



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

## Foreword

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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 stock feed 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

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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

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### 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 B<sub>1</sub> and B<sub>2</sub>, 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 effects 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 27°C, two conditions are met. Firstly, the natural resistance of the maize plant to fungi in general is compromised; and secondly, the relatively heat-tolerant *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 35°C, the 'water activity' ( $a_w$ ) 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.

## Fumonisin

Fumonisin are another group of chemically related mycotoxins, the most common and most toxic called fumonisin B<sub>1</sub> (FB<sub>1</sub>), with FB<sub>2</sub> and FB<sub>3</sub> common in lower concentrations. Fumonisin 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 fumonisin 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 fumonisin. 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 fumonisin.

### What conditions make fumonisin 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 fumonisin (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 fumonisin, 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.



**Figure 2 Starburst pattern on *F. verticillioides* infected maize** (Source: [American Phytopathological Society](#))

## Zearalenone

Zearalenone is a non-steroidal estrogenic mycotoxin that has been implicated in some forms of infertility in pigs, cattle, sheep and possibly other animals. It has not been proven to affect human health. In maize, zearalenone is primarily produced by *Fusarium graminearum*, a fungus responsible for causing ear and stalk rots. *F. graminearum* also causes head blight of wheat, and roasting wheat and maize is a common cause of increased infection in both crops if climatic factors suit. Rice is also potentially susceptible, but no problems have been observed in Australian production regions. The fungus has been isolated from stalk rot of sorghum, while inoculum also persists in pasture grasses rotated with maize in a few high rainfall localities. Provided that inoculum is

present on crop residues in soil, infection of maize occurs at flowering, facilitated by cool, wet weather at this time.

## 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.



**Figure 4** *Helioverca armigera* damage to a cob (Source: [Ecoport Picture Databank](#))

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 ( $a_w$ ) is  $<0.88$ . Consequently, significant amounts of *Fusarium* mycotoxins will not be produced during maize storage in Australia – fumonisin, 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 ( $a_w$  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 (*Rhizopertha 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 ( $A_w$ ) 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](#))

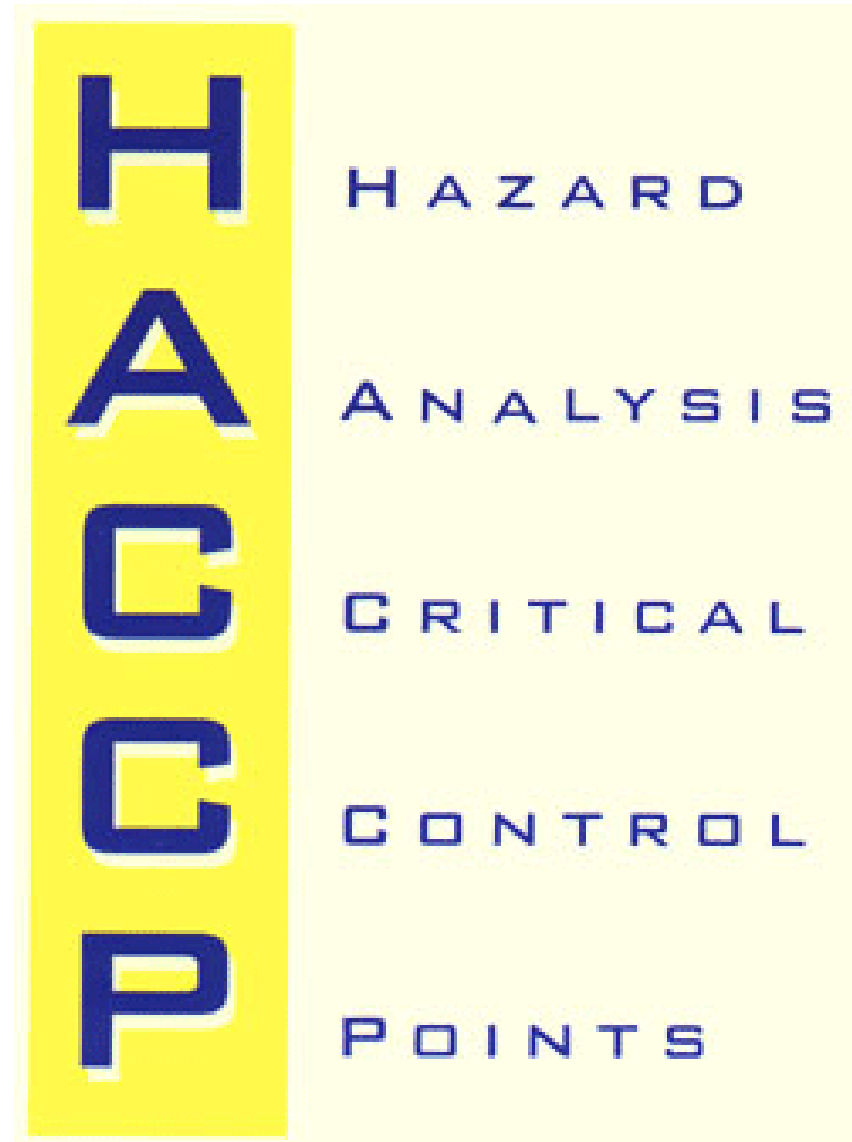
## What is HACCP?

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**

<i>Principle</i>	<i>Description</i>
Conduct a hazard analysis.	A detailed step by step diagram of the process is prepared, identifying where significant hazards occur.
Determine critical control points	Critical Control Points (CCPs), points at which the hazards can be controlled, are identified throughout the process.
Devise a monitoring programme.	A method of monitoring hazards is critical in any HACCP programme to ensure these remain under control at the critical control points
Establish critical limits.	These are limits that must be adhered to in the monitoring system if risk is to be minimised
Devise a monitoring programme.	Monitoring is critical in any HACCP programme to ensure control points remain under control
Define corrective actions.	If a hazard is shown to be outside the set critical limits, corrective measures must be implemented
Establish verification procedures.	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.
Develop documentation and record keeping.	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.

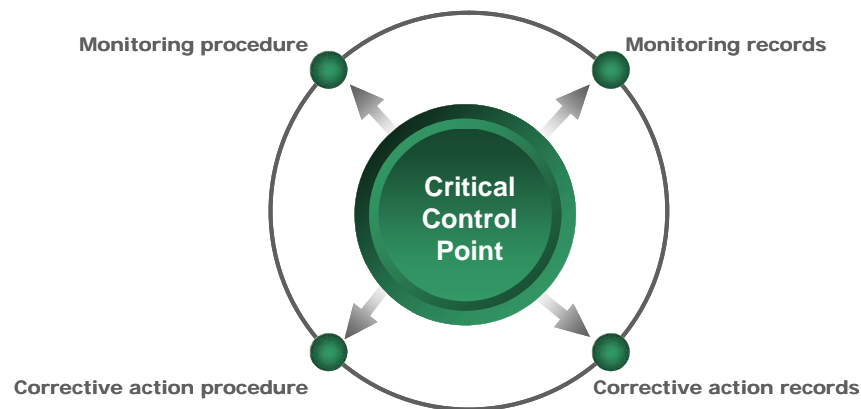
## Critical Control Points

The most important items in any HACCP 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.

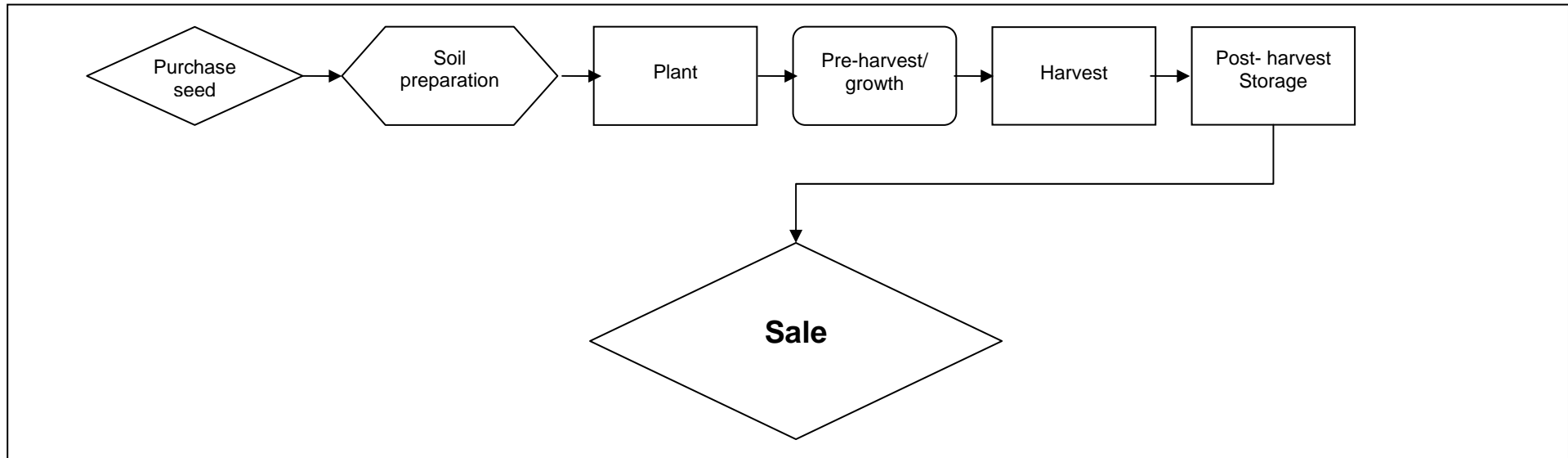
**Chemical-** the presence of harmful chemicals not related to biological entities, such as pesticides

**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.



### Task

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 2 Hazard analysis**

<i>Step</i>	<i>Answer</i>	<i>Hazard</i>
Purchase seed grain	Q1) No	
	Q2) Yes	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 <i>F. graminearum</i> purchase for planting on the Atherton Tableland)
Storage of seed	Q1) No	
	Q2) No	
Soil preparation	Q1) No	
	Q2) Yes	Soil contaminated with <i>Fusarium graminearum</i> inoculum from previous wheat crop
		Soil contaminated with <i>Aspergillus flavus</i> inoculum from trash of previous crops
		Soil of uneven depth or moisture holding capacity due to field levelling over different soil types or rocky outcrops.
Planting	Q1) No	
	Q2) Yes	Planting time could expose developing kernels to high temperatures & low precipitation at anthesis and the following 20 days
Pre-harvest/ Growing	Q1) Yes	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
	Q2) No	
Harvest	Q1) No	
	Q2) Yes	Damage to kernels from harvester
		Kernels insufficiently dried and susceptible to damage
		Rainfall or high humidity around harvest risks high moisture
Storage	Q1) Yes	Moisture content of kernels excessive
		Insect attack, allowing fungi to penetrate kernel
		Insufficient aeration, allowing moisture migration and fungal growth
		Storage container contaminated with dusts containing high concentrations of fungal spores
	Q2) No	

## Determining Controls, Critical Control Points & Good Agricultural Practice

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### 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 3 Defining Controls, GAPs and CCPs**

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

\*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**

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

### 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**

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

### 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.

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

## Verification

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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 fumonisin. 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 fumonisin 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](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**

<i>Mycotoxin (mg/kg)</i>	<i>Milling</i>	<i>Prime</i>	<i>Feed #1</i>	<i>Feed #2</i>
Total aflatoxins	0.005	0.015	0.02	0.08 (0.02 B <sub>1</sub> )
Total fumonisins	2	5	10	40



## 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 (eg. '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.

**Table 7 Verification plan**

<i>Mycotoxin</i>	<i>Laboratory</i>	<i>Sampling</i>	<i>When?</i>
Aflatoxins B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub>	"Acculab", Brisbane	<ul style="list-style-type: none"> <li>10 x 200g samples from each truck taken using the spear sampling method.</li> <li>Samples from 10 trucks combined, mixed well and divided using riffle divider into 4 x 5 kg samples.</li> <li>All 5 kg of each sample ground in a Romer Mill on the finest setting; 200g sub-sample taken before</li> <li>One sample submitted to lab, other kept by stakeholders.</li> </ul>	Immediately prior to storage or sale
Fumonisin: B <sub>1</sub> , B <sub>2</sub> , B <sub>3</sub>	"Acculab", Brisbane	<ul style="list-style-type: none"> <li>10 x 200g samples from each truck taken using the spear sampling method.</li> <li>Samples from 10 trucks combined, mixed well and divided using riffle divider into 4 x 2 kg samples.</li> <li>All 2 kg of each sample ground in a Romer Mill on the finest setting; 200g sub-sample taken before assay</li> <li>One sample submitted to lab, other kept by stakeholders.</li> </ul>	Immediately prior to storage or sale

## 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**

Step in process	Hazard	Good Agricultural Practice
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 <i>F. graminearum</i> purchased for planting on the Atherton Tableland)	Select seed in accordance to advice from reputable seed dealer
Soil preparation	Soil contaminated with <i>Fusarium</i> inoculum from previous wheat crop	Avoid rotating wheat and maize crops in susceptible areas
	Soil contaminated with <i>Aspergillus</i> inoculum from crop residues	Plough trash into soil, ensuring good soil/plant contact
	Soil of uneven depth or moisture holding capacity due to field levelling over different soil types or rocky outcrops	Prepare maps of fields showing shallow areas, that can be monitored for stress and harvested separately – aerial photography with NDVI imagery
Planting	Planting time could expose developing kernels to high temperatures & low precipitation during kernel development	Avoid planting times which will lead to the period of anthesis and the following 20 days occurring in periods of hot, dry weather.
Harvest	Rainfall or high humidity around harvest risks high moisture	Check weather reports and harvest earlier if necessary
Storage	Storage container contaminated with dusts and residues containing high concentrations of fungal spores	Decontaminate container before storage

**Table 9 HACCP plan**

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

## 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 10 Extra CCPs for export hazards**

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

## Links

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- **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  
<http://www2.dpi.qld.gov.au/fieldcrops/8606.html>
- **Maize: NSW planting guide**  
[http://www.dpi.nsw.gov.au/\\_data/assets/pdf\\_file/90273/maize-nsw-planting-guide-2006-07.pdf](http://www.dpi.nsw.gov.au/_data/assets/pdf_file/90273/maize-nsw-planting-guide-2006-07.pdf)
- **Maize Association of Australia**  
<http://www.maizeaustralia.com.au/>
- **The Cob-** magazine of the Maize Association of Australia  
<http://www.maizeaustralia.com.au/cob.htm>
- **Transport Information Service: cargo loss prevention information from German insurers**  
[http://www.tis-gdv.de/tis\\_e/ware/getreide/mais/mais.htm](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  
<http://archive.gipsa.usda.gov/reference-library/handbooks/aflatoxin/aflatoxin-hb.pdf#search=%22usda%20aflatoxin%20handbook%22>
- **Practical Procedures For Sampling Grain At Farm Sites And Remote Locations-** Grain Inspection Packers & Stockyards Administration  
[http://archive.gipsa.usda.gov/pubs/practical\\_sampling.pdf](http://archive.gipsa.usda.gov/pubs/practical_sampling.pdf)
- **NATA.**  
<http://www.nata.com.au>



## Further reading

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- **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  
<http://www.agric.nsw.gov.au/reader/general-farm-practices/manage-on-farm-grain-store>
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