for example in proximity to the QLD-NSW border ranges, conditions become more conducive for growth of the mould that produces zearalenone, nivalenol and deoxynivalenol, *Fusarium graminearum*, but even so, significant contamination of crops is quite unusual.

As previously noted, parts of the north Queensland tablelands feature a cool, persistently wet climate during maize silking and maturation, and zearalenone and nivalenol contamination can be common. Genetic variations in, and distribution of, *F. graminearum* isolates mean that while both areas experience zearalenone contamination, nivalenol tends to occur in northern Queensland and deoxynivalenol in southern Queensland. In this region, aflatoxin occurs only rarely in maize, and is limited to the hotter, drier parts, such as the Mareeba tableland, although further study is warranted as maize production is extending into the hot, wet lowlands of this region.

Australian maize does not seem to experience the amount of insect damage common in parts of the USA. The predominant insect pest in Australian pre-harvest maize is the ear worm, *Helicoverpa armigera* (Hübner). Eggs of this species are common on maize during silking and the larvae develop in the cob, leaving the kernels susceptible to fungal invasion. Control of this pest is difficult in maize due to costs and the difficulty in reaching the target through large canopies.
Another pest known to affect Australian maize is common armyworm, *Mythimna convecta* Walker (Lepidoptera: Noctuidae). In Australia, mycotoxin contamination appears to be more related to climate than to insect attack, with incidents of medium to high contamination occurring in undamaged grain, but more investigation is certainly warranted. One study in northern Qld did not indicate increased zearalenone in maize infected with *F. graminearum* as a result of severe insect damage (*Spodoptera* sp.). Control of insect pests should be approached using Integrated Pest Management (IPM) programs which are available from local agricultural advisors.

Harvest

Mycotoxin production during the actual harvest operation is unlikely, unless the process is interrupted and prolonged by rain; however contamination with soil-borne spores and damage to kernels may make mycotoxin formation more likely during storage. Mechanical harvesters can cause damage to kernels and leave them more vulnerable to fungal invasion. Mechanical damage is more likely to occur when grain is insufficiently dried before harvest, an uncommon situation in Australia, where it is more common to allow grain to dry to storage conditions before harvest. Another hazard is unexpected precipitation or high humidity during harvest. If these conditions are forecast or expected to occur around harvest, early harvest should be considered. The most critical factor during harvest is accurate determination of moisture content, and ensuring that the entire crop meets desired moisture targets. Removal of trash and weeds is also very important, since admixture will compromise air flows in storage. Further information can be found in the *Managing on-farm grain storage CD-ROM* published by Value Added Wheat CRC Limited and available through the NSW Department of Primary Industries.

Storage

The factors conducive to fungal growth during storage are primarily related to the amount of inoculum present, temperature, relative humidity, moisture content and insect activity. Fungal infection usually occurs prior to harvest, but can also occur from dormant fungal spores present in grain dust residues in storage silos, which can also be transported through grain by insects or rodents.

Mycotoxin production in storage is also governed by moisture content and temperature. *Fusarium* species grow best at moisture levels of 30 – 40%, as in the developing maize kernel, and will not grow if water activity (a<sub>W</sub>) is <0.88. Consequently, significant amounts of *Fusarium* mycotoxins will not be produced during maize storage in Australia – fumonisins, zearalenone, DON and nivalenol are predominantly pre-harvest problems. Aflatoxin, on the other hand, can be both a pre-harvest and post-harvest problem. *Aspergillus* species are most competitive at lower moisture activities (a<sub>W</sub> 0.80 – 0.92; 16 – 20% moisture at 30°C), and so pre-harvest invasion is associated with premature drying of maize kernels as a consequence of heat stress or physical damage. Avoiding aflatoxin production in storage involves ensuring that the water activity of the maize is kept below 0.70, which corresponds to 14% moisture at 30°C.
The climate in major Australian grain production regions means that elevated temperatures (>30°C) in storage are routinely experienced, making the moisture content of stored grain critical. Even if the moisture content is in the range of 14-15%, at 30°C moisture migration and accumulation due to temperature differentials at the grain surface can easily provide pockets of maize with 16-18% moisture, favouring rapid growth of Aspergillus species and aflatoxin (and ochratoxin) production. Conversely, maize stored (and maintained) at 10 - 20°C is very unlikely to support significant aflatoxin production, since moisture content must be at 17% before the water activity allows A. flavus growth, and any growth will be very slow at these temperatures. Good aeration is essential when ambient temperatures are high, but is only effective when the external air has a relative humidity <80% and temperature of <20°C, so aeration is usually carried out at night.

Insects also play a role in rendering stored maize susceptible to fungal invasion. There are five major insect pests of stored cereal grain in Australia; moths (Angoumois, Tropical warehouse and Indian moths), weevils (Sitophilus spp.), the lesser grain borer (Rhyzopertha dominica), flour beetles (Tribolium castaneum), the saw-toothed grain beetle (Oryzaephilus surinamensis) and flat grain beetles (Cryptolestes spp.). Moths and the sawtooth grain beetle multiply rapidly at temperatures between 30-35°C and humidities between 75-80%.

The most effective and widely accepted method of control of insect invasion is prevention, through using airtight storage, hygiene, aeration, controlled atmosphere and drying. Market restrictions and grain-specific chemical registrations limit other pest control options. Phosphine fumigation is accepted in cereals by all markets; dichlorvos and other residual pesticides are only acceptable to non-restricted markets. With pest species becoming resistant to commonly used organophosphate chemicals, alternative chemical registrations for use in grain are expected in the future. Check with your intended market before using any chemical treatment in stored grain. There are many sources of information on control of insects in storage, some of which are listed at the end of this guide.

Transport and export

The hazards associated with mycotoxin production, during transport and export, are effectively the same as those occurring in stored grain. Maize should be sound, and as free as possible of lightweight grain, cracked grain and contaminants. Ensure that only food grade containers are used, and that they are clean and free of grain residues and dust, which can be heavily contaminated with fungal spores. Once these prior conditions are met, the primary reason for fungal growth and mycotoxin production during transport is moisture migration and accumulation within sealed containers, often held at tropical summer temperatures for several weeks, which can cause condensation to form on the grain. Acceptable moisture content for maize decreases as ambient temperature increases. At 40°C, the water activity (Aw) of maize with 14% moisture rises to 0.75, and at 50°C to 0.8 (the minimum for A. flavus growth), so maize that might be subject to such temperatures during transport should be dried to 12 – 13% moisture. During export, the risks can be minimised by ensuring shipping containers are placed on lower decks to avoid temperature fluctuations and including moisture absorbing materials in containers during transport. Commercial products are available for this purpose, based on silica gel or diatomaceous earths.

Figure 5 Cargo damage during maritime transport: mouldy, agglomerated and germinated corn (Source: Transport Information Service, Germany)
What is HACCP?

HACCP (Hazard Analysis Critical Control Point) is a well known quality control framework, developed to ensure “absolute food safety” for US astronauts and used internationally for quality control in the food industry. There is a significant amount of research currently supporting the use of HACCP planning in primary production and specifically in the grain industry; and HACCP has been endorsed by the World Health Organisation and Codex Alimentarius for minimising mycotoxin contamination in grain.

HACCP is a logical process which analyses each step in production and identifies controls critical in minimising contamination. Applying these controls ensures that risk is managed throughout the entire supply chain, not just in the end product. Documented monitoring of critical control points contributes to quality assurance and allows purchasers to select product from agents who have followed appropriate management procedures.

Each of these critical control points is assigned an acceptable limit and a method for testing. Test results are recorded for quality assurance purposes and the HACCP plan is documented and, ideally, certified by an appropriate body.

HACCP has been accepted by the Food and Agriculture Organisation of the United Nations (FAO) and the International Agency for Atomic Energy (IAEA) as an appropriate process for mycotoxin control, and a Manual on this has been published by the joint FAO/IAEA Training and Reference Centre for Food and Pesticide Control. The principles of HACCP can be readily applied to managing the various hazards identified above in the Australian maize industry.
### Principles of HACCP
HACCP has seven basic principles, as described in the table below.

**Table 1 Principles of HACCP**

<table>
<thead>
<tr>
<th>Principle</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conduct a hazard analysis.</td>
<td>A detailed step by step diagram of the process is prepared, identifying where significant hazards occur.</td>
</tr>
<tr>
<td>Determine critical control points</td>
<td>Critical Control Points (CCPs), points at which the hazards can be controlled, are identified throughout the process.</td>
</tr>
<tr>
<td>Devise a monitoring programme.</td>
<td>A method of monitoring hazards is critical in any HACCP programme to ensure these remain under control at the critical control points.</td>
</tr>
<tr>
<td>Establish critical limits.</td>
<td>These are limits that must be adhered to in the monitoring system if risk is to be minimised.</td>
</tr>
<tr>
<td>Devise a monitoring programme.</td>
<td>Monitoring is critical in any HACCP programme to ensure control points remain under control.</td>
</tr>
<tr>
<td>Define corrective actions.</td>
<td>If a hazard is shown to be outside the set critical limits, corrective measures must be implemented.</td>
</tr>
<tr>
<td>Establish verification procedures.</td>
<td>Verification that the HACCP plan is achieving the desired target is necessary. At this point, analysis of the final product is usually required. If controls are found to exceed critical limits, immediate action is necessary to identify the CCP at which failure has occurred. This may mean new CCPs are identified, critical limits are adjusted or the monitoring programme is altered.</td>
</tr>
<tr>
<td>Develop documentation and record keeping.</td>
<td>A successful HACCP programme relies on comprehensive documentation of procedures and records. This will usually involve a flow diagram of the process; the hazard and risk assessment; and a list of CCPs, methods to monitor the hazard, and critical limits for the monitoring programs. Ongoing records of monitoring and corrective action must be kept for consultation as well as the results of verification. Operation requirements for staff and records of staff training should also clearly documented and available. An audit of a HACCP system will include an examination of all this documentation and must be satisfactory should accreditation be desired.</td>
</tr>
</tbody>
</table>
Critical Control Points

The most important items in any HACPP plan are the critical control points (CCPs). CCPs are identified by applying a set of stringent criteria to each hazard identified in the hazard analysis step of the process.

One of the greatest criticisms of HACCP to date has been the complexity and time consuming nature of the paperwork. In a small operation such as a maize storage facility, the plan should be uncomplicated and need not include large amounts of paperwork requiring document control. A good HACCP plan should include no more than six to eight CCPs.

Other primary components revolve around the CCPs and include a documented monitoring procedure of the action to be taken, the person responsible, when and how often the procedure needs to occur; as well as records of monitoring results and documented corrective action with associated records, as illustrated below.

A hazard analysis is a step by step analysis of your process, critically identifying hazards that may cause your product to become unsafe.

Conducting a hazard analysis

When conducting a hazard analysis you need to consider:

- Your product/s
- The end users of your product
- Your users’ expectations and specifications
- To what purpose the product will be put

When conducting this hazard analysis, consider your own situation in light of the information provided above in the section on ‘Mycotoxin-related hazards in the Australian maize industry’.

Hazards and risks

Before you can conduct your hazard analysis, it is important to understand the difference between the terms “hazard” and “risk”. Often these terms are used interchangeably but in the context of risk management are two separate concepts.

Hazard: a situation that has the potential to cause harm; for example ‘Aspergillus flavus colonies in broken kernels in stored maize’, or ‘temperature fluctuation’ in stored maize.

Risk: the likelihood of a specific hazard causing harm; for example, the likelihood that a high aflatoxin concentration arising from the hazard of ‘Aspergillus flavus colonies’, could cause rejection of the maize by an end-user, or product recalls, or harm to consumers, or litigation, etc.

Types of hazards

Hazards fall into one of three general categories:-

- **Biological**: related to the presence of biological organisms or their by-products.
- **Chemical**: the presence of harmful chemicals not related to biological entities, such as pesticides
Physical hazards caused by foreign materials or environmental conditions

Mycotoxin contamination is not only a result of biological hazards such as the presence of fungal spores, also known as inoculum, but also of physical hazards such as temperature and soil nutrient deficiencies.

Task

Write down a list of all the steps in your own production or supply chain in the space below, from the time that you either decide to grow maize, up to the time when the maize leaves your possession. This can most easily be done in a flow chart format as illustrated below.

Consider each stage in your flow chart. For each stage, ask the following questions:

Q1) Can fungal infection or mycotoxin contamination of maize either occur or increase at this stage?

Q2) Can a decision at this point affect mycotoxin contamination occurring at a later stage?

If the answer to either question is yes, describe the conditions that might lead to this occurring. These are hazards.
<table>
<thead>
<tr>
<th>Step</th>
<th>Answer</th>
<th>Hazard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purchase seed grain</td>
<td>Q1) No</td>
<td>Hybrid unsuitable for local conditions</td>
</tr>
<tr>
<td></td>
<td>Q2) Yes</td>
<td>Hybrid unsuitable for planned market</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hybrid unsuitable for expected planting window</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hybrid susceptible to local diseases (e.g. hybrid susceptible to <em>F. graminearum</em> purchase for planting on the Atherton Tableland)</td>
</tr>
<tr>
<td>Storage of seed</td>
<td>Q1) No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Q2) No</td>
<td></td>
</tr>
<tr>
<td>Soil preparation</td>
<td>Q1) No</td>
<td>Soil contaminated with <em>Fusarium graminearum</em> inoculum from previous wheat crop</td>
</tr>
<tr>
<td></td>
<td>Q2) Yes</td>
<td>Soil contaminated with <em>Aspergillus flavus</em> inoculum from trash of previous crops</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soil of uneven depth or moisture holding capacity due to field levelling over different soil types or rocky outcrops.</td>
</tr>
<tr>
<td>Planting</td>
<td>Q1) No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Q2) Yes</td>
<td>Planting time could expose developing kernels to high temperatures &amp; low precipitation at anthesis and the following 20 days</td>
</tr>
<tr>
<td>Pre-harvest/ Growing</td>
<td>Q1) Yes</td>
<td>Low soil moisture leading to plant stress during kernel development</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insufficient soil nutrients leading to plant stress during kernel development</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insect attack leading to damaged kernels</td>
</tr>
<tr>
<td></td>
<td>Q2) No</td>
<td></td>
</tr>
<tr>
<td>Harvest</td>
<td>Q1) No</td>
<td>Damage to kernels from harvester</td>
</tr>
<tr>
<td></td>
<td>Q2) Yes</td>
<td>Kernels insufficiently dried and susceptible to damage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rainfall or high humidity around harvest risks high moisture</td>
</tr>
<tr>
<td>Storage</td>
<td>Q1) Yes</td>
<td>Moisture content of kernels excessive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insect attack, allowing fungi to penetrate kernel</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insufficient aeration, allowing moisture migration and fungal growth</td>
</tr>
<tr>
<td></td>
<td>Q2) No</td>
<td>Storage container contaminated with dusts containing high concentrations of fungal spores</td>
</tr>
</tbody>
</table>
Determining Controls, Critical Control Points & Good Agricultural Practice

Controls
Controls are an action that can be applied at a point in the production process to prevent, eliminate or reduce the risk of a hazard contributing to the undesired outcome – in our case, mycotoxin contamination of maize.

Good Agricultural Practice
Good agricultural practice (GAP) in this context includes all agronomic and crop management factors that can contribute to maximum production of maize of the highest quality. Some of these are more critical than others and also require regular monitoring and control – these are amenable to use of the HACCP system. Those that involve simple choices and decisions, but not ongoing control and monitoring remain important as GAP, but are not amenable to HACCP.

Task
For each hazard you previously identified, ask yourself the following question:

Does a control exist at this step to prevent or minimise mycotoxin contamination or fungal infection?

Extend the table you created above, and write the answer to this question and the control measure you would adopt.

Critical Control Points
Critical Control Points (CCPs) are points in the process at which a control can be applied to prevent, eliminate, or reduce a hazard to acceptable levels. For instance, it is known that excess moisture in storage creates conditions conducive to fungal growth and, therefore, mycotoxin production. Excess moisture in storage must be controlled at the point of entry into storage as well as during storage, so these are both Critical Control Points.

Not all the hazards you identified in the previous step will be CCPs. There will be points in your process at which you can minimise mycotoxin contamination through good agricultural practice. The defining point of the CCP is that it is critical in minimising contamination and is therefore must be monitored. A primary requirement of a CCP is that the control applied is measurable.

Task
For each control you suggested in the following step, ask:

Can the outcome of the control be measured?

A CCP is not about measuring mycotoxin levels. In most cases a CCP will be a physical variable such as temperature or moisture.

The stages in your process where the controls to which you can answer “yes” occur are Critical Control Points or CCPs. Other steps are Good Agricultural Practice (GAP). Note CCPs and other GAPs in your table.
<table>
<thead>
<tr>
<th>Step in process</th>
<th>Hazard</th>
<th>Control</th>
<th>Measurable?</th>
<th>CCP or GAP?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purchase seed grain</td>
<td>Hybrid unsuitable for local conditions</td>
<td>Yes- select seed in accordance to advice from reputable seed dealer</td>
<td>No</td>
<td>GAP</td>
</tr>
<tr>
<td></td>
<td>Hybrid unsuitable for planned market</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hybrid unsuitable for expected planting window</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hybrid susceptible to local diseases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil preparation</td>
<td>Soil contaminated with <em>Fusarium</em> inoculum from previous wheat crop</td>
<td>Yes- avoid rotating wheat and maize crops in susceptible areas</td>
<td>No</td>
<td>GAP</td>
</tr>
<tr>
<td></td>
<td>Soil contaminated with <em>Aspergillus</em> inoculum from trash from previous</td>
<td>Yes- plough trash into soil, ensuring good soil/plant contact</td>
<td>No</td>
<td>GAP</td>
</tr>
<tr>
<td></td>
<td>crops</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Soil of uneven depth or moisture holding capacity due to field levelling</td>
<td>Yes- prepare maps of fields showing shallow areas that can be monitored for stress and harvested separately -- aerial photography with NDVI images.</td>
<td>No</td>
<td>GAP</td>
</tr>
<tr>
<td></td>
<td>over different soil types or rocky outcrops.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Planting</td>
<td>Planting time could expose developing kernels to high temperatures &amp;</td>
<td>Yes- avoid planting times which will lead to the period of anthesis and the following 20 days occurring in periods of hot, dry weather.</td>
<td>No</td>
<td>GAP</td>
</tr>
<tr>
<td></td>
<td>low precipitation during kernel development</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-harvest/ Growing</td>
<td>Low soil moisture leading to plant stress during kernel development</td>
<td>Yes- irrigate</td>
<td>Yes</td>
<td>CCP</td>
</tr>
<tr>
<td></td>
<td>Insufficient soil nutrients leading to plant stress during kernel</td>
<td>Yes- fertilise</td>
<td>Yes</td>
<td>CCP</td>
</tr>
<tr>
<td></td>
<td>development</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insect attack leading to damaged kernels</td>
<td>Yes- integrated pest management</td>
<td>Yes</td>
<td>CCP</td>
</tr>
<tr>
<td>Harvest</td>
<td>Damage to kernels from harvester</td>
<td>Yes- dry maize in field to 14% moisture</td>
<td>Yes</td>
<td>CCP</td>
</tr>
<tr>
<td></td>
<td>Rainfall or high humidity around harvest</td>
<td>Yes- check weather reports and harvest earlier</td>
<td>No</td>
<td>GAP</td>
</tr>
<tr>
<td>Storage</td>
<td>Moisture content of kernels excessive</td>
<td>Yes- do not store until kernels dry</td>
<td>Yes</td>
<td>CCP</td>
</tr>
<tr>
<td></td>
<td>Insect attack, allowing fungi to penetrate kernel</td>
<td>Yes- integrated pest management</td>
<td>Yes</td>
<td>CCP</td>
</tr>
<tr>
<td></td>
<td>High ambient humidity and temperature</td>
<td>Yes- aerate grain to control temp and humidity</td>
<td>Yes</td>
<td>CCP</td>
</tr>
<tr>
<td></td>
<td>Storage container contaminated with old grain residues containing</td>
<td>Yes- thoroughly clean and decontaminate container before storage</td>
<td>No</td>
<td>GAP</td>
</tr>
<tr>
<td></td>
<td>high concentrations of fungal spores</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Normalised Difference Vegetation Index*
Critical limits, monitoring & corrective action

Critical limits

In the previous section, you identified which points in your process had control measures for mycotoxin contamination and fungal infection that could be measured. Critical limits are the minimum criteria you set for your measurement. Essentially they define what is considered a “safe” or an “unsafe” product at that point in the process. In our previous example, at the “storage” step, mycotoxin contamination/ fungal infection is controlled by ensuring maize is dry before storage. An appropriate critical limit for maize in most Australian conditions would be to ensure moisture content is below 14%, since maize with levels above 14% is at risk of moisture migration leading to the development of fungal colonies. An appropriate critical limit for maize going into extended storage and/or transport at high temperatures would be a moisture content of 12 – 13%.

Task

For each Critical Control Point and the associated control measure/s you identified in the previous section, identify a critical limit. An example is shown below. Critical limits are not necessary for GAPs because you have previously identified them as not being measurable.

Table 4

<table>
<thead>
<tr>
<th>Step/ CCP</th>
<th>Hazard</th>
<th>Control</th>
<th>Critical Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-harvest/ Growing</td>
<td>Low soil moisture leading to plant stress during kernel development</td>
<td>Irrigate</td>
<td>Lower limit of critical A_w (check with your agronomist or extension staff for an exact value)</td>
</tr>
<tr>
<td></td>
<td>Insufficient soil nutrients leading to plant stress during kernel development</td>
<td>Fertilise</td>
<td>N, P &amp; K applications as recommended for hybrid by local agronomists (insert the values)</td>
</tr>
<tr>
<td></td>
<td>Insect attack leading to damaged kernels</td>
<td>Integrated pest management (IPM) plan</td>
<td>Insect population within acceptable limits as determined by control program</td>
</tr>
<tr>
<td>Harvest</td>
<td>Damage to kernels from harvester</td>
<td>Harvest when kernels are dry</td>
<td>Moisture content ≤ 14%</td>
</tr>
<tr>
<td>Storage</td>
<td>Moisture content of kernels excessive</td>
<td>Do not store until kernels dry</td>
<td>Moisture content ≤ 14%</td>
</tr>
<tr>
<td></td>
<td>Insect attack, allowing fungi to penetrate kernel</td>
<td>IPM plan</td>
<td>No evidence of insect or rodent infestation using inspection protocols specified in IPM plan</td>
</tr>
<tr>
<td></td>
<td>High ambient humidity and temperature</td>
<td>Aerate grain to control temperature and humidity</td>
<td>Temperature &amp; humidity within limits recommended in industry literature</td>
</tr>
</tbody>
</table>

Monitoring

A regular, documented monitoring programme is necessary to ensure your product remains safe at each Critical Control Point. A monitoring programme defines the measurement that must take place, the frequency of the measurement and the person responsible for conducting the measurement. The way a control is measured will vary depending on what you are measuring and the technology or equipment available to you. The interval between measurements depends on the type of control and the amount of variation likely to occur in relation to the set critical limits.
Table 5 CCP monitoring plan

<table>
<thead>
<tr>
<th>Step/CCP</th>
<th>Hazard</th>
<th>Control</th>
<th>Critical Limit</th>
<th>Monitoring</th>
<th>Frequency</th>
<th>Person</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-harvest/Growing</td>
<td>Low soil moisture leading to plant stress during kernel development</td>
<td>Irrigate</td>
<td>Lower limit of critical $A_w$ (check with your agronomist or extension staff for an exact value)</td>
<td>Measure soil moisture and record</td>
<td>Weekly on Monday morning</td>
<td>AW</td>
</tr>
<tr>
<td></td>
<td>Insufficient soil nutrients leading to plant stress during kernel development</td>
<td>Fertilise</td>
<td>N, P &amp; K applications as recommended for hybrid by local agronomists (insert the values)</td>
<td>Fertiliser applied (appropriate for soil type and hybrid); dates, amounts and type recorded</td>
<td>As recommended for hybrid</td>
<td>FN</td>
</tr>
<tr>
<td></td>
<td>Insect attack leading to damaged kernels</td>
<td>Integrated pest management (IPM) plan</td>
<td>Insect population within acceptable limits as determined by control program</td>
<td>Visual inspection and sample, with results recorded</td>
<td>Weekly</td>
<td>AW</td>
</tr>
<tr>
<td>Harvest</td>
<td>Damage to kernels from harvester</td>
<td>Harvest when kernels are dry</td>
<td>Moisture content ≤ 14%</td>
<td>Measure and record grain moisture</td>
<td>Prior to harvest</td>
<td>AW</td>
</tr>
<tr>
<td>Storage</td>
<td>Moisture content of kernels excessive</td>
<td>Do not store until kernels dry</td>
<td>Moisture content ≤ 14%</td>
<td>Measure and record grain moisture</td>
<td>Immediately prior to storage</td>
<td>AW</td>
</tr>
<tr>
<td></td>
<td>Insect attack, allowing fungi to penetrate kernel</td>
<td>IPM plan</td>
<td>No evidence of insect or rodent infestation using inspection protocols specified in IPM plan</td>
<td>Visual inspection with results recorded</td>
<td>Weekly</td>
<td>FN</td>
</tr>
<tr>
<td></td>
<td>High ambient moisture and temperature</td>
<td>Aerate grain to control temperature and humidity</td>
<td>Temperature &amp; humidity within limits recommended in industry literature</td>
<td>Measure and record humidity, ambient temperature and airflow inside storage and at air intake.</td>
<td>Daily during storage</td>
<td>FN</td>
</tr>
</tbody>
</table>

**Corrective action**

If the product is found to fail a CCP measurement, it is important that corrective actions can be instigated until the product meets requirements.

For example, there is a large amount of natural variation in moisture levels in a load of maize. To allow for this, moisture should be tested from a significant number of samples every time a load of maize is put into storage.

Your monitoring programme will specify how you collect samples and how many samples you will test to be sure you get a representative result. It will also specify how you will test moisture and the level at which you will instigate corrective action.

In this case, unless maize going into storage has a moisture level of 14% or less, it is not safe to go into storage. Another form of drying must be instigated before it meets requirements and can be stored safely. Your plan will specify what form of drying, how long to do it for and when to test for moisture again.
**Task**
For each CCP, assign a corrective action should your results be outside the respective critical limit.

<table>
<thead>
<tr>
<th>Step/CCP</th>
<th>Hazard</th>
<th>Control</th>
<th>Critical Limit</th>
<th>Monitoring</th>
<th>Frequency</th>
<th>Person</th>
<th>Corrective action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-harvest/</td>
<td>Low soil moisture leading to plant stress during kernel development</td>
<td>Irrigate</td>
<td>Lower limit of critical $A_w$ (check with your agronomist or extension staff for an exact value)</td>
<td>Measure soil moisture and record</td>
<td>Weekly on Monday morning</td>
<td>AW</td>
<td>Additional irrigation; record amounts</td>
</tr>
<tr>
<td>Growing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insufficient soil nutrients leading to plant stress during kernel</td>
<td>Fertilise</td>
<td>N, P &amp; K applications as recommended for hybrid by local agronomists (insert the values)</td>
<td>Fertiliser applied (appropriate for soil type and hybrid); amounts and type recorded</td>
<td>As recommended for hybrid</td>
<td>FN</td>
<td>Additional fertilizer; records amount added</td>
</tr>
<tr>
<td></td>
<td>development</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insect attack leading to damaged kernels</td>
<td>Integrated pest</td>
<td>Insect population within acceptable limits as determined by control program</td>
<td>Visual inspection and sample, with results recorded</td>
<td>Weekly</td>
<td>AW</td>
<td>Apply pesticide in accordance with IPM plan</td>
</tr>
<tr>
<td></td>
<td></td>
<td>management (IPM) plan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harvest</td>
<td>Damage to kernels from harvester</td>
<td>Harvest when</td>
<td>Moisture content ≤ 14%</td>
<td>Measure and record grain moisture</td>
<td>Prior to harvest</td>
<td>AW</td>
<td>Delay harvest until kernels sufficiently dried</td>
</tr>
<tr>
<td></td>
<td></td>
<td>kernels are dry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Storage</td>
<td>Moisture content of kernels excessive</td>
<td>Do not store</td>
<td>Moisture content ≤ 14%</td>
<td>Measure and record grain moisture</td>
<td>Immediately prior to storage</td>
<td>AW</td>
<td>Dry mechanically</td>
</tr>
<tr>
<td></td>
<td></td>
<td>until kernels dry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insect attack, allowing fungi to penetrate kernel</td>
<td>IPM plan</td>
<td>No evidence of insect or rodent infestation using inspection protocols specified in IPM plan</td>
<td>Visual inspection with results recorded</td>
<td>Weekly</td>
<td>FN</td>
<td>Apply pest control methods in accordance with IPM plan</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>High ambient humidity and temperature</td>
<td>Aerate grain to</td>
<td>Temperature &amp; humidity within limits recommended in industry literature</td>
<td>Measure and record humidity, ambient temperature and airflow</td>
<td>Daily during storage</td>
<td>FN</td>
<td>Adjust aeration- time of day or airflow to achieve desired temperature and humidity.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>control temperature and humidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Verification

Verification that the HACCP plan is successfully controlling mycotoxin contamination is necessary. At this point, some chemical analysis of the product is required to confirm that your plan is achieving your goal of minimising mycotoxin contamination. Providing your plan is working, this should only need to occur at occasional points, and usually only to meet a stringent end-use like milling or export. Your testing frequency should rise following any season where conditions outside of your control increased the risk of contamination.

If contamination is found to exceed limits, immediate action is necessary to identify the step or steps at which failure has occurred. This may mean new CCPs are identified, critical limits are adjusted or the monitoring program is altered.

Maize is subject to contamination by a number of different mycotoxins, so you will need to decide which mycotoxins to test for, which laboratory you are going to use and how often you will conduct verification. At harvest, aflatoxin will usually be the most important mycotoxin to assay, followed by fumonisins. Assay for zearalenone and trichothecenes would only be warranted in maize grown in a few cool, wet districts and where Fusarium graminearum is common (presence of visually damaged kernels with a pink to deep purple discoloration often indicates infection and growth of this fungus). The only mycotoxin likely to increase in storage is aflatoxin, so provided that fumonisins has been assayed at harvest, only aflatoxin warrants further testing. In a few isolated cases, if severe moulding has occurred, ochratoxin testing might be considered (and this might be required for export to certain markets like the EC).

Sampling

Mycotoxin contamination does not occur uniformly in every kernel. The number of infected kernels in a load of maize may be as little as 0.1%, yet still result in mycotoxin levels exceeding desired limits. This means that obtaining a representative sample of the load is critical in getting an accurate estimation of the extent of contamination. Samples sent for analysis should be a composite of sub-samples taken from every part of a load or bin of maize. One recommended method is to sample during loading by passing a cup through a moving stream of grain at a standard interval, such as every minute. The Grain Inspection, Packers and Stockyards Administration (GIPSA, an agency of the United States Department of Agriculture), provides a description of some practical methods for sampling grain on farm. In their Aflatoxin Handbook, GIPSA recommends the following minimum sample sizes for maize. Smaller sample sizes can result in seriously inaccurate estimates of the actual content of aflatoxin in a load. It has been estimated that sampling contributes up to 90% of error to a test result. The European Mycotoxin Awareness Network has produced a fact sheet on the theory and basic criteria for sampling. It can be found on the Web at http://193.132.193.215/eman2/fsheet6_3.asp.

Appropriate methods for sampling and sub-sampling for analysis have been documented in ‘Supply Chain & Export Protocols for Managing Mycotoxins in Australian Maize’, available on the Maize Association of Australia website (http://www.maizeaustralia.com.au).

Mycotoxin tests

Maize samples are assayed for mycotoxins by a number of different tests, including Enzyme-Linked Immunoassay (ELISA), high performance liquid chromatography (HPLC) and thin layer chromatography (TLC). Each test varies in accuracy, specificity and variability as well as speed of analysis, complexity and cost. All tests will vary when conducted multiple times, and exhibit further variation when conducted by different analysts in different laboratories. This variation is described by the "confidence limit". This +/- figure is shown on laboratory reports to indicate the uncertainty inherent in the final reported value. It is very important to discuss these aspects with the staff of your chosen laboratory in order to ascertain if the method used will be sufficiently accurate for your purpose. This uncertainty about results must be factored into your risk management. For example, if you need to ensure that your maize will meet a 5 ug/kg limit, and the method shows a variability of +/- 0.002 mg/kg, you might need to set your acceptance standard at 0.003 mg/kg in order to minimise the risk of another laboratory finding 0.005 mg/kg or more. The National
Association of Testing Laboratories (NATA) certifies those laboratories that can demonstrate the accuracy and proficiency of their measurements. It must be recognised that this confidence limit only takes into account the potential variability in the laboratory analysis; it does not include the variation attributable to sampling. Bear in mind that sampling can contribute up to 90% of error in an assay, so the actual variation of the mycotoxin in your entire load or harvest is going to be much higher than the confidence limit of the assay method alone.

Table 6 National Association of Commodity Marketing Agencies trading standards for mycotoxins in maize

<table>
<thead>
<tr>
<th>Mycotoxin (mg/kg)</th>
<th>Milling</th>
<th>Prime</th>
<th>Feed #1</th>
<th>Feed #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total aflatoxins</td>
<td>0.005</td>
<td>0.015</td>
<td>0.02</td>
<td>0.08 (0.02 B₁)</td>
</tr>
<tr>
<td>Total fumonisins</td>
<td>2</td>
<td>5</td>
<td>10</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 7 Verification plan

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Laboratory</th>
<th>Sampling</th>
<th>When?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxins B₁, B₂, G₁, G₂</td>
<td>“Acculab”, Brisbane</td>
<td>• 10 x 200g samples from each truck taken using the spear sampling method.</td>
<td>Immediately prior to storage or sale</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Samples from 10 trucks combined, mixed well and divided using riffle divider into 4 x 5 kg samples.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• All 5 kg of each sample ground in a Romer Mill on the finest setting; 200g sub-sample taken before</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• One sample submitted to lab, other kept by stakeholders.</td>
<td></td>
</tr>
<tr>
<td>Fumonisins: B₁, B₂, B₃</td>
<td>“Acculab”, Brisbane</td>
<td>• 10 x 200g samples from each truck taken using the spear sampling method.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Samples from 10 trucks combined, mixed well and divided using riffle divider into 4 x 2 kg samples.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• All 2 kg of each sample ground in a Romer Mill on the finest setting; 200g sub-sample taken before</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• One sample submitted to lab, other kept by stakeholders.</td>
<td></td>
</tr>
</tbody>
</table>

Task

Using the examples as a guide, decide on the verification procedures you will use to ensure your plan is effective. Remember to specify how you will sample, what you want to test, which laboratory you will send your samples to as well as when and how often you will verify. A link to NATA accredited laboratories is provided at the end of this Guide; the lab listed in the examples is not an operating business. Enter the name of the mycotoxin you are interested in testing for (e.g. ‘aflatoxin’) into the keywords field to return the list of accredited laboratories. Not all of these laboratories will be commercial labs offering a public testing service— you will need to scroll through the list.
Documentation and records

A successful HACCP programme relies on comprehensive documentation of procedures and records. This will usually involve a flow diagram of the process; the hazard and risk assessment; a list of identified GAPs you intend to follow; and a list of CCPs, critical limits and monitoring programmes. Ongoing records of monitoring and corrective action must be kept for consultation as well as the results of verification. Operation requirements for staff and records of staff training should also clearly documented and available. An audit of your HACCP system will include an examination of all this documentation and must be satisfactory should accreditation be desired.

Tasks

- Record each of the GAPs you identified
- Print out your completed HACCP plan
- Prepare documents to keep records of each CCP you monitor, allowing space for the person who took the measurement to initial and date their entry and record any corrective action they may have had to instigate.
- Start records of all staff training
- Design a document to keep records of verification
- Make records of all operating instructions

Table 8 GAPs to minimise mycotoxin contamination

<table>
<thead>
<tr>
<th>Step in process</th>
<th>Hazard</th>
<th>Good Agricultural Practice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purchase seed</td>
<td>Hybrid unsuitable for local conditions</td>
<td>Select seed in accordance to advice from reputable seed dealer</td>
</tr>
<tr>
<td>grain</td>
<td>Hybrid unsuitable for planned market</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hybrid unsuitable for expected planting window</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hybrid susceptible to local diseases (eg. hybrid susceptible to <em>F. graminearum</em> purchased for planting on the Atherton Tableland)</td>
<td></td>
</tr>
<tr>
<td>Soil preparation</td>
<td>Soil contaminated with <em>Fusarium</em> inoculum from previous wheat crop</td>
<td>Avoid rotating wheat and maize crops in susceptible areas</td>
</tr>
<tr>
<td></td>
<td>Soil contaminated with <em>Aspergillus</em> inoculum from crop residues</td>
<td>Plough trash into soil, ensuring good soil/plant contact</td>
</tr>
<tr>
<td></td>
<td>Soil of uneven depth or moisture holding capacity due to field levelling over different soil types or rocky outcrops</td>
<td>Prepare maps of fields showing shallow areas, that can be monitored for stress and harvested separately – aerial photography with NDVI imagery</td>
</tr>
<tr>
<td>Planting</td>
<td>Planting time could expose developing kernels to high temperatures &amp; low precipitation during kernel development</td>
<td>Avoid planting times which will lead to the period of anthesis and the following 20 days occurring in periods of hot, dry weather.</td>
</tr>
<tr>
<td>Harvest</td>
<td>Rainfall or high humidity around harvest risks high moisture</td>
<td>Check weather reports and harvest earlier if necessary</td>
</tr>
<tr>
<td>Storage</td>
<td>Storage container contaminated with dusts and residues containing high concentrations of fungal spores</td>
<td>Decontaminate container before storage</td>
</tr>
<tr>
<td>Step/ CCP</td>
<td>Hazard</td>
<td>Hazard Analysis</td>
</tr>
<tr>
<td>----------------</td>
<td>-----------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Pre-harvest/ Growing</td>
<td>Low soil moisture leading to plant stress during kernel development</td>
<td>Irrigate</td>
</tr>
<tr>
<td></td>
<td>Insufficient soil nutrients leading to plant stress during kernel development</td>
<td>Fertilise</td>
</tr>
<tr>
<td>Harvest</td>
<td>Damage to kernels from harvester</td>
<td>Harvest when kernels are dry</td>
</tr>
<tr>
<td>Storage</td>
<td>Moisture content of kernels excessive</td>
<td>Do not store until kernels dry</td>
</tr>
<tr>
<td></td>
<td>Insect attack, allowing fungi to penetrate kernel</td>
<td>IPM plan</td>
</tr>
<tr>
<td></td>
<td>High ambient humidity and temperature</td>
<td>Aerate grain to control temperature and humidity</td>
</tr>
</tbody>
</table>
Special requirements for exporting maize

Maize shipped overseas may endure extreme conditions of heat and humidity and may also be subject to strict standards applying to mycotoxin contamination. In recent years problems have occurred with mycotoxin contamination of exported maize exceeding overseas standards. For this reason, a protocol has been developed to advise the maize industry on important methods to minimise mycotoxin contamination occurring during shipping; 'Supply Chain & Export Protocols for Managing Mycotoxins in Australian Maize', available on the Maize Association of Australia website (http://www.maizeaustralia.com.au). This protocol should be consulted to ensure that both exporter and buyer achieve the best quality result. The following table describes additional CCPs for exported maize.

<table>
<thead>
<tr>
<th>Step/CCP</th>
<th>Hazard Analysis</th>
<th>Control</th>
<th>Critical Limit</th>
<th>Monitoring Monitoring</th>
<th>Frequency</th>
<th>Person</th>
<th>Corrective action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Export</td>
<td>Moisture migration during transport</td>
<td>Moisture check before grain loaded into container</td>
<td>Maximum moisture 12% (or other limit specified by protocols)</td>
<td>Moisture checked and recorded</td>
<td>Before container sealed</td>
<td>KR</td>
<td>Mechanically dry</td>
</tr>
<tr>
<td>Export</td>
<td>Include desiccant material in container</td>
<td>Appropriate amount per tonne of grain as recommended</td>
<td>Visual check and results recorded</td>
<td>Before container sealed</td>
<td>DB</td>
<td>Insert desiccant material and sign off</td>
<td></td>
</tr>
<tr>
<td>Export</td>
<td>Ambient temperature very high during shipping</td>
<td>Reduce temperature by shipping containers on lower decks</td>
<td>Contract with shipping company</td>
<td>Written into shipping contract, no top stowage</td>
<td>Prior to shipping</td>
<td>DB</td>
<td>Delay shipping until requirements can be met</td>
</tr>
</tbody>
</table>

Table 10: Extra CCPs for export hazards
Links

- Manual on the Application of the HACCP System in Mycotoxin Prevention and Control-
  http://www.fao.org/docrep/005/y1390e/y1390e00.htm
- A Guide to Maize Production in Queensland- Qld DPI&F
- Maize: NSW planting guide
- Maize Association of Australia
- The Cob- magazine of the Maize Association of Australia
- Transport Information Service: cargo loss prevention information from German insurers
  http://www.tis-gdv.de/tis_e/ware/getreide/mais/mais.htm
- European Mycotoxin Awareness Network
  http://www.mycotoxins.org/
- The Aflatoxin Handbook- Grain Inspection Packers & Stockyards Administration
- Practical Procedures For Sampling Grain At Farm Sites And Remote Locations- Grain Inspection Packers & Stockyards Administration
- NATA.
  http://www.nata.com.au
Further reading

- Storing, Handling & Drying Grain (2004) Queensland Department of Primary Industries
  http://www.publish.csiro.au/nid/18/pid/5397.htm#description

- Managing on-farm grain storage CD-ROM Value Added Wheat CRC Limited

- Microbiological facts and fictions in grain storage - Ailsa Hocking, Food Science Australia
  http://sqr1.csiro.au/aptc2003/10_hocking.pdf#search=%22aflatoxin%20corn%20OR%20maize%20storage%22

- Avoid aflatoxin poisoning of livestock, and the potential for residues in milk and meat - Qld DPI&F
Appendix B

Risk management for mycotoxin contamination of Australian maize

L. K. BricknellA,B,D, B. J. BlaneyC and J. NgA

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BPresent address: Faculty of Sciences, Engineering & Health, Central Queensland University, Bruce Highway, North Rockhampton, Qld 4702, Australia.
CQueensland Department of Primary Industries and Fisheries, Locked Mail Bag 4, Moorooka, Qld 4105, Australia.
D Corresponding author. Email: l.bricknell@cqu.edu.au

Abstract. Recent incidents of mycotoxin contamination (particularly aflatoxins and fumonisins) have demonstrated a need for an industry-wide management system to ensure Australian maize meets the requirements of all domestic users and export markets. Results of recent surveys are presented, demonstrating overall good conformity with nationally accepted industry marketing standards but with occasional samples exceeding these levels. This paper describes mycotoxin-related hazards inherent in the Australian maize production system and a methodology combining good agricultural practices and the hazard analysis critical control point framework to manage risk.

Introduction

Mycotoxins are toxic products of secondary metabolism produced by a range of fungi on a wide variety of substrates. Past investigations into Australian maize, as well as data collected by millers and manufacturers, have identified aflatoxins, fumonisins, ochratoxin A, trichothecenes [including nivalenol (NIV) and deoxynivalenol (DON)] and zearalenone in Australian maize (Blaney 1981, 2004; Connole et al. 1981; Blaney et al. 1984, 1986, 2006). This is of concern because of the risk they pose to human and animal health (Pitt and Tomaska 2001, 2002; Council for Agricultural Science and Technology 2003; Whitlow and Hagler 2003).

The National Agricultural Commodities Marketing Association (NACMA) has formulated trading standards for aflatoxins and fumonisins in maize, shown in Table 1. While these are not standards enforceable by law, they have been widely accepted by industry and it is to be expected that they will be used in most domestic contracts.

In recent years, the Australian maize crop has experienced several cases of mycotoxin contamination causing disruption to maize marketing (Blaney et al. 2006). Despite only affecting a small proportion of Australian maize, these incidents have indicated a need for an industry-wide system to ensure Australian maize meets the standards of all domestic users and export markets.

This paper provides preliminary data from a survey of Australian maize produced between 2004 and 2006 and discusses factors associated with contamination. With this as a basis, we describe mycotoxin-related hazards inherent in the Australian maize production system and propose potential controls for these hazards.

Mycotoxin occurrence in Australian maize

In 2003, industry monitoring identified an outbreak of fumonisin and aflatoxin contamination in maize received for milling (Blaney et al. 2006). At this time, although members of the manufacturing sector conducted in-house monitoring, there had been no systematic review of the entire Australian maize crop over several seasons. In response to industry concern, we conducted an extensive survey of maize across all growing regions of Australia between 2004 and 2006.

The detailed results of these surveys will be published separately. Five-kg samples of shelled maize were requested from growers, seed companies and bulk handlers; with samples received ranging between 500 g and 20 kg. Samples were ground in entirety and subsampled in a Romer Mill. Milled maize was assayed using 2-dimensional, thin-layer chromatography for aflatoxins, ochratoxin A and zearalenone (Blaney et al. 1984). For fumonisins, milled samples were quantified using high performance liquid chromatography (AOAC International 1998; Shephard 1998).

Preliminary results for aflatoxins and fumonisins are summarised in Table 2, showing that aflatoxin and fumonisin contamination is widespread across Australian maize growing regions. Aflatoxins were detected in 25% of samples (>0.001 mg/kg), and fumonisins were detected in 66% of samples (>0.1 mg/kg). Nevertheless, over 85% of all samples complied with the NACMA standard for milling grade maize. Ochratoxin A was not detected in any of the samples and zearalenone was detected in only a few, almost entirely in maize originating from the Atherton Tableland region in Far North
Queensland (Qld) – the only part of Australia where this is common (Blaney et al. 2006).

These results indicate that geographic region plays an important part in determining the type of mycotoxin contamination that occurs, probably due to climatic differences. Distribution also appears to be related to a combination of other factors including soil type, humidity, availability of inoculum and season; relationships which will be explored in a future paper.

Aflatoxins appear to be the mycotoxins of most concern in Australian maize (Blaney et al. 2006), based on their implications to human and animal health (IARC 1993) and widespread occurrence, but our results indicate that this is mainly for companies supplying the human food and pet food markets that are aiming to meet the NACMA milling standard of 0.005 mg/kg. Fumonisins are of secondary concern, but do require regular monitoring and management owing to their potential carcinogenicity and proven negative health effects in animals (Gelderblom et al. 1988; Diaz and Boermans 1994; Gelderblom et al. 2002; Gelderblom et al. 2004).

Mycotoxin-associated risk factors in Australian maize

Fungi on crops can produce mycotoxins in the field, during handling and in storage. The conditions required for the production of mycotoxins are complex and involve a combination of those favourable to fungal infection, growth and mycotoxin formation. Not all mycotoxins require the same combination of conditions.

Aflatoxins

In Australian maize, aflatoxins are most often produced by Aspergillus flavus. A. flavus can start to grow in maize of lower moisture content [16% at 35°C; water activity (A_w) ~0.8] and at higher temperatures (12–43°C; optimum 30°C) than many other fungi found on field crops (Diener and Davis 1987), and for this reason it was originally classified as a ‘storage fungus’. The combination of drought stress and high ambient temperatures has been well established as the primary environmental factor leading to aflatoxin contamination in the growing crop (Trenk and Hartman 1970; Bruns 2003; Munkvold 2003). Although aflatoxin research in maize has mostly been conducted in the US, our results support similar principles. The critical period for aflatoxin production begins ~20 days after anthesis (Bruns 2003) and, if average day/night temperatures exceed 27°C, two conditions are met. First, the natural resistance of the maize plant to fungi in general is compromised; and second, the relatively heat-tolerant A. flavus has the advantage over other fungi present. At this stage, windblown fungal spores can enter through the silks. These temperatures also fall within the optimum conditions for aflatoxin production (Diener and Davis 1987).

Physical damage to the ear from insects (especially boring insects) or birds is also a critical factor in aflatoxin contamination, since it exposes the endosperm to premature drying and A. flavus invasion. Once fungal growth has begun, it can continue until the moisture content of the grain reduces below 14% and so, if environmental conditions do not ensure rapid drying, delaying harvest can increase contamination (Munkvold 2003; Kaaya et al. 2005).

Good agricultural practice (GAP) for managing aflatoxin in growing maize involves selection of sowing times to avoid extreme temperatures during the critical period of kernel formation, maintaining irrigation evenly across fields, good nutrition, insect control, early harvest, minimising light-weight material at harvest, and drying to <14% moisture before storage by either preharvest natural or postharvest mechanical means.

Aflatoxins 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. If these pockets reach 16% with average temperatures around 35°C, the A_w of maize reaches the minimum of 0.80 at which A. flavus can start to grow (Sanchis and Magan 2004). Initially, the fungus will grow in the very small proportion of infected kernels, but this growth releases more moisture from the maize and eventually the fungus will rapidly spread into adjacent sound kernels. This process is accelerated by storage insects. GAP for aflatoxin

<table>
<thead>
<tr>
<th>Region</th>
<th>n</th>
<th>+ve^A Milling</th>
<th>Prime</th>
<th>Feed 1</th>
<th>Feed 2</th>
<th>Exceeds</th>
<th>+ve^B Milling</th>
<th>Prime</th>
<th>Feed 1</th>
<th>Feed 2</th>
<th>Exceeds</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0</td>
<td>46</td>
<td>8</td>
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<td>3</td>
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<tr>
<td>Burnett, Qld</td>
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<td>141</td>
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<tr>
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<td>117</td>
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<td>68</td>
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<td>1</td>
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<td>1</td>
<td>8</td>
<td>75</td>
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<tr>
<td>MIA (NSW)^C</td>
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<td>62</td>
<td>57</td>
<td>8</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>15</td>
<td>68</td>
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<tr>
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<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

^A Level of reporting >0.1 mg/kg.
^B Level of reporting >0.001 mg/kg.
^C MIA, Murrumbidgee Irrigation Area.
management in stored maize include 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.

**Fumonisins**

Many *Fusarium* species are associated with ear rot and stalk rot in maize. The most common species in Australian maize is *Fusarium verticillioides* (previously called *F. moniliforme*) which is presumed to be the main source of fumononisins (Munkvold and Desjardins 1997). However, *F. proliferatum*, *F. thapsinum* and *F. nygamai* have also been isolated from ear-rotted maize, and are on record as capable of producing fumonisins.

*F. verticillioides* is considered ubiquitous in maize and is systemic in the maize plant but seems to grow rapidly and increase fumonisin concentrations only when the plant is stressed (Munkvold and Desjardins 1997; Jackson and Jablonski 2004). While drought is a significant factor in fumonisin contamination, the association with very high temperatures is not as strong as with aflatoxin.

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 (Munkvold 2003; Jackson and Jablonski 2004). Physical and insect damage to the kernel can also increase fumonisin contamination (Munkvold and Desjardins 1997; Bruns 2003) increasing access to the endosperm. Different maize hybrids could vary in susceptibility to fumonisin, but more research is needed in this area (Jackson and Jablonski 2004). When serious fumonisin contamination does occur, our ad hoc analysis has shown that more than 90% can occur in the lightweight fraction and is thus removable by gravity grading. This is supported by Johansson et al. (2006) and Munkvold and Desjardins (1997) although the latter qualify that the method is not completely effective. Because *F. verticillioides* requires a minimum moisture content of 18% and relative humidity of ~95%, fumonisins are unlikely to increase in maize postharvest.

**Zearalenone, DON and NIV**

In maize, zearealenone, DON and NIV are primarily produced by *F. graminearum*, a fungus responsible for causing ear and stalk rots. *F. graminearum* also causes head blight of wheat, and rotating wheat and maize is a common cause of increased infection in both crops if climatic factors suit (Codex Alimentarius Commission 2003). Provided that inoculum is present on crop residues in soil, infection of maize occurs at flowering and is facilitated by cool, wet weather at this time (Blaney 2001). These conditions are uncommon in the main Australian maize-growing regions, although exceptions include parts of the Atherton Tableland area and wet coastal areas like the Northern Rivers district of New South Wales (NSW) (Blaney et al. 1984, 1986, 2006).

In the main Australian maize production areas, these mycotoxins do not appear to warrant specific controls but if necessary this could involve reduced stubble retention and avoiding maize–wheat rotation. On the Atherton Tableland, effective management involves use of the hybrids specifically developed for disease resistance in that region.

**Hazards inherent in the Australian maize supply chain**

Some factors increasing risk of contamination such as weather variables are not entirely controllable, although there are good GAPs that will assist. Other factors such as insect pressure and storage conditions can be controlled. One framework for risk management is the Hazard Analysis Critical Control Point (HACCP) system. Codex Alimentarius, in its ‘Code of Practice for the Prevention and Reduction of Mycotoxins in Cereals’, identifies mycotoxin related hazards at each stage of cereal production in line with GAP and HACCP principles. A similar framework is used below, describing generic hazards as well as those specific to different Australian regions.

**Pre-sowing**

Pre-sowing planning should include attention to several critical steps in minimising mycotoxin contamination. The first step lies in reducing exposure to infection though reducing the available fungal inoculum. Fungal spores remain dormant in soil from crop to crop and from year to year, present in layers of infected crop residues. Increasing adherence to no-till cultivation aimed at preserving topsoil, can increase soil contamination with fungal spores, requiring a trade-off between mycotoxin control and soil conservation.

Rotating crops that share susceptibility to specific fungi increases the availability of inoculum in shared fields. Wheat and maize share a susceptibility to some *Fusarium* spp., particularly *F. graminearum*. Rotating these two crops increases the availability of inoculum and subsequent zearealenone, NIV and/or DON contamination in these crops, particularly if there is rainfall during anthesis and persistently moist conditions during maturation (Blaney 2001). Such conditions rarely occur in the main grain production regions of Australia, although they did occur in 1999–2001 at a few localities on the Liverpool Plains of NSW (Southwell et al. 2003).

While GAP can reduce the availability of inoculum, it is impossible to eliminate it altogether. Selection of a hybrid adapted for local conditions and suitable for the proposed end-use is a key decision. For example, the Qld Department of Primary Industries and Fisheries (QDPI&F) has had a long-term breeding program in North Qld to develop hybrids tolerant to *Fusarium* spp. infection, and in this region selection of appropriate hybrids may prove to be the most effective way to minimise zearealenone and NIV contamination. While no hybrids are currently available specifically for aflatoxin and fumonisin resistance, hybrids with increased resistance to insect attack and increased drought tolerance could be less susceptible. It has been known for many years that hybrids with long cobs with tight husk cover are more resistant to insect attack than other hybrids and experience less aflatoxin contamination (Bruns 2003). Other varieties are more tolerant to drought and thus experience less stress in dry conditions. In the United States (US) there has been some success in identifying inbred genotypes for aflatoxin resistance, although the majority of these lack traits that make them suitable for commercial purposes (Betran et al. 2002;
Betran and Isakeit 2004). Early maturing hybrids common in the Midwestern corn belt of the US were trialled in Mississippi to avoid the high temperatures commonly occurring in the grain filling stage in that state; however, these early maturing varieties had looser husks that made cobs susceptible to insect attack and subsequent aflatoxin contamination and the trial was not successful (Betran and Isakeit 2004).

New techniques in genetic engineering are aimed at improving resistance to toxigenic fungi and their toxins. The first commercially available transgenic variety is Bt (Bacillus thuringiensis) corn, which has proven partly resistant to aflatoxin contamination through resistance to certain boring insects (Hammond et al. 2004; Munkvold and Muntzen 2004; Williams et al. 2005). The Australian maize industry’s voluntary genetically modified organism free policy means that genetically engineered hybrids are not currently available to Australian producers and, given that early maturing hybrids have proven ineffective in climatic conditions similar to Australia’s in the US, GAP will remain the only option to minimise aflatoxin contamination in the near future.

**Sowing**

Timing sowing dates to avoid high temperatures and/or drought stress during the period of kernel development and maturation could be an important precaution in the prevention of both aflatoxin and fumonisin contamination. The QDPI&F is using computer modelling to assist growers to schedule sowing and harvesting dates by predicting potential aflatoxin contamination in maize based on existing and historical climatic conditions (Chauhan et al. 2006).

**Preharvest/growing**

Australia’s climate poses specific challenges in terms of mycotoxin control. Many maize growing areas of Australia, including the Murrumbidgee Irrigation Area (MIA), central west of NSW and central Qld can experience high temperatures and low precipitation during the maize growing season. Maize crops in these areas are irrigated but aflatoxin problems still occur occasionally in parts of crops if irrigation is uneven or if soil is shallow in spots due to field levelling for flood irrigation. The risk increases if crops are planted in December, when the soil is shallow in spots due to field levelling for flood irrigation. Another hazard is unexpected precipitation or high humidity during harvest operation, when the developing ear can be exposed to very high January/February temperatures, often exceeding 35°C.

Although less often subject to such high temperatures, crops in the central Burnett, south Burnett and Darling Downs in Qld are often rain-fed (Robertson et al. 2003) and have regularly suffered stress over the last 10 seasons. Surveys indicate more frequent aflatoxin contamination in these areas, particularly in the central Burnett. Our data indicate aflatoxin contamination of grain produced in these areas is more common than elsewhere. Data from modelling also show that in some regions during summer, even full irrigation may not provide sufficient water to the growing ear to combat the extreme evaporation rates from high temperature and dry winds (Chauhan et al. 2006). When sufficient irrigation is not available and long-term climate predictions indicate below average rainfall, maize may not be an appropriate crop and producers should consider alternatives.

The conditions in north-eastern NSW and the southern Darling Downs in south-east Qld are more moderate in terms of temperature and rainfall, and aflatoxin contamination is rarely a problem. Less data exist for fumonisins in these areas but our surveys show no more contamination than in other regions. As the climate becomes cooler and moister, for example in proximity to the Qld-NSW border ranges, conditions become more conducive for growth of the mould that produces zearalenone, NIV and DON, F. graminearum, but even so, significant contamination of crops is quite unusual.

As previously noted, parts of the north Qld tablelands feature a cool, persistently wet climate during maize silking and maturation, and zearalenone and NIV contamination can be common. Genetic variations and distribution of F. graminearum isolates mean that while both areas experience zearalenone contamination, NIV tends to occur in northern Qld and DON occurs in southern Qld. In this region, aflatoxin occurs only rarely in maize, and is limited to the hotter, drier parts, such as the Mareeba Tableland, although further study is warranted as maize production is extending into the hot, wet lowlands of this region.

Australian maize does not seem to experience the amount of insect damage common in parts of the US. The predominant insect pest in Australian preharvest maize is the ear worm, Helicoverpa armigera (Hübner) (Murray and Miles 2003). Eggs of this species are common on maize during silking and the larvae develop in the cob, leaving the kernels susceptible to fungal invasion. Treating infestations of this species in growing maize is difficult owing to the difficulty in reaching the target through large canopies (O’Keeffe 2006). Another pest known to affect Australian maize is common armyworm, Mythimna convecta Walker (Lepidoptera: Noctuidae) (Hardwick 2006). In Australia, mycotoxin contamination appears to be more related to climate than to insect attack, with incidents of medium to high contamination occurring in undamaged grain, but more investigation is certainly warranted. One study in northern Qld did not indicate increased zearalenone in maize infected with F. graminearum as a result of severe insect damage (Spodoptera sp.) (Blaney et al. 1986). Control of insect pests should be approached using integrated pest management programs, which are available from local agricultural advisors.

**Harvest**

Mycotoxin production during the actual harvest operation is unlikely, unless the process is interrupted and prolonged by rainfall, but mechanical harvesters can cause damage to kernels and leave them more vulnerable to fungal invasion. Contamination with soilborne spores and damage to kernels may make mycotoxin formation more likely during storage.

Mechanical damage is more likely to occur when grain is insufficiently dried before harvest, an uncommon situation in Australia, where it is more common to allow grain to dry to storage conditions before harvest. However, over-drying maize can lead to the kernel becoming brittle and susceptible to damage (Munkvold 2003).

Another hazard is unexpected precipitation or high humidity during harvest. If these conditions are forecast or expected to occur around harvest, early harvest should be considered. The most critical factor during harvest is accurate determination of moisture content, and ensuring that the entire crop meets desired moisture targets. Removal of trash and weeds is also very important, as admixture will compromise air flows in storage.
Storage

The factors conducive to fungal growth during storage are primarily related to the amount of inoculum present, temperature, relative humidity, moisture content and insect activity. Fungal infection usually occurs before harvest, but can also occur from dormant fungal spores present in grain dust residues in storage silos, which can also be transported through grain by insects or rodents.

Mycotoxin production in storage is also governed by moisture content and temperature. While fumonisins, zearalenone, DON and NIV are predominantly preharvest problems in Australia, aflatoxin can be both a preharvest and postharvest problem. Avoiding aflatoxin production in storage involves ensuring that the A_w of the maize is kept below 0.70, which corresponds to 14% moisture at 30°C (DPI&F 2005a).

The climate in major Australian grain production regions means that elevated temperatures (>30°C) in storage are routinely experienced, making the moisture content of stored grain critical. Even if the moisture content is in the range of 14–15%, at 30°C moisture migration and accumulation due to temperature differentials at the grain surface can easily provide pockets of maize with 16–18% moisture, favouring rapid growth of Aspergillus species and aflatoxin (and ochratoxin) production. Conversely, maize stored (and maintained) at 10–20°C is very unlikely to support significant aflatoxin production. Good aeration is essential when ambient temperatures are high, but is only effective when the external air has a relative humidity <80% and temperature of <20°C (Shapira 2004). For this reason aeration is usually best carried out at night.

Insects also play a role in rendering stored maize susceptible to fungal invasion. There are five major insect pests of stored cereal grain in Australia; moths (Angoumois, Tropical warehouse and Indian moths), weevils (Sitophilus spp.), the lesser grain borer (Rhyzopertha dominica), flour beetles (Tribolium castaneum), the saw-toothed grain beetle (Oryzaephilus surinamensis) and flat grain beetles (Cryptolestes spp.) (DPI&F 2004). Moths and the sawtooth grain beetle multiply rapidly at temperatures between 30–35°C and humidities ranging between 75–80% (DPI&F 2004). Controlling temperature and humidity with aeration not only reduces mould growth, and thus mycotoxin production, but also insect populations.

The most effective and widely accepted method of control of insect invasion is prevention, through using airtight storage, hygiene, aeration, controlled atmosphere and drying. Market restrictions and grain-specific chemical registrations limit other pest control options. Carbaryl can be used a protective treatment for grain to be used on-farm or in feed grain but residues are not accepted in grain intended for human consumption. Phosphine fumigation is accepted in cereals by all markets; dichlorvos and other residual pesticides are only acceptable to non-restricted markets. With pest species becoming resistant to commonly used organophosphate chemicals, alternative chemical registrations for use in grain are expected in the future (DPI&F 2005b).

Transport and export

The hazards associated with mycotoxin production during transport and export, are effectively the same as those occurring in stored grain. Maize should be sound and as free as possible of lightweight grain, cracked grain and contaminants. Ensure that only food grade containers are used, and that they are clean and free of grain residues and dust, which can be heavily contaminated with fungal spores. Once these prior conditions are met, the primary reason for fungal growth and mycotoxin production during transport is moisture migration and accumulation within sealed containers. These containers are often held at tropical summer temperatures for several weeks, which can cause condensation to form on the grain.

Acceptable moisture content for maize decreases as ambient temperature increases. At 40°C, the A_w of maize with 14% moisture rises to 0.75, and at 50°C the A_w rises to 0.8 (the minimum for growth of A. flavus), so maize that might be

<table>
<thead>
<tr>
<th>Step</th>
<th>Hazard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purchase seed grain</td>
<td>Hybrid unsuitable for local conditions</td>
</tr>
<tr>
<td>Soil preparation</td>
<td>Soil contaminated with excessive F. graminearum inoculum from previous wheat crop</td>
</tr>
<tr>
<td>Sowing</td>
<td>Sowing time could expose developing kernels to high temperatures and low precipitation at anthesis and the following 20 days</td>
</tr>
<tr>
<td>Preharvest/growing</td>
<td>Low soil moisture leading to plant stress during kernel development</td>
</tr>
<tr>
<td>Harvest</td>
<td>Kernels insufficiently dried and susceptible to damage</td>
</tr>
<tr>
<td>Storage</td>
<td>Moisture content of kernels excessive</td>
</tr>
</tbody>
</table>

Table 3. Mycotoxin-related hazards in the maize supply chain
subject to such temperatures during transport should be dried to 12–13% moisture. During export, the risks can be minimised by ensuring shipping containers are placed on lower decks to avoid temperature fluctuations and including moisture absorbing materials in containers during transport. Commercial products are available for this purpose, based on silica gel or diatomaceous earths.

In response to this issue, a protocol for managing mycotoxins in maize intended for export has been compiled and is being promoted by the Maize Association of Australia for wide adoption across the industry.

**An Australian risk-based mycotoxin management system**

Mycotoxins cannot be easily eliminated from grain once contamination has occurred. It can be difficult to predict when contamination will occur and when it does, mycotoxins can be distributed extremely irregularly, both in maize growing in the field and in stored maize. If not detected before reaching the end-use, the costs can be very high in terms of rejected product, trade embargos and product recalls.

There are two ways to approach this problem. First, we can assume that contamination is beyond our control and perform multiple mycotoxin tests on each load of maize at harvest, each load sold from storage, and in each batch of final product. Alternatively, we can apply a quality control system at all stages of production, transport and storage, to minimise contamination, and limit mycotoxin tests to the occasional confirmatory assay.

A quality control system incorporates many of the specific measures already in place in most well run maize growing, processing, transport, storage and marketing operations, particularly with respect to moisture control and storage. A formal quality control system includes appropriate documentation assuring that maize has been subject to appropriate care throughout its history. Although most stakeholders try to maintain a good quality product, without documentation there is no way to assure a purchaser that GAP has been followed and that the risk of contamination is, therefore, low.

The Food and Agriculture Organisation of the United Nations has published a manual on the application of the HACCP system in mycotoxin prevention and control (FAO 2001), but the case studies and examples in that document relevant to maize are for conditions in South-East Asia rather than Australia. The risk factors for maize grown under Australian conditions are in many cases different to those described in these examples. Environmental parameters are critical in mycotoxin production and Australian conditions also significantly vary from those in the major maize growing centres of the US and Canada.

In the northern states of the US and in Canada, maize is often harvested at higher moisture contents. In the lower ambient temperatures of these northern latitudes this does not present a significant problem (Abbas et al. 2002), but in Australia this would lead to a high risk of aflatoxin contamination occurring during storage owing to high ambient temperatures in storage. In South-East Asia, high relative humidity means maize is harvested at high moisture content and dried postharvest before storage. The major Australian maize growing areas are more subject to low relative humidities, making preharvest drying the normal procedure.

In response to the identified hazard of mycotoxins in Australian maize and the lack of a suitable management tool adapted to Australian conditions, we have developed a guide book for Australian maize producers applying the principles in the Codex Alimentarius Code of Practice for minimising mycotoxins in cereals of GAP and combine them with HACCP principles of quality control. The guide acknowledges the fact that the grower has the best understanding of their own process/production line. Consequently, we have not prescribed a specific detailed plan, but instead a process to assist operators to develop their own plan, using examples specific to Australian conditions and the maize industry. An example of hazards
Table 5. Example of a possible Hazard Analysis Critical Control Point (CCP) plan for minimising mycotoxin contamination in maize

<table>
<thead>
<tr>
<th>Step/CCP</th>
<th>Hazard analysis</th>
<th>Critical limit</th>
<th>Monitoring</th>
<th>Frequency</th>
<th>Corrective action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preharvest/growing</td>
<td>Low soil moisture leading to plant stress during kernel development</td>
<td>Available soil moisture</td>
<td>Measure soil moisture and record</td>
<td>Weekly</td>
<td>Irrigate; record amounts</td>
</tr>
<tr>
<td></td>
<td>Insufficient soil nutrients leading to plant stress during kernel development</td>
<td>Available soil nutrients</td>
<td>Fertiliser applied (appropriate for soil type and hybrid); amounts and type recorded</td>
<td>As recommended for hybrid</td>
<td>Add fertiliser; record amount</td>
</tr>
<tr>
<td></td>
<td>Insect attack leading to damaged kernels</td>
<td>Integrated pest management (IPM) plan</td>
<td>Inspect for insects and record results</td>
<td>Weekly</td>
<td>Apply pesticide in accordance with IPM</td>
</tr>
<tr>
<td>Storage</td>
<td>Moisture content of kernels excessive at point of storage</td>
<td>Kernel moisture content</td>
<td>Measure and record grain moisture</td>
<td>Immediately before storage</td>
<td>Dry mechanically</td>
</tr>
<tr>
<td></td>
<td>Insect attack, allowing fungi to penetrate kernel</td>
<td>IPM plan</td>
<td>Inspect for pests and record results</td>
<td>Weekly</td>
<td>Control pests in accordance with IPM</td>
</tr>
<tr>
<td></td>
<td>High ambient humidity and temperature</td>
<td>Aeration</td>
<td>Measure and record humidity, ambient temperature and airflow</td>
<td>Daily during storage</td>
<td>Adjust aeration – time of day or airflow</td>
</tr>
</tbody>
</table>

identified in a fictional Australian maize producing operation is provided in Table 3.

In the guidebook, once the grower has identified hazards in their operation, they are guided through the process of identifying appropriate control measures. These control measures are then designated to be either GAPs or HACCPs. Examples of GAPs are given in Table 4. For those controls considered critical, the grower is directed through the process of defining critical limits; and developing a monitoring program for critical control points. An example of the resultant HACCP plan is shown in Table 5.

Conclusion

Our survey results indicate that while mycotoxins are often present at low levels, in general Australian maize is of good quality. Aflatoxin is the mycotoxin of greatest concern, primarily to manufacturers of human food products and pet food. Despite this, with the worldwide move towards total quality control and risk management, it is to the maize industry’s benefit to manage mycotoxin contamination during production, rather than rely on industry and/or regulatory standards that apply to the end product. While it is not possible to eliminate mycotoxin contamination, it is possible to minimise contamination by using effective risk management strategies.

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Appendix C

AFLATOXINS IN MAIZE AND MAIZE-BASED FOOD PRODUCTS: IMPLICATIONS OF CLIMATE CHANGE

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Abstract
Mycotoxins are toxic chemicals produced naturally by a wide range of fungi. The best known of these, the aflatoxins, are potent liver carcinogens in both human and animal subjects. It is well known overseas that mycotoxins occur in maize and their occurrence is related to exposure of the developing kernels to high temperatures and drought stress. Australian-grown maize is used in both human food products and animal feed. An analytical survey was conducted to ascertain the extent of mycotoxin contamination of maize growing in the major Australian maize growing regions. The survey which included North Queensland, the South Burnett, the Darling Downs, northern and central NSW and the Murrumbidgee Irrigation areas over the 2004, 2005 and 2006 growing seasons, is the largest and most comprehensive survey to date. Samples were analysed for a range of mycotoxins, including aflatoxin B1. Concentrations of aflatoxin contamination were correlated with climate data provided by the Australian Bureau of Meteorology and a significant relationship with periods of low precipitation was identified. The implications of this correlation in the context of climate change in Australia are discussed.

INTRODUCTION
Mycotoxins are toxic products of secondary metabolism produced by a range of fungi on a wide variety of substrates, including food products and animal feed. Several are known or suspected to be toxic to humans and animals. The toxic effects of these compounds have been known for centuries; in the Middle Ages, when rye bread was a dietary staple, the biblical staff of life became known as the sceptre of death as a result of the outbreaks of hallucinations, manic depression, gangrene, abortion, decreased fertility and painful convulsive death. These symptoms were caused by ergot, a mycotoxin produced in grain colonised by Claviceps purpureum.

The most widely known and best researched mycotoxins are the aflatoxins. Their existence was first postulated in 1960, after an outbreak of disease that killed more than 100,000 young turkeys on poultry farms in England. The disease was named “Turkey X Disease” and investigations were immediately instigated. The cause of the disease was subsequently identified as contaminated peanut meal from Brazil used as cheap poultry feed. The fungal contaminant was identified as Aspergillus flavus and the toxin named aflatoxin by virtue of its origin.

In 2001 and again in 2003, Australian maize growers experienced outbreaks of mycotoxin contamination that significantly affected the industry. As a result, the Grains Research and Development Corporation awarded a grant to
the National Research Centre of Environmental Toxicology and the Queensland Department of Primary Industries & Fisheries to investigate the extent of mycotoxin contamination of Australian grown maize and develop strategies for managing future outbreaks. This paper reports on some of the findings of this project, specifically discussing them in the context of climate change and the risk to health.

**AFLATOXINS AND HEALTH**

Aflatoxins are known to be acutely toxic. LD$_{50}$ values range between 0.5 and 10mg/kg body weight, depending on the species, age and nutritional status of the animal under investigation (Watson 1998) and outbreaks of acute toxicosis have occurred from infected commodities in regions of Africa (Lewis et al. 2005) and India (Brown 1999). One of the largest and most recent outbreaks of acute poisoning occurred in Kenya in 2004 as a result of consumption of contaminated maize, leading to 317 cases of acute aflatoxicosis and 125 deaths (Lewis et al. 2005).

Aflatoxins are also considered potent carcinogens, mutagens and teratogens, primarily affecting the liver in humans (IARC 2002). They have been found to be carcinogenic and teratogenic in animals and are also implicated in impairment of protein formation, blood coagulation, weight gain and immunogenesis (Hell 1997). In 1988, the International Agency for Research on Cancer (IARC) classified aflatoxins as carcinogenic to humans (Group 1).

Human exposure to aflatoxins occurs predominantly through the consumption of peanuts and maize, dietary staples in many tropical counties (IARC, 2002). Dietary aflatoxin exposure is considered to be an important risk factor in the development of hepatocellular cancer in some regions of the world (Sudakin 2003).

Aflatoxin M$_1$ occurs in cow’s milk as a result of the metabolism of aflatoxin B$_1$ and commonly occurs when dairy cows are fed contaminated grain. In Australia to date this has not posed a problem, as Australian dairy cows are usually put to pasture, although this mycotoxin has been detected in Australian milk on rare occasions when milk producers have used peanut meal as a cheap source of supplementary feed.

**AFLATOXINS IN AUSTRALIAN-GROWN MAIZE**

Maize is grown as a summer crop in Australia, usually rotated with wheat over the winter period in the Murrumbidgee Irrigation Area (MIA). Other important maize growing areas of Australia include the Atherton Tablelands, South Burnett, Darling Downs, Liverpool Plains and NSW Highlands. Maize in Australia is used primarily for human food, pet food and stock feed. Other uses include starch manufacture and “green chop” or silage.
Aflatoxins are also known to be present in Australian maize, although usually at low frequency and at concentrations less than 5µg/kg (Blaney, O’Keeffe & Bricknell 2008). Occasionally, however, outbreaks of more severe contamination can occur. Examples of such outbreaks occurred in 2001 and again in 2003, when levels of aflatoxin between 200-300 µg/kg were detected in maize produced for milling purposes (Blaney, O’Keeffe & Bricknell 2008).

Aspergillus sp. are known to favour the heat and drought stress associated with warmer climates (Whitlow Jnr & Hagler Jnr 2003) and the combination of drought and high ambient temperatures has been proven to be the primary environmental factor leading to pre-harvest aflatoxin contamination in the southern maize growing areas of the United States (Abbas et al. 2002; Bruns 2003; Payne 1992). Similar conditions prevail in most Australian maize-producing regions.

Despite this relationship being well-documented, it has proven extremely difficult to model mycotoxin contamination occurring in the field. Pre-harvest mycotoxin contamination occurs heterogeneously in the field; a small number of infected kernels can contribute sufficiently to render an entire harvest contaminated (Blaney, O’Keeffe & Bricknell 2008). In addition to highly variable air temperatures and rainfall, the availability of inoculum is a crucial factor- contamination cannot occur without it, no matter how conducive the climatic conditions.

Over the 2004-2006 seasons, concentrations of aflatoxin contamination proved to be correlated with low rainfall during kernel development (p<0.01). In 2006, the Burnett area of Queensland was significantly more likely to produce maize unsuitable for milling purposes than any other maize-producing region in Australia (p<0.05). This corresponded with lower daily rainfall averages over the kernel development period. Regions using irrigation reported significantly lower levels of aflatoxin contamination (p<0.01) as did areas with higher rainfall (p<0.01). Temperature could not be proven to play a part, as all maize growing areas reported temperatures well over 30°C during the relevant kernel development periods.

**IMPLICATIONS OF CLIMATE CHANGE ON AUSTRALIAN MAIZE**

Climate change is expected to have significant impacts upon Australia. Our continent is predicted to experience increases in ambient temperature and more frequent episodes of drought (Hennessy, Macadam & Whetton 2006). These conditions clearly not only favour aflatoxin contamination but also induce plant stress, making the plant more susceptible to fungal infection.

Climate change is also tipped to cause more frequent extreme climate events such as droughts and episodes of extreme temperature (Hennessy, Macadam & Whetton 2006). This would indicate that episodes such as the 2001 and
2003 outbreaks of severe aflatoxin contamination experienced in Australia will also occur more frequently.

Climate change may make growing dryland maize in some Australian regions unprofitable and farmers may turn to more drought-resistant crops. The dryland maize-growing regions in South East Queensland are expected to grow warmer, with more hot days. A decline in annual rainfall is expected to occur, coupled with higher evaporative demand (Hennessy, Macadam & Whetton 2006). This may reduce the availability of Australian grown grain, causing an increase in imported maize to meet demand. Alternatively, if farmers continue to produce maize, irrigation will be required in greater volumes to meet the need for milling grade maize. Given the current water shortage and projected reductions in annual precipitation, this is unlikely to be a sustainable choice. Even in maize-producing areas customarily using irrigation, reduced water allocations may lead to maize being considered an unviable crop. By 2030, it is predicted that NSW will suffer increased water stress, with little change in rainfall but higher evaporative demand (Hennessy, Macadam & Whetton 2006).

Maize considered unsuitable for milling purposes would in many cases remain suitable for stock feed. A significant increase in the amount of maize available for this purpose would have the potential to reduce prices. Another use of contaminated maize is as a source of material for the production of biofuel, although the limited size of Australia’s maize industry would be unlikely to make this a profitable exercise.

**POTENTIAL CONSEQUENCES FOR AUSTRALIAN CONSUMERS**

In Australia, the only mycotoxin currently regulated is aflatoxin B1, and only in peanuts (Government of Australia 2008). Until recently a specific standard existed for aflatoxins in all other food products, but this standard was removed in 1999 as part of an overhaul of the Australian and New Zealand Food Standards Code. Standard A12 of the *Food Standards Code* also does not include mycotoxins in the general requirement requiring unspecified contaminants to be absent from all food products, as they are not classified as “contaminants” under the provisions of the Code.

In the 1999 review of Standard A12, it was recommended that the specific 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” (ANZFA, 1999). *Codex Alimentarius* recommends that “contaminant levels in foods shall be as low as reasonably achievable” and that “maximum levels shall only be set for those foods in which the contaminant may be found in amounts that are significant for the total exposure of the consumer”. The position of ANZFA was that the Australian Market Basket Survey had failed to detect aflatoxin in foods other than peanuts and thus, it appears, did not believe that the contaminant could occur in amounts significant for the total exposure of the consumer.
This failure to detect aflatoxin in Australian foods is not indicative of the contamination of maize-based foods at the time because the Authority did not choose to sample and analyse a range of maize-based foods for aflatoxin contamination. In more recent surveys, should maize-based foods have been analysed for aflatoxin contamination, the current practice of Australian manufacturers to test incoming loads of raw maize for a range of mycotoxins and reject those not meeting the voluntary National Agricultural Commodities Marketing Association (NACMA) trading standard for milling grade maize (Table 1) would probably ensure the same result. A survey of a range of foods containing significant proportions of maize was carried out as part of our study and results appear to support this assumption, with no domestically-produced foods containing detectable aflatoxin levels.

**TABLE 1 NATIONAL AGRICULTURAL COMMODITIES MARKETING ASSOCIATION TRADING STANDARDS FOR MAIZE**

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Milling</th>
<th>Prime</th>
<th>Feed 1</th>
<th>Feed 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total aflatoxins (µg/kg)</td>
<td>5</td>
<td>15</td>
<td>20</td>
<td>80 (not more than 20µg/kg B1)</td>
</tr>
<tr>
<td>Total fumonisins (mg/kg)</td>
<td>&lt;2</td>
<td>5</td>
<td>10</td>
<td>40</td>
</tr>
</tbody>
</table>

While the application of the NACMA trading standards appears to protect the consumer from significant dietary exposure through maize-based food products, the same cannot be said for imported commodities. Of the foods tested as part of this study, only one product tested positive for mycotoxins. This product, puffed corn imported from the USA, contained fumonisin B1 at concentrations up to 4ppm. It is worth noting that this concentration is significantly above the US Advisory Standard for fumonisin in food products. While fumonisin concentrations have not yet been investigated with respect to climate change, this example serves to illustrate the vulnerability of the Australian market to unscrupulous dealers seeking to take advantage of Australia’s lack of regulation to offload product unsuitable for sale in home markets.

If maize continues to be farmed but mycotoxin levels increase, harvests found to be unacceptable for milling purposes have a high probability of being sold for stock feed. Reduced rainfall may lead to lack of pasture and contaminated maize may be utilised for supplementary feed for dairy cattle, presenting obvious risk of the contamination of milk with aflatoxin M1. Australia has no standard for aflatoxin in milk or milk products. Additionally, milk powder also carries the potential for contamination with aflatoxin M1 and is permitted for import from all areas certified as free from foot & mouth disease provided an import permit is granted (AQIS 2008). Once again, even if dairy feed were to be regulated, the lack of a food standard would leave Australia potentially vulnerable to import of contaminated product.
CONCLUSION

While “killer cornflakes” may not be precisely around the corner, it is clear that climate change potentially carries a risk to consumers of maize-based food products and the maize industry as a whole. Careful monitoring of Australian-grown maize and imported maize-based food products in the future will be necessary to determine if these potential risks have become a reality.

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Appendix D

Managing mycotoxins in maize: case studies

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Abstract. Mycotoxin contamination of Australian maize is neither common nor extensive, but has the capacity to seriously disrupt marketing. Low to moderate levels of aflatoxins and fumonisins can be widespread in some seasons, but zearalenone, nivalenol and deoxynivalenol are usually confined to small growing localities. Possible approaches to such situations were tested by an analysis of several case studies. It is concluded that communication and coordination across the industry, prediction and prevention of contamination, rapid detection and assessment of contamination, effective use of contaminated maize and breeding for resistance comprise a useful set of strategies for managing mycotoxins in maize.

Introduction

It is not always possible to produce maize free of mycotoxins, because the fungi responsible are always present, requiring only suitable conditions for growth and mycotoxin production. However, it is practical to ensure that the extent of contamination meets acceptable standards for different uses, whether that is milling for human food, manufacturing purposes such as gluten extraction, or incorporation into pet foods and stock foods. This paper examines the problem of mycotoxins in Australian maize to clarify the underlying causes of failure to meet market specification through an analysis of several case studies and provides suggestions to assist industry to find solutions.

Mycotoxin occurrence in Australian maize

Information about mycotoxin contamination of maize has been obtained from some targeted mycotoxin surveys in certain regions, from industry quality testing programs, and from investigations into occasional episodes of livestock poisoning by animal health laboratories. Plant disease control and maize breeding programs also provide information on the prevalence of mycotoxinogenic fungi.

Aflatoxins

Aflatoxins are usually present at low frequency and concentration (0.001–0.005 mg/kg) in maize grown in subtropical and temperate regions of Queensland (Qld) and New South Wales (NSW), but occasional samples can contain higher concentrations, up to 0.2 mg/kg (Blaney 1981). Invasion of maize by the fungi Aspergillus flavus and A. parasiticus is favoured by high temperatures, insect attack and premature drying of the ear during filling. Once the fungus has invaded certain kernels, aflatoxin production is then favoured by persistent high humidity during grain maturation, and very high concentrations can quickly develop if the grain is stored at 16–20% moisture (Blaney and Williams 1991). Preharvest contamination can involve a very small number of kernels, yet provide enough aflatoxin to significantly contaminate an entire crop. In moist, hot storage, the fungus can quickly spread to adjacent sound maize kernels. Hence, critical control steps for aflatoxin include: avoiding planting situations (region and time) and rainfall/irrigation systems that subject the developing kernel to high temperatures (35–40°C); control of insects; and harvest and storage at recommended moisture contents (<14%).

Ochratoxins

Ochratoxin is quite uncommon in Australian maize, although traces are occasionally detected (0.001–0.003 mg/kg). The causative fungus in maize is generally considered to be A. ochraceus although identification of other ochratoxin-producing fungi that used to be grouped with A. ochraceus (Frisvad et al. 2004), and production of ochratoxin by some isolates of A. niger has raised some uncertainty about the point. Ochratoxin production by Qld isolates of A. ochraceus was reported by Conolle et al. (1981). This fungus is less prevalent than aflatoxin-producing fungi, and seems to prefer slightly higher moisture contents, which are most commonly provided once moisture migration is well underway in stored maize. Control steps are similar to those for aflatoxin.

Fumonisins

Fumonisins are produced by Fusarium verticillioides, F. proliferatum, F. thapsinum and F. nygamai. These fungi all occur in Australian maize, but F. verticillioides appears to be the main source of fumonisins. F. verticillioides was previously called F. moniliforme, but the latter is now considered to include several related fungi (Seifert et al. 2003). F. verticillioides causes kernel rot, but is now considered an endophyte that is present in apparently sound grain (Williams et al. 1992). Low concentrations of fumonisin (0.2–1 mg/kg) are consequently very common (Bryden et al. 1995). Increased stress due to water restrictions and insect attack has been associated with increased
ear rot in NSW (Watson et al. 2006). Occasionally, very high concentrations (>100 mg/kg) of fumonisin can be produced, albeit in visually rotted kernels (Shanks et al. 1995). The cause is not clear, although hybrid susceptibility and climate are involved. Until these factors are explored, control measures cannot be fine tuned, but selecting suitable hybrids for each region and not restricting water during grain maturation will certainly help.

**Zearalenone**

Zearalenone can be produced by several *Fusarium* spp., but the main producer in maize is the ear- and stalk-rot pathogen *F. graminearum*, often associated with a deep purple colouration of infected kernels (Blaney et al. 1984b). The fungus is present on crop debris in the soil and release of spores, and infection of developing maize ears during silking, are both favoured by moderate temperatures and persistent high humidity at that time. Thus, infection is higher in situations when persistently moist and overcast conditions occur during maize silking. Such conditions tend to be limited to the higher-rainfall regions of the Far North QLD tablelands and the northern rivers region of NSW. Even in these minor growing regions, samples do not often exceed 1 mg/kg (Blaney et al. 1986), the level that can affect pigs (Blaney et al. 1984a). Zearalenone contamination can be limited through the use of hybrids resistant to *F. graminearum*.

**Nivalenol and deoxynivalenol**

The trichothecene mycotoxins, nivalenol (NIV) and deoxynivalenol (DON), are produced in maize by *F. graminearum*, which can also produce zearalenone. As explained above, this fungus is only common in Australia on the cool, wet tablelands of Far North QLD, where for reasons not completely clear, the fungus produces mainly NIV. In southern QLD and in NSW, the fungus produces mainly DON, also called vomitoxin (Blaney and Dodman 2002). It is very unusual for NIV and DON to exceed 1 mg/kg, a level reducing feed intake by pigs (Williams and Blaney 1994). Control of NIV and DON is best achieved with resistant hybrids in higher risk areas, but suitable crop rotations and removal of crop residues can also assist in lower risk areas.

**Overview of current mycotoxin surveillance**

Mycotoxin testing is regularly carried out by organisations in the milling and pet food industries, and by some stock food manufacturers if a problem is suspected. Data provided to the authors of testing results over the last 5–10 years by some of these organisations, are consistent with conclusions from surveys (Bricknell et al. 2008) that the major proportion of Australian maize meets the most stringent milling standards, and that all but a very few of the remaining crops are suitable as stock feed. Aflatoxins are of most concern, particularly for companies supplying the human food (millers) and pet food markets, who are using a standard of 0.005 mg/kg. Increasing drought and high temperatures associated with global warming are increasing the risks. Less data have been collected on fumonisins, but these also require regular monitoring. There are some localities where the risk of contamination with certain mycotoxins is always higher (such as zearalenone and NIV on wetter parts of the Atherton Tableland), and seasons where the aflatoxin risk increases (such as the impact of drought on rainfed crops in hotter localities in central QLD).

Despite these localised and seasonal risks, there are no indications over the last 30 years that mycotoxin contamination has ever been so excessive that it could not be managed, at least potentially, in a way that achieved satisfactory outcomes for both the producer and the end-user of maize. Problems in managing situations that have arisen in the past appear to be due to several factors. These are:

1. Lack of information about mycotoxins in a form that is accessible and easily understood by industry participants. Related to this is the ‘outrage factor’ arising from the shock of finding unexpected contamination, through not knowing how to respond to that situation and who to discuss it with in order to find a resolution.
2. The sporadic seasonal nature of contamination, and inability to predict situations where the risk of contamination increases. Sometimes, this is compounded by failure to use good storage and transport practices to avoid increases in mycotoxin contamination.
3. The current inability to test maize for contamination within the current truck turn-around times for grain deliveries to end-user, and the inappropriateness of general grain quality standards for assessing mycotoxin contamination. Related to this is the availability of cost-effective mycotoxin testing methods.
4. Failure to set contractual standards for mycotoxin concentrations that are practicable and appropriate for the intended end-use, based on solid scientific data on tolerances of livestock to mycotoxins, and internationally accepted limits for maize used as human food. Related to this is lack of awareness of, and failure to meet, the expectation of international trading partners in respect to mycotoxin levels.
5. Use of maize hybrids with innate susceptibility to certain fungi in high risk localities.

**Proposed management strategies**

From 2003–06, the Grains Research and Development Corporation (GRDC) supported a project on managing mycotoxins in maize, conducted by the authors and other officers of the Qld and NSW Departments of Primary Industries (DPI) and the Universities of Qld and Sydney. This project set the basic hypothesis that mycotoxins in maize can be managed by addressing five broad strategies that relate to the factors discussed above. Under the guidance of a steering group comprised of a cross section of industry participants, the project team engaged in various activities aimed at providing the tools to help industry address these strategies, as listed below:

**Strategy 1 – communication and coordination across the industry**

Activities included: devising a communication plan to ensure distribution of relevant information to key industry and regulatory authorities, based on a detailed stakeholder analysis; undertaking a formal risk analysis of the food safety hazards from mycotoxins, based on known and projected hypothetical levels of contamination; adapting the guidelines for good agricultural practice for managing mycotoxins in grain
published by the Codex Alimentarius Commission (2003) to the specifics of mycotoxins in Australian maize; and developing information packages on managing mycotoxins in maize.

Success criteria for this strategy were that the project team and steering group worked effectively, that a national strategy was endorsed by stakeholders, and that information on managing mycotoxins in maize was distributed and adopted across the industry.

**Strategy 2 – prediction and prevention of contamination outbreaks**

Activities included: investigating outbreaks of contamination to determine key contributing factors; identifying the fungi involved in diseases of maize that give rise to mycotoxin contamination; and developing a model to predict mycotoxin contamination of maize from climatic variables, starting with an approach similar to that used for aflatoxin in peanuts (Rachaputi et al. 2002).

Success criteria for this strategy were that the epidemiology and aetiology of the plant pathogens producing mycotoxins were well understood, that control measures were available, and that maize growers and other industry participants were able to predict seasons with a high risk of contamination, and took measures to minimise the impact of this on their operation.

**Strategy 3 – rapid detection and assessment of contamination**

Activities included: developing sampling protocols appropriate to Australian maize; compiling and promulgating information on physical indicators of contamination; investigating near infrared analyser technology for rapid assessment of contamination (Dowell et al. 2002); validating sampling plans and analytical methods for mycotoxins of interest; maintaining a list of Australian laboratories that were accredited for performing mycotoxin assays; and assaying maize from all major production regions during the project (three to four seasons).

Success criteria for this strategy were that a suite of sensitive, specific and rapid assay methods and sampling protocols were available to industry for testing maize; and that detailed information was obtained on mycotoxin contamination of the Australian maize crop over four seasons.

**Strategy 4 – effective use of contaminated maize**

Activities included: collating available data on tolerances of livestock to different mycotoxins, and providing these data to industry; performing risk assessments on the potential for reduced livestock production by different levels of contamination; and helping to establish industry and regulatory standards for mycotoxins in maize, based on good science, which balanced the ability of growers to produce quality grain with the requirements of end-users.

Success criteria for this strategy were that standards for acceptable levels of mycotoxins in maize were established and incorporated into livestock feeding practices, and that markets accepted these standards and responded in an economically rational manner.

**Strategy 5 – breeding maize for mycotoxin resistance**

Activities included: collecting data that might indicate variable susceptibility of maize cultivars to mycotoxin contamination; and developing germplasm combining resistance to certain mycotoxigenic fungi with other desirable characteristics, for incorporation into commercial cultivars.

Success criteria for this strategy were that mycotoxin minimisation was incorporated into objectives of maize breeding programs, and that cultivars with appropriate resistance to mycotoxins were planted in higher risk situations.

**Testing the strategies: case studies**

The appropriateness of these management strategies was tested via case studies of contamination incidents that arose over the previous few years. These cases provide examples of the problems that can arise and lessons for their effective resolution.

**Case study A – aflatoxins in central NSW**

In 2001, levels of aflatoxin described as ‘extremely high’ (0.2–0.3 mg/kg) were detected in some maize grown in ‘central NSW’ by member companies of the Australian Food and Grocery Council (AFGC). The confidential report raised the concern that the matter could develop into a serious food scare if not handled with sensitivity. Members were all advised to be extra vigilant in regard to aflatoxin, to ensure appropriate screening procedures (not specified) were in place, and to advise members and regulatory authorities if high levels of aflatoxin were detected. With hindsight, the reaction appeared excessive, as the problem was confined to a very small locality, affected by crop flooding, and where high moisture storage was involved.

In examining the case response, it is clear that the problem was identified and appropriately communicated across those industry participants in the AFGC. What was not done was predicting the problem in the first place, quickly defining the extent of contamination within the overall picture of good quality grain, specifying what screening procedures should be adopted, what standards should be met for what end-use, what should happen in case of dispute, and advising the growers about their rights and responsibilities in the matter. The response was constrained by natural concern over potential adverse publicity, which is a continuing dilemma for all industries. Our opinion is that concealing information about contamination might have short-term benefits, but in the long run, simply impairs credibility and leaves the whole industry vulnerable. A strong case can be made that Australia is in a good position in regard to mycotoxins compared with many other countries – mainly because of climatic patterns, dry harvests and fewer storage problems – and stands to benefit from a full and open scrutiny of grain quality. There is natural concern that instances of contamination are not blown out of proportion, but this should not occur if the industry can produce evidence of responsible testing, and managing incidents as they arise.

**Case study B – fumonisins in the Murrumbidgee irrigation area**

In April 2003, a milling company in the Murrumbidgee irrigation area (MIA) rejected a large number of deliveries of contracted maize because of high fumonisin contents – some also had excessive aflatoxin concentrations. It was proposed to offer the maize to local feedlots, but there was concern on both sides about acceptable concentrations for this purpose (and of
that stress in relation to *F. verticillioides* March/April. While ‘stress’ clearly contributed, the timing of irrigation water and noticed quality problems on harvest in growers had ‘pushed the system’ a bit by stretching out humidity for the following few days. Two weeks after this, some 2003, and crops received ~40 mm rain on 21 February with high December 2002, 32 mm of storm rain fell at the start of January 1991; Williams livestock production was also provided (Blaney and Williams Detailed information on tolerances of livestock to mycotoxins involved were radio interviews, addresses to farmer groups, and circulated to maize industry participants across Australia. Also those who needed to know and appropriate decisions were made the risks were clarified, accurate information was provided to which might be more stress susceptible. 

Additionally, recommendations are to plant on time (to sow late September), to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties, to adjust irrigation intervals (but not extend them), to manage nitrogen application (avoid excess), and avoid softer varieties. 

At this local level, the contamination episode was managed quite well after the initial shock – the problem was recognised, the risks were clarified, accurate information was provided to those who needed to know and appropriate decisions were made by most stakeholders. A positive outcome was the subsequent establishment of levels for aflatoxins and fumonisins in trading standards of the National Agricultural Commodities Marketing Association (NACMA 2003). These standards are shown in Table 1. Ongoing needs identified were better prediction of mycotoxin problems, and faster (and cheaper) assay methods. 

**Case study C – aflatoxins in central Qld**

In mid 2004, the project team detected aflatoxin in a large number of small (0.5 kg) ‘grower samples’, supplied by a bulk handler, grown on one farm in central Qld. Concentrations ranged up to 0.24 mg/kg, but averaged 0.045 mg aflatoxin B1/kg. This level exceeded the Qld stock food regulation limit of 0.02 mg aflatoxin B1/kg for ‘grain, crushed grain and seeds’ (Anon. 2003). The average level would meet the limit of 0.05 mg/kg for ‘stock food for beef cattle, horses or sheep’, but the regulation did not specify a process whereby grain became stock food for beef cattle, horses or sheep.

It was recognised that the samples tested were too small to accurately represent the aflatoxin content of bulk maize. According to the Aflatoxin Handbook of the Grains Inspection, Packers and Stockyards Administration (GIPSA), a minimum of 2 pounds (908 g) should be taken per truckload (USDA 2003).

Even then, the aflatoxin content of that sample might vary between 0.003 and 0.039 mg/kg, if the ‘true’ concentration in the truck was 0.02 mg/kg. Obviously, a 1-kg sample might be satisfactory for detecting potential contamination, but for regulatory purposes, larger samples (5–10 kg per truckload) need to be taken. The entire 5-kg sample must be milled before subsampling, and certain mills like the Romer mill are available for this purpose. The logistics of testing such large samples have been addressed by certain milling companies in Australia, but not by many other maize end-users.

The supplier, once aware of the potential problem, elected to place the grain under quarantine, and also submitted larger samples representing bulk maize from that region. These samples all met the Qld stock food standard for grain of 0.02 mg B1/kg, suggesting substantial dilution by other negative deliveries of maize. Although the regulations were apparently met, it was recognised that some portions of the bulk maize could have higher concentrations, so to minimise risk the maize was sold to a cattle feedlot, and this appeared to have been an appropriate course of action.

This case study raises several learning points. First, the industry now has sufficient evidence to indicate that mycotoxin testing, at least for aflatoxin and fumonisin, should be regularly performed, although the frequency of this might be low except in certain high risk circumstances. Now that the maize industry, via NACMA, has set mycotoxin standards for maize, pressure will increase for suppliers to provide evidence that their product meets those standards! Second, appropriate sampling procedures

### Table 1. Aflatoxin and fumonisin limits for maize sold under National Agricultural Commodities Marketing Association (NACMA) contracts

<table>
<thead>
<tr>
<th>NACMA grade</th>
<th>Milling</th>
<th>Prime</th>
<th>Feed 1</th>
<th>Feed 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxins (B1 + B2 + G1 + G2) (mg/kg)</td>
<td>0.005</td>
<td>0.015</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>Fumonisins (B1 + B2 + B3) (mg/kg)</td>
<td>2</td>
<td>5</td>
<td>10</td>
<td>40</td>
</tr>
</tbody>
</table>
for aflatoxin must be used. Third, it is important to debate the question of whether government regulations are still required if industry sets its own standards. If regulations are to be retained, it is important that these be harmonised with industry (NACMA) standards. Another important question for processors (e.g. grit or gluten or stock feed manufacturers) is whether standards should be applied to incoming maize or to the final products, since processing can either reduce or concentrate mycotoxins in different product streams. These questions involve all the maize industry, and cross-industry forums such as was hosted by the MAA in Brisbane in October 2006 (Cogswell 2006) provide the opportunity to resolve these matters.

Case study D – aflatoxins in an export maize consignment

In January 2005, a single container of bulk maize from the MIA was rejected on arrival in Japan for aflatoxin residues. Japan has a limit of 0.005 mg total aflatoxins/kg, and the container tested at 0.027 mg/kg. The Australian Department of Agriculture, Fisheries and Forestry was notified of this by the Japanese Ministry of Health, Labour and Welfare, and requested to investigate the cause of the incident, to introduce measures to reduce contamination and to ensure that it did not happen again. Under an ‘enhanced inspection order,’ the next 300 maize shipments or all shipments over the next 3 years would be tested for aflatoxin.

The investigation was a good example of cooperation at the national level, being coordinated by members of the Grains Council, MAA, NSW DPI, Qld Department of Primary Industries and Fisheries (DPI&F), and the GRDC, and revealed the following story. The maize was grown under irrigation in 2003–04 over a particularly hot and dry summer in the MIA – conditions known to favour A. flavus invasion. Harvesting took place during unusually cool and showery conditions and the harvest moisture content ranged from 13.5–16% (14% is regarded as the maximum safe level for storage). Noticing some quality problems, the owner gravity-graded the maize and ~90% of physically damaged grain was removed. Follow-up testing by our project as part of the trace-back investigation found 0.002 mg aflatoxins/kg in graded grain, and 0.005 mg/kg in ungraded grain – clear indication of the presence of the fungus, although aflatoxin levels were probably acceptable before shipment. However, the grain was then placed in bulk in non- aerated transport containers, which spent several weeks on docks (both in Australian and Japan), and on ships at temperatures ranging up to 50°C, before testing was conducted in Japan. Under these extreme conditions, any slight excess of moisture becomes concentrated into pockets through the alternate heating and cooling of container sides, an ideal situation for aflatoxin production by the fungus.

As a consequence of this case, Australian exporters have been made aware of Japan’s increased testing regimen, and the MAA has recommended all exporters test for mycotoxins before export (in addition to existing testing being carried out by milling companies) and to fully document the test results. Another key lesson is the need to manage moisture levels in stored maize at all times. In shipping containers, maize in bags is of lower risk than bulk maize since migrating moisture will condense outside the bags, and inert adsorbents like diatomaceous earth in the container will remove some condensation (there are commercial products for this purpose). Containers should be carried in the hold of ships, not on deck where it can be hotter. These measures have been implemented by grain exporters, and a large number of shipments have since been accepted. It has become clear that several additional issues need to be negotiated and inserted into contracts between exporter and importer. These should define how containers are to be sampled and tested and which standards will apply, and limit the time between arrival in the importing country and testing to avoid further deterioration.

Even with these precautions in place, some serious risks remain: first, that some occasional or first-try exporter might send untested maize overseas, either through ignorance or overconfidence, and put all Australian grain markets at risk; and second, that the aflatoxin testing process used by certain laboratories itself might be insufficiently rigorous to ensure that certain batches will meet a stringent limit of 0.005 mg aflatoxin/kg (see the requirements for testing discussed in case study C). At least the latter risk can be reduced if clients specify an appropriate sampling system like the GIPSA system, and only use laboratories that can supply evidence of method validation and an accreditation system like that of the National Association of Testing Authorities. All of these recommendations have been incorporated into supply chain and export protocols for maize, which the MAA is proposing for wide adoption across the industry (Cogswell 2006).

Case study E – effective use of contaminated maize screenings

In mid 2004, a sample of maize screenings was submitted to the authors by a grower in mid-west NSW. Alert to visible damage and the possibility of mycotoxin contamination, his agent had gravity graded several hundred tonnes of lightweight material out of a 30000 t crop. We detected 0.06 mg aflatoxins/kg and well over 200 mg fumonisins/kg in the screenings. The most lenient NACMA standard for maize used in stock food is 0.08 mg/kg aflatoxins, and 40 mg/kg fumonisin.

Our advice to this grower was that there was a high risk of toxicity if the undiluted material was fed to livestock. If he intended to feed the grain to his own mature beef cattle or sheep, it should be diluted substantially or used only as a feed supplement. The aflatoxin level should be tolerated by adult ruminants, but the fumonisin content was too high. Ruminants are tolerant to fumonisins compared with horses and pigs, but reduced production has been reported in dairy cows fed 75 mg fumonisin B1/kg for 14 days (Richard et al. 1996), and evidence of liver damage in feeder calves given 148 mg total fumonisins/kg for 30 days (Osweiler et al. 1993). Consequently, it would seem best to feed no more than 1–2 kg of these screenings/animal.day to cattle.

The grower was warned that the material must not be fed to horses, which are very susceptible to fumonisins (EU 2005), nor to pet species of unknown susceptibility. Given this information, the grower declined to feed his own stock but accepted an offer of $115/t for the material (cf. $195/t for sound maize), which was incorporated into mineral supplement blocks. Such blocks are used mainly for cattle and sheep, which are relatively resistant to fumonisins and aflatoxins, and the formulation is usually designed to limit intake to <0.2 kg/day (maybe a 50-fold...
appropriate, providing all industry participants understand the proposed strategies for managing mycotoxins are generally reasonable, low-risk decisions in the circumstance. A set of guidelines for maximum aflatoxin and fumonisin content of food for various livestock and pet species was published in *The Cod* (Kopinski and Blaney 2006).

Other options explored included the use of ‘mycotoxin-binding’ agents, but we were unable to find any scientific evidence that these were effective with fumonisin, so the benefit to cost ratio was doubtful. Directing grain to ethanol production plants is another avenue, but the by-product of distillers grain retains much of any mycotoxins present in the original grain, so the hazard remains. In summary, effective use of contaminated grain means to get the best economic dividend (Blaney and Williams 1991) and despite adding a cost, accurate mycotoxin assay can minimise the risk of an adverse outcome.

**Case study F – breeding for resistance to mycotoxin-producing fungi**

Almost 40 years ago, a maize breeding program was set up in tropical north Qld by DPI&F to develop hybrids suitable for the particular climate of the northern Tablelands, which features a persistently wet and often cool growing and maturation period. This climate was conducive to many diseases affecting yields and quality, and the breeding program led by Ian Martin at Kairi Research Station has gradually eliminated many of these. *F. graminearum*, *F. verticillioides* and other *Fusarium* species were common causes of stalk and ear rots of maize in the early 1980s, and zearalenone contamination was very common in surveys conducted at the time (Blaney et al. 1986). Since that time, the breeding program has greatly reduced the extent of *F. graminearum* ear rots, and also zearalenone contamination, judging by our recent surveys. The hybrids might be resistant to fumonisin contamination as well, but this hasn’t been fully investigated. The message is clear — breeding for resistance to certain fungi is a vital strategy in managing mycotoxins, and this characteristic should be as important in breeding targets as yields and other agronomic values.

The major breeding companies are aware of these issues, but the demand for mycotoxin resistance needs to come from the market place. Rightly or wrongly, some hybrids are being linked to increased fumonisin contamination, and this needs further investigation. Research into sources of fumonisin resistance is well underway in other countries (Clements et al. 2004; Butron et al. 2006). It is noted that Bt hybrids have been reported to have some resistance to fumonisin contamination in the USA through increased resistance to boring insects (Munkvold and Butzen 2004). There is a possibility that breeding for drought resistance might have a positive impact on aflatoxin susceptibility.

The message to growers from this case study is to choose hybrids appropriate for each region, and to take account of the potential impact of a stressful season on mycotoxin contamination and eventual market suitability. Adjusting planting times and plant populations can also reduce stress and decrease risks of mycotoxin contamination (Chauhan et al. 2008).

**Conclusions**

An examination of the case studies above indicates that the proposed strategies for managing mycotoxins are generally appropriate, providing all industry participants understand the issues involved and work together to achieve objectives. The more these issues are discussed, the more likely it is that solutions will present. To be pragmatic, any particular industry participant is more likely to retain the necessary information once they have had to deal with the problems these situations create, or at least to adopt and routinely apply the necessary mycotoxin management processes. We consider that the Hazard Analysis Critical Control Point framework is most suitable for managing the known risks within industry operations (Bricknell et al. 2008). Managing the unknown risks such as the impact of variable weather patterns on mycotoxins does require more research, and climatic modelling to predict aflatoxin contamination in maize is feasible (Chauhan et al. 2008). Research also needs to continue on disease control relevant to mycotoxins (Watson et al. 2006), and on rapid assessments methods for detecting contamination. All participants in the industry have an important role to play – managing mycotoxins in maize is too serious an issue to be ignored.

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**References**


Mycotoxins in maize

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Appendix E

Mycotoxins in Australian Maize

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Mycotoxins known to occur in Australian maize include aflatoxins, fumonisins, ochratoxin A, zearalenone, deoxynivalenol (DON) and nivalenol, all known or suspected to be toxic to humans and animals. Samples from the 2004 and 2005 seasons were collected and assayed for the presence and level of mycotoxin contamination. The survey will be continued in 2006.

Results

Significant variations in the type and levels of contamination occurred relating to climate, region and season, with climate being the predominant factor. Regions experiencing high temperatures and low rainfall experienced aflatoxin contamination. Zearalenone contamination occurred predominantly in areas of North Queensland where temperatures were cooler and humidity higher during kernel maturation. Fumonisins were ubiquitous, although lower concentrations tended to occur in regions experiencing dry conditions during kernel maturation and immediately prior to harvest. Aflatoxins and fumonisins are the mycotoxins of primary concern, with occasional high levels occurring in samples from all maize growing areas.

Despite this, the Australian maize crops of 2004 and 2005 were of a high standard, with >80% of samples meeting the National Agricultural Commodities Marketing Association (NACMA) standards for milling maize and 98% meeting the standards for stock feed.

The difficulty in predicting when and where high levels of contamination will occur highlights the need for an industry wide risk management system for mycotoxin contamination to ensure Australian maize meets the standards of all domestic users and export markets.
Appendix F

There has been no comprehensive research into the levels of fumonisins in Australian maize. We are conducting the largest survey to date of contamination by fumonisins and other mycotoxins in the crops of 2003, 2004 & 2005. We have refined the method for fumonisin assay to improve throughput during routine monitoring.

Whole maize samples were milled, extracted in methanol:water and cleaned up using Strong Anion Exchange Solid Phase Extraction (SAX- SPE). Extracts were reconstituted in acetonitrile:water and derivatised with o-phthaldialdehyde (OPA)-2-mercapto ethanol before being loaded onto a reverse phase HPLC C18 column with fluorescence detection as per the AOAC standard method.

Previously, problems relating to the stability of the derivatising agent have required derivatisation to occur less than three minutes prior to the sample being manually loaded onto the column. In this project it was found that decay of the derivatising agent occurred consistently, allowing multiple samples to be loaded using an autosampler with pre-column derivatisation. Despite the sample being loaded approximately nine minutes following derivatisation, the decay of the derivative was consistent, effectively improving throughput and precision of the assay over manual injection.

The limit of detection for fumonisin B₁ increased from 0.1 ppm to 0.2 ppm but as the relevant Australian trading standard for fumonisin in milling grade maize is 2 ppm, this was considered acceptable.

Survey results to date indicate that the majority of samples from 2004 and 2005 met trading standards for milling (86%) and animal feed (11%) with <3% exceeding these standards.