

MINIMIZING THE RISKS OF MYCOTOXINS UTILIZING THE HACCP CONCEPT

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Abstract

*Mycotoxins are toxic compounds produced by the secondary metabolism of toxic moulds in the *Aspergillus*, *Penicillium* and *Fusarium*, etc. genera occurring in food commodities and foodstuffs. The range and potency of mycotoxins makes this group of naturally occurring toxins an ongoing animal health hazard and a constant risk for contamination of the food supply (Hermann, 2002).*

Mycotoxins are difficult, and sometimes impossible to eliminate. The best way to control their presence is prevention. The occurrence can be prevented by reducing the moisture content of food products and controlling storage conditions such as temperature and/or relative humidity (Weidenborner, 2001). Hazard Analysis Critical Control Points (HACCP) offers suitable tools to control mycotoxins by preventing their formation.

Our work identifies the hazards, evaluates the risks and recommends control measures to reduce and eliminate the risks of mycotoxins presence in cereals, including GAPs (good agricultural practices) and the following steps from crop to storage.

Keywords: *mycotoxins, HACCP, GAP, GMP, hazard identification, hazard characterization risk characterization*

Introduction

Mycotoxins are toxic compounds produced by the secondary metabolism of toxic moulds in the *Aspergillus*, *Penicillium* and *Fusarium*, etc. genera occurring in food commodities and foodstuffs. The range and potency of mycotoxins makes this group of naturally occurring toxins an ongoing animal health hazard and a constant risk for contamination of the food supply (Hermann, 2002).

Many mycotoxins and toxicogenic fungi of economic importance were discovered because they were associated with mycotoxicoses affecting humans (ergotism, liver cancer, yellow rice disease, alimentary toxic aleukia

(ATA), Balkan endemic nephropathy (BEN), red mold toxicosis) or animals (turkey-Xdisease from aflatoxin, porcine nephropathy from ochratoxin A, vulvovaginitis in pigs from zearalenone, equine leukoencephalomalacia and porcine pulmonary edema from fumonisins) (Hermann, 2002).

Understanding of the toxic properties of mycotoxins, short-term, sub-chronic, chronic and carcinogenic studies at various dosage levels should be conducted in a variety of animal species, under controlled conditions. Also some studies are needed to assess reproductive effects and a battery of genotoxicity studies (Barlow *et al.*, 2002).

In addition, studies need to be conducted to determine the absorption, distribution, metabolism, and excretion of mycotoxins. Special studies, designed to gain a better understanding of the underlying mechanisms of the most significant health effects observed, may also be conducted. It is preferred that toxicity studies follow international testing guidelines, such as those established by the OECD (OECD, 2000).

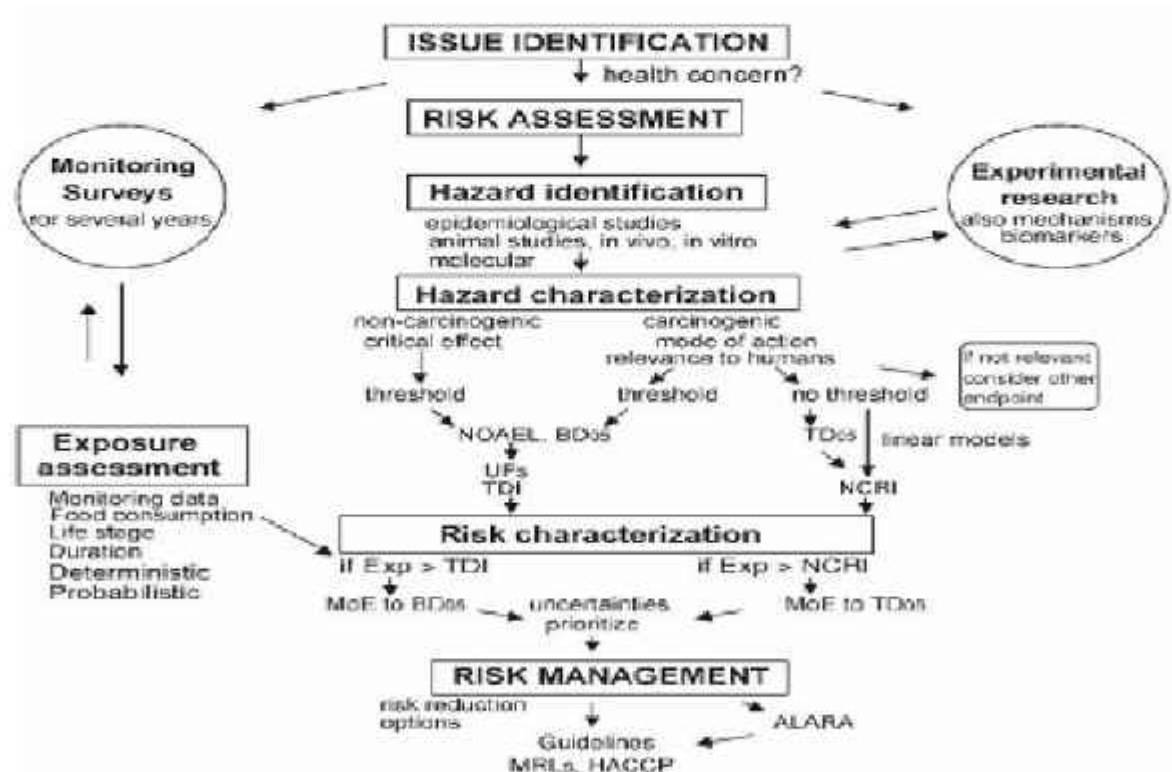


Fig.1: Showing the iterative process involved in Issue Identification, Risk Assessment (hazard identification, hazard characterization, exposure assessment, risk characterization and Risk Management) of mycotoxins in foods and the relationship to monitoring surveys and toxicological and epidemiological research (Magan *et al.*, 2004)

Methods for risk assessment

Currently, the term *risk assessment*, in the context of food safety, is used by many organizations to describe the process of assessing the health risks from a variety of agents that can be present in food. Thus, in order to derive potential human health risks, one assesses exposure to toxic substances in food and combines this with information on the hazards of these same substances, as identified in animal and human studies (WHO, 1999). For chemical agents such as mycotoxins, risk assessment consists of *hazard identification*, involving a complete toxicological and epidemiological assessment; *hazard characterization*, involving an assessment of dose response and extrapolation to humans; *exposure assessment*; and *risk characterization* (figure 1).

HACCP study for mycotoxin control

The application of HACCP to the control of mycotoxins is already well established. If we consider the last end of food production, i.e. where materials enter a food processing plant and a finished product results, here mycotoxins are typically well understood (for relevant industry sectors) and are treated as one of the hazards to be considered in the development and maintenance of a HACCP plan. There will typically be 'approved supplier' schemes in place for incoming materials, including permissible limits for mycotoxin content, backed up by documentation. In many cases, the materials entering in such facilities have been subjected to some degree of processing and are quite distinct from the raw materials from which they were originally derived. Applications of HACCP principles to the earliest stages of food production; to the crop in the field, at harvest and the stages which follow on, including primary storage and primary processing are needed. In fact we will be taking a holistic view 'from field to table' or 'from plough to plate' and we will consider the potential for HACCP to play a wider role in food production – beyond the areas where it has become an industry standard.

Why HACCP for mycotoxin control?

HACCP takes a proactive approach to hazard control, an approach of prevention is better than cure', and this is a particularly appropriate attitude to take with mycotoxins. The presence of mycotoxins in a finished product is

often the result of events and circumstances affecting commodities much earlier in the food chain. A preventative approach for the entirety chain of could therefore represent a very effective strategy. There are two other reasons for taking a proactive approach to mycotoxin control.

- Mycotoxins tend to be stable compounds that are difficult to remove once formed; in particular they tend to survive many of the processing stages involved in food manufacture.

- Mycotoxin analysis is typically complicated, expensive and time-consuming.

These factors mean that a QC approach to mycotoxin control, involving extensive end-point testing, is not currently economically feasible or practical.

Mycotoxins as hazards

In order to develop HACCP plans for mycotoxin control, we must first establish them as significant hazards within the food industry. In fact, it is relatively easy to identify at least some mycotoxins as important health hazards, mainly due to research that has been carried out relatively recently.

Although mycotoxins are chemical entities, they are classed as biological hazards because their presence is always as a direct result of fungal contamination at some point in the system. This is despite the fact that, in some instances, mycotoxins may be present in a commodity where no trace of the fungus responsible remains. This can occur because, mycotoxins are typically very stable, and can survive processes that will eliminate the fungi that originally produced them.

There is a relatively large number of compounds produced by fungi that are classed as mycotoxins, and of these around ten are currently considered a threat to human health. All of these are associated with specific food groups, and are produced under certain circumstances, and sometimes at specific stages in commodity production. For example, the trichothecene mycotoxins including nivalenol (NIV) deoxynivalenol (DON) and T-2 toxin are produced in growing cereal crops by fungi belonging to the genus *Fusarium*. Contamination is therefore a field event. However, the mycotoxin ochratoxin A (OTA), produced by the fungus *Penicillium verrucosum*, again occurs on cereals, but generally only during storage of the harvested grains. This reflects physiological differences between the producing fungi and the ecological niches they occupy in nature. Some mycotoxins are associated with geographical locations. This is generally the case for mycotoxins that are formed in growing crops and may reflect the requirements of the

producing fungus, the host plant, or both. However, mould growth and toxin production may also occur on food products (bread, fruit jams, cheese, etc.). The fungi growing on the processed product may differ considerably from those occurring on the raw material, due to changes in the composition of the substrate and the requirements of the producing fungi. In fact, moulds that grow on bread are not necessarily the same as those that grow on unprocessed cereals and may produce mycotoxins that are not expected in cereals.

There is generally understanding of the importance of mycotoxins, both in terms of acute and chronic disease, and in many countries permissible limits for relevant food groups are either planned or already in place.

There is currently a large number of data available which details the mycotoxin hazards associated with a wide range of commodities. However, for the purposes of developing a HACCP plan, where published data is lacking, mycotoxin analysis and surveillance of the product and the relevant supply chain will become necessary.

The complete elimination of mycotoxin contaminated commodities is not possible at this time. The elaboration and acceptance of a General Code of Practice by Codex will provide guidance for all countries in attempting to control and manage contamination by various mycotoxins. In order for this Code of Practice to be effective, it will be necessary for the producers in each country to consider the general principles given in the Code, taking into account their local crops, climate, and agronomic practices, before implementation of the Codex provisions. It is important for producers to realize that good agricultural practices (GAP) represents the primary defense against contamination of cereals with mycotoxins, followed by the implementation of good manufacturing practices (GMP) during the handling, storage, processing, and distribution of cereals for human food and animal feed.

The recommendations for the reduction of mycotoxins in cereals are divided into two parts: recommended practices based on Good Agricultural Practice (GAP) and Good Manufacturing Practice (GMP); a management system to consider in the future is Hazard Analysis Critical Control Point (HACCP) principles.

National authorities should educate producers regarding the environmental factors that promote infection, growth and toxin production in cereal crops at the farm level. Emphasis should be placed on the fact that the planting, pre-harvest and post-harvest strategies for a particular crop will

depend on the climatic conditions of that particular year, taking into account the local crops, and traditional production conditions for that particular country or region. There is need to develop quick, affordable and accurate test kits and associated sampling plans that will allow testing of grain shipments. Procedures should be in place to properly handle, through segregation, reconditioning, recall or diversion, cereal crops that may pose a threat to human and/or animal health. National authorities should support research on methods and techniques to prevent fungal contamination in the field and during harvest and storage.

Recommended practices based on Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) planting

Consider developing and maintaining a crop rotation schedule to avoid planting the same commodity in a field in two consecutive years. Wheat and maize have been found to be susceptible to *Fusarium* species and they should not be used in rotation with each other. Crops such as potato, other vegetables, that are not hosts to *Fusarium* species should be used in rotation to reduce the inoculum in the field.

When possible, prepare the seed bed for each new crop by plowing under or by destroying or removing old seed heads, stalks, and other debris that may have served, or may potentially serve as substrates for the growth of mycotoxin-producing fungi. In areas that are vulnerable to erosion, no-till practices may be required in the interests of soil conservation.

Utilize the results of soil tests to determine if there is need to apply fertilizer to assure adequate soil pH and plant nutrition to avoid plant stress.

When available, grow seed varieties developed for resistance to seed-infecting fungi and insect pests. Only seed varieties recommended for use in a particular area of a country should be planted in that particular area.

Crop planting should be timed to avoid high temperature and drought stress during the period of seed development and maturation.

Avoid overcrowding of plants by maintaining the recommended row and intra-plant spacing for the species/varieties grown. Information concerning plant-spacing may be provided by seed companies.

Pre-harvest

Minimize insect damage and fungal infection in the vicinity of the crop by proper use of registered insecticides, fungicides and other appropriate practices within an integrated pest management program.

Control weeds in the crop by use of mechanical methods or by use of registered herbicides or other safe and suitable weed eradication practices.

Minimize mechanical damage to plants during cultivation.

If irrigation is used, ensure that it is applied evenly and that all plants in the field have an adequate supply of water. Irrigation is a valuable method of reducing plant stress in some growing situations. Excess precipitation during anthesis (flowering) makes conditions favorable for dissemination and infection by *Fusarium* spp.; thus irrigation during anthesis and during the ripening of the crops, specifically wheat, barley, and rye, should be avoided.

Plan to harvest grain at low moisture content and full maturity. Delayed harvest of grain already infected by *Fusarium* species may cause a significant increase in the mycotoxin content of the crop.

Before harvest time, make sure that all equipment, which is to be used for harvesting and storage of crops, is functional. A breakdown during this critical period may cause grain quality losses and enhance mycotoxin formation. Make sure that the equipment needed for moisture content measurements is available and calibrated.

Harvest

Containers (e.g., wagons, trucks) to be used for collecting and transporting the harvested grain from the field to drying facilities, and to storage facilities after drying, should be clean, dry and free of insects and visible fungal growth before use and re-use.

Avoid mechanical damage to the grain and avoid contact with soil during the harvesting operation. Minimize the spread of infected seed heads, chaff, stalks, and debris on the ground where spores may inoculate future crops.

During the harvesting operation, the moisture content should be determined in several spots of each load of the harvested grain since the moisture content may vary considerably within the same field.

Immediately after harvest, determine moisture levels of the crop; where applicable, dry the crop to the moisture content recommended for storage of that crop. Samples taken for moisture measurements should be as representative of the lot as possible. To reduce the variation of moisture content within a lot, the grain may be moved to another facility (or silo) after the drying process.

Cereals should be dried in such a manner that damage to the grain is minimized and moisture levels are lower than those required to support mold

growth during storage (generally less than 15%). This is necessary to prevent further growth of a number of fungal species that may be present on fresh grains, especially *Fusarium* species.

Harvested cereals should be cleaned to remove damaged kernels and other foreign matter. Kernels containing symptom less infections cannot be removed by standard cleaning methods. Seed cleaning procedures, such as gravity tables, may remove some infected kernels. More research is needed to develop practical procedures for separating symptom less infected kernels from those that are not infected.

Storage

In the storage environment dangerous fungi are usually those species capable of growing in relatively dry conditions. Fungi that are able to grow in these conditions are termed "xerophilic" or "xerotolerant", depending on whether they grow best at relatively dry conditions (xerophilic). One factor of risk is that once growth is initiated the metabolic water produced by the growing fungus can perpetuate and amplify the process, and even allow the development of other, more moisture dependent fungal species.

Avoid wet, harvested commodities for more than a few hours prior to drying or threshing to lessen the risk of fungal growth. Sun drying of some commodities in high humidity may result in fungal infection. Aerate the commodities by forced air circulation.

Make sure that the storage facilities include dry, well-vented structures that provide protection from rain, drainage of ground water, protection from entry of rodents and birds, and minimum temperature fluctuations.

Crops to be stored should be dried to safe moisture levels and cooled as quickly as possible after harvest. Minimize the amount of foreign materials and damaged kernels in stored grains. The mycotoxin level should be monitored using appropriate sampling and testing programs. The important parameter when considering moisture and fungal growth is not water content, but water "activity" (a_w).

Water activity is a measure of the fraction of the water content of a material which is "free"- since in any natural material water will partition into "bound" and "free" states. "Bound" water is attached chemically or physically to the material, and is therefore not available to support fungal growth. The remainder is "free" water, which is immediately available for fungal growth. The value of water activity of a sample is directly related to the equilibrium relative humidity (ERH) the sample can generate by the

expression ($a_w = ERH/100$), and water activity is therefore usually measured using an electronic hygrometer or psychrometer, which actually measures ERH. Water activity is measured on a scale from 1.0 (pure water) to 0.0 (completely desiccated material). The growth limit for the most xerophilic fungal types is around 0.7. The relationship between water content and water activity for a given material can be determined by constructing "moisture sorption isotherms" which are graphs of moisture content plotted against water activity. Once the relationship between water content and water activity is known, "safe" water activity levels can be translated into "safe" moisture contents. This is important in the commodity supply situation because the concept of moisture content is far more readily understood, and can be measured without the rather specialized equipment needed to assess water activity accurately. It is important to note that water activity is a function of temperature, so moisture sorption isotherms are always constructed at a specified temperature. In fact, for a given water content, water activity increases with increasing temperature. The practical implication for commodity storage is that a temperature rise may lead to an increase in water activity to unsafe levels, and allow the initiation of fungal growth. This is why it is important to control temperature in the storage situation.

Transport from storage

Transport containers should be dry and free of visible fungal growth, insects and any contaminated material. Transport containers should be cleaned and disinfected before use and re-use and be suitable for the food transport. The use of registered fumigants or insecticides may be useful. At unloading, the transport container should be emptied and cleaned.

Shipments of grain should be protected from additional moisture by using covered or tarpaulins. Avoid temperature fluctuations and measures that may cause condensation on the grain, which could lead to moisture increase and consequent fungal growth and mycotoxin formation.

Avoid insect, bird and rodent infestation during transport.

Conclusion

For bagged commodities, ensure that bags are clean, dry and stacked on pallets or incorporate a water impermeable layer between the bags and the floor.

Where possible, aerate the grain by circulation of air through the storage area to maintain proper and uniform temperature levels throughout the storage area. Check moisture content and temperature in the stored grain at regular intervals during the storage period.

Measure the temperature of the stored grain at fixed time intervals during storage. A temperature rise of 2-3°C may indicate microbial growth and/or insect infestation. Separate the infected grains and send samples for analysis. Lower the temperature in the remaining grain and aerate. Avoid using infected grain for food or feed production.

Use good cleaning procedures to minimize the levels of insects and fungi in storage facilities. This may include the use of suitable, registered insecticides and fungicides or appropriate alternative methods. It is necessary to select only chemicals that will not interfere based on the intended end use of the grains and should be strictly limited.

The use of a suitable, approved preservative (e.g., organic acids such as propionic acid) may be beneficial. These acids are effective in killing various fungi and thus prevent the production of mycotoxins in grains intended only for animal feed. Document the harvesting and storage procedures implemented each season by making notes of measurements (e.g., temperature, moisture, and humidity) and any deviation or changes from traditional practices. This information may be very useful for explaining the cause(s) of fungal growth and mycotoxin formation during a particular crop year and help to avoid similar mistakes in the future.

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