Supply Chain & Export Protocols for Managing Mycotoxins in Australian Maize

Purpose: Development of these protocols was commissioned by the Maize Association of Australia. The purpose is to enhance the reputation for quality of Australian maize, to better meet the needs of end-users, and to facilitate export marketing by highlighting all steps that should be taken by a prospective exporter, in order to ensure that shipments meet internationally accepted standards for aflatoxins, fumonisins and other mycotoxins. The protocols provide a guide that can succeed with the input and support of all industry stakeholders.

Summary of protocols:

1. *Determine requirements of end-user, or importer and the importing country*
   - 1.1. Which mycotoxin standards will apply?
   - 1.2. What grain sampling protocols will apply?
   - 1.3. Which mycotoxin assay methods will be used?
   - 1.4. How will the assay results be interpreted (pass/fail criteria)?
   - 1.5. What MRLs apply for grain storage chemicals?
   - 1.6. How many days after arrival will ownership and risk pass to importer?
   - 1.7. Draw up contract to incorporate these requirements.

2. *Identify maize from crops with low risk of aflatoxin/other mycotoxins*
   - 2.1. Source from growers using effective, documented quality standards.
   - 2.2. Source from well-grown crops not exposed to heat and water stress.
   - 2.3. Conduct confirmatory mycotoxin assay before purchase.
   - 2.4. Use a laboratory that is NATA certified for mycotoxin assay.
   - 2.5. Decide if maize is suitable for export on basis of test results.

3. *Check that crop is harvested and stored in good conditions*
   - 3.1. Avoid harvesting lightweight maize and extraneous material.
   - 3.2. Check moisture at intake, using appropriate sampling protocols. Dry grain if necessary to below 14% moisture.
   - 3.3. Select appropriate type of storage and ensure aeration and/or good ventilation.
   - 3.4. Manage night-day air flows to avoid moisture condensation.
   - 3.5. Control storage insects, using appropriate methods. If using chemicals, ensure compliance with MRLs of importing country.
   - 3.6. Gravity grade grain to remove light and damaged kernels.
   - 3.7. Assay to ensure mycotoxin concentration is well below target level.

4. *Specify and monitor transport/shipping conditions*
   - 4.1. Identify a suitable carrier and the best route.
   - 4.2. Choose Food Grade containers and inspect for cleanliness and suitability.
   - 4.3. Load grain and insert moisture absorbents.
   - 4.4. Protect container from excessive heat during transport (no top stowage).

5. *Oversee protocols for hand-over of shipment to client*
   - 5.1. Ensure importer is notified on arrival.
   - 5.2. Ensure mycotoxin testing is conducted as soon as possible after arrival.
   - 5.3. Review effectiveness of these export protocols and modify as required.

Introduction

Mycotoxins can be produced by certain moulds growing in maize. Mycotoxins cause disease in humans, companion animals and livestock, and are domestically and internationally regulated. A short review of the occurrence of mycotoxins in Australian maize is an APPENDIX to these protocols.

Australia exports about $11 million of maize each year, compared to a total production worth about $77 million. Australian maize is generally of high quality in regard to mycotoxins by comparison with maize produced in other countries, yet there have been recent trade problems when containers of maize failed to meet the quality standards expected for aflatoxin upon arrival at destination. Whether the contamination occurred at source or during transport is not clear, but many factors could have contributed. Domestic end-users of maize are also concerned about the risks to their enterprises from mycotoxins.

It is desirable that a consistent and coordinated approach is taken to detect contaminated maize and ensure that it is diverted away from human food and sensitive markets but this is difficult with production and marketing spread among many different groups and regions. Some sections of the industry regularly test maize for mycotoxins, but other sections are unaware of the potential problem, leaving the industry vulnerable to incidents of contamination.

Accordingly, the Maize Association of Australia proposes to establish a set of protocols to guide all participants in the maize supply chain, including potential exporters of maize, in order to minimise the risks. This document provides a list of proposed protocols, with background and supporting information.

Proposed supply chain & export protocols, and supporting information

1. Determine requirements of end-user, or importer and the importing country

1.1. *Which mycotoxin standards will apply?*

1.1.1. Which mycotoxins are of concern, and what is the standard that needs to be met?

Aflatoxins are most important. Standards can refer only to aflatoxin B1, or to total aflatoxins (B1+B2+G1+G2). A common limit is <0.005 mg total aflatoxins/kg for human food, and this is the industry standard for milling maize in Australia. There is no Australian standard for aflatoxins in maize, but these must be ‘As Low As is Reasonably Achievable’ (the ALARA principle). The ALARA principle recognises the difficulties in trying to ensure that all maize is entirely free of aflatoxins (even though Australian maize is generally of better quality than that produced in other countries, because of fewer storage problems).

The effects of processing also must be considered in deciding which standard to apply. Grits contain less aflatoxin than the feed maize, since aflatoxin tends to be concentrated in the surface layers of the grain. Aflatoxin is not oil-soluble, so maize oil does not have problems. Aflatoxins can be associated with protein, so gluten can have somewhat higher concentrations than the source maize, but maize products used as thickeners or additives are substantially diluted in the final product. Baking processes also cause some reductions. All this should be taken into account in the risk assessment. It is proposed that in many cases, the Prime maize standard of 0.015 mg aflatoxins/kg is perfectly adequate for raw maize ingredient.

Maize for stockfeed is allowed to contain up to 0.05 mg/kg aflatoxin B1 under Queensland Regulations and the maize industry has set limits for various maize grades. These regulations are soundly based on known tolerances of different animal species to aflatoxin, and it would be economically unwise to apply unnecessarily stringent limits to feed maize with the additional costs inevitably involved.

1.1.2. For export, mycotoxin standards vary between countries, and import regulations often allow little room for negotiation. Aflatoxins are most widely regulated, at limits ranging from 0.005 to 0.02 mg aflatoxins/kg. The specific regulations that apply in that country for imported grain must be clarified in consultation with the importer. An importing country might apply the human standard regardless of intended use because of local regulations.

1.1.3. Fumonisins are moderately common in maize in most countries, but not yet widely regulated. A limit of 2 mg fumonisins/kg is the industry standard for milling maize in Australia, and although 1 mg/kg has been discussed in some trade forums, lower limits appear unlikely to be applied by our trading partners at present.

1.1.4. Zearalenone, nivalenol and deoxynivalenol are quite uncommon in Australian maize, except in a few coastal regions of southern Qld, northern NSW and the tablelands of far north Qld. They are not internationally regulated, but 1 mg/kg is the most stringent advisory standard in the USA, with higher concentrations accepted for tolerant classes of livestock. A limit lower than this would be difficult to justify by an importing country.

1.1.5. Ochratoxin A is regulated in grain in a few countries like the EC and Canada, but is rare in Australian maize without storage problems. It is not currently regulated domestically or internationally but 0.005 mg/kg is being considered.

1.1.6. A few other mycotoxins (cyclopiazonic acid, citrinin, moniliformin, T2-toxin) have been detected in maize elsewhere, but there are no indications that these are significant in Australian maize.

1.2. What grain sampling protocols will apply?

1.2.1. Mycotoxins can have an extremely irregular distribution in bulk maize, and aflatoxins are probably the worst in this regard. This arises because only a small proportion of ears in a maize field can be contaminated, and the resultant contaminated kernels (which can represent <0.1% of total kernels) are not evenly distributed through bulk grain during the harvest and storage processes. This problem can be compounded if any further mould growth and aflatoxin production occurs in storage – since this occurs in small pockets where moisture has condensed.

1.2.2. *Fusarium* mycotoxins (fumonisins, zearalenone, nivalenol, deoxynivalenol) also have an irregular distribution, though less so than aflatoxins. However, in this case the contaminated kernels are often lightweight (see 3.1.2 below), and the augering and transport operations cause these to segregate and accumulate in pockets.

1.2.3. The usual purpose of sampling is to determine the average mycotoxin content of the given batch of maize, and determination of what this 'batch' consists of can be an important decision. In some cases, the batch will be a truckload or a single container, but it might be a bulk grain silo. Such a decision should realistically be based on the total amount sold or processed, be that continuous milling or batch processing. However, it is recommended that any batch exceeding 300 tonne should be treated as more than one batch, and sampled accordingly.

Once this is decided, it is essential that the sampling is systematic, and that the sampling design (sample sizes and frequency) has been validated to match the variability of the mycotoxin distribution. It should also be random, in that it does not over-sample or under-sample from any pockets of maize of different appearance. The extremely irregular distribution of aflatoxins dictates that a large number of small samples are taken in a

systematic way, and well mixed before the final sample is drawn. The Grain Fungal Diseases and Mycotoxin Reference published by the Technical Division of the Grain Inspection, Packers and Stockyards Administration (GIPSA) of the USDA gives details of sampling designs for maize. (www.gipsa.usda.gov).

1.2.4. ***Sampling bulk maize:*** The size of the sample submitted to the laboratory must be much larger when sampling mycotoxins than for moisture or protein. For example, if the purpose is to accurately detect aflatoxin, GIPSA has determined that the optimum sample size for maize is a minimum of 10 pounds (4 kg), which must be ground before a 500 g analytical sample is taken. Certain types of continuous flow mill are required, such as the Romer mill. Details of grinders are given in the Aflatoxin Handbook, published by GIPSA (www.gipsa.usda.gov).

A study quoted by GIPSA has shown that for a bulk batch of maize of average content of 0.020 mg aflatoxins/kg, several 500 g samples could range from 0.001 to 0.050, several 1 kg samples from 0.003 to 0.039, 2.2kg samples from 0.008 to 0.032, and 4 kg samples from 0.012 to 0.028 mg/kg. Observations in Australia support similar variability, so the conclusion is clear that if any reliability is to be attached to an aflatoxin assay conducted on bulk grain, then a minimum of a 4 kg sample comprised of a large number of smaller samples should be ground and well mixed before assay. Bulk maize is best sampled from the grain stream during loading or unloading, at a rate not less than 100 g/tonne. The combined samples are then well-mixed and the composite or 'submitted' samples withdrawn with a riffle divider.

1.2.5. ***Sampling from trucks:*** While the 10 lb (4 kg) sample is the GIPSA standard for maize in bulk, a smaller 2 lb (0.9 kg) sample was established by GIPSA for domestic deliveries by truck and rail car because of industry concerns over the increased costs of inspection. (Grain Fungal Diseases & Mycotoxin Reference, p25, www.gipsa.usda.gov). It is likely that similar decision will be made in Australia. However, the consequences if these are conducted on the smaller samples on the reliability of aflatoxin assay results must be clearly understood.

Let us assume that a potential grain exporter is seeking to meet a standard of 0.005 mg aflatoxins/kg with a high degree of confidence, and intends to test each truckload of maize at delivery into bulk store. Also assuming a similar aflatoxin variability as in the previous example, then several assay results from a truckload of average content of 0.004 mg aflatoxin/kg could range from 0.002 to 0.006 mg/kg if several 4 kg samples were taken and ground before assay, but from <0.001 to 0.010 mg/kg if only 500 g samples were ground before assay. It can be seen how the smaller sample could produce a very misleading result.

Another way to view this problem is to examine the consequences of grinding only 1 kg, and obtaining a single assay result of <0.001 mg/kg for truckload 'M' of maize. If we are dealing with a large number of incoming truckloads (A-Z) of maize, with 80% of these being negative for aflatoxin (<0.001 mg/kg), then it is highly probable that repeat assays on 1 kg samples from truckload M will also be <0.001. However, there remains a small possibility that the 'true' value for truck M is 0.004 mg/kg, and that repeat assays could range from <0.001 to 0.010 mg/kg. On the other hand, if only 50% of truckloads A-Z were <0.001, then there is a high probability that a single assay result of <0.001 could be from a positive load of 'true' value of 0.006 mg/kg and further assay results might range up to 0.013 mg/kg. If, in this same scenario of 50% negative truckloads, a 4 kg sample was ground for assay, then we greatly reduce the risk that our <0.001 result will later be tested at >0.004 mg/kg.

To summarise, if 80% of truckloads are negative for aflatoxin, grinding 1 kg samples might be an acceptable risk if the aflatoxin target is <0.004 mg/kg. However, if only 50% of truckloads are likely to be negative, then we should grind a 4 kg sample to have an

acceptable risk of making a correct decision in regard to meeting a <0.005 mg/kg standard.

Fortunately, surveys of Australian maize indicate that most maize in most growing regions in most seasons would meet the criterion of having >80% of deliveries <0.005 mg aflatoxin/kg. Consequently, an end-user with stringent requirements like an exporter can reduce the risk at source by not procuring maize for export purposes from regions where there is a greater risk of aflatoxin (eg rainfed crops grown in very hot conditions), and by inspecting each crop for indicators of stress before contracting to purchase.

When sampling from trucks and containers, samples must be drawn using a sample probe or 'trier' in order to reach different depths and specific positions according to set patterns. The sampling frequency should also be specified, but one sample of about 100g for each tonne of maize should be the absolute minimum starting point. For a 25 tonne truck, this will result in a minimum of one 2.5 kg sample. As explained above, minimum samples of 4 kg must be ground and sub-sampled to achieve a reasonable measure of aflatoxin content, but in the interests of speed and throughput at grain intake, samples of 1 – 2 kg might be used for rapid screening processes such as % damaged grain and BGYF testing (or perhaps NIR) aimed at assessing relative risk of serious contamination. These results can then guide decisions on accepting or rejecting or 'segregate, hold and assay' decisions to minimise risks at intake.

It is emphasized that maize intended for domestic stockfeed can contain up to 0.080 mg aflatoxins/kg (NACMA Feed Grade 2), and as 99% of Australian maize deliveries meet that standard or better, smaller samples and much less frequent testing can be justified. The premium paid for milling grade and export quality maize should take into account the need for, and costs of, the additional mycotoxin tests required.

1.2.6. ***Sampling bagged maize*** also should be systematic and specified. In this case, it should also be agreed that a single composite sample is to be taken from a minimum of 10% of the bags, and of a minimum of 4 kg should be ground before assay.

1.3. Which mycotoxin assay methods will be used?

1.3.1. The most precise methods are based on high performance liquid chromatography (HPLC), which can measure individual mycotoxins, but these require expensive equipment and highly skilled staff, so they are a little slower and more costly than other methods. HPLC can be used for most mycotoxins, although different mycotoxins require different extraction processes.

1.3.2. Immunoassay methods such as enzyme-linked-immuno-assays (ELISA) are highly specific, sensitive and robust, but less accurate and precise (more variable) than HPLC. These are the most commonly used methods because less equipment is required. They might not measure individual mycotoxins. For example, aflatoxin test kits usually measure an approximation of 'total' aflatoxins B1, B2, G1 & G2, since not all are detected with equal sensitivity. These are very suitable for first-line testing of largely negative samples, but not as satisfactory for precise determination of concentrations in positive samples. Assays must be routinely run in duplicate because of variations across ELISA plates. Care has to be taken to ensure that sample extracts remain in the quantifiable range. ELISA kits are available for all mycotoxins of interest.

1.3.3. Thin layer chromatography (TLC) (and variations) is still used for screening large numbers of mainly negative samples. Highly specific in skilled hands but less precise than HPLC. Skill is required, but less expensive equipment than HPLC. Individual mycotoxins (aflatoxins, ochratoxin, zearalenone) can be assayed, often in a single operation. Fumonisin and trichothecenes are not as well suited to assay by TLC.

1.3.4. To summarise, ELISA can provide the fastest result for routine screening of large numbers of mainly negative samples, but HPLC is the method of choice when the highest accuracy and precision are required. It is very important to discuss with the chosen laboratory which method will be used and the 'confidence limits' of that method. These 'confidence limits' show the normal variability of the method itself which is separate to the sampling variability discussed above.

1.4. ***How will the assay results be interpreted (pass/fail criteria)?***

1.4.1. Some countries specify 'negative' for certain mycotoxins. This usually relates to the sensitivity of the method they regard as standard. Find out what this is!

1.4.2. How will the importing authority deal with a positive test? Often, it is the practice to assay a duplicate sample as a check on the method. Other authorities and laboratories might take several additional samples for testing. Will multiple test results be averaged, or will one sample exceeding the standard cause rejection? The latter criterion might be impossible to meet, given normal variation in aflatoxin content within bulk maize. In addition, it is important to agree on what the 'batch' consists of, that the testing purports to represent – is it the entire container, parts of it, or several containers? (see 1.2.3).

1.4.3. Will a duplicate sample be retained for an independent assay if case of dispute? Is the regulatory agency or emd-user prepared to use different methods to test the result?

1.5. Draw up contract to incorporate these requirements

1.5.1. Decide on a plan to meet the client's requirements. When will mycotoxin tests be performed, how often and at what cost? The contract price must include the costs of all mycotoxin assays.

1.5.2. There is much maize available on the world market. Aflatoxin contamination is a serious trade barrier for maize grown in most countries of south-east Asia. Developed importing countries expect to pay a premium for a guarantee that Australian maize is free of aflatoxin and other mycotoxins. You must be able to provide that guarantee.

2. Identify maize from crops with a low risk of aflatoxin/other mycotoxins

2.1. ***Source from growers using effective documented quality standards***

2.1.1. Documentation is your best guarantee that your supplier understands your requirements in regard to minimising risks of contamination. It requires a commitment on their part to oversee the process and to assume responsibility for it.

The World Health Organisation and Codex Alimentarius have supported the Hazard Analysis and Critical Control Point (HACCP) system for quality control. A guide to preparing a Quality Plan based on HACCP principles for Australian maize will soon be available on the Maize Association website. (www.maizeaustralia.com.au)

2.2. ***Source from crops not exposed to heat and water stress***

2.2.1. Suitable hybrids should be selected for the region and grown at optimal spacing to minimise water stress. This might require a trade-off in terms of some reduction in potential yield. Good nutrition and insect control are also important.

2.2.2. For aflatoxin control, sowing time should be adjusted so that early kernel development does not coincide with extreme summer temperatures (January/February). Climatic modelling using historic regional weather data has provided some guide to aflatoxin risk.

For example in the Burnett region, early-maturing hybrids grown under rainfed conditions will on average have a lower aflatoxin risk when planted in October/November than slower hybrids, but when planted in December/January, the early-maturing hybrid have the higher risk. Maize grown under irrigation has much lower risk, but inadequate irrigation due to shallow soil patches or uneven field levelling can provide stressed patches, which if exposed to January temperatures in the MIA will have a high risk of aflatoxin.

2.2.3. Overall, the risks of aflatoxin are lowest in adequately irrigated crops and in cooler locations of northern NSW, southern Qld and the tablelands of far-north Qld.

2.2.4. Increased fumonisin contamination has also been associated with stress from uneven and inadequate irrigation, inadequate nutrition, and insect damage.

2.2.5. Inspect the crop in the field. Some indicators of stressed crops and higher mycotoxin risk are: variable growth; poor weed control; high % of insect damage; obvious mould and cob rots, apart from on the tip; other signs of disease such as stem rot, loose ears and boiled smut; and dull (not bright) grain. This is the best time to reduce your risks by not purchasing for milling or exporting- it is much more suitable for ruminant feed.

2.3. ***Conduct confirmatory mycotoxin assay before contracting to purchase.***

2.3.1. This step would be most applicable when purchasing maize in bulk from another supplier, rather than directly from farm. Ensure appropriate protocols for grain sampling are followed – large samples of 4 kg must be collected and milled before assay to produce a reliable result (See section 1.2.4 above).

2.3.2. Testing prior to harvest is occasionally done, but the irregularity of mycotoxin contamination will not permit an accurate estimate. It might be useful to test stressed portions of a maize field, as a ‘worst case’ scenario. A large number of ears would need to be taken in a systematic manner, shelled, mixed and a large sample ground before assay.

2.3.3. For maize being moved either into or out of storage, the best process is to take regular samples from the grain stream. For large tonnages, a rate of about 100 g per tonne is suggested, to form a composite sample of 30 kg per 300 tonne. This is mixed well and reduced using a riffle divider until four samples, each of 4 kg, are taken. One is submitted to the laboratory, one retained by the potential exporter or his agent, one retained by the grower, and the fourth sent to the potential importer. All samples must be kept cool and dry and in clean conditions.

2.4. Use a laboratory that is NATA certified for mycotoxin assay

2.4.1. Obtaining an accurate result in any laboratory depends on the interaction of many factors: effective management with knowledge of client’s needs, skilled and well-trained staff, regularly maintained and calibrated equipment, validated methods and techniques, and regular practice on reference samples of known mycotoxin content. Any of these factors can and do go awry at times. Certification with the National Association of Testing Authorities (NATA) provides a safeguard that the operation of the laboratory is regularly checked by other experts in laboratory practice and also that its analytical performance is satisfactory in comparison to other laboratories.

A list of laboratories certified for aflatoxin and other mycotoxin assays can be obtained by searching the NATA website (www.nata.asn.au). Costs of NATA accreditation in terms of additional check sample assays, extensive documentation, calibration, staff training, and fees can add 30-40% to the costs of assays, but can pay off in increased reliability of the results.

2.4.2. As the client, you need to discuss your needs with the chosen laboratory, and to ascertain if the assay method to be used has sufficient accuracy and precision for your purpose (see section 1.3). You can also ask for details of their certification, and evidence of method validation. Ensure that the chosen laboratory is aware of these protocols, and will abide by your requirements, particularly in regard to grinding of large samples before sub-samples are drawn for assay.

2.5. Decide if maize is suitable for end-use, such as export

2.5.1. On the basis of the results obtained, and your confidence about the result arising from your decisions over sampling and methods, decide if the maize is suitable for export, or better used for stock feed. Providing sampling protocols and methodology are up to scratch (section 1.2.5), a negative mycotoxin test of <0.001 mg/kg should provide good assurance that the maize will pass inspection at the other end. However, if any aflatoxin is detected, even at low levels <0.005, consider the greater risk of further fungal growth during transport in a container in tropical conditions.

3. Check that the crop has been harvested and stored in good conditions

3.1. avoid harvesting lightweight maize and extraneous material

3.1.1. Harvest as soon as grain is mature and sufficiently dry, and when rainfall is not expected. Delaying harvest can increase aflatoxin (and possibly fumonisins) in previously stressed and infected crops.

3.1.2. Mould growth in maize tends to first consume the starch and oil fractions, leaving a less dense fibrous kernel. Studies have shown that a major proportion of mycotoxins can be present in the lightweight fraction of grain. Minimise harvest of lightweight grain by increasing air flows during shelling.

3.1.3. Avoid trash and weed seeds in grain. These greatly impair storage, since such material is often moister than the maize, and because it blocks aeration channels in the grain.

3.1.4. Avoid harvesting stressed patches of plants when these are apparent. Such maize could be harvested separately for less-demanding markets or ploughed in.

3.2. Check moisture at intake, using appropriate sampling protocols

3.2.1. Ensure that the samples for moisture determination are taken is a representative manner. Take as many samples as is feasible and in any batch of maize which you suspect of varying from the norm. It will be better to take several moisture determinations as a check on variability.

3.2.2. Use any of the standard devices for moisture determination, but ensure that they are properly calibrated, operated correctly and well maintained. Keep records of the calibrations.

3.3. Dry grain if necessary

3.3.1. Maximum moisture content for maize in the Australian climate is 14%, but it might be necessary to aim at 12-13% to reduce the risk that some grain pockets could exceed 14%.

3.3.2. If any significant portions exceed 14%, drying is required. Do not rely on mixing during harvesting and augering into silos to 'blend' wet and dry grain to meet target levels of <14%: most often little such blending actually occurs and moist grain will remain segregated.

3.3.3. Drying procedures, air temperatures and flow rates, will depend on the type of storage involved and ambient temperature and humidity. Too high a heat will affect grain quality. Advice should be sought from producers of drying and storage equipment, and from grain storage experts.

3.4. *Select appropriate type of storage and manage it well*

3.4.1. General hygiene of storage facilities cannot be emphasized enough. Accumulated grain dusts and caked residues can contain extremely high concentrations of fungal spores and mycotoxins, and also harbour storage insects. Only 1 kg of such material could raise the aflatoxin content of 20 tonne of clean grain to >0.005 mg/kg when it dislodges and falls into clean grain, and it can also form a nucleus of new fungal growth. Thoroughly clean all storage containers and equipment like augers and belts on which grain residues accumulate, after each use. Completely empty silos on a rotational basis for this purpose, so that walls and struts can be effectively cleaned.

3.4.2. Do not keep storage vessels for maize sealed without aeration – even maize at <14% moisture will release some moisture as a result of heating and cooling (see 3.5), and this will accumulate and start mould growth in sealed silos.

The ability to seal silos is very important for fumigation, but once fumigation is completed, regular aeration of any grain is essential (see 3.5). Long experience suggests that good aeration is particularly important for maize, compared with wheat and barley.

Do not confuse this process with sealed high-moisture maize storage (>20%), which relies on rapid fermentation to control moulds – such grain is only suitable for stockfeed.

3.4.3. Vertical silos used to store maize have had problems from 'racheting', in which expansion of the metal silo wall in high temperatures allows the grain to settle, followed by contraction that compresses the grain. This expansion and contraction under pressure fatigues the metal over time and can lead to buckling and collapse. This can be avoided by regularly drawing grain from the bottom of the silo and replacing it in the top to relieve the pressure. Some experienced organisations now store maize in sheds, with tunnel aeration to bypass the problem.

3.5. Manage night-day air flows as appropriate for ambient temperatures to avoid moisture condensation

3.5.1. Even maize stored at <14% moisture will release some humidity into the air under the influence of temperature changes. Cooling of the metal silo wall and roof at night promotes condensation of this moisture, which will then begin to accumulate in pockets and support mould growth. Maintaining a steady air flow through the grain at certain times is the only way to prevent this. Consult grain storage experts for correct flows for your storage facilities.

3.5.2. Aeration will not work if ambient air is hot and humid. Only aerate grain when relative humidity is < 80% and temperature is < 20^{o}C (usually at night). This can be set automatically.

3.6. Control storage insects, using appropriate chemicals

3.6.1. Apart from general effects on grain quality, storage insects promote mould growth by transporting spores into the kernels they damage, and by releasing additional moisture from the grain as they consume the starch. Keeping grain cool with proper aeration is an important control on insect activity.

3.6.2. Use pictorial guides and expert opinion to correctly identify the insects present. Fumigation and chemical treatments should be carefully targeted against these pests, using chemicals approved for the purpose.

3.6.3. Ensure that the chemicals are used appropriately and that the grain will comply with Maximum Residue Limits (MRLs) of an importing country.

3.7. Gravity grade grain to remove light and damaged kernels

3.7.1. Tests have shown that a high proportion of mycotoxins present in some batches of maize can be in the broken and lightweight grain fractions (see 3.1.2). This particularly applies to heavily contaminated loads, for example, grading of several batches of maize from the MIA and central NSW that were heavily contaminated with fumonisin and aflatoxin, reduced concentrations by over 70%, and allowed its use as stockfood.

3.7.2. It is less clear as to how effective grading is on maize containing lower aflatoxin concentrations (0.005 – 0.010 mg/kg) for the purpose of meeting a milling standard. However, grading is still recommended for milling and export maize, since any broken grain is much more vulnerable to infection and mould growth during storage.

3.8. Assay to ensure mycotoxin concentration is well below target level

3.8.1. Conduct mycotoxin assay of cleaned grain just prior to shipping (see sections 2.3 and 2.4). Carefully document all sampling procedures and the results obtained in case of dispute.

3.8.2. From the detailed explanation of variations in sampling and assay of maize for mycotoxins (sections 1.2 and 1.3), it can be seen that there will always remain some risk that a laboratory in another country will obtain a higher result than the assay performed before shipping. We must add to this the risk of some increase during transport.

In regard to the sampling and analytical risks, it should be clear that these will be much higher in a shipment of maize assayed at 0.004 mg aflatoxins/kg in Australia, than one assayed at <0.001 mg/kg.

In regard to the risks of mould growth during shipping, a sample assayed at 0.004 mg aflatoxin/kg definitely contains kernels infected with the *Aspergillus* fungus, and one assayed at <0.001 might not. Once again, the risks are much greater with the former shipment.

The costs of shipping and consequences if a shipment is rejected dictate the precaution of only shipping maize which has no detectable level of aflatoxin.

3.8.3. For fumonisin and other *Fusarium* mycotoxins, there is less variability as a result of sampling and assay than with aflatoxin (provided these are performed correctly), and

these *Fusarium* fungi will not grow in maize stored in these conditions. Consequently, a result of <50% of the target level prior to shipping should cover the risk.

4. Specify and monitor transport/shipping conditions

4.1. Identify suitable carrier and the best route

4.1.1. The key message is speed and reliability. The longer that sealed containers sit on docks and shipboard in tropical temperatures, the greater is the risk of aflatoxin development.

4.2. Inspect containers for cleanliness & suitability

4.2.1. Ensure these have been thoroughly cleaned and disinfected. Use only food-grade containers, and ensure they do not have any gaps and leaks through which water or insects can enter.

4.3. Load grain & insert moisture absorbents

4.3.1. Compare risks for bagged versus bulk grain. Bagged grain will allow more air movement, and reduce condensation. Bag material could have an effect – Hessian and similar absorbent bags will allow better aeration, but synthetic woven bags can shed dripping moisture.

4.3.2. Insert absorbants to reduce humidity and minimise condensation. These are commercially available and based on diatomaceous earth (bentonites, zeolites) or silica-gel. They can come with adherent strips for attachment to sides of container. One such product is ‘container dri’ (www.sud-chemie.com).

4.4. Protect container from excessive heat during transport

4.4.1. Maize should be regarded as a perishable food product. Containers should be loaded shortly prior to shipping, and not left on wharves for long periods in summer.

4.4.2. On ship, containers should be loaded below deck, where it is cooler (‘no top stowage’).

5. Oversee protocols for hand-over of shipment to client

5.1. Ensure importer is notified on arrival.

5.1.1. Determine when the shipment will arrive at destination, and request the importer to oversee container storage until quarantine procedures are completed. Request importer to facilitate clearance when possible.

5.2. *Ensure mycotoxin testing is conducted as soon as possible after arrival*.

5.2.1. Advise the importer that this is perishable material that needs to be placed into ventilated storage as soon as possible. It must not be left in the container for long periods.

5.2.2. Advise local authorities that any required mycotoxin testing needs to be done quickly, so that the material can be placed in suitable storage

5.2.3. Determine at which stage the responsibility for any further potential deterioration passes to importer – this should be covered in the contract and insured.

5.3. ***Review effectiveness of these protocols and modify as required***.

5.3.1. This set of protocols will only remain useful if any deficiencies and possible improvements are brought to the notice of the Maize Association of Australia.

Acknowledgement

Much of this information has been drawn together as part of a project called 'Managing mycotoxins in maize' led by the Queensland Department of Primary Industries and Fisheries in collaboration with the NSW Department of Primary Industries, University of Qld, University of Sydney, and supported by the Grains Research & Development Corporation.

Comments to:

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Mycotoxins in Australian Maize

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This is a brief overview of current knowledge of mycotoxins in Australian maize. A comprehensive document with references is available from the author, or from the Maize Association of Australia.

Aflatoxins

The aflatoxins are a group of compounds produced by *Aspergillus flavus* and *A. parasiticus.* When chemically extracted and viewed under ultraviolet light, two fluoresce with a blue colour (aflatoxins B1 & B2) and two with a green colour (G1& G2). Aflatoxin B1 is one of the most potent liver carcinogens known, and has been associated as a co-carcinogen with hepatis B in the high incidence of liver cancer in parts of Africa and south-east Asia. Maize and peanuts grown in Africa and south-east Asia have serious aflatoxin contamination problems. Aflatoxins can also cause acute affects if ingested in high doses, such as occurred in Kenya during 2004 when consumption of aflatoxin contaminated maize affected 317 people, with 125 deaths. Natural cases of human disease caused by aflatoxin have never been recorded in Australia, although livestock have been poisoned in the past. It is clearly important that management systems are in place to ensure exposure to aflatoxin is minimised, and that Australian maize can be demonstrated to meet international standards.

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. *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 invade.

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 flowering, and if average temperatures exceed 27°C and approach 32°C, two conditions are met: firstly, the natural resistance of the maize plant to fungi in general is compromised; and secondly, the relatively heat-tolerant *A.flavus* has the advantage over other fungi present. At this stage, windblown fungal spores (*A. flavus* spores are highly resistant to desiccation) can enter through the silks. Physical damage to the ear from insects (especially boring insects) or birds also is a critical factor in aflatoxin contamination, since it exposes the endosperm to premature drying and *A. flavus* invasion. Aflatoxin contamination is often limited to ears in a small section of a field, for example if soil depth is shallower and/or irrigation across the field is uneven. After harvest, most aflatoxin can be present in a tiny proportion of kernels (eg 0.1%) 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 avoid extreme temperatures during the critical period of kernel formation, maintaining irrigation evenly across fields while monitoring any sections where soil holds less moisture, good nutrition, insect control, early harvest, minimising light-weight material at harvest, and drying (if necessary) to <14% moisture before storage.

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 night-day air flows as appropriate for ambient temperatures to avoid moisture condensation; and controlling insects with appropriate chemicals.

Ochratoxin A

This mycotoxin is produced in maize by *Aspergillus ochraceus*. Ochratoxin A is known to cause kidney damage and suppress the immune system in animals, as well as inducing DNA damage in laboratory animals. To date there is no conclusive evidence that the toxic effects of ochratoxin A are the same in humans as in laboratory animals, but the latter evidence was sufficient for the International Association for Research into Cancer to classify ochratoxin A as a possible human carcinogen.

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 all been negative. Ochratoxin A in maize is also uncommon in the USA, where high concentrations (1-7 mg/kg) have only been associated with maize that has undergone extensive mould growth in storage. A similar instance was observed in southern Queensland some years ago, but most indications are that ochratoxin does not present a serious risk to Australian maize quality nor to other Australian grain crops. Even in parts of south-east Asia where aflatoxins are common in maize due to slow sun-drying and storage problems, ochratoxins appear to be only occasional contaminants

While it can be concluded that *A. ochraceus* is far less common than *A. flavus* in maize, the occasional case of contamination is hard to predict, since little is known about factors controlling *A. ochraceus* infection. In laboratory cultures, *A. ochraceus* grows over a similar range of temperature and moisture as *A. flavus*, but there are apparently other factors limiting toxin production in field maize. These factors could include survival of spores on soils (relative resistance to desiccation), ability to invade the developing ear, and ability to compete with other fungi for damaged kernels. 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 mycotoxins that commonly occur in maize, but not in other grains. The most common and most toxic is fumonisin B_1 (FB_1), usually accompanied by FB_2 and FB_3 in lower concentrations. Fumonisins are particularly toxic to horses, where they cause liquefaction of the brain known as equine leucoencephalomalacia. This disease has been reported in many countries, including Australia. Pigs fed fumonisins suffer pulmonary oedema, but no cases have been confirmed in Australia. Whether or not fumonisins have a role in human disease is still being investigated, but they have been associated with human oesophageal cancer and diseases resulting from inhibition of sphingolipid biosynthesis. They are sufficiently toxic and common in maize to warrant control.

Several *Fusarium sp.* are associated with ear rot and stalk rot in maize. The most common species in Australian maize is *Fusarium verticillioides*, which is presumed to be the main source of fumonisins. *F. verticillioides* is systemic in the maize plant, but seems to grow rapidly and increase fumonisin concentrations only when plant defences are impaired. *F. verticillioides* requires a higher moisture content than *A.flavus* and is less heat tolerant. While drought stress is a significant factor in fumonisin contamination, the association with very high temperatures is not as strong as with

aflatoxin. Irregular water availability (which can occur at the edges of irrigated fields) can produce sudden contraction and expansion of the pericarp, causing a 'starburst' pattern of fine cracks which appears to be associated with increased growth of *F. verticillioides* and production of fumonisins. Insect damage can also increase fumonisin contamination. Physical damage increases access to the endosperm, and stress might also reduce the activity of a beneficial maize fungus *Acremonium zeae*. 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 a major proportion can be in the lightweight fraction, and removable by gravity grading. Because *Fusarium* species require a moisture content of 30-40% and relative humidity of ~95%, fumonisins are extremely unlikely to increase in maize post-harvest.

Zearalenone, Deoxynivalenol & Nivalenol

These mycotoxins are considered together as they are all produced by *Fusarium graminearum*, a fungus responsible for causing ear and stalk rots in maize. *F. culmorum* and other *Fusarium* species might also be involved in DON and/or ZEA production in cooler locations.

Zearalenone (ZEA) is a non-steroidal estrogenic mycotoxin that has been implicated in some forms of infertility in pigs, cattle, sheep and possibly other animals. Although suspected at times, ZEA has not been proven to affect human health.

Deoxynivalenol (DON, also referred to as vomitoxin), nivalenol (NIV) and their acetyl derivatives are collectively known as Type B trichothecenes. The trichothecene group also includes Type A trichothecenes such as T-2 toxin and diacetoxyscirpenol, but these have not been identified as significant in Australian maize. Acute exposure to trichothecenes induces anorexia (reduced appetite) 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. Humans have been affected in other countries (Europe, Japan, Korea) but not Australia. To date there is no evidence that NIV and DON are either carcinogens or mutagens.

Problems with livestock production from *F. graminearum* mycotoxins are uncommon in Australia, but the infertility syndrome in pigs caused by ZEA has been reported, as has the vomiting and feed refusal syndrome caused by DON and NIV. The effects observed in livestock depend on the ratio of ZEA to DON/NIV present in the grain. These livestock cases have occurred on the Atherton tableland of far-north Qld and in the coastal and hinterland districts both sides of the Qld-NSW border.

Infection of maize by *F. graminearum* is favoured by cool, wet conditions during flowering and grain maturation, which are common in Australia only on wetter localities (Kairi, Malanda, Herberton) on the tablelands of far-north Queensland and the north coast of NSW (Casino, Kyogle, Grafton). Research indicates that infection in far-north Queensland produces NIV and ZEA while infection with the same species in northern New South Wales tends to produce DON and ZEA. *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, which did occur on the Liverpool Plains in 1999/2000. To emphasize the point, DON, NIV and ZEA are far more common in maize in wet, cooler regions such as parts of New Zealand than in Australia, and DON has been responsible for widespread economic losses in North America, particularly in wheat. DON and NIV are more concentrated in visually damaged grain, often with a dark reddish purple discolouration.

In the main Australian maize production areas, ZEA, DON and NIV are likely to be occasional contaminants at low levels. If specific controls become necessary, this would 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 *F. graminearum* problems become significant.

End Document