

BREAKING THE MOLD – IMPACTS OF ORGANIC AND CONVENTIONAL FARMING SYSTEMS ON MYCOTOXINS IN FOOD AND LIVESTOCK FEED

An Organic Center State of Science Review

by Charles M. Benbrook Ph.D. Chief Scientist



ACKNOWLEDGEMENTS

Many people contributed information, ideas, and insights in the evolution of this State of Science Review (SSR). Thanks to Dr. Joe Cummins for helpful information on the major mycotoxins found in food and the fungi causing them. Mary-Howell Martens, a member of The Organic Center's "Scientific and Technical Advisory Committee" (STAC), helped make the case for this SSR and shared important information at several stages. Thanks also to the Chairperson of STAC, Dr. Kathleen Merrigan, and other STAC members who reviewed the document. Your many constructive suggestions led to important additions and revisions.

Lisa Bell did an excellent job in editing both the full SSR, as well as the Executive Summary. Thanks to Karen Lutz Benbrook for another good job in preparing both the Executive Summary and full SSR for publication. We also appreciate the support of the very capable professional staff at Selkirk Press in Sandpoint, Idaho, where the executive summary was printed.

Last, thanks to the many donors that have and continue to support the work of The Organic Center. Your contributions make possible the in-depth research and analytical work that is required to produce each "State of Science Review."

Chuck Benbrook Chief Scientist The Organic Center

Table of Contents

| MANAGING MYCOTOXINS – A CRITICAL CHALLENGE FOR FARMERS AN | ملا 1 |
|--|----------|
| THE FOOD INDUSTRY | 1 |
| AN OVERVIEW OF MYCOTOXIN DIVERSITY. TOXICITY AND REGULATION | ON3 |
| Why Do Fungi Produce Mycotoxins? | |
| Overview of Mycotoxins and the Fungi Producing Them | 4 |
| Major Mycotoxins Posing Food Safety Risks | 5 |
| Impacts of Weather and Grain Handling Practices on Mycotoxins in Food | |
| Moisture Levels | |
| Heat Stress | 10 |
| Plant Genetics | 10 |
| Fertilization Sources and Levels | |
| Harvest and Handling. | |
| Health Risks Following Mycotoxin Consumption | 13 |
| Cancer | |
| Mycotoxins and Antioxidants | 14 |
| Kidney and Liver | 14 |
| Fetal Development and Infants | 15 |
| Breast Milk | 16 |
| Livestock | 17 |
| Regulation of Mycotoxins in Food | 17 |
| The Basis for Regulation of Mycotoxins in Food | 18 |
| Major Differences Exist in Mycotoxin Standards Worldwide | |
| Mycotoxin Standards and High-Exposure Episodes | 20 |
| | |
| FREQUENCY AND LEVELS OF MYCOTOXIN IN CONVENTIONAL AND | |
| ORGANIC FOODS | |
| Direct Comparisons | |
| Ochratoxin | |
| Deoxynivalenol (DON) | |
| Studies on Fumonisin and DON | 25 |
| Patulan | 26 |
| Learaienone | 26 |
| Overview of Dublished Studies | 20 |
| Understand of Management of Management of the Angel of Picks and Disks in Comments and One | |
| Indirect Measures of Mycotoxin Contamination and Risks in Conventional and Org | anic |
| | ····.30 |
| Widely Cited Contamination Episodes Used to Support the Assertion that Organic | Foods |
| Are More Prone to Mycotoxin Contamination than Conventional Foods | |
| U.K. Spice and Cornmeal Surveys | |
| Genetically Modified Corn and Mycotoxins | |
| Other Instances and Episodes | |
| IMDACTS OF CONVENTIONAL AND OPCANIC FARMING SVSTEMS ON | |
| IVILAGI 5 OF CONVENTIONAL AND ORGANIC FARMING 5151 EMIS ON MVCOTOVIN CONTAMINATION | 2 F |
| Minotonin Dialy Eastern Associated with Convertineal Easterners | |
| Mycoloxiii Nisk Factors Associated with Conventional Farming Systems | |

| Mycotoxin Risk Factors Associated with Organic Farming Systems | 7 |
|---|---|
| Microbial Diversity | 7 |
| Disease Suppressive Soils | 7 |
| Compost Tea | 8 |
| Resistance to Heat Stress | 8 |
| Overview of Farming System Impacts | 9 |
| What Others Have Concluded | 1 |
| EINDINGS AND CONCLUSIONS | 2 |
| Findings and Least of Contention in Oreanic and Contentional East (2010) | 2 |
| Frequency and Levels of Contamination in Organic and Conventional Foods42. | 2 |
| Impacts of Organic and Conventional Production Systems on Mycotoxin Risks | 4 |
| RESEARCH, MONITORING AND OUALITY CONTROL PRIORITIES4 | 5 |
| Identify and Target Potential "Hot" SPots in the ORganic Food Supply 4 | 6 |
| Coordinating Research and Policy Initiatives in a Volatile Regulatory Environment | 7 |
| | |
| GLOSSARY | 0 |
| | |
| ABBREVIATIONS | 1 |
| | |
| REFERENCES | 2 |
| | |

LIST OF TABLES

| TABLE 1. SPECIFIC MYCOTOXINS OF IMPORTANCE IN MANAGING FOOD SAFETY THAT ARE FORMED BY DIFFERENT CENERA OF FUNCI |
|---|
| SAFETT THAT ARE FORMED DT DIFFERENT GENERA OF FUNGI |
| TABLE 2. ALLOWABLE LIMIT'S FOR MYCOTOXINS IN FOOD AND ANIMAL |
| FEED IN SELECTED COUNTRIES |
| TABLE 3. OVERVIEW OF STUDIES COMPARING MYCOTOXIN LEVELS IN |
| CONVENTIONAL AND ORGANIC FOOD |
| TABLE 4. MYCOTOXINS FOUND IN THE UNITED KINGDOM'S SAMPLING OF |
| SPICES |
| TABLE 5. DIFFERENCES IN THE FACTORS CONTRIBUTING TO MYCOTOXIN |
| RISKS ON CONVENTIONAL AND ORGANIC GRAIN AND LIVESTOCK FARMS |
| |

MANAGING MYCOTOXINS -- A CRITICAL CHALLENGE FOR FARMERS AND THE FOOD INDUSTRY

The world would be a very different place without fungi. These organisms play vital roles as decomposers, breaking down all sorts of organic matter from roots and leaves to crop residues and wood, as well as the bodies of dead mammals, fish, and insects. The process of decomposition releases the nutrients stored within decaying organic matter. Through this invaluable service, fungi help provide the foundation for the diversity of species living within an ecosystem and the capacity of one generation of life to sustain the next.

Most fungi pose little or no risk to humans and many are delicacies – morel and chanterelle mushrooms are two of the most well known. Patches of fungal spores create the distinctive flavor and blue splotches in blue cheese, and without fungi, there would be no beer or wine. *Penicillium* and *Streptomyces* fungi produce antibiotics widely used in treating bacterial infections in humans and animals, and many other fungi produce antibacterial mycotoxins that help plants avoid and/or slow the progression of bacterial infections.

However, a few fungi are poisonous, even deadly, to humans, like the *Aminita muscaria* mushroom. Others produce molds and mold spores that can trigger human allergies, aggravate asthma and other respiratory problems, and lead to many other mild to serious health problems. Many fungi thrive by attacking plants, trees, or insects and slowly consume their tissues. Others break down the integrity of cell walls, causing damage that often proves fatal. This is sometimes a good thing, such as when the *Bassiana beauveria* fungi attack Colorado potato beetles in a farmer's field.

Under certain environmental conditions, some fungal species that can infect grains like wheat and corn, fruits and vegetables, peanuts, tea, and spices, produce toxic byproducts called mycotoxins. Aflatoxin is the most toxic mycotoxin and is among the most widely distributed and well known. But it is just one of dozens of mycotoxins that can, and sometimes do reach dangerous levels in food. While more than 300 species of fungi with the ability to produce mycotoxins have been identified, many more species no doubt exist and await discovery and characterization. Fortunately, only about 20 mycotoxins produced by five genera of fungi (Aspergillus, Penicillium, Fusarium, Alternaria, and Claviceps) are found periodically in food at levels posing threats to people (Steyn 1995). Still, mycotoxins cost American agriculture between \$630 million and \$2.5 billion annually, largely because of market rejection of grain that contains mycotoxins at levels above either government or company standards (Vardon et al., 2003; Wu 2004).



Peanut affected by aflatoxin. Published with the permission of the Department of Primary Inidustries & Fisheries, Queensland, Australia

There is nothing theoretical about the human health risks posed by mycotoxins in food. Each year around the world thousands of mild to serious food poisoning episodes are traced to mycotoxin-contaminated food. Some result in multiple deaths and many serious illnesses. In addition, mycotoxins

are a recurring threat to livestock, since the grain-based products fed to farm animals are sometimes contaminated seriously enough to disrupt reproduction or normal growth. Mycotoxins consumed by milk-producing animals can show up in milk, cheese, and other dairy products. Human breast milk also sometimes contains mycotoxins (Skaug et al., 1998; Skaug et al., 2001; Turconi et al., 2004). Low levels of exposure to mycotoxins have been shown to disrupt fetal development in a range of animal experiments (Wangikar et al., 2004b; Wangikar et al., 2004a; Wangikar et al., 2005).

The storage of corn, and rice under allow them to get wet of mycotoxin cause but there are thousands different mycotoxins can into the food supply. Agriculture Organization United Nations estimates of the world's grain supply mycotoxins. Fortunately, cases, the levels in food too low to trigger health developed world, grain and storage, and food control procedures are in that this remains the case.



grains like wheat, conditions that is a common contamination, of ways that find their way The Food and (FAO) of the that 25 percent contains some in all but isolated as consumed are problems. In the harvest, drying safety quality place to assure

Mold in wheat storage bin. Photo: Winnipeg Cereal Reseach Centre, Agriculture and Agri-Food Canada

Serious mycotoxin problems are more common in the developing world. Storage facilities are often rudimentary and leaky, allowing grains and other stored foods to get wet. People sometimes are forced to choose between moldy grain and no grain. Poor rural populations typically suffer greater impacts from mycotoxin exposure than urban populations, in part because urban food supplies are more closely monitored and regulated, and are stored under safer conditions. As the volume of agricultural commodities traded between regions and countries grow, so does the diversity of mycotoxin problems confronting the food industry, scientists, and governments in both the developed and developing world.

In recent years some individuals and organizations critical of organic farming have claimed that organic food and animal feed are more frequently and heavily contaminated with mycotoxins than conventional food and feed. These assertions often arise in the course of public debate over the costs and benefits of organic farming, in contrast to the spraying of crops with pesticides or the planting of genetically engineered plant varieties. Isolated instances where mycotoxins were detected in organic or "naturally" grown food are cited in support of the sweeping claim that mycotoxins in organic food pose greater food safety risks than mycotoxins in conventional food. Studies showing that genetically engineered, insect-protected field corn is less prone to mycotoxin contamination than corresponding conventional corn are also frequently put forth as evidence that organic farming poses greater mycotoxin risk than "modern" agriculture that utilizes genetically engineered seeds. The assertion that organic food and animal feed is more prone to mycotoxin problems is typically linked by critics of organic farming to the fact that organic farmers do not spray their crops with synthetic chemical fungicides, and as a result, fungal populations are presumed to be higher on organic farms. This State of Science Review (SSR) analyzes the basis and validity of this assertion. It presents the results of studies that have compared mycotoxin levels in conventional and organic foods grown and stored under similar circumstances. The factors unique to organic farming systems that may increase or decrease relative vulnerability to mycotoxin contamination are highlighted.

Last, this SSR describes steps that all farmers and food processors should take to assure that mycotoxin exposure episodes are both infrequent and inconsequential. Our goal throughout is to identify insights from each system of farming and food processing that will help all farmers and the food industry more effectively prevent mycotoxins from reaching potentially damaging levels.

AN OVERVIEW OF MYCOTOXIN DIVERSITY, TOXICITY, AND REGULATION

Fungi are ubiquitous in the environment and primarily utilize nonliving organic matter as sources of energy for growth and reproduction. Fungal growth is typically triggered by wet conditions and is accelerated by heat, moisture stress, and humidity. Some fungi require continued wet conditions to thrive, others keep growing after moisture levels drop.

There are many different species of fungi. Each has evolved to exploit a given combination of environmental conditions. For this reason, wherever there is heat, some moisture, and a source of nonliving organic matter (i.e. decomposing crop residues), there will be fungi.

Why Do Fungi Produce Mycotoxins?

When a fungal spore comes into contact with organic material, it sends out filament-like structures called hyphae, which help attach the fungus to its new home. When the fungus senses conditions are right (a trigger often linked to moisture levels), it initiates the decomposition process by secreting enzymes into its new food source. These enzymes break down complex organic molecules in the host tissues into simpler molecules that are more readily available to the fungi, as well as to other microorganisms.

As fungal growth breaks down host material, the digested nutrients are classified into two categories: primary and secondary metabolites. Primary metabolites encompass cellulose and other carbon-based compounds that are used by the fungi and other microorganisms for growth and reproduction.

The secondary metabolites produced by fungi during the course of digestion are called mycotoxins. Fungi produce these biochemicals for a wide array of reasons, many of which remain unknown. Mycotoxin production tends to increase when fungal growth rates slow down and as fungi move toward dormancy. In such instances, mycotoxin production appears to be a defensive reaction. The purpose of the mycotoxins might be to combat the factors reducing the growth rate of fungi. Alternatively, fungi may produce mycotoxins to protect dormant molds and fungal spores from other, surviving fungal species and bacteria. Perhaps mycotoxins help protect molds from adverse environmental conditions (too cold or dry), or from the lack of some essential nutrient in the substrate on which the mold is growing.



Fungal hyphae on a leaf. Photo coutesy of Kevin MacKenzie, University of Aberdeen (c)

The strong bitter taste of moldy plant matter and tendency to induce nausea on species higher up the food chain may protect the infected material from being consumed by animals, including humans. It is obviously an advantage to fungi that humans tend to discard moldy food and feed, and that animals learn to avoid spoiled food. All these explanations of why fungi produce mycotoxins share a common denominator – attempts by fungi to survive and thrive by gaining or retaining a competitive edge within environments crowded by a host of organisms trying to thrive off available moisture, warmth, and nutrients.

Overview of Mycotoxins and the Fungi Producing Them

Fungi vary greatly in terms of what organisms and tissues they attack and how. Mycotoxins are a byproduct of fungal growth. Mycotoxin potency and production varies widely across classes of fungi. Higher organisms, including humans, are not specific targets of molds or mycotoxins, although one potential role of mycotoxins may be to deter animals from eating infected food. Still, both farm animals and people periodically fall victim to the biochemical warfare waged between species of fungi and bacteria competing to exploit the nutrients bound up in dead and decaying organic matter.

"Mycotoxins produced by fungi play a major role in the biochemical warfare that unfolds among competing species in virtually every environment on Earth"

Different fungi produce different types and levels of mycotoxins depending upon the substrate the mold is growing on. Molds are nothing more than growing masses of fungi. In most cases, mycotoxins are produced and transmitted within the spores created by molds. The viability of the next generation of fungi is entirely dependent on the competitiveness and environmental fate of spores. Therefore, it makes sense that fungi would provide their spores with as many biochemical tools as possible, some of which are classified as mycotoxins, and that mycotoxin production and levels would somehow be linked to factors placing fungi under stress.

The fungal species of *Aspergillus, Penicillium, Fusarium, Alternaria*, and *Claviceps* are the main producers of mycotoxins that periodically pose food safety risks (Steyn, 1995), as shown in Table 1. The fungal species within each genera of fungi can produce multiple mycotoxins, and each mycotoxin is designed to play a unique role, or roles, in response to specific combinations of circumstances. This report focuses on the mycotoxins that appear most frequently in food: aflatoxins, ochratoxin, fumonisins, deoxynivalenol, patulin, and the ergot alkaloids.

TABLE 1. SPECIFIC MYCOTOXINS OF IMPORTANCE IN MANAGING FOOD SAFETY THAT ARE FORMED BY DIFFERENT GENERA OF FUNGI

| Aspergillus | Penicillum | Fusarium | Alternaria | Claviceps | |
|---|--------------------|----------------------|--------------------------|-----------------|--|
| Aflatoxin B ₁ | Patulin | Deoxynivalenol (DON) | Tenuazonic acid | Ergot alkaloids | |
| Aflatoxin G ₁ | Ochratoxin A (OTA) | Nivalenol | Alternariol | | |
| Aflatoxin M ₁ | Citrinin | Diacetoxyscripenol | Alternariol methyl ester | | |
| Ochratoxin A (OTA) | Penitrem A (PA) | Т-2 | | | |
| Sterigmatocystein | Cyclopiaoznic acid | Fumonisins | | | |
| Cyclopiazonic acid | | Moniliform | | | |
| | | Zearalenone | | | |
| Source: Derived from information in Bennett et al., 2003; Steyn, 1995 | | | | | |

Mycotoxins are classified in a number of different ways (Bennett et al., 2003). Physicians tend to classify mycotoxins in relation to the illnesses they cause, while medical researchers focus on the organs they affect in humans – neurotoxins, immunotoxins, hepatotoxins, nephrotoxins, etc. Organic chemists favor categories based on chemical structures, while biochemists prefer to focus on the biosynthetic pathways that produce the mycotoxins. Mycologists prefer to classify mycotoxins based on the fungi that produce them.

Fungi produce compounds other than mycotoxins that are toxic to other organisms. Some secondary metabolites of fungi are antibiotics, while others play a role in triggering plant diseases and are called phytotoxins. Fungi also produce ethanol, which is not regarded as toxic at the levels produced in the natural environment.

Major Mycotoxins Posing Food Safety Risks

<u>Aflatoxin</u> The most dangerous of all mycotoxins to humans – aflatoxin -- has attracted worldwide attention since its discovery in 1960. It is a powerful toxin that attacks genes and can trigger

liver damage and cancer. Two types of mold - *Aspergillus flavus* and *Aspergillus parasiticus* - can produce different strains of aflatoxin. Aflatoxin B_1 is the most common strain found in food and is the most carcinogenic of the aflatoxins studied to date (Steyn, 1995). It is regarded by many scientists as the most potent carcinogen known to man (Bennett et al., 2003).

Aspergillus flavus is widespread in soil, and moldy grains and nuts are commonly contaminated with the fungus. About half of all known Aspergillus flavus strains produce mycotoxins. Aflatoxin production is stimulated by moisture and high temperature. At least 13 different types of aflatoxin are produced by different fungal species. The most potent is aflatoxin B_1 .

Other *Aspergillus* species are benign and are used in a number of food manufacturing processes. There is a cluster of species referred to as *Aspergillus* section *Nigri* that are a common source of extracellular enzymes and organic acids used in food processing (Abarca et al., 2004). Several of these strains have been granted "Generally Recognized as Safe" status by the U.S. Food and Drug Administration (FDA). Other *Aspergillus Nigri* strains can produce ochratoxin A, a dangerous mycotoxin.

<u>Ochratoxin</u> Ochratoxin is primarily found in grains, nuts, beans, and dried fruits and is usually associated with the storage of such foods. Barley is particularly vulnerable to ochratoxin contamination, and some wines can also contain ochratoxin. Pork is one of the major dietary sources of ochratoxin and swine become contaminated via animal feed (Bennett et al., 2003; Fink-Gremmels 1999).

Ochratoxin is produced mainly by *Aspergillus* fungal species and can damage the kidney, can cause cancer and immune suppression. It is readily absorbed through the gastrointestinal track (Fung et al., 2004). For this reason it is among the mycotoxins most commonly found in cow's milk and human breast milk (Marquardt et al., 1992). Ochratoxin makes its way into cow's milk through the consumption of grain-based animal feeds and concentrates, and into human breast milk through a wide range of human foodstuffs and beverages (Miraglia et al., 1995; Turconi et al., 2004).

The importance of milk in the diets of infants and children, coupled with the potent developmental toxicity of ochratoxin, makes any detectable presence of ochratoxin in milk a cause for concern.

<u>Patulin</u> Many fungi within the genera *Penicillium* can produce patulin, which was first isolated and studied as an antimicrobial compound in the 1940s (Bennett et al., 2003). Fruit with bumps, bruises, and insect feeding damage is far more likely to contain patulin than undamaged fruit and visibly rotten portions of apples can have "extraordinarily high" levels (Beretta et al., 2000).

Patulin is most often found in apples and processed foods and drinks made from apples. It is regularly found in unfermented apple juice, but does not survive the fermentation of apple juice into apple cider products (Trucksess et al., 2001). In 2000, a scientific review body convened by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) recommended a provision maximum tolerated daily intake (PMTDI) for patulin of 0.4 micrograms per kilogram (ug/kg) of body weight per day. (See Appendix 1 for an overview and explanation of the units of measure used in this report). Exposure patulin can lead to congestion and edema of the heart, liver, and intestines, and may also increase cancer risk (Fung et al., 2004).

Fusarium Mycotoxins The fungal genus *Fusarium* is very broad, encompassing hundreds of species. Fortunately, only a few Fusarium species produce mycotoxins that can pose food safety risks

(Moss 2002). The major mycotoxins of concern produced by *Fusarium* fungi include fumonisins and the trichothecenes (e.g., T-2 toxin, deoxynivalenol, and zearalenone).

Virtually all corn grown throughout the world is contaminated with low levels of the fungi *Fusarium moniliforme*, also referred to as *Fusarium verticilliodes*. This fungus attacks both vegetative and reproductive tissues in corn plants and causes the disease commonly referred to as "ear rot." It also produces fumonisin B_1 (FB₁), one of the most widespread mycotoxins in the food supply worldwide (Steyn, 1995).

In the United States, concern over FB_1 contamination in corn is growing, in part because European food companies and governments have intensified their testing for this mycotoxin and have recently set a standard far more restrictive than the "guidance level for industry" set by the FDA. The website of Pioneer Hi-Bred International, the leading supplier of hybrid corn varieties around the world, contains a summary of its corn research priorities and states under "Disease Resistance" –

"Recently, grain quality concerns have prompted Pioneer researchers to test hybrids for susceptibility to fungi that produce ear molds and mycotoxins. Future products will have improved resistance to ear mold fungi, as well as other important diseases." (Pioneer Hi-Bred International, 2005).

It is unusual for levels of FB_1 to increase in storage, except for when storage facilities are leaky and lead to wet spots in stored grain (Moss 2002). The separation and removal of screenings from grain prior to storage can substantially reduce FB_1 levels in grain when removed from storage (Moss 2002).

 FB_1 is a potent inhibitor of long chain fatty acid formation, competing with a key enzyme that forms sphingolipids. It disrupts the normal biochemistry of lipid formation, particularly in the liver. A joint FAO/WHO Expert Committee on Food Additives has identified a No Observed Adverse Effect Level (NOAEL) for FB₁ based on liver effects, and set the value 0.2 mg/kg per day. Applying a standard 100-fold safety factor to this NOAEL leads to a provisional maximum tolerable daily intake (PMTDI) of 0.002 mg/kg.



Oral lesions located in the laryngeal mound, tongue and mandibular mucosa of 11-week old turkey due to T-2 mycotoxins in the feed. Photo coutesy of Dr. Nati Elgin

Exposure to FB_1 has been associated with increased rates of human esophageal cancer and a host of problems in livestock consuming contaminated corn-based feeds.

<u>Trichothecene Mycotoxins</u> The mycotoxins produced by *Fusarium* species include the trichothecenes nivalenol, deoxynivalenol, and the T-2 toxin. These mycotoxins are common in several foods and target mammalian cells that are actively dividing, such as those in the small intestine, thymus, spleen, bone marrow, and testes (Poapolathep et al., 2004a). In addition, pregnant animals and developing fetuses can be harmed by exposure to trichothecene mycotoxins. The T-2 toxin is a potent mycotoxin that has triggered devastating outbreaks of alimentary toxic aleukia (ATA). Exposure to the T-2 toxin can suppress the immune system and cause irreversibly damage to bone marrow. The FAO/WHO expert committee has established a PMTDI for the T-2 toxin of 0.006 mg/kg per day.

<u>Deoxynivalenol (DON)</u> Deoxynivalenol, or DON, is one of the most common *Fusarium*based mycotoxins found in human foodstuffs. It is also called vomitoxin, because of its impact on farm animals consuming contaminated feed. The T-2 toxin is about 14-times more toxic than DON in terms of acute lethality (i.e., based on LD-50s). While less toxic then related trichothecene mycotoxins, DON is much more frequently found in barley, corn, safflower, wheat, and mixed animal feeds than the more toxic T-2 and FB, toxins. The fungal pathogens producing DON cause ear rot in corn and head blight in wheat, two of the most common sources of DON in the food supply. Rain during the flowering period in small grains clearly increases the risk of DON contamination.

DON suppresses the immune system of mice at doses as low as 0.25 mg/kg per day and pigs are even more sensitive (Moss 2002). Given the presence of DON in a high percentage of grain based foods in many countries, toxicologists are concerned that persistent, low-level DON exposure may suppress the ability of the human immune system to suppress common bacterial infections such as those cased by the food-borne bacterial pathogens Salmonella, Campylobacter, or Listeria (Moss 2002).

Zearalenone Zearalenone is a secondary metabolite of *Fusarium graminearum* species. Unlike other mycotoxins, zearalenone is virtually non-toxic to mammals following acute ingestion, and has an acute LD-50 of over 10,000 ppm in rats (i.e., 1 percent of the diet). Nonetheless, it is extremely potent in other ways, since it resembles a key hormone produced

estradiol, and as a result, can disrupt the human endocrine system. As little as 0.0001 ppm of zearalenone has been shown to create a detectable, hormone-related uterogenic response in female swine (Bennett et al., 2003). In other words, zearalenone exposure has been shown to impact reproductive processes at a dose that is 100 million times less than the lethal dose in a rat LD-50 study.

Some countries, including the United States, allow conventional cattle producers to add low levels of zearalenone to feed mixtures. In cattle, it acts as a growth promoter because of its hormonal activity and has been shown to improve feed efficiency. In humans, a PMTDI level of 0.1 ug/kg per day has been proposed by the Nordic Council of Ministers (Moss 2002).

Ergot Alkaloids The oldest recognized form of poisoning from mycotoxins is associated with Claviceps purpurea contamination of rye flour and dates back to the Middle Ages (Steyn, 1995). Claviceps species typically occur in fields growing grasses or small grains. In the developed world, ergot alkaloids have largely been eliminated as a routine cause of human disease because of advances in grain handling, but isolated outbreaks continue to emerge. Ergotism also remains a problem in cattle in some developing countries.

by human ovaries, 17β-



Ergotized grass can be recognized by the presence of sclerotia, that protrude from the florets resembling black or dark brown seeds. Fungal tissue is hidden between the glumes and only the presence of sticky exudate (honeydew) containing hyaline conidia signals the disease. Photo: Gary Odvody, Texas A&M Research and Extention Center, Corpus Christi, TX

The Organic Center

Impacts of Weather and Grain Handling Practices on Mycotoxins in Food

Fungi can grow almost anywhere under a wide array of environmental conditions. Fortunately, not all fungi produce measurable levels of mycotoxins. In many instances, fungi thrive and produce molds, but little or no mycotoxin is formed. The synthesis of mycotoxin by a fungus is more heavily dependent on specific weather and environmental conditions than is fungal growth (Drusch et al., 2003). Scientists around the world are actively researching why some fungal growths produce mycotoxins and others do not.

Moisture Levels

The moisture level of grain at harvest is a critical variable driving mycotoxin formation. Wet conditions, followed by hot and dry periods can trigger or worsen mycotoxin production by some fungi, especially aflatoxin.

Ochratoxin reached a maximum level of 0.24 ppm after 20 weeks in wheat initially stored at 19 percent moisture, yet no ochratoxin was found in wheat first stored at 15 percent moisture (Abramson et al., 1990; Abramson et al., 1992). In another wheat study, ochratoxin levels in wheat stored at 19 percent moisture were about 100-times higher than when grain was first stored at 15 percent moisture (Abramson et al., 1992).

A detailed study of mycotoxins in grain grown in Finland in a very wet season was undertaken (Eskola et al., 2001). Fusarium molds in stored rye, wheat, barley, and oats were common, especially in the rye samples, yet relatively few samples contained mycotoxins, and levels found were "very low," falling in the range 5 to 111 micrograms per kilogram (ug/kg) of grain (Eskola et al., 2001).

Significant differences in DON and ochratoxin A levels in organic wheat were detected in sampling conducted in 1997 and 1998 in Germany, reflecting major differences in moisture conditions between the two years during the flowering period (Birzele et al., 2000). The study assessed the impact of grain moisture levels at harvest and during storage on DON levels. Prolonged rains during the harvest season in 1998 forced some farmers to harvest grain at higher than desired moisture levels. This study focused on the impacts of post-harvest handling, drying, and storage on DON levels in stored wheat.

DON levels were on average almost three-times higher in the wetter of the two years (1998). At harvest, the mean DON level in 1997 was 111 ug/kg, and in 1998, it reached 280 ug/kg. None of the samples tested in 1997 exceeded the proposed guideline level for DON in wheat of 500 ug/kg. In 1998, DON levels in 9 percent of the samples exceeded this standard (Food and Agriculture Organization 2004; Lo Curto et al., 2004; Tafuri.A. et al., 2004)Birzele et al., 2000). DON levels rose 13-fold during the first two weeks of storage in 1997, reaching 1,326 ug/kg. The authors note that the increase in DON levels during suboptimal storage cannot be predicted solely by the levels of DON on grain entering storage.

No buildup in ochratoxin A levels were detected in either year in grain that went into storage at 17 percent moisture, although at moisture levels of 19 percent and above, studies have documented buildup of ochratoxin A in stored grain. Grain that contains detectable levels of ochratoxin A is typically visibly contaminated with fungal mycelium. None of the samples tested contained ochratoxin A in

excess of the 5 ug/kg limit for raw grains set by the E.U. (FAO, 2004).

Research in Denmark on grain handling and storage on organic farms has also pointed directly to the importance of on-farm storage facilities and methods. Improper handling of grain was shown in several seasons to produce severe problems with ochratoxin A contamination (Elmholt, 2003). Two farms with home-built, forced air (but unheated) dryers experienced ochratoxin A levels far above the EU limit of 5 ug/kg, and the drying system itself was found to be a significant source of contamination. A third farm with a well-designed, modern, hot-air drying system that included an efficient vacuum cleaning system with filters was able to prevent problems with ochratoxin A contamination (Elmholt, 2003).



Growth of *P. vertucosum* in rye after 100 days at 20 C and 22% moisure content. Photo: Danish Research Centre for Organic Farming

In the wet year of 1998, much of the grain harvested in Germany went into storage at 20 percent or more moisture, well above typical levels. In Norway, grain is harvested in the 20 percent to 35 percent moisture range under normal conditions. To prevent mycotoxin problems, grain must be quickly and evenly dried to 14 percent moisture or less, a process that is routine on farms with modern and well-managed drying and storage facilities. Without such facilities though, it is very difficult for farmers to manage moisture levels in wet grain to prevent the build up of DON in stored grain (Birzele et al., 2000).

Heat Stress

Several studies have shown that plants experiencing stress from excessive heat are more vulnerable to fungal and mycotoxin infections (Abbas et al., 2002a). Sun scald, for example, can cause mycotoxin infection in some crops. In tomatoes after harvest, *Alternaria* species can proliferate in sundamaged tissues, causing black rot lesions (Hasan 1996). These infections tend to produce a number of mycotoxins, including alternariol (AOH), alternariol monomethyl ether (AME), and tenuazonic acid (TA). Different temperatures favored the production of different mycotoxins; 28 degrees Celsius for AOH, 21 degrees for TA, and 14 degrees for aflatoxin species (Hasan 1996).

Drought stress is typically associated with aflatoxin contamination of crops in the field (Bennett et al., 2003). Droughty conditions coupled with some insect damage, followed by wet, humid weather near harvest can optimally set the stage for the fungi capable of producing aflatoxin. *Penicillium* species with the ability to produce ochratoxins tend to thrive in cooler climates with temperatures as low as 5 degrees centigrade, while *Aspergillus* species are the most common source of ochratoxins in tropical areas.

Plant Genetics

Crop cultivars are known to have a significant impact on susceptibility to mycotoxins. In general, varieties that are resistant to fungal attacks during the growing season are also less likely to become contaminated with mycotoxins, although mycotoxin problems can still arise during storage (Edwards 2004a).

Research in Germany assessed the impact of 12 winter wheat cultivars on DON levels and found very significant varietal differences (Doll et al., 2002). The percent of samples across varieties testing positive ranged from 17 percent to 100 percent, and the median level of DON detected varied from 160

ug/kg to 2,390 ug/kg. The highest level of DON found in the Bussard variety of wheat was 180 ug/kg, while the highest levels in the Ritmo and Aron varieties were over 11,000 ug/kg, or more than 60-times higher (Doll et al., 2002).

A study in Canada found a significant impact of barley cultivar on ochratoxin levels (Abramson et al., 1987). Plant varieties bred to better withstand heat stress tend to have significantly lower levels of fumonisins and aflatoxin. A similar study compared aflatoxin and fumonisin levels in a range of corn hybrids, comparing commercial lines to experimental, highly-resistant lines. Under high heat conditions, the corn lines with a high level of resistance contained 91 percent lower levels of mycotoxins (Abbas et al., 2002b). Aflatoxin levels were reduced 74 percent in the resistant lines under moderate heat stress. These and other studies suggest that farming systems that tend to lessen the severity of heat stress suffered by plants may also reduce the frequency and levels of mycotoxin contamination.



^cMaxum' field pea - Nitrogen 90-150 lbs per acre. Photo: Jeromy Biazzo, USDA-ARS, U.S. Plant, Soil and Nutrition Lab, Ithaca, NY

Fertilization Sources and Levels

There is strong evidence that high levels of readily available nitrogen fertilizer can promote mycotoxin formation in cereal grains and row crops, compared to crops produced in organic systems (Birzele et al., 2002; Gunst et al., ; Scalera 2002) . In addition, the form of fertilizer has also been shown to make a difference in the frequency and/or levels of mycotoxins in grain crops (Edwards 2004b). Various mechanisms have been noted as possible explanations for these differences and include:

- Changes in the rate of decomposition of crop residues;
- Physiological stress on fast-growing plants;

• An enhanced supply of readily available energy sources for fungal pathogens; and

• Alterations in the crop canopy structure that give rise to higher levels of fungal infections and/or mycotoxin formation.

Evidence showing that fertilization methods and

levels can impact mycotoxin formation is important because of major differences in how organic and conventional farmers provide plant nutrients to growing crops. In general, conventional farmers apply enough nitrogen (N) fertilizer in a readily available form early in the crop year to eliminate a shortage of nitrogen and a possible constraint to yields, even under optimal growing conditions. In most years, such a level of fertilization is well in excess of crop needs.

Organic farmers, on the other hand, use legumes, cover crops, manure, compost, and relatively slow release fertilizers to meet crop nitrogen needs. Typically, lower levels of nitrogen are applied per acre compared to nearby conventional farms, and a significant share of the nitrogen is or becomes bound up in decaying organic matter, and hence tends to be released more slowly. Because of these differences, excessive supply of readily available nitrogen does not contribute to mycotoxin formation on organic farms to the degree it does on conventional farms.

Harvest and Handling

The condition of food at harvest plays a key role in determining mycotoxin risks. Food can be, and often is free of mycotoxins at harvest, only to become contaminated during handling, storage, and processing. Food grown on both conventional and organic farms is vulnerable to contamination by mycotoxin-producing fungi after harvest.

In order to prevent mycotoxin problems in fruit and fruit-based products, it is important to carefully select fruit without signs of rot, and thoroughly wash and sort the fruit before consumption or processing. These steps are critical in preventing problems, since mycotoxins are not appreciably removed in the manufacturing of fruit juices (Drusch et al., 2003).



Fusarium fungi commonly infect wheat crops and produce several mycotoxins, especially DON. A study was carried out in four states. It focused on wheat infected with *Fusarium*, to test the impact of wheat milling, processing, and baking on DON levels (Abbas et al., 1985). DON was found throughout all milling fractions, although concentrations varied from a high of 21 parts per million (ppm) in bran, to a low of 1 ppm in break flour. Cleaning grain and milling facilities and equipment did not appreciably reduce DON levels. Baking reduced DON levels by up to two-thirds, but never removed it entirely (Abbas et al., 1985).

A detailed study was carried out in California tree nut crops, in order to develop management strategies to meet stringent international standards for aflatoxin. Scientists determined that most of the fungal species colonizing nuts undamaged by insects were present at low levels on the surface of the nuts, rather than inside the nuts. They also found that each type of nut tends to be colonized with a unique combination of fungal species, and that several fungi are typically present, as opposed to contamination with a single species (Bayman et al., 2002). This finding suggests that fungi are constantly competing with a number of species to sustain growth in a given microenvironment, like on the shell of a walnut. Accordingly, any factors that are likely to impact the diversity of species present, as well as the dynamics of their interactions, are likely to have an impact on the distribution and levels of mycotoxins.

The time of year can also impact mycotoxin levels. A survey of 580 liters of milk in Mexico found aflatoxin in 13 percent of the samples. There was a significant correlation between aflatoxin levels and the time of year. Levels were higher in the autumn, compared to the winter and spring (Carvajal et al., 2003).

Health Risks Following Mycotoxin Consumption



Mycotoxin poisoning from ingestion of contaminated food has been recognized around the world since the beginning of agriculture and has taken a large toll on humans and farm animals. Mycotoxins cause a range of immunological effects, can damage several vital organs, especially the kidneys, liver, and gastrointestinal tract, and can cause cancer.

The health effects of mycotoxins on humans and farm animals have been extensively studied. A search on the National Library of Medicine's PubMed database identified 8,098 citations using the keywords {mycotoxin or mycotoxins} and {health or health effects}, and another 8,168 citations involving studies on animal health. Many excellent reviews have been published on the toxicity and health effects of the major mycotoxins contaminating food; several are cited in this section, which provides only a brief introduction to the extensive literature on mycotoxin health effects.

A recent review entitled "Health Effects of Mycotoxins: A Toxicological Review" concluded that liver disease, damage to the gastrointestinal tract, and cancer were the most common and serious consequences of exposure to mycotoxins in food (Fung et al., 2004). The review summarizes the literature on each of the major mycotoxins appearing in food, as well as studies on the effects of mycotoxins on the pulmonary, neurological, immunological, kidney and liver systems.

While food is the most common source of mycotoxin exposure for the general public, farmers and



Moldy Grain. Photo: Cereal Research Centre - Agriculture and Agri-Food, Canada

people working in grain handling and milling facilities can suffer from high acute exposures, typically through the air. The inhalation of dust containing mycotoxins can cause a variety of toxic effects in humans, including acute and chronic respiratory disease (Bunger et al., 2004). Extreme cases can lead to death.

Inhalation exposure is also a confounding variable in many studies of mycotoxin health effects in agricultural settings and among workers on farms and in grain handling facilities. Much more research and monitoring are necessary in order to understand the scope and importance of inhalation exposures to mycotoxins.

Cancer

Liver cancer is the fifth most prevalent cancer in the world, and 80 percent of the cases are in the developing world. The primary causes of liver cancer in the developing world are the hepatitis B virus and aflatoxin. The two combined can be an especially dangerous mix. Limiting the contamination of foodstuffs by aflatoxin is a particularly important target for public health (Wild et al., 2000).

Mycotoxins have also been implicated as a cause of esophageal cancer. Mycotoxins in corn were studied in the Linxian region in China where high rates of esophageal cancers have been documented. A variety of mycotoxins were found in 100 percent of the 107 corn samples tested (Hsia et al., 1988). The average levels were very high – 757 nanograms per gram (or 757 micrograms per kilogram) of nivalenol (NIV) and 5,379 nanograms per gram (5,379 ug/kg) of DON, leading the authors to suggest a possible association between mycotoxin consumption and throat cancer. The European Union standard for DON in cereal-based products is 500 ug/kg, while the U.S. limit on DON in wheat-based products ready for consumption is 1,000 ug/kg (Food and Agriculture Organization, 2004).

In a related study, the same team showed that NIV induces benign and malignant tumors in mice (Hsia et al., 2004). The team documented very high levels of NIV and DON mycotoxins in 97 samples of wheat flour, barley, and corn collected from farms in the Linxian and Cixiang provinces in China. No DON was found in samples of rice from the United States. The team reported that –

"The mean levels of NIV in three main dietary foods in those two high-risk areas were estimated at 400 to 800-fold higher than that in the USA."

Mycotoxins and Antioxidants

A number of studies have explored the interactions of mycotoxins and antioxidants (Atroshi et al., 2002). Several have found that certain antioxidants have the ability to moderate the toxic impacts of mycotoxins. For example, the antioxidants coenzyme Q10 mixed with l-carnitine partially counteracted the impacts of mycotoxins on bacterial growth curves (Atroshi et al., 1998).

The beneficial impact of antioxidants on mycotoxin damage is likely linked, at least in part, to their ability to scavenge reactive oxygen species, thereby protecting cell membranes from mycotoxininduced damage. A sophisticated experiment using rats fed radio-labeled DON, with and without antioxidant supplementation in their diets, demonstrated a strong protective effect of antioxidants on the damage caused by DON (Rizzo et al., 1994).

In research in rats, antioxidant-rich cabbage seed extract and garlic were given to pregnant rats that were also exposed to fumonisin (Abdel-Wahhab et al., 2004). Both the cabbage and garlic provided a protective effect against the profound developmental effects caused by the fumonisin in the control animals. The authors attribute this protective effect to:

- Inhibition of microsomal lipid peroxidation;
- Enhancement of antioxidant-driven detoxification systems; and/or
- Scavenging free-radicals.

Kidney and Liver

Fumonisin causes cancers of the liver or kidney along with blood disorders and pulmonary edema in farm and experimental animals. Fumonisin B_1 in corn fed to pigs causes pulmonary edema (Becker et al., 1995). In humans, deoxynivalenol causes loss of appetite at low levels and vomiting at higher levels, and also damages the immune system.

Fetal Development and Infants

A number of studies have documented profound impacts of mycotoxins on fetal development, both in humans and livestock, and on young animals when mycotoxins are present in milk.

Scientists explored the effects of prenatal exposures to ochratoxin A and aflatoxin B_1 (AFB₁) alone and in combinations in an important study involving rats (Wangikar et al., 2004b). A range of dose levels was tested. Each was delivered via gastric intubation (a tub inserted into the stomach) from days 6-15 of gestation. The scientists set the dosage levels in part based on a study of ochratoxin A and AFB₁ levels in commercially available laboratory feeds. Out of 321 feed samples tested, 83 were positive for ochratoxin A and 117 were positive for AFB₁. The lowest dosage rates were chosen to reflect the highest mycotoxin levels present in feed, while the highest dose tested had been determined to be a "maximum tolerated dose" for pregnant rats (i.e., the highest dose tested in an experiment that did not trigger adverse impacts).

Ochratoxin A and AFB_1 fed alone caused dose-dependent maternal problems during gestation (the impacts worsened at higher doses). The number of animals aborted rose and the bodyweights of pups at birth declined.

Among the pups in the ochratoxin A group, there were several significant developmental abnormalities mostly impacting the head and face. Several skeleton and skin anomalies were observed, even at the lowest dose tested (0.125 mg/kg), as well as in the control treatment. Only the higher doses showed statistically significant differences to the control group. Pups were born with incomplete closure of the skull, lack of eye openings, abnormal ribs, sternum, and bent spinal cords, and deformed thoracic cages (chests) (Wangikar et al., 2004a). In addition, there were several soft tissue anomalies involving parts of the brain, thyroid, and reproductive systems (Wangikar et al., 2004a).

Similar, but less serious developmental impacts were observed only at the highest dose of aflatoxin B_1 tested. In these pups, a range of cardiac development defects was also observed (Wangikar et al., 2004b; Wangikar et al., 2004a). The scientists concluded that "the histopathological alterations observed in the fetal organs indicated that these mycotoxins caused direct effects on the fetuses" and were not associated with maternal toxicity (Wangikar et al., 2005).

Unexpectedly, the developmental impacts on pups of the two mycotoxins together were substantially less severe than when each was fed alone. In the group fed both mycotoxins, for example, all pups had properly formed skulls, in contrast to the ochratoxin A fed group, where incomplete closure of the skull was observed. The authors speculate that in the group of mothers fed both mycotoxins, the presence of the AFB1 interfered with the uptake of ochratoxin A, hence "rendering it less available to cause brain and face anomalies" (Wangikar et al., 2005).

In discussing the significance of their findings to humans, the authors state that there is "...no data on dose and teratogenic effects of these mycotoxins in humans." They also note that both mycotoxins have been detected in human umbilical cord blood samples, and that once in a fetus, the mycotoxins would have a relatively long half-life. In light of evidence that pregnant women are routinely exposed to low levels of these mycotoxins alone and in combinations, they conclude that more research on these effects in humans is "...demanded" (Wangikar et al., 2004b). In April 2005 the same team of scientists published a similar study carried out in rabbits that produced evidence of developmental effects much like those found in their work with rats. This study established a dose of 0.1 mg/kg as the minimal teratogenic dose in rabbits (Wangikar et al., 2005).

The risk of mycotoxin-induced adverse effects to fetuses is clearly a function of the timing of exposures and levels of exposure. There is evidence that most species, including humans, have developed an aversion to the consumption of spoiled, moldy food (Scalera 2002). Such a conditioned response clearly will reduce the likelihood of high-level exposures to some mycotoxins in most cases, but not to most mycotoxins that find their way into baked or processed foods, beverages, meat and milk.

Breast Milk

Several studies have shown that human milk sometimes contains specific mycotoxins near or even above the levels that would expose a nursing infant to levels above currently estimated tolerable daily intakes (TDIs) (Skaug et al., 1998; Skaug 1999b; Skaug et al., 2001). For example, a study in Italy assessed aflatoxin and ochratoxin A in the breast milk of 247 women. While aflatoxin was found in only one sample, ochratoxin A was present in 85.7 percent of the breast milk samples tested (Turconi et al., 2004). Bread was the only food group that showed a statistically significant correlation between consumption levels and ochratoxin A levels in breast milk. In a study of 80 Norwegian women, the breast milk of 21 percent contained ochratoxin A. Women who consumed liver paste (liverwurst, liver pate), juices of all kinds, breakfast cereals, processed meats, and cakes were most likely to test positive for ochratoxin A in their breast milk (Skaug et al., 2001).

On the fourth and sixth day of nursing, 71 percent and 60 percent respectively of infants were exposed to ochratoxin A over the provisional tolerable daily intake level of 0.2 nanograms per kilogram of body weight (Turconi et al., 2004). The authors note studies in four other European countries, two other studies in Italy, and a study in Sierra Leone, showing comparable or higher levels of ochratoxin A in breast milk.

Moreover, studies in rats and mice have shown that trichothecene mycotoxins ingested by a pregnant animal freely move through the placenta into the growing fetus. The levels reached in whole tissues in the fetus were 150 percent or more of those in maternal blood in one study (Poapolathep et al., 2004b). In addition, Poapolathep et al. (2004) also showed that trichothecenes move from a suckling dam to her babies via milk, and reach levels comparable to that in maternal blood within six hours. In the case of nivalenol, the levels in the plasma of suckling mice actually exceeded maternal plasma levels after 24 hours.

Patulin is known to damage genes, cause birth defects, and can lead to immune and neurological dysfunction when ingested at toxic doses. A preliminary maximum tolerated dose of 0.4 micrograms of patulin per kilogram of body weight has been established by the European Union, leading to limits in fruit juices, concentrates, and fermented drinks of 50 ug/kg (FAO, 2004). The limit is lowered in solid apple products to 25 ug/kg, and lowered further to 10 ug/kg for baby food and apple juice and apple-based products marketed to infants and young children. The U.S. guidance level for all apple-based products is 50 ug/kg.

Multiple samples of apples and apple-derived products were found to have patulin levels below the tolerable intake level in nearly all cases. Levels were high in sections of apples that were visibly rotting (Beretta et al., 2000).

Livestock

Several mycotoxins are known to reduce the digestibility of grains and forages by livestock. Ochratoxin had the largest effect of any mycotoxin in one study (Abdelhamid et al., 1990; Abdelhamid et al., 1992). Other mycotoxins can act as antibiotics in the rumen of livestock, increasing feed digestibility by reducing the levels of harmful bacteria.

In livestock in North America, the five leading mycotoxins present in feed that impair growth and disrupt reproduction are aflatoxin, zearalenone, DON, ochratoxin, and ergot (Diekman et al., 1992). In pigs, mycotoxins impair liver and kidney function, and delay blood clotting and immune responses. Even limited exposures to *Fusarium* mycotoxins in corn fed to pregnant swine can result in embryonic loss and disruption of normal reproductive cycling for an extended period of time (Long et al., 1983).

Ruminants are more resistant to aflatoxins than other livestock, but mycotoxins can pass through milking animals and appear in milk, a problem that is periodically monitored in the developed world (Hollinger et al., 1999). Dairy farmers and processors in the United States conduct periodic sampling of milk shipments for both mycotoxins and antibiotics. *Fusarium* mycotoxins are known to be powerful teratogens, disrupting normal embryonic development (Diekman et al., 1992; Green et al., 1990; Long et al., 1983; Wangikar et al., 2004b; Wangikar et al., 2004a; Wangikar et al., 2005).

Regulation of Mycotoxins in Food

Many developed countries have established standards or guidelines governing mycotoxins in food and at the international level, the Codex Alimentarius Commission has developed a detailed regulatory guidance framework for the monitoring and control of mycotoxins. Still, there are no widely acceptable standards for many combinations of specific mycotoxins in specific foods.

In addition, the official status and impact of "guidance levels," versus "allowable levels," versus "standards" varies significantly around the world. The United States is among a set of countries that have established "guidance levels" that are intended to trigger actions by private companies if and when the levels are exceeded. Such levels are, however, not enforced and are not legally enforceable by any government authority.

The European Union and several countries in Europe have established enforceable "standards" for several food-mycotoxin combinations. Food found to contain mycotoxins above an existing standard is deemed adulterated and when possible, is recalled from the market. Further enforcement actions, including penalties, can be imposed on the company selling such food.

In addition to the mycotoxin standards currently in force in the European Union, several additional mycotoxin-food-specific standards are under development, and are typically referred to as "proposed" or "provisional" maximum allowable levels. Some countries in Europe consider a "proposed" allowable level to be essentially the same as a standard. For example, the United Kingdom Food Standards Agency withdrew several cornmeal products from the market that were found to contain fumonisins above the proposed EU standard of 0.5 mg/kg (Food Standards Agency 2005).

As global agricultural and food trade grows, the mycotoxin guidelines and standards of the developed world are increasingly applied to food grown and produced in the developing world. A review of the results of mycotoxin monitoring programs in the United States, Great Britain, and

throughout Europe highlights the disproportional representation of imports from the developing world among the most worrisome cases of mycotoxin-contaminated food.

The Basis for Regulation of Mycotoxins in Food

There are two basic steps in setting allowable levels in human food of any toxic chemical or biological contaminant. In the case of mycotoxins, the first step entails identifying the biological effect of concern that may be triggered by exposure to a specific mycotoxin, and setting a maximum acceptable daily exposure level to the mycotoxin based on available toxicological data. The hope is that exposures below this level will not produce the adverse effect.

The second step entails the establishment of limits in food of a specific mycotoxin designed to prevent people from being exposed above the maximum acceptable level. There is a big difference – and a lot of complicated toxicology and mathematics – in going from an estimate of a maximum allowable (and hopefully safe) dose, to estimates of the highest acceptable levels in food, which is a concentration thought to pose little or no risk. (For more on the units of measure typically used in studies of mycotoxin food safety risks, see Appendix 1).

In establishing acceptable daily exposure levels, regulatory authorities and expert bodies assess all toxicological data that is available on a compound or contaminant in order to determine the adverse impact that occurs at the lowest dose. They then establish a "tolerable" or "acceptable" dose, based on the findings in that study. They do this by converting the dose rate in the study that produced no impacts (expressed usually as micrograms of chemical per kilogram of diet, or parts per million) into a maximum daily exposure level, based on how much feed the animal consumed in a typical day during the study. The resulting estimate is a maximum tolerable daily exposure level (typically expressed in micrograms of chemical per kilogram of body weight).

Estimates of maximum daily exposure are then typically divided by a safety factor to account for differences between how humans and experimental animals respond to exposures to chemicals. The result is a key regulatory benchmark that is called different things by regulatory and scientific bodies around the world including:

- TDI, or tolerable daily intake;
- PMTDI, provisional maximum tolerable daily intake;
- ADI, acceptable daily intake (typically includes a safety factor);
- Reference dose (RfD).

Established daily intake limits for mycotoxins are mostly based on animal data and reflect risks one mycotoxin at a time. Given that monitoring data typically find more than one mycotoxin present in food, the need to consider the additive and/or synergistic impacts of exposure to multiple mycotoxins has been recognized (Creppy 2002).

The second step in regulating mycotoxins requires estimation of the maximum level of mycotoxin that can be in a given food without delivering a dose to people in excess of the maximum tolerable daily intake. Such a level is typically expressed as a concentration in food – micrograms of mycotoxin per kilogram of food (ug/kg), which is the same as parts per billion (ppb) of mycotoxin in the food. This level is estimated through a simple mathematical formula: (maximum allowed concentration) multiplied by (amount of food consumed in a day) equals the maximum tolerable dose for a person of known size. This maximum dose, in turn, is calculated by multiplying the maximum tolerable dose rate,

which is typically expressed in micrograms of mycotoxin per kilogram of body weight, by the weight of a typical consumer. In calculating safe levels for adults, regulators usually use 60 kilograms as the standard body weight, and when estimating safe levels for infants or children, they use 3 to 20 kilograms.

Major Differences Exist in Mycotoxin Standards Worldwide

Important differences exist in the regulation of mycotoxins within the developed world and in particular, between the United States and the European Union. In general, US "guidance levels" are less strict, are not enforceable, and are not as comprehensive as E.U. standards, which are enforceable and can lead to food recalls. According to an expert in international food safety standards at the University of Pittsburgh, most of the corn grown in the United States has fumonisin levels between 0.5 mg/kg and 2 mg/kg, which would place a major portion of U.S. corn exports in jeopardy if the relatively strict standard under review by the European Union of 0.5 mg/kg for fumonisin B_1 plus fumonisin B_2 were adopted internationally (Vardon et al., 2003; Wu 2004). The current U.S. "guideline" for fumonisin in corn is 2.0 mg/kg, but is not binding on food companies or exporters. Because of the relatively lax standards in the United States on several mycotoxins, Wu (2004) concludes that –

"Among industrial nations, the United States would experience the heaviest economic losses from more precautionary mycotoxin hazards." (Wu, 2004).

| Musstaria | Trans of East an East | Limits in Food or Feed (ug/kg or ppb) | | | | |
|--|--|---------------------------------------|----------------|-------|-------|--|
| IviyColoxiii | Type of rood of reed | United States | European Union | Japan | China | |
| Aflatoxin | All foods, except milk | 20 | 2 to 15 | 10 | | |
| Aflatoxin | Rice products | 20 | 10 | 10 | 10 | |
| Aflatoxin M ₁ | Milk | 0.5 | 0.05 | 10 | 0.5 | |
| Aflatoxin | Most feed for finishing animals, except swine | 300 | 20 | 20 | | |
| Aflatoxin | Feed for young animals | 20 | 10 | 10 | | |
| Fumonisin B ₁ B ₂ B ₃ | Whole or partially degermed dry milled corn products | 4,000 | 1,750* | | | |
| Fumonisin B ₁ B ₂ B ₃ | Popcorn | 3,000 | 1,750* | | | |
| Fumonisin $B_1B_2B_3$ | Degermed dry milled corn products | 2,000 | | | | |
| Ochratoxin A | Dried vine fruits | | 10 | | | |
| Ochratoxin A | Cereal grains | | 5 | | | |
| Deoxynivalenol (DON) | Finished wheat for humans | 1,000 | 500 | 1,100 | 1,000 | |
| Deoxynivalenol (DON) | Grain and byproducts, ruminants | 10,000 | | 4,000 | | |
| Deoxynivalenol (DON) | Grain for swine | 5,000 | | 1,000 | | |
| Patulin | Apple juice and concentrates | 50 | 50 | 50 | | |
| Patulin | Apple products labeled for children | 50 | 10 | | 50 | |
| Zearalenone | Animal feeds | | | 1,000 | | |

TABLE 2. ALLOWABLE LIMITS FOR MYCOTOXINS IN FOOD AND ANIMAL FEED IN SELECTED COUNTRIES

Several examples of differences in standards across countries are apparent in Table 2, which provides an overview of regulatory standards governing the presence of major mycotoxins in key foods.

With the exception of patulin in apple juice, E.U. standards for mycotoxins in food and feed are two to fifteen-times lower than those in the United States. Unlike other countries, the European Union has established limits for ochratoxin A in two types of food, while the United States is the only country that has set formal "guidance" limits on fumonisin B_1 in corn based products.

An international effort has been underway for several years to harmonize mycotoxin food safety standards. This effort has focused on standardizing risk assessment methods and data, setting maximum acceptable daily intakes for people and livestock, and establishing limits on specific mycotoxins in food. Countries within the European Union are also working to harmonize EU-wide standards, policies, and monitoring programs (Creppy 2002). As evident in the table, much work remains, not just in harmonizing standards, but also in establishing standards for mycotoxin-food combinations not currently covered.

Mycotoxin Standards and High-Exposure Episodes

In corn and corn-based products intended for human consumption, China has established a maximum allowed standard of 20 micrograms per kilogram (ug/kg) of aflatoxin B_1 (Li et al., 2001). A study in Guangxi province found that 76 percent of corn samples tested for aflatoxin exceeded this standard, and implicated exposure to this mycotoxin as a cause of the elevated levels of hepatocellular carcinomas in the region (Li et al., 2001). The average daily intake of aflatoxin B_1 in the high-risk area was estimated to be 184 micrograms, or 3.7 micrograms per kilogram of body weight. Remarkably, this level of exposure is 3.2-times higher than the dose killing 50 percent of the rats in an acute toxicity study.

Li et al. (2002) have also studied DON in wheat samples in Henan province, following an outbreak of red mold intoxication. They found that 70 percent of the samples tested exceeded the Chinese DON standard of 1,000 micrograms per kilogram, some by a factor of 14 (Li et al., 2002). The mean level of DON in the sampled wheat was 2.8 times the allowable standard, indicative of a serious contamination episode. The authors explain that these exceptionally high levels of DON occurred because of wet conditions during wheat harvest, coupled with favorable conditions for *Fusarium* growth during winter storage period.

While mycotoxin levels and poisoning episodes in the developing world are far more serious than in developed nations, concerns persist in several developed countries over specific mycotoxins and routes of exposure. A team of scientists in the Netherlands calculated a provisional maximum daily acceptable intake of DON of 1.1 microgram per kilogram of body weight (Pieters et al., 2002). Based on this limit, the team proposed a DON concentration limit of 129 micrograms per kilogram (ug/kg) of wheat, a level designed to protect the health of children who are heavy consumers of wheat based products (Pieters et al., 2002). The standard for DON in finished wheat products in the United States is 1,000 ug/kg, about eight-times higher than the level proposed by the Dutch team.

From 1998 through early 2000, the average level of DON in wheat in the Netherlands was 446 micrograms per kilogram. During this period, a probabilistic risk assessment projected that 80 percent of one-year-old children consumed DON over the 1.1 microgram per kilogram provisional standard and that 20 percent consumed more than twice the standard (Pieters et al., 2002).

In an update of their 2002 study, the Dutch team conducted a more sophisticated exposure assessment based on more recent data on DON levels in wheat and determined that the average one-year-old child consumed about one-half the provisional DON standard of 1.1 microgram per kilogram of body weight (Pieters et al., 2004). The child at the 95th level of consumption consumed 1 microgram of DON per kilogram of body weight, just below the provisional standard, leading the team to conclude that "no clear adverse health effects will be associated with the exposure (February 2000-December 2002) to DON in the Netherlands" (Pieters et al., 2004). The authors leave the clear impression that exposures in the 1998-early 2000 period might be associated with health effects in some children.

In part because of concerns raised in the Pieters studies, the Dutch government took on the task of conducting a very extensive survey of foods tested for *Fusarium* mycotoxins. The results of more than 35,000 samples of food tested across the European Union were collected and studied. DON in wheat and wheat-based products was by far the most prevalent mycotoxin in the tested foods. While the average intakes for most population groups were well below the provisional standard, children who are heavy consumers of wheat-based products had estimated exposures very close to the provisional standard (Schothorst et al., 2004).

FREQUENCY AND LEVELS OF MYCOTOXIN IN CONVENTIONAL AND ORGANIC FOODS

Concern over mycotoxins in food is increasing as a result of growth in global agricultural trade and greater understanding of the role of mycotoxin exposures in triggering human and animal health problems. In this section we report the findings of studies that have directly compared mycotoxin levels in foods derived from agricultural commodities grown under conventional and organic production systems. The majority of these studies focus on wheat and other grain-based products, the foods that tend to have the highest levels of mycotoxins and account for the largest share of estimated daily exposures.

While hundreds of mycotoxins exist in nature, it is fortunate that relatively few are regularly found in food at levels that pose food safety risks. Indeed, a significant share of all foods contains very low levels of one, and often several, mycotoxins, but none at levels posing risks to otherwise healthy people. For this reason, the challenge facing all farmers and the food industry is managing mycotoxin levels, not eliminating them. One key ingredient in the management of mycotoxin risk is understanding the conditions that can trigger unusually high levels of mycotoxin production, and then managing farming and food systems to avoid those conditions.

Most governments in the developed world carry out some sort of mycotoxin monitoring and have established standards governing acceptable levels of specific mycotoxins in different foods (see section II, E, which focuses on regulation). A few governments have begun to differentiate between conventional and organic foods in their surveillance and food safety monitoring programs. Some differences have been reported in the levels and frequency of certain mycotoxins in some foods, during specific monitoring periods.

Differences found in government mycotoxin monitoring studies, however, shed little light on the core question of whether organic food is more or less prone to mycotoxin contamination. This is because government programs test too few samples at any one time to reach reliable conclusions regarding differences in mycotoxin levels in organic versus conventional foods. Moreover, government monitoring is not random. A food poisoning episode or cluster of human health problems linked to diet, or animal growth or reproductive problems on farms, sometimes lead to the discovery of unusually high mycotoxin levels in food or animal feeds, and triggers an ad hoc, short-term monitoring study by a government agency.

In addition, the kind of testing for mycotoxins carried out by governments cannot determine whether food became contaminated with a mycotoxin on the farm, as a result of some combination of farming practices and environmental conditions, or after harvest, as a result of how the food was handled, stored, transported, or processed. When mycotoxins are found in multi-ingredient processed foods, it is also not always clear which ingredient was the source of the contamination.

University scientists and research organizations in Europe have conducted the majority of peerreviewed, published studies that have compared mycotoxin contamination in organic versus conventional foods produced in the same region in the same year, and sometimes on similar soils. Comparative studies that seek to identify impacts on mycotoxin frequency and levels need to ideally control for plant genetics, soil types, harvest and storage methods, and the weather and other environmental factors. To our knowledge, there is not a single such ideal comparative study of mycotoxin levels in the current scientific literature.

Direct Comparisons

Nine studies were identified in an extensive search of the peer-reviewed literature that report direct comparisons of mycotoxins in conventional and organic foods purchased and/or grown at the same time, in the same region. While these studies are not carefully controlled comparative farm-level trials, they collectively provide key insights into the differences in the frequency of mycotoxins in food available at the retail level, as well as average levels of mycotoxins found in food. All these studies were carried out by European research teams and reflect food production in the 1997 through 2002 seasons.

Ochratoxin

Ochratoxin is a mycotoxin produced predominantly by *Aspergillus* species that is mainly found in grain, nuts and dried fruits, wine, certain fruit juices, pork and dairy products. It usually emerges as a problem as a result of how raw agricultural commodities and animal feeds are stored prior to consumption by livestock, or before the commodities are processed into a form consumed by people.

Several studies have directly compared ochratoxin levels in conventional and organic foods in the hope of identifying some management strategies that will reliably lessen the occurrence and severity of contamination episodes. Typically, ochratoxin A levels are reported, since this is the most common form of ochratoxin found in food, as well as the most toxic to humans.

An Italian team of scientists analyzed 422 samples of conventional, Integrated Pest Management (IPM) grown, and organic cereals, as well as cereal derivatives like flour and bakery products, for ochratoxin A contamination (Biffi et al., 2004a). All commercial flours and derivatives contained ochratoxin A concentrations well below the legal limit (3 micrograms per kilogram). Data on ochratoxin A levels in conventional and organic foods were reported in five cases involving at least two positives in either the organic or conventional foods tested.

Ochratoxin A levels were higher in the conventional wheat flour, hard wheat baby food, and corn

flour by a factor of 2.5, 8.7, and 4.6 respectively compared to the corresponding organic samples. In one case, conventional samples contained detectable levels while organic samples did not, and in another case, the organic samples contained ochratoxin while the conventional samples did not. Despite these product-specific differences, the authors conclude that "no important difference was found between the two types of agricultural practice when all types of cereal derivatives were considered together."

Another Italian team analyzed the presence of mycotoxins in conventional and organic cocoa products purchased from retail stores (Tafuri.A. et al., 2004). They focused on ochratoxin A because of its reproductive and developmental toxicity and because cocoa is a food commonly consumed by children. They also note that in Europe, ochratoxin A is the mycotoxin most frequently present in food and that more than 90 percent of the people and swine in the European Union have detectable levels in their blood (Tafuri.A. et al., 2004). Nine of 18 samples of cocoa powder had no detectable ochratoxin A, with a detection limit of 10 ng/kg. Nine of 14 conventional samples were positive (64 percent), while none of the four organic samples were positive. Four of the positive conventional samples were above the suggested legal limit of 0.5 ug/kg, leading the authors to suggest further research on mycotoxins in this important ingredient in children's snack foods.

Cereal-based baby food products from grain grown on conventional, IPM-managed, and organic farms in Italy were analyzed for ochratoxin A levels (Beretta et al., 2002). Most contained ochratoxin below the limit of detection, although 16.8 percent contained detectable levels and four samples (3.4 percent) had levels above the very strict standard permitted in baby food, two from conventional grain and two from organic grain (Beretta et al., 2002). The study concludes, however, that there is no significant risk to children who occasionally consume food contaminated at the observed levels. Still, the authors call for stricter controls in order to identify and reject batches of cereal-based baby-food products that contain unusually high ochratoxin A levels.

Ochratoxin is sometimes found in the milk from cows that have consumed contaminated grain or feed concentrates. Norwegian cow's milk and baby formula from organic and conventional sources has been assessed for ochratoxin contamination (Skaug 1999a). No toxin was found in any of the conventional or organic-derived infant formulae. The toxin was detected in 6 out of 40 conventional milk samples (15 percent) and 5 out of 47 organic milk samples (10.6 percent). In addition, the highest level detected in conventional milk was twice the highest level detected in organic milk (Skaug 1999a). The author concluded that the levels of ochratoxin in some cow's milk in this study would result in exposures over the recommended maximum daily intake (5 nanograms per kilogram of body weight per day), especially among children who are heavy milk drinkers (Skaug 1999a).

The same scientist also studied ochratoxin in mother's milk in Norway, to compare levels in different regions and to determine whether breast-feeding would reduce average ochratoxin exposure levels compared to cow's milk. Ochratoxin was found in 33 percent of the human milk samples tested, and at generally higher levels than in cow's milk. The highest level found in breast milk was over twice the maximum level found in conventional milk (Skaug et al., 1998).

Milk is not the only beverage that contributes significantly to ochratoxin A exposure. An Italian team studied ochratoxin A in white and red wines, and found all red wines had detectable levels. They note that monitoring studies show detectable levels of ochratoxin in the blood of over 50 percent of the Italian population, and that the FAO has estimated that about 15 percent of ochratoxin A intake is from contaminated wine (Lo Curto et al., 2004; Tafuri.A. et al., 2004). The team tested 7 red and 16 white wine samples grown in 2000 in three regions using different viticultural systems. The systems varied

significantly in terms of the number of conventional fungicide treatments made, ranging from none to eleven applications. Some samples were not treated with any fungicide, including sulfur.

Major differences were found in the frequency and levels of ochratoxin found across the three regions, pointing to environmental sources of fungi capable of producing ochratoxin A. Very low levels, or no ochratoxin A was detected in the white wine samples. The authors noted that in the red wine samples tested, the levels of ochratoxin A detected could be interpreted as an indicator of the efficacy of fungicide treatments in controlling the fungi producing ochratoxin A. The two red wine samples not sprayed with fungicides contained 2.0 and 0.71 ug/kg of ochratoxin A, compared to an average of 0.18 ug/kg in the five samples treated with nine to 11 fungicide treatments, plus at least two sulfur applications. The European Union standard for ochratoxin A in dried fruits is 10 ug/kg (Food and Agriculture Organization ; Lo Curto et al., 2004; Tafuri.A. et al., 2004).

Deoxynivalenol (DON)

Extensive research has been carried out in Germany over the last decade focusing on the levels of deoxynivalenol (DON) and other trichothecene mycotoxins in cereal-based products, including baby food. Dr. Margit Schollenberger and colleagues at the Institute of Animal Nutrition, University of Hohenheim in Stuttgart, have conducted several studies on DON levels in foods and how organic and conventional production systems have impacted DON contamination levels. This team has carried out one of the most scientifically rigorous, ongoing mycotoxin sampling programs in the world.

In sampling carried out in 1998, the team found lower DON levels in baby food, as well as in cookies and cake, compared to bread (Schollenberger et al., 1999). Foods produced from cereals grown under organic management contained DON less frequently and at lower average levels (Schollenberger et al., 1999). In 1999, the same team tested 60 samples of wheat flour collected from stores and mills in southwest Germany. Wheat flour milled from grain grown on conventional farms contained higher levels of DON than flour from wheat grown under organic production systems (Schollenberger et al., 2002).

During the same period, 101 bread samples were collected from bakeries and food stores within 50 kilometers of the city of Stuttgart (Schollenberger et al., 2003). The breads fell into four groups based on their grain content: at least 90 percent wheat flour, at least 90 percent rye-based flour, breads with rye content between 51 percent and 89 percent, and mixed grain breads with wheat between 50 percent and 89 percent of the flour were tested for DON levels. DON was found in between 79 percent and 100 percent of the breads tested within the different groups. All wheat bread samples contained DON. The median content of DON across the 101 samples was 134 nanograms per kilogram. The mean level in wheat bread (169 ug/kg) was almost six-times higher than the level in rye (29 ug/kg).

Median DON levels in mixed wheat breads made from grain grown using conventional methods were more than four-times higher than the median level in mixed breads from organic flour (Schollenberger et al., 2003). The median DON level in conventional mixed wheat breads was 232 ug/kg, while the organic level was 52 ug/kg. This statistically significant difference was similar to the differences found in DON levels in organic and conventional grain sampled previously in the same region. The team points out, however, that factors in addition to the DON level in conventional and organic flour may impact DON levels in bread. These factors might include other ingredients used in the bread making process, or differences in mixing and baking procedures.

Research by another team of German scientists compared DON levels in winter wheat grown in the Rhineland region in 1997 and 1998 (Birzele et al., 2002). The weather conditions in 1998 were wetter and more conducive to *Fusarium* infection than the 1997 season, which was hotter and dryer. In 1997, 30 percent of the conventional wheat samples not sprayed with fungicides tested positive for DON, while 9 percent of the organic samples were positive. The mean levels of DON in the conventional samples were 250 ug/kg, 2.5-times higher than in the organic samples. In 1998 testing, the frequency of detections in the conventional samples rose to 50 percent, and 17 percent in the organic samples, with mean levels of 485 ug/kg in the conventional samples and 300 ug/kg in the organic samples.

A study in the Thuringia region of Germany also found higher infection rates in conventional wheat and rye compared to organic wheat and rye in research carried out in 1998 (Doll et al., 2002). In wheat, 69 percent of the conventional wheat samples tested positive for DON, at a mean level of 1,540 ug/kg, while 54 percent of the organic samples were positive, at a mean level of 760 ug/kg. While the DON levels in rye were markedly lower, the differences between the conventional and organic samples were greater. Conventional rye tested positive 3.1-times more frequently than the organic samples, at a mean level that was 3.8-times higher than in the organic samples (Doll et al., 2002).

Studies on Fumonisin and DON

Fusarium fungi are the most common and costly pathogens attacking wheat and corn crops around the world. Fumonisin B_1 is the most common mycotoxin produced by the *Fusarium* species that infect agricultural crops, although several other closely related mycotoxins are produced by *Fusarium* fungi, including DON.

Conventional and organic Italian foodstuffs were purchased in retail markets and included 27 batches each of conventional and organic maize-based products (popcorn, flour, polenta, biscuits, breakfast products); 44 batches of conventional wheat-based products and 36 batches of organic wheat-based products (flour, bran, biscuits, bread, and flour); 13 and 11 batches of conventional and organic rice-based foods; and, 17 and 29 batches of conventional and organic mixed-grain products (Cirillo et al., 2003).

Samples were tested for the *Fusarium* toxins fumonisin and deoxynivalenol. Across the 101 samples of conventional and 101 batches of organic foods tested, 82 percent of the organic and 85 percent of the conventional foods contained DON. Across the types of foods tested, DON levels were higher in the conventional batches compared to organic batches of foods, except for in rice-based products (85 percent conventional, 91 percent organic). Fumonisin B_1 was found in 31 percent of the foods from conventional fields and 20 percent in samples from organically-grown grain (Cirillo et al., 2003). The highest median deoxynivalenol levels were found in conventional rice-based foodstuffs, while the highest median level of fumonisin B_1 was found in conventional maize-based foodstuffs.

The Cirillo et al. study found that median DON levels were the same in conventional and organic maize and mixed-based products, but conventional wheat and rice products contained 1.7 and 3.2-times higher DON levels than the corresponding organic batches of food. Across all 202 samples, there was no difference in median DON levels, while fumonisin B_1 levels were 1.2-times higher in conventional batches and fumonisin B_2 levels were 1.7-times higher in the organic batches tested. An organic wheat-based product had the highest level of fumonisin B_2 . In rice-based products, DON appeared more frequently in organic products, but the median level in conventional rice-based products was about

three-times higher (207 ug/kg versus 65 ug/kg). Overall, the organic foodstuffs contained consistently lower contamination levels than conventional foodstuffs (Cirillo et al., 2003). The authors note that their findings are comparable to those reported from similar research in Germany (Schollenberger et al., 2002; Schollenberger et al., 2003; Schollenberger et al., 2005) and France (Malmauret et al., 2002).

Patulin

Patulin is produced by *Penicillium* and *Aspergillus* and sometimes is present at dangerous levels in fruit juices and processed fruit products. It is sometimes referred to as "blue mold rot" in apples and is regarded as an indicator of food quality.

In an Italian study, 11 of 44 samples of apple-based products tested positive for patulin. No significant difference in patulin levels was found between organic and conventional apple products (Ritieni 2003), while three samples from orchards following "integrated" pest management systems were free of patulin contamination. The published report does not provide any information regarding the production practices and pest management systems used in the "integrated" orchards, making it difficult to interpret this aspect of the study's findings.

Zearalenone

The study in the Thuringia region of Germany also compared zearalenone levels in conventional and organic winter wheat samples. Far fewer samples tested positive for zearalenone, compared to DON, and the mean level was far lower. The conventional samples tested positive 1.8-times more frequently, and the mean zearalenone level in the conventional samples was 74 ug/kg, compared to 47 ug/kg in the organic samples.

Multiple Mycotoxins

Scientists in Finland studied 16 *Fusarium* and *Aspergillus* mycotoxins in selected organic and conventional grain-based products, including baby food, from the Finnish and Italian markets. The level of contamination was low overall and no differences were found between the conventional and organic foods (Jestoi et al., 2004). Baby food contained lower levels of mycotoxins (mean 47 micrograms per kilogram), compared to other food products (99 micrograms per kilogram) (Jestoi et al., 2004).

A broad study done by a French team analyzed heavy metals, nitrates and mycotoxins in a range of organic and conventional foods including milk, meat, eggs, vegetables, and cereals (Malmauret et al., 2002). No statistically significant differences in mycotoxin contamination were found between 192 samples of organic and conventional foods. Nearly all conventional and organic wheat samples were contaminated with DON, as was the case with German and Italian monitoring. The authors report that on average organic apples were not more contaminated with patulin than conventional apples, although one organic sample was found to contain a very high level of patulin (1,240 ug/kg) that exceeded the proposed maximum level established by the European Union (50 ug/kg for adults; 10 ug/kg for infants and children).

The study's basic conclusion was that there is "no conclusive evidence whether conventional products are more or less safe than organic ones" in terms of heavy metal, nitrate, and mycotoxin contamination. The two foods accounting for the greatest share of mycotoxin related risks – wheat

contaminated by DON and apple juice with patulin – warrant more intensive monitoring according to this French research team (Malmauret et al., 2002).

French researchers carried out a probabilistic risk assessment of DON exposure levels from 11 organic and 11 conventional wheat samples, drawing on the findings in the Malmauret et al. (2000) study. Monte Carlo simulation was carried out based on food consumption survey data collected by the French national agricultural research agency (INCA). The estimates of exposure were below the established provisional maximum tolerable daily intake of 1 ug/kg body weight per day, assuming a 60 kilogram adult, except for 95th and above levels of the per capita exposure distribution for consumers of organic wheat products (Food and Agriculture Organization 2004; Lo Curto et al., 2004; Tafuri.A. et al., 2004)Leblanc et al., 2002). Still, the study did not produce statistically significant differences in exposure levels by mode of production, given that all consumers regularly consumed cereal-based products and that the distribution of DON levels were different in the limited number of conventional and organic samples included in the simulation (Leblanc et al., 2002). DON was found more frequently in the 11 conventional samples, but were present at a higher mean level in the organic samples (Malmauret et al., 2000).

Overview of Published Studies

Nine studies have been published in the peer-reviewed literature comparing mycotoxin levels in conventional and organic foods. These studies report mycotoxin frequencies and/or levels in 24 matched pairs of conventional and organic food with 2 or more positive samples in either the organic or conventional foods tested. Two assessed patulin in apples, one focused on ochratoxin A in milk, and 21 assessed four mycotoxins in grain-based products, as shown in Table 3. Some of the studies did not report full data on the frequency of detection of average residue levels; these cases are noted with an "NR" (Not Reported) in the table.

The sixth and seventh columns in the table show the ratio of the conventional samples testing positive compared to the percent positive of the organic samples; and the ratio of the mean (and median in one case, see the table's notes) levels in the conventional samples compared to the organic samples. Numbers greater than one in these columns indicate that the conventional foods were relatively more contaminated than the organic samples, and values less than one are cases where organic samples were relatively more contaminated.

It is important to stress that the vast majority of the positive samples of both organic and conventional foods were well below current acceptable levels. The few samples in these seven studies that were over the current limits that have been set, or limits that are under consideration by the European Union, were still very low and did not trigger serious food safety concerns.

Mycotoxins were detected more frequently in the conventional samples compared to the organic samples. There was one case where some conventional samples tested positive, while none of the organic samples were positive, making it impossible to calculate a ratio of the percent positives (ochratoxin A in cocoa); and there was one case where only organic samples tested positive, again making it impossible to calculate a ratio of the percent of samples testing positive (patulin in apples).

The average ratio value of the percent positives was 1.5, suggesting that conventional samples were found positive about 50 percent more often, compared to the corresponding organic samples.

It was possible to compare the average levels of mycotoxins found in conventional and organic samples in 20 of the 24 cases (see column seven in Table 3). The ochratoxin A average levels in milk were not reported, but the author stated in the paper that there was "no difference" in mean levels. Accordingly, there is a value of one for this study in the column reporting the ratio of the conventional mean level to the organic mean level (Skaug, 1999a). In two cases involving the conventional samples, there were no mycotoxins found above the limits of detection, while positives were found in the organic samples. No ratio could be calculated in these cases, nor in the two cases where no mycotoxins were found in the organic samples, while mean levels were reported for the conventional samples. Across the 20 cases, the levels reported in conventional food exceeded those in organic food by a factor of 2.2.

"Conventional samples of food contained mycotoxins about 50% more frequently than the organic samples in a set of comparison studies, at average levels a little over twice as high"

Nine studies reporting 24 comparisons of mycotoxins in conventional and organic foods is far too limited a sample to draw any firm conclusions. Taken together the studies are reassuring, in that the levels of mycotoxins found in the vast majority of cases in organic and conventional foods are low and do not give rise to significant food safety concerns.

The evidence also clearly supports the conclusion that foods with relatively high mycotoxin levels – whether organic or conventional – were grown and/or harvested during unusually wet conditions, or were dried and stored after harvest in a less than optimal way. The importance of weather conditions and post-harvest management in determining the frequency and average levels of mycotoxins is emphasized repeatedly in the published literature, including these nine studies.

Last, these nine studies, coupled with dozens of other published surveys of mycotoxin levels in specific foods that did not present data on both organic and conventional samples, point to a relatively small number of food-mycotoxin combinations that contribute significantly to overall dietary exposure to mycotoxins. The major mycotoxin-food combinations that warrant ongoing research, as well as attention by farmers, grain millers, and the food industry, include:

- Ochratoxin A in milk and grain-based products intended for human consumption, as well as pork products;
- Deoxynivalenol in wheat, barley, and rice-based products;
- Fumonisins in corn-based products intended for humans and in animal feeds;
- Patulin in apple-based products, especially processed products;
- Aflatoxin in nuts, especially imported nuts, and peanut-based products.

TABLE 3. OVERVIEW OF STUDIES COMPARING MYCOTOXIN LEVELS IN CONVENTIONAL AND ORGANIC FOOD

| Manataria and Eard | Percent Samples | | Average Level in | | Ratio Conventional | | Source |
|--|-----------------|---------|------------------|---------|--------------------|------------------|--------------------------------|
| Mycotoxin and Food | Positi | ve | Food (u | g/ kg) | to C | Organic | |
| | Conventional | Organic | Conventional | Organic | 70 POSITIVE | Levels in rood | |
| | 10** | 10** | 0.12.4 | 0.052 | 214. | 25 | D:((: , 1 2004 |
| Wheat Flour | NR** | NR** | 0.134 | 0.053 | NA+ | 2.5 | Biffi et al., 2004 |
| Hard Wheat Baby Food | NR** | NK** | 0.234 | 0.027 | NA+ | 8.,7 | Biffi et al., 2004 |
| Corn Flour | NR** | NR** | 0.17 | 0.037 | NA+ | 4.6 | Biffi et al., 2004 |
| Rice Baby Food | NR** | NK** | ND* | 0.228 | NA+ | NA+ | Biffi et al., 2004 |
| Multicereal Baby Food | NR** | NR** | 0.159 | ND* | NA+ | NA+ | Biffi et al., 2004 |
| Сосоа | 64% | 0% | 0.43 | ND* | NA+ | NA+ | Tafuri et al., 2004 |
| Milk | 15% | 10% | NR** | NR** | 1.4 | 1: No Difference | Skaug, 1999a |
| DEOXYNIVALENOL | Conventional | Organic | Conventional | Organic | % Positive | Levels in food | |
| Mixed Grain Bread | 96% | 82% | 184 | 62 | 1.2 | 3.0 | Schollenberger et. al. 1999 |
| Grain-based Products | 85% | 82% | 65 | 65 | 1.04 | 1.0 | Cirillo et al., 2003 |
| Wheat-based Products | 77% | 69% | 65 | 38 | 1.1 | 1.7 | Cirillo et al., 2003 |
| Rice-based Products | 85% | 91% | 207 | 65 | 0.9 | 3.2 | Cirillo et al., 2003 |
| Wheat | 91% | 55% | 55 | 106 | 1.7 | 0.5 | Malmauret et al., 2002 |
| Barley | 80% | 60% | 41 | 69 | 1.3 | 0.6 | Malmauret et al., 2002 |
| Wheat | 69% | 54% | 1,540 | 780 | 1.3 | 2.0 | Doll et al., 2002 |
| Rye | 34% | 11% | 490 | 130 | 3.1 | 3.8 | Doll et al., 2002 |
| Wheat (1997) | 30% | 9% | 250 | 100 | 3.3 | 2.5 | Birzele et al., 2002 |
| Wheat (1998) | 50% | 17% | 485 | 300 | 2.9 | 1.6 | Birzele et al., 2002 |
| FUMONISIN B ₁ | Conventional | Organic | Conventional | Organic | % Positive | Levels in food | |
| Grain-based Products | 20% | 31% | 80 | 67 | 0.6 | 1.2 | Cirillo et al., 2003 |
| Corn-based Products | 30% | 44% | 345 | 185 | 0.7 | 1.9 | Cirillo et al., 2003 |
| FUMONISIN B ₂ | Conventional | Organic | Conventional | Organic | % Positive | Levels in food | |
| Grain-based Products | 38% | 32% | 90 | 150 | 1.2 | 0.6 | Cirillo et al., 2003 |
| Corn-based Products | 22% | 32% | 20 | 120 | 0.7 | 0.2 | Cirillo et al., 2003 |
| PATULIN | Conventional | Organic | Conventional | Organic | % Positive | Levels in food | |
| Apple-based Products | 28% | 35% | 24.8 | 28.3 | 0.6 | 0.9 | Ritieni, 2003 |
| Apples | 0% | 33% | ND* | 5 | NA* | NA* | Malmauret et al., 2002 |
| ZEARALENONE | Conventional | Organic | Conventional | Organic | % Positive | Levels in food | |
| Wheat | 7% | 4% | 74 | 47 | 1.8 | 1.6 | Doll et al., 2002 |
| Notes: ND*= None Detected; NA* = Not applicable or cannot be calculated; NR** = Not Reported; 1. The Cirillo et al. study reported only median concentration levels, not average or mean levels. | | | | | | | |

The Organic Center

Indirect Measures of Mycotoxin Contamination and Risks in Conventional and Organic Foods

Other methods have been used to project exposures and risks associated with mycotoxins in the diet. A recent study by an Italian team presents a comparative risk assessment of multiple hazards in organic and conventional wheat (Finamore et al., 2004). The team developed a new immunological assay to compare the impacts of chemical toxins in conventional foods in contrast to the mycotoxins and other natural toxins found in both conventional and organic food.

Conventional and organic wheat-based diets were fed to groups of rats. Eight varieties of conventional and organic wheat were grown under carefully controlled conditions and were mixed in equal parts in preparing the rations. The organic wheat contained 3-X the concentration of mycotoxins, compared to the conventional wheat, although levels in both sources of feed were regarded as within acceptable levels.

Two sensitive markers of immunological responses were measured in the rats. One involves the degree of proliferation in lymphocytes found in the intestine and in the spleen. The second is based on the short-term response of the liver to the presence of toxins ingested via food.

Stress, disease, and hunger are among many factors that can impair an animal's ability to detoxify or otherwise overcome exposure to toxins (Finamore et al., 2004). The team decided to study how protein energy malnutrition (PEM) impacted the animals' response to toxins in the diet, so the rats were divided into a well-nourished group (WN) and a PEM group and fed the conventional or organic wheat for 30 days.

The scientists analyzed lymphocyte proliferation in the two groups of rats. They did this through the use of a cell culture medium composed of either fetal calf serum (FCS) or the rat's own serum (RS). They stimulated rat lymphocyte cells in each culture medium with a chemical known to trigger cell division in order to mimic the proliferative response.

They found no differences in the proliferative response of lymphocytes cultured with FCS in rats fed organic versus conventional wheat, under either well-fed or PEM conditions. The proliferative response of lymphocytes cultured with the rat's own serum, however, was depressed in PEM rats on conventional feed compared to the animals fed organic wheat. The authors attribute the effect to pesticides and possibly other contaminants in the conventionally grown wheat.

The results showed that under the conditions of this study and in animals under stress because of inadequate food intake, conventionally grown wheat poses a higher risk for impaired immune system response compared to wheat that is organically grown. The authors highlight the fact that the toxic impacts of synthetic chemicals and/or other toxins in the conventional wheat elicited a greater biological response than the mycotoxins in the organic wheat.

A study was carried out in the United Kingdom to assess the health impacts of dietary choices in a population of 11,000 health-conscious people. The study population included 4,627 vegetarians and 6,699 people consuming whole-meal bread on a daily basis (Key et al., 1996). Over three-quarters reported frequent and heavy fruit and vegetable consumption. The health status of these people was tracked for 16.8 years. Compared to the rest of the U.K. population, the subjects in this study had reduced mortality from heart disease, cerebrovascular disease, and all causes combined (Key et al., 1996).

While the study produced no definitive explanation of the cause of the differences noted in health outcomes, the results suggest that mycotoxins are not significantly contributing to these diseases in the United Kingdom, since mycotoxin exposures are likely higher in people who are heavy consumers of whole-grain breads and fruits and vegetables. Alternatively, the study population may have been exposed to higher levels of mycotoxins through their diet, but remained healthier because of higher antioxidant intakes and other, generally health-promoting life style choices.

Widely Cited Contamination Episodes Used to Support the Assertion that Organic Foods Are More Prone to Mycotoxin Contamination than Conventional Foods

Since the mid-1990s, the risks, benefits, and costs of genetically-modified (GM) crops have been a focal point of public discussion on the future of farming and food system technological innovation. In Europe, the debate has come to focus on a comparative assessment of the likely outcomes associated with two trajectories for change – one rooted in specialization, relatively more dependent on chemical inputs and animal drugs, and inclined to embrace GM technology and seeds, and the second, founded on organic and/or sustainable farming systems that minimize the need for pesticides, exploit crop and farming system diversity, and avoid use of, or reject outright current-generation genetically- engineered crops, animals, and production inputs.

Mycotoxin issues have been brought into this debate by proponents of biotechnology who feel that the potential risks and benefits of organic farming have not received the same level of critical scrutiny directed toward GM crops and food. The Center for Global Food Issues (a program of the Hudson Institute) has led the effort in the United States to raise concerns about mycotoxins in organic food (e.g., see Avery et al., 2003).

Proponents of biotechnology have cited two types of information in support of their assertions that organic farming increases the likelihood of mycotoxin food safety problems and risks, while GM-crop agriculture reduces such risks: (1) government surveys of mycotoxins in conventional and organic food; and (2) data on levels of mycotoxins in GM-corn engineered to resist damage from European corn borers, compared to non-GM varieties of corn grown in conventionally managed systems without pesticides, and often with artificially added insects. No published study has compared natural insect damage in corn and mycotoxin levels in organically managed fields, compared to nearby fields planted to GM-corn.

U.K. Spice and Cornmeal Surveys

Those hoping to raise doubts about the safety of organic food typically cite two United Kingdom surveys of mycotoxins in conventional and organic food. Both surveys were carried out by the U.K. Food Standards Agency (FSA). One focused on imported spices and the second on corn meals.

The FSA tested 61 samples of imported spices after receiving a notification from Hungary reporting aflatoxin levels in paprika (a type of ground red pepper) over the European Union allowable level of 5 ug/kg for aflatoxin B1 and 10 ug/kg for total aflatoxins. Samples of pepper, paprika, and chili powder were collected from a wide cross-section of warehouses, packing houses, supermarkets, and small specialty shops. The product name on the label included the word "organic" in three cases, and "natural"

in two cases (Food Standards Agency, 2003). No information was given regarding whether the organic and natural samples were certified, or complied with any given set of standards. In the report on its testing, the FSA grouped together the organic and "natural" samples and labeled the group "organic."

Table 4 summarizes the findings in the 56 conventional samples and the five samples in which the term organic or natural was used on the product's label. The average levels of aflatoxin B_1 in both conventional and organic spices were low compared to the applicable limit – 5 ug/kg for aflatoxin B_1 . The European Union has established a limit of 10 ug/kg of ochratoxin A in dried fruits, but has yet to finalize a standard applicable to spices. Average levels of ochratoxin A in both conventional and organic spices were close to the limit established for dried fruits. According to the FSA, only two of the positive samples "could have caused consumers to exceed an exposure of 5 ng/kg of bw/day" (Food Standards Agency, 2003). One (the highest) was conventional and the second was labeled organic.

TABLE 4. MYCOTOXINS FOUND IN THE UNITED KINGDOM'S SAMPLING OF SPICES

| | Average Level 1 | Found (ug/kg) | Maximum Value Found (ug/kg) | | |
|--|--------------------------|---------------|-----------------------------|--------------|--|
| | Aflatoxin B ₁ | Ochratoxin A | Aflatoxin B ₁ | Ochratoxin A | |
| Organic and "Natural" Samples (5) | 2.6 | 12.9 | 6.8 | 47.7 | |
| Conventional Samples (56) | 1.9 | 9.0 | 13.9 | 152.2 | |
| | | | | | |
| Ratio Organic to Conventional | 1.3 | 1.4 | 0.5 | 0.3 | |
| Source: Calculated from Table 4. "Survey of Spices for Aflatoxins and Ochratoxin A." Food Standards Agency, United Kingdom | | | | | |

The levels found were highly variable. The fourth and fifth columns in the table report the maximum level found in conventional and organic samples. In the case of aflatoxin B_1 , the highest level in an organic-labeled sample was about half the maximum level in the conventional samples. The maximum level of ochratoxin found in a sample labeled organic was about one-third the maximum in a conventional sample.

Overall, the five organic and "natural" spices contained marginally higher levels than the conventional spices – by a 30 percent margin in the case of aflatoxin B_1 and 40 percent in the case of ochratoxin A. Given the very small sample size and the very large standard deviation in the levels, these differences are clearly not statistically significant.

The Food Standards Agency also assessed mycotoxins in corn-based products in a special survey in 2003 involving 292 samples. This survey identified two samples of maize (corn) meal with unusually high levels of total fumonisins (4,712 ug/kg and 20,435 ug/kg), both labeled organic. The FSA issued a notice to withdraw these products from the market, although it stated in its September 10, 2003 press release that there is "unlikely to be any immediate risk to health" associated with the withdrawn brands of corn meal.

These two relatively high samples triggered further Food Standards Agency testing of 30 samples of maize products, of which 10 were found to contain total fumonisins over the proposed European Commission level of 500 ug/kg. Six of the 10 were organic, and four were conventional. Again, the FSA stated in its September 26, 2003 press release announcing that these 10 brands had been withdrawn from the market that "there is unlikely to be any significant risk to health if these products are consumed."

In January 2005 the Food Standard Agency released the full results of its 2003 sampling in a 55-

page report entitled "Survey of maize-based retail products for mycotoxins" (Food Standards Agency, 2005). The 292 samples included sweet corn, corn on the cob, corn oil, corn flour, polenta, maize meal, tortillas, and maize-based snacks. No samples were found to contain either aflatoxin or ochratoxin A over legal limits. The only worrisome findings involved fumonisins.

The majority of samples were conventional (they were not labeled as organic), and for several of the foods tested there were no or very few organic samples collected. Sweet corn and corn on the cob were the most intensively sampled foods, accounting for 60 of the 292 samples. Five of the 60 sweetcorn/corn on the cob samples were labeled organic. Six of the conventional samples contained total fumonisins over 100 ug/kg, with a maximum value of 230.7 ug/kg, while the highest level found in an organic sample was 48.6 ug/kg. The organic samples averaged about 32 ug/kg of total fumonisins, well below the 400 ug/kg proposed limit currently under review by the European Union.

The second most intensively sampled type of corn-based food was corn flake cereal. There were only two organic samples out of a total of 38. The organic samples contained 51.2 ug/kg and 468.5 ug/kg of total fumonisins. Eight of the 36 conventional corn cereal samples contained total fumonisins over 100 ug/kg (values in ug/kg were 420, 333, 285, 243, 185, 169, 145, 106). The most heavily contaminated conventional sample contained nearly the same level as the organic sample with the highest level.

In its summary report, the FSA stated: "The dietary exposures were below established safety guidelines, indicating that consumption of these products does not pose an appreciable risk to human health." In summarizing the findings on conventional and organic maize-based products, the FSA said:

"Although the levels are superficially similar, there are not enough data to statistically determine if foods based on conventionally-grown maize are generally higher or lower in mycotoxins content compared with foods produced from organically–grown maize." (Food Standards Agency, 2005)

Genetically Modified Corn and Mycotoxins

In one of its public statements on the recall in the United Kingdom of 10 maize meal products, including six labeled organic, the Center for Global Food Issues states that –

"Ironically, biotech corn produces the safest corn meal of all. The U.S. Agricultural Research Service has found that fumonisin levels in Bt corn (genetically engineered to contain a natural insecticide) is 30 to 40 times lower in pesticide-protected conventional corn." (Avery et al., 2003)

Bt corn has been shown in several studies to reduce fumonisin levels compared to conventional corn that suffers damage as a result of European corn borer (ECB) feeding (Clements et al., 2003; Munkvold 2003). When corn borers damage the top part of the growing ear of corn, the wound site provides a point of entry for *Fusarium* spores and can trigger the growth of fungi, which in turn can produce fumonisin. In much of the U.S. corn crop, the levels of fumonisin remain well below acceptable limits on account of a lack of ECB feeding damage and the efficient drying facilities on most commercial farms.

The key Agricultural Research Service study on Bt corn and fumonisin levels cited by the Center for Global Food Issues found fumonisin levels in grain from <1 ug/gram to 210 ug/gram (Clements et al., 2003; Munkvold 2003). The average level in Bt-hybrids was 8 ug/g, and 10 ug/g in non-Bt hybrids. The authors state that Bt-corn will generally reduce ECB feeding damage and fumonisin levels compared to hybrids that are relatively susceptible to ECB feeding damage, but not when compared to the more resistant non-GM hybrids. Munkvold (2003) cites studies in Iowa where fumonisin levels were ten-times higher in fields with moderate ECB pressure. But in field studies in 55 locations in 11 states assessing corn damage under natural ECB population levels, Bt corn reduced fumonisin levels on average by onehalf. A tenfold reduction was recorded only in locations where the corn plants were manually infested with corn borers (Munkvold, 2003).

None of the research to date has compared ECB damage to corn and fumonisin levels in comparably well-managed fields of conventional corn hybrids and *Bt*-corn grown by organic and conventional farmers. Published research clearly supports three conclusions:

- Insect-damaged corn is more vulnerable to fumonisin contamination than undamaged corn.
- *Bt*-corn hybrids suffer little or no ECB feeding damage, but still often become infected with *Fusarium* fungi.
- Proper drying and storage of corn will prevent substantial buildup of fumonisin levels in corn in the vast majority of cases where some level of infection occurs in the field prior to harvest.

Farmers producing organic field corn manage ECBs largely through crop rotation, by balancing nutrient supplies relative to plant needs, and through biological control processes. Researchers at Ohio State University have found that the generally lower levels of readily available plant nutrients on well-established organic farms tend to discourage feeding of ECB larvae, and can also discourage adult moths from landing on plants in organic fields.

Still, organically produced field corn does sometimes suffer ECB feeding damage and will, in some years, contain fumonisins at harvest. If such grain is not properly dried, handled, and stored, the potential exists for fumonisin levels to rise during storage to levels that may be dangerous to the animals fed the corn, or people if the corn is manufactured into products for direct human consumption. This same potential exists on conventional farms, but to a somewhat lesser degree on farms planting *Bt*-corn hybrids.

Other Instances and Episodes

A report from "Science in Africa" indicated that patulin was present in commercial apple products in Africa and claimed that a study on organically produced apple cider has found "levels up to 40,000 micrograms per liter" (Theunissen 2002). Since one liter of apple juice weighs about 1,000 grams, the apple juice sample cited by Theunissen would have contained 0.004 percent patulin by weight. No explanation was offered regarding why, how, or when the organic juice had become contaminated. Clearly, the presence of such levels, if true, suggest some extraordinary circumstances. In an Italian study of patulin in apple products, the mean of the positive samples was 26.7 microgram per liter (Ritieni 2003) – about 1,500-times lower than the highly contaminated organic juice samples in Africa.

The author of the "Science for Africa" article used the unsubstantiated finding of an organic apple juice sample with 40,000 micrograms per liter to make general claims about the unsafe practices in organic agriculture. We are not aware of a single peer-reviewed report documenting such an

extraordinary high level of patulin in any food. Still, this unusual organic apple juice sample from Africa is often cited by critics of organic agriculture as evidence that organic production practices lead to higher levels of mycotoxin contamination.

IMPACTS OF CONVENTIONAL AND ORGANIC FARMING SYSTEMS ON MYCOTOXIN CONTAMINATION

Mycotoxins are produced by certain species of fungi that sometimes infect and grow on agricultural crops. Mycotoxins in animal feed can show up in milk, eggs, and other animal products. The question at the heart of this State of Science Review is –

Are there factors intrinsic to organic and/or conventional production systems that increase susceptibility to fungal infection and the production of mycotoxins?

Two widely accepted conclusions deserve emphasis:

1. Inappropriate post-harvest handling and storage of conventional and organic food greatly increases the risk of mycotoxin contamination and has little or nothing to do with how the food was produced in the field.

2. Unusual and largely uncontrollable environmental conditions play an important role in triggering a significant portion of the serious mycotoxin contamination episodes that have occurred, regardless of how food was grown.

Much work is needed to fully understand how farming practices – conventional or organic – impact mycotoxin formation, even in a single production system and region. To develop such understanding comprehensively, across all crops, regions, and farming methods, will take a century or two.

There has been virtually no research in the United States specifically on the impact of organic farming systems on the factors either leading to, or preventing mycotoxin contamination. There has been modest research on fungal disease control on organic farms. There has been substantial work around the world on the general ecological impacts of organic farming practices on soil quality, nutrient cycles, soil microbial diversity and the soil food web, and biological control of insects. Accordingly, it is possible now to only offer plausible explanations of the ways that organic farming practices and systems may be impacting mycotoxin formation. Much more work will be needed to prove cause and effect relationships.

Mycotoxin Risk Factors Associated with Conventional Farming Systems

Many conventional farming systems control fungal infections by applications of fungicides, although for many species of fungi on lower-value crops, there are no cost-effective pesticide treatments available. For the most part, this is the case with *Fusarium* infections in wheat and other small grains. On fruits and vegetables, however, fungicides are frequently used to prevent disease losses. Often two to four fungicides are applied on higher-value crops like tomatoes, apples, potatoes, and grapes, especially in

hot, humid areas. Four to six fungicide applications are common, and some crops are treated with 10 or more fungicide applications.

The use of fungicides and other types of pesticides is obviously a major difference between conventional and organic systems. While fungicides usually control certain fungal species, they tend to work for one to a few weeks and rarely control all fungal species in an agroecosystem. Many studies have shown that some fungicides dramatically reduce fungal infections, yet do not decrease mycotoxin levels (Edwards et al., 2001; Jennings et al., 2000; Menniti et al., 2003). In addition, some fungi can actually utilize fungicides as a food source. In one study, treatment with dithane Z-78, an ethylene-bis-dithiocarbamate fungicide (EBDC), actually stimulated production of aflatoxin (Hasan 1994). The concentration of the mycotoxin nivalenol increased 16-fold in wheat treated with a combination of the fungicides tebuconazole and triadimenol, despite reducing the severity of Fusarium infection (D'Mello et al., 1998; Lutz et al., 2003b).

Treatment of cereal fields with the strobilurin fungicide azoxystrobin increased DON levels in several field trials, even though it also reduces *Fusarium* infection rates (Jennings et al., 2000). Work in Europe has shown that complex weather-disease-cropping system interactions can occur in grain fields that are treated with fungicides, sometimes leading to little or no change, or even increases in mycotoxin levels (Hope et al., 2003). Other studies on azoxystrobin and other fungicide applications on wheat suggest that changes in *Fusarium* fungal species diversity and population dynamics occur that favor DON-producing species, thereby leading to increased DON levels in treated fields (Edwards et al., 2001; Jennings et al., 2000).



In a review article on agricultural practices and mycotoxins, Edwards (2002) notes that sub-lethal applications of fungicides can sometimes stimulate mycotoxin formation. This likely occurs because the fungi are stressed by the fungicide, but not killed. The production of mycotoxins is a normal response to stress in many fungal species. In addition, research has shown that resistance to fungicides in one fungal species can both stimulate mycotoxin production in other species and alter the concentrations of different mycotoxins (D'Mello et al., 2001).

Insecticides also are known to sometimes serve as a food source for certain fungal species. Organophosphate (OP) and carbamates insecticides, in particular, sometimes stimulate growth of certain fungi both in the soil and on plants. *Fusarium oxysporum* has been shown to grow on five pesticides that serve as a phosphorous source; one study found a more than 50 percent increase in growth of Fusarium fungi in the presence of these pesticides (Hasan 1999).

In one sense, the capacity of fungi to utilize pesticides as a food source is a positive thing, since it accelerates the breakdown of pesticides and reduces their half-lives on plant tissues and in the soil. But this advantage also is a disadvantage from the perspective of minimizing fungal infections in crop plants and reducing the frequency of mycotoxin contamination.

Many studies have shown that individual fungicides reduce the populations of certain fungi, while stimulating the growth of others, either directly or as a result of lessened competition from other fungal species for food sources. Overall, the use of fungicides almost certainly reduces the frequency and severity of mycotoxin contamination in some crops in certain years, but rarely will fungicides eliminate the risk of fungal infection and mycotoxin buildup. In the majority of crops, however, fungicides are never or are not routinely applied, or do not control a broad enough spectrum of fungal pathogens to substantially reduce mycotoxin contamination risk.

Harvest time, and post-harvest infection will always remain critical periods when farmers must take the appropriate precautions to avoid allowing fungi to gain a foothold in stored commodities, regardless of how they were grown.

Mycotoxin Risk Factors Associated with Organic Farming Systems

In any given agricultural region, organic and conventional farmers must find ways to sustain soil fertility and manage locally adapted fungal species. In the case of managing fungal pathogens, the wetter the season, the more difficult this task generally proves to be.

Conventional farmers generally rely on prevention, genetics and chemistry to manage fungal infections, while organic farmers tend to rely on the plant's ability to control fungal infections when grown with optimal rather than maximal nutrient supply, supported by prevention, genetics and the management of interactions among fungal species and between fungi and bacteria. Organic fruit and vegetable farmers also rely to some extent on spraying fungicides, but their choice of products is limited to those that are non-synthetic and of natural origin, like cooper-based fungicides, oils, and sulfur. Because of the limited supply of fungicides, some organic farmers have relied excessively on cooper and sulfur-based products. Frequent and heavy applications of these fungicides on organic farms lead to the same sort of problems experienced on conventional farms where fungicides bear most of the disease control burden in crops vulnerable to serious economic losses from plant diseases.

Microbial Diversity

It is known that each variety of fruits, vegetables, and nuts grown in a certain region is likely to be colonized by a unique combination of fungal species, and moreover, that several fungi are typically going to be present and growing at any given time (Bayman et al., 2002). These fungi must constantly compete with a number of species to sustain growth in a given microenvironment; the more species present, the more likely it becomes that no species will dominate and reach population levels high enough to produce detectable levels of mycotoxins.

Given that organic farming systems tend to promote greater diversity in microbial communities and more complex food webs, both below and above the ground, ongoing competition among fungal species, and between fungi and bacteria, should help assure that no one fungal species reaches dangerous levels. There is evidence from European research that supports this hypothesis (Brandt et al., 2005).

In addition, recent research points to biological control benefits from low levels of mycotoxin contamination in some crops. Deoxynivalenol (DON) is the major mycotoxin formed by several *Fusarium* species. In addition to its toxicity to mammals, DON has been shown to have significant biological activity against other plant pathogens, some with potential to produce other mycotoxins (Lutz et al., 2003a). As a result, the greater diversity of fungal species in organic fields may lead to low levels of a variety of mycotoxins, which in turn play a positive role in biological control of other pathogens.

Disease Suppressive Soils

Several management practices are commonly used on organic farms to build soil organic matter levels. The most common are the application of compost and other organic materials, and the planting of cover crops, which are subsequently worked into the soil. These practices build soil organic matter by enhancing the diversity of soil microbial communities and creating more complex food webs that can support both greater diversity in organisms and higher population levels of many beneficial organisms. The goal is to create what is called a "disease suppressive" soil (Mazzola 2002).

Suppressive soils are characterized by low levels of root and plant disease, despite the presence of multiple virulent pathogens and a susceptible host crop. Such soils prevent plant diseases primarily through biological processes including competition for nutrients, antibiosis (the production of antibiotics or other natural biochemicals that are toxic to other life forms), and stimulation of plant defense mechanisms via a process called systemic acquired resistance, or SAR. For example, non-pathogenic *Fusarium* species and fluorescent *Pseudomonas* species play key roles in suppressing pathogenic *Fusarium* species, including those that produce mycotoxins (Mazzola 2002). Organic management systems, in particular, encompass many of the practices known to support the proliferation of non-pathogenic fungal species and as a result, can trigger higher levels of competition among more diverse mixtures of fungal and bacteria species.

The tendency of soils on organic farms to sustain more diverse microbial communities, as well as higher levels of microbial activity, likely explains why many organic farmers are able to routinely harvest disease-free produce and grains, despite the presence of pathogenic strains in the surrounding area and no use of synthetic fungicides. The lower level of readily available nitrogen on organic farms is another factor that can reduce fungal infections and mycotoxin risk.

Crops are often most vulnerable to fungal attack toward the end of their growth cycle as harvest time approaches. This is when fungal pathogens can gain a foothold on maturing fruit and grain, especially in the presence of some wound or damage to plant tissues or fruit. Initial infection can set the stage for problems, especially if weather conditions prove conducive to fungal growth. Again, the relatively greater diversity of microorganisms colonizing leaf surfaces, the skins of fruit and vegetables, and other crops on organic farms can help moderate the increases in fungal populations late in the season, even when conditions are favorable for fungi. The risk of serious mycotoxin problems will be lessened as a result. Lessened, but not eliminated, just like on conventional farms where fungicides are routinely applied.

Compost Tea

Many organic fruit and vegetable farms now apply compost teas to crop leaves and fruit. These teas are liquid solutions containing high levels of microorganisms extracted from actively maturing compost. The purpose of applying compost tea is to inoculate the surface of plant leaves and maturing fruit with high levels of relatively benign microorganisms, in the hope they will effectively compete with and suppress the populations of pathogenic fungi.

A tactic with impacts similar to compost tea is under development for application in wheatproducing areas. It entails applying to growing wheat antagonistic strains of fungi that depress the growth of pathogenic strains of *Fusarium* species, thereby reducing DON levels. Reductions of over 50 percent in DON levels in the field have been accomplished after application of an antagonist (Schisler et al., 2002).

Resistance to Heat Stress

Plants experiencing stress from excessive heat and/or other environmental factors are more vulnerable to fungal and mycotoxin infections (Abbas et al., 2002a). Plant varieties bred to withstand heat stress tend to have significantly lower levels of fumonisins and aflatoxin. Accordingly, farming systems that tend to lessen the severity of heat stress may also tend to reduce the frequency and levels of mycotoxin contamination.

Soils on long-established organic farms tend to have higher levels of organic matter and lower bulk density than nearby farms managed conventionally. As a result, they tend to take in and hold moisture more effectively than otherwise similar soils that have been managed conventionally. For this reason, at least in some regions during some seasons, plants growing on organic farms are likely to suffer less from heat-related stresses. This should also lessen mycotoxin risks.

Overview of Farming System Impacts

Weather is clearly a major factor driving mycotoxin risks that impacts organic and conventional farmers in roughly the same way. Avoiding physical damage to grain during harvest, assuring that grain is ripe and not too damp at harvest, quick and consistent drying prior to storage, and the use of clean and dry storage facilities are equally vital on organic and conventional grain farms.

In several other ways, however, conventional and organic farming systems differ in the degree to which they may allow mycotoxins a foothold in harvested crops, or set the stage of mycotoxins to proliferate during the storage of grain or animal feed. Two German scientists reviewed 50 studies addressing factors impacting mycotoxin contamination levels on conventional and organic grain and livestock farms. They produced a table summarizing how 13 factors generally impacted the risk of mycotoxin contamination (Paulsen et al., 2002). Table 5 presents their conclusions.

| TABLE 5. DIFFERENCES IN | N THE FACTORS CONTRIBU | TING TO MYCOTOXIN | | | |
|---|------------------------|-------------------|--|--|--|
| RISKS ON CONVENTIONAL AND ORGANIC GRAIN AND LIVESTOCK FARMS | | | | | |
| | Conventional Farms | Organic Farms | | | |
| Weather | Equal Impact | Equal Impact | | | |
| Site Conditions | Equal Impact | Equal Impact | | | |
| Storage/Drying | Equal Impact | Equal Impact | | | |
| Plant Variety | Negative | Positive | | | |
| Tillage | Negative | Positive | | | |
| Crop Rotation | Negative | Positive | | | |
| Use of Growth Regulators | Negative | Positive | | | |
| Fertilization | Negative | Positive | | | |
| Population Density | Negative | Positive | | | |
| Fodder | Negative | Positive | | | |
| Fungicides | Positive | Negative | | | |
| Presence of Weeds | Positive | Negative | | | |
| Livestock Bedding | Positive | Negative | | | |
| Source: Paulsen and Weissman, 2002. | | | | | |

Three of the most important factors – weather, site conditions, and storage – impact both systems equally. Organic farming systems tend to lower mycotoxin risks relative to conventional systems because of seven factors included in the table, and conventional farms pose lower relative risks because of two factors.

The authors explain that organic farmers tend to plant grain varieties with longer stems because they are less concerned about lodging ("lodging" occurs when grain grows too tall and falls over). Higher levels of fertilization on conventional farms trigger faster growth of grain plants, which can lead to lodging and require applications of plant growth regulators and fungicides (Paulsen et al., 2002). For this reason, conventional farmers are more likely to choose dwarf varieties, where the flowers and stems of grain are closer to the ground, and hence somewhat more likely to become contaminated by fungal spores in the soil. Research in Switzerland has also shown that higher levels of fertilization on conventional farms can stimulate the growth of mycotoxin-producing fungi, especially *Fusarium* species (Gunst et al., 2001).

Crop rotations and tillage – key practices on organic farms – tend to reduce innoculum levels in the soil. Paulsen et al. (2002) point out that the typically lower plant densities on organic farms, coupled with varieties producing longer, stronger stems, creates more stable cell walls that are marginally less vulnerable to fungal infections. Research in Switzerland demonstrated a significant impact of tillage system and rotations on the risk of *Fusarium* infections and DON contamination in winter wheat. Moldboard plowing, use of a disc-cultivator, and no-tillage systems were compared. The highest incidence of disease (42 percent infection) and highest DON levels (up to 6 parts per million) occurred in the no-tillage plots with maize or wheat as the previous crop (Krebs et al., 2000).

Fodder (forage-based animal feed) is identified as an advantage for organic systems because organic farms tend to produce a higher percentage of their feed on the farm, and bring less grain and forage onto the farm that was grown elsewhere. The authors conclude that this higher level of self-

Breaking the Mold

sufficiency in feed lessens the risk of bringing mycotoxins onto the farm from feed grown elsewhere.

Use of fungicides is the key factor on conventional farms that can reduce fungal pathogen pressures compared to organic farms. The generally higher weed populations on organic farms provide alternate hosts for fungi, and hence increases mycotoxin risk compared to conventional farms. Some research has shown, however, that fungicide applications can actually stimulate mycotoxin production (Edwards, 2002).

Last, the bedding and husbandry requirements on organic farms tend to produce substrates that can support fungal growth and increase mycotoxin risks. Paulsen and WeiBmann conclude their paper by stating –

"Organic farming is not generally more endangered by the risk of contamination of the products with mycotoxins than other farming systems. Knowledge about the influence of litter beddings on mycotoxin exposure of livestock is rare. Due to restrictions on silage additives and fungicides, organic farms are limited in their possibilities to prevent and to cure fungal diseases. But the organic production system offers several important factors for lowering infections with mycotoxin producing fungi." (Paulsen et al., 2002).

The conclusions drawn by other scientists, organizations and meetings of experts regarding the relative risks of mycotoxin contamination in organic and conventional foods appear in the section"What Others Have Concluded."

What Others Have Concluded

The "Sense of the Congress" statement from the "First World Congress on Organic Food," held in March 2004 at Michigan State University, states –

"Organic farming systems represent greater biodiversity in above and below ground habitats. These agroecological communities provide a buffering capacity for the soil and plant surfaces, and therefore may lower the likelihood of establishment of food borne enteric pathogens or plant pathogens. There is evidence that organically grown plants have stronger natural protection against plant pathogens than conventional ones, and this may also be the case for enteric bacteria, but needs to be verified."

In a recent review of the impact of organic farming on food quality and safety presented at an international conference in 2005, two European scientists stated that:

"Due to the high intrinsic resistance to diseases, organic products tend to have low incidence of infections with mycotoxin-forming fungi and storage diseases. This reduces the risk of toxic contaminations, both with mycotoxins and with natural plant toxins (phytoalexins)." (Brandt et al., 2005).

The summary of a review of the impact of fungicide treatments on mycotoxin levels states -

"...the overall evidence concerning the effectiveness of fungicides [in reducing mycotoxin

contamination] is contradictory and in certain cases somewhat unexpected. In particular, at sub-lethal doses of a number of fungicides...mycotoxin production from Fusarium phytopathogens may increase." (D'Mello et al., 1998).

A review of organic food safety and quality issues was carried out during the 22nd Food and Agriculture Organization (FAO) regional conference for Europe in 2003. The meeting report states that

"...it cannot be concluded that organic farming leads to an increased risk of mycotoxin contamination. It is important to emphasize that good agricultural practice, handling and storage practices are required in organic and conventional agriculture to minimize the risk of mold growth and mycotoxin contamination." (Food and Agriculture Organization, 2000).

Two Swiss scientists published a review of mycotoxins in organic food and offered these conclusions regarding the impacts of conventional and organic systems on the risk of mycotoxin infections –

"The available data do not suggest that the organic farming system does, as a general rule, facilitate higher contamination levels than conventional farming systems. The hypothesis that the prohibition of efficient agrochemicals leads to higher contamination risk is highly speculative, and there is, at least in cereals, evidence that the opposite is likely." (Tamm and Thurig, 2002).

FINDINGS AND CONCLUSIONS

The presence of mycotoxins in food is an inevitable and unavoidable consequence of the existence of fungi. Mycotoxins in food at levels posing risks to livestock and people are infrequent and preventable. Serious mycotoxin problems are most likely when adverse weather conditions strike a region close to harvest, especially when farmers have inadequate equipment and facilities to harvest crops in a timely manner and lack the ability to dry them quickly and evenly to an optimal level of moisture for storage. The lack of clean and dry storage space also can open the door to fungal infection and the production of molds and mycotoxins.



In the developed world, advances in food handling and safety procedures have largely eliminated the consumption of food with high and potentially dangerous levels of mycotoxins. This is not the case in the developing world. Most people who suffer serious health problems from dietary exposures to mycotoxins consume food that is at least partially spoiled, with visible signs of mold or rot, and do so because they have no other choice and the alternative is chronic hunger, if not starvation.

Today, in the United States and other developed countries, the food industry does not sell rotten, moldy produce, and most people do not eat produce if it goes bad in the home. For these reasons, the greater risk for mycotoxin contamination comes through processed foods and grain-based products, which tend to mask the presence of mycotoxins. Fruit juices can contribute significant levels of certain mycotoxins to the human diet. Animal products sometimes are another hidden source of mycotoxin exposure, especially pork-based products and milk and other diary products.

Frequency and Levels of Contamination in Organic and Conventional Foods

Several research teams in Europe have carried out comparative surveys of the frequency and levels of mycotoxins in conventional and organic foods, leading to several peer-reviewed studies reporting results and/or assessing levels of risk (Biffi et al., 2004b; Birzele et al., 2000; Birzele et al., 2002; Brandt et al., 2005; Cirillo et al., 2003; Doll et al., 2002; Jestoi et al., 2004; Leblanc et al., 2002; Schollenberger et al., 1999; Schollenberger et al., 2002; Schollenberger et al., 2005; Skaug et al., 1998; Skaug 1999a). The results are surprisingly consistent.

Averaged across 24 direct comparisons of mycotoxins in conventional and organic foods in published studies, mycotoxins were detected in conventional food about 50 percent more often than in corresponding organic food.

Mycotoxin levels in conventional food averaged a little over twice as high as in the corresponding organic foods. Ten of the comparisons involved wheat or wheat-based products produced in Europe. Mycotoxin levels were higher in the conventional wheat samples in eight of the 10 comparisons, equal in one, and the levels were higher in the organic samples in one study.

The probable explanations for the generally higher levels of mycotoxins in the conventional wheat crops grown in Europe is the routine use of high levels of nitrogen fertilizer, and fungicide applications to prevent disease losses. Several studies have demonstrated that input intensive, high-yield cereal management systems heighten the susceptibility of wheat and other cereal grains to fungal pathogens and can actually stimulate mycotoxin production, even in fields where fungicides have reduced the incidence of fungal infection. The likely role of fungicides in explaining the consistently higher levels of mycotoxins in European grain crops is ironic, given the claim by some critics of organic farming that conventional food is less frequently contaminated with mycotoxins **because** of the use of fungicides.

The nine published studies providing the 24 comparisons of mycotoxins in organic and conventional foods reflect crops produced, processed and sold in the late 1997-2001 period. Substantial growth and technological change has occurred in the organic food industry in Europe since this period, likely impacting risks of mycotoxin contamination in several ways.

Only minor and inconsistent differences between mycotoxin levels in conventional and organic foods were found in three major surveys carried out by the United Kingdom's Food Standards Agency (FSA). These surveys focused on spices, corn-based products, and baby foods. Survey results, coupled with detailed human health risk assessments, led the FSA to emphasize that the levels of mycotoxins in food are generally very low and pose little or no risk to consumers.

Moreover, in its most recent report on mycotoxins, the FSA reports no statistically significant differences between levels of mycotoxins in conventional and organic corn-based foods, despite widely publicized, earlier findings of unusually high mycotoxin levels in several samples of organic corn-based products.

The FSA surveys and academic research have identified isolated samples of food with unusually high levels of specific mycotoxins, and more such findings are inevitable. These "hot" samples have and will continue to include both organic and conventional foods. All the evidence reviewed in this SSR supports the conclusion that conventional and organic foods are both vulnerable to mycotoxin contamination and that the conditions triggering unusually high contamination levels impact conventional and organic food in roughly the same way. In the vast majority of cases described in the scientific literature, "hot" samples are weather related and caused by how crops are harvested, prepared for storage, and stored.

It is also clear that relatively few food-mycotoxin combinations account for the majority of dietary risk associated with mycotoxins. These food-mycotoxin combinations warrant closer attention throughout the food chain and will almost certainly receive more intense focus in the years ahead by scientists and regulators worldwide:

- Ochratoxin A in milk and grain-based products intended for human consumption, as well as pork products;
- Deoxynivalenol in wheat, barley, and rice-based foods;
- Fumonisins in corn-based products intended for humans, as well as in animal feeds;
- Patulin in apple-based products, especially processed products;
- Aflatoxin in nuts and peanut-based products.

Impacts of Organic and Conventional Production Systems on Mycotoxin Risks

Organic and conventional farming practices do not fundamentally alter any of the environmentrelated factors that play such a major role in determining mycotoxin risks. Farming system alternatives, including organic farming and the planting of genetically-modified seeds, can sometimes alter the ecology of farming systems enough to change the mix and levels of fungi and bacteria competing within ecological niches, and such changes might, in turn, impact the presence and levels of mycotoxins in food at harvest.

Conventional farming systems increase the risk of fungal infections through a lack of diversity and reliance on monocultures, and because of heavy use of readily available fertilizers. Conventional farmers also can apply a broader and more effective array of fungicides than organic farmers, which often dramatically suppresses populations of certain fungal pathogens, including some capable of producing mycotoxins. The effectiveness of fungicides is, however, a double-edged sword. Repeated use of fungicides can:

• Accelerate the emergence of new, resistant fungal strains that might also pose or lead to greater risk of mycotoxin production;

- Reduce the biodiversity of fungal species colonizing plant tissues or fruit, thereby sometimes increasing the chance that a given mycotoxin-producing species will be able to proliferate if environmental conditions turn favorable for it;
- Undermine the efficacy of fungal antagonist technologies and management strategies (a

fungal antagonist is a non-pathogenic species of fungi that compete with pathogenic and/or mycotoxin producing strains) (Edwards, 2004b);

- Shift the population mix of fungi such that a decrease in the levels of a less harmful mycotoxin is accompanied by an increase in the levels of more toxic mycotoxins (D'Mello et al., 1998); and
- Impose stress on fungal species that are only partly susceptible to the fungicides applied on a farm, and in this way trigger certain fungi to produce mycotoxins.

Organic farming systems reduce the prevalence of serious fungal infections, and hence mycotoxin risks, by promoting diversity in the fungi and bacteria colonizing plant tissues and living in the soil. With a diversity of such species in an ecosystem, it remains more likely that there will be intense competition across species, even under variable weather and environmental conditions. Resiliency within fungal and bacterial populations lessens the risk that mycotoxin-producing fungi will become dominant and reach levels triggering potentially serious mold and mycotoxin infections. A disadvantage of organic farming systems is the inability to rely on synthetic chemicals to quickly reduce fungal populations, and hence the need to:

- Pay closer attention to prevention;
- Select the most resistant plant varieties;
- Manage farming systems to enhance the contribution of microbial biocontrol to disease prevention.

All in all, however, the advantages of organic farming practices likely equal if not exceed any disadvantages in terms of mycotoxin management, at least on the majority of well-managed organic farms. The evidence is strong that organic production of small grains, especially wheat, can reduce the frequency and severity of mycotoxin contamination compared to conventional farms, even including conventional wheat farms applying fungicides.

Science and innovation on the farm have great potential to more fully develop and exploit the biological advantages in organic farming systems, while the cost and difficulty of bringing an effective new fungicide to the market continues to grow. The rapid evolution of wheat fungal pathogens in Europe that are resistant to the highly effective, relatively new strobilurin fungicides (e.g., azoxystrobin) is another reminder that fungicides can trigger ecological changes with important consequences, ranging from loss of product efficacy to higher levels of mycotoxins.

The majority of fungicides discovered and commercialized in the last five years work through very specific and narrow modes of action. As a result, modern-day fungicides are vulnerable to the development of new fungal strains that are less susceptible, and eventually become fully resistant. As the degree of resistance increases in a population of fungal pathogens, more and more fungi are likely to be treated with sub-lethal doses, setting the stage for higher levels of mycotoxin formation. For this reason, the impact of fungicide use on resistance, and in turn, the impacts of resistance on mycotoxin levels, deserve ongoing attention.

RESEARCH, MONITORING, AND QUALITY CONTROL PRIORITIES

At this time, mycotoxin monitoring studies and research show no consistent differences in mycotoxin levels, frequency, or risks in organic versus conventional foods. While mycotoxins pose little or no risk in most of the food supply, some key foods do regularly contain mycotoxins and under some conditions, levels can rise high enough to pose human and animal health risks.

For this reason all segments of the food and agriculture system owe the public a concerted effort to steadily reduce the risks of mycotoxins in food. This effort must continuously shape day-to-day activities on the farm and in food processing and manufacturing plants. The discovery of more effective and reliable preventive strategies deserves the ongoing attention of scientists and engineers.

Our assessment of mycotoxin risks in food leads us to conclude that more can and should be done to better understand where mycotoxin risks can arise in the food supply, as well as the factors enhancing the vulnerability of particular foods to possibly serious mycotoxin contamination. With the benefit of such insights, organic farmers and food companies will be able to reduce both the frequency and levels of mycotoxins in finished food products. Such food safety innovation, if acted upon across the food supply, will help assure that mycotoxin contamination episodes are rare and inconsequential. Consumers will benefit significantly as a result.



Identify and Target Potential "Hot" Spots in the Organic Food Supply

Much of the research on mycotoxins and organic farming systems has been conducted in Europe under farming conditions quit different than in the United States. Better information from research in the United States is needed on whether and to what extent mycotoxins are finding their way into organic foods. This information is essential in order to target efforts to improve the sophistication of quality control procedures. The collection and careful assessment of this sort of information will remain a necessary investment in building and holding consumer confidence in the safety of organic food.

The organic community should not wait for others to take on this challenge and should not judge the adequacy of its efforts and investments by those made by other segments of the food industry, or by the government.

Research shows that a few mycotoxins in just a few types of food account for the majority of dietary exposure to mycotoxins. The organic community needs better information to identify whether organic farming or food processing practices and procedures in the United States are in any way heightening the risk of serious mycotoxin contamination. The first step is an obvious one –

Samples of organic and conventional milk, apple juice, corn meals, and whole wheat bread purchased in retail markets around the country should be tested for the mycotoxins most commonly found in each type of food.

No new information is needed to identify grain harvest, drying, and storage as critical steps and stages in the annual effort to minimize the risk of mycotoxin infections.

Most serious mycotoxin contamination episodes involve grains and are triggered or made worse by inclement weather. The first line of defense in preventing serious mycotoxin problems is assuring that on-farm grain harvest, drying, and storage equipment and facilities are up to the task, properly maintained, and used with appropriate skill. The organic grain milling and livestock industries have the most to gain from steady improvement in the on-farm management of mycotoxins, and hence leading companies in these industries should work together to fund themselves, or encourage the USDA to carry out –

A survey of grain harvest, drying, and storage facilitates and practices on organic grain farms, with special attention to their capacity to reduce moisture levels quickly and evenly.



The organic grain and livestock industries should also work with organic certifiers to develop a mechanism to periodically assess the adequacy of onfarm grain harvest, drying, and storage facilities and procedures. The results of the survey recommended above will provide critical direction in developing such a mechanism.

In our review of steps to increase average intakes of antioxidants through organic farming and food processing, The Organic Center highlighted significant, near-term opportunities to reduce the portion of

antioxidants lost during food manufacturing and processing. As a result, we recommended additional research on novel food processing methods that show potential in preserving the antioxidants that are in crops when they are harvested.

The same need -- and potential -- exists in managing mycotoxins, and future research projects should be designed to address impacts of novel processing aids and technologies on both antioxidants and mycotoxin levels.

Coordinating Research and Policy Initiatives in a Volatile Regulatory Environment

To remain competitive in international markets and build consumer confidence, conventional and organic farmers and food manufacturers in the United States must steadily expand mycotoxin margins of safety by making full and effective use of science and technology. Better methods are needed to anticipate and prevent possible future problems, and to screen foods for mycotoxins as they move from the field to the consumer, or from the field to a food manufacturer and then to the consumer.

Compared to the United States, the European Union has more comprehensive and stricter mycotoxin regulations in place or under development. Disparity between U.S. standards and policies and those in the European Union will likely grow in the near term and may become a factor impacting access to the European market for U.S.-produced organic and conventional foods. In addition, the European Union is investing much more heavily in the new science that will drive innovation in the management and regulation of mycotoxins.

The Organic Trade Association, the Organic Materials Review Institute, the Organic Farming Research Foundation, and The Organic Center each have key roles to play in creating a well-coordinated institutional effort to accelerate food safety innovation across the organic food community. These organizations need to work together in identifying mycotoxin research and monitoring priorities, and in assuring that mycotoxin food safety policies and regulations are science-based, cost-effective, and targeted. To assist the organic community in complying with emerging regulations and meeting challenges within the always-changing international marketplace, organizations working with and on behalf of organic farmers and food companies should –

Monitor changing laws and public policies impacting allowable levels of mycotoxins in food in individual countries and the European Union, with special attention to how new standards might begin to impact the flow of organic commodities in international commerce.

There is great potential to reduce the risks of mycotoxins in organic food through innovation on the farm and in food processing. The impact of new technologies, production inputs, and manufacturing processes on the risk of mycotoxin contamination should be systematically appraised prior to approval for incorporation in organic farming and food processing. Once approved, the impacts of novel inputs and processes on food safety and quality should be periodically monitored.

Scientists have barely begun to systematically explore how organic farming practices and systems impact the risk of fungal infections in plants. This is a highly promising area of research, both to advance the reliability of disease management practices on organic farms, and to facilitate the transfer of innovative techniques from organic farmers to conventional producers. Both public agencies and private organizations should –

Build assessment of the impacts of organic and conventional farming practices on the production of mycotoxins into research focused on plant disease management.

In particular, new research is needed on the impacts of fungicides on mycotoxin levels in fresh produce and grains. Fungicide use on conventional farms clearly lowers certain fungal populations and in most cases decreases the short-term risk of mycotoxin contamination, but there are exceptions and longer-run impacts are not as clear and warrant further study.

Organic farmers have just a few natural ingredient-based fungicides to choose from and sometimes use individual products more intensively than conventional farmers. The mycotoxin-related impacts of fungicide use deserve more systematic focus by researchers on both conventional and organic farms..

Competition among and across diverse populations of fungi, bacteria, and other microorganisms on and around the plants growing on organic farms is a critical component of successful organic farming disease management. Still, weather, soil conditions, or management practices sometimes tip the scales in favor of one type of fungi, triggering the need for fungicide applications or other interventions. Organic farmers need better information to understand the steps they can take in the face of active fungal infections to prevent crop losses, without undermining the rich diversity of microorganisms that helps keep infections down and mycotoxins out of food.

New science is likely to push U.S. mycotoxin limits lower, especially for foods marketed to infants and young children. Research teams in several European and Asian countries are using state-of-the-art toxicological protocols and assays to study whether and how mycotoxins can disrupt fetal development and trigger abnormal patterns of growth in infants and children. Recent results reported in studies involving rats, mice, rabbits, and humans are worrisome.

Established daily intake limits for mycotoxins are mostly based on animal data and reflect risks one-mycotoxin-at-a-time. Given that monitoring studies often detect more than one mycotoxin present in food samples, the need to consider the additive and/or synergistic impacts of exposure to multiple mycotoxins has been recognized.

To assist the U.S. organic community, public health specialists, risk assessors, and regulators stay abreast of the science driving the regulation of mycotoxins, the government should –

Provide an up-to-date overview of: (1) the tolerable daily intakes (TDIs) for mycotoxins in different countries, for different segments of the population and for farm animals; (2) the toxicological science supporting TDIs; and (3) the methods used in different countries and by international organizations to establish maximum allowable levels in specific foods for specific mycotoxins.

Endnote

A substantial body of evidence supports the conclusion that organic food is no more frequently or seriously contaminated with mycotoxins than conventional food. Consumers should rest assured that farmers and the food industry in the United States – organic and conventional alike – are currently doing a good job in preventing serious mycotoxin contamination in the nation's food supply.

Still, many mysteries remain in the world of mycotoxins: why fungi produce them, what controls their production, how farmers and the food industry can detect and avoid them, and their health effects. Like all farmers and food companies, organic farmers and manufacturers of organic food must view the minimization of mycotoxin risks as a daily challenge and a long-run mission. New tools and more knowledge are needed, and will greatly help in the ongoing struggle to keep mycotoxins out of the food supply and off the minds of consumers.

GLOSSARY

Alimentary toxic aleukia (ATA) - A disease in humans caused by a lack of leukocytes arising from food poisoning. The disease begins with nausea and vomiting, and can progress to a stage where bone marrow is affected and there is a failure to generate new blood cells.

1-carnitine – A compound involved in the transfer of acyl groups across the inner mitochondria membrane.

Coenzyme Q10 - A lipid-soluble electron-transporting coenzyme synthesized by the shikimic pathway. It is an essential component in the respiratory chain.

Disease suppressive soils – A soil containing disease pathogens that does not trigger disease symptoms in growing plants. A number of mechanisms can help create a disease suppressive soil including microbial biological control, the production of antibiotics, competition for nutrients and moisture, and direct predation among soil microorganisms.

Fungal spore – Small, usually single-celled reproductive bodies that are highly resistant to dehydration and heat and are capable of growing into a new fungal organism.

Hepatotoxin – A natural substance or chemical toxic to the liver.

Hyphae -- Single threads emitting from a fungal body that attaches fungi to a substrate.

Immunotoxin – A natural substance or chemical toxic to the immune system, or which impairs immune function.

Lymphocytes – Spherical cells that serve a variety of roles in immune response.

Mycelium – The network or mass of discrete hyphae that forms the body of a fungus.

Mycologist – A person who studies molds and the fungi producing them.

Mycotoxin – Secondary metabolites produced by certain fungi in response to stress or to enhance survival.

Nephrotoxin – A natural substance or chemical toxic to the kidney.

Neurotoxin - A natural substance or chemical agent toxic to the brain and/or nervous system.

Sphingolipids – Any class of lipids containing sphingosine, a long-chain amino diol (an organic compound containing two hydroxyl groups). Sphingolipids play an important structural role in cell membranes.

Uterogenic – Formed in the fetus.

ABBREVIATIONS

- $AFB_1 Aflatoxin B_1$
- Bt Bacillus thuringiensis
- DON Deoxynivalenol
- EBDC Ethylene-bis-dithiocarbamate fungicides
- ECB European corn borer
- EU European Union
- FAO Food and Agriculture Organization of the United Nations
- FB_1 Fumonisin B_1
- FDA U.S. Food and Drug Administration
- FSA United Kingdom Food Standards Agency
- GM Genetically modified

LD-50 – Lethal dose, 50% (the dose in an acute toxicology study at which one-half of the test organisms are killed)

- NIV Nivalenol
- NR Not reported
- OP Organophosphate insecticides
- PEM Protein energy malnutrition
- PPB Parts per billion (i.e., micrograms per kilogram)
- PPM Parts per million (i.e. milligrams per kilogram)

PMTDI – Provision maximum tolerated daily intake (usually measured in micrograms of chemical substance per kilogram of human body weight)

- SAR Systemic acquired resistance
- SSR State of Science Review
- TDI Tolerated daily intake
- WHO World Health Organization
- WN Well nourished

REFERENCE LIST

- Abarca, M. L., Accensi, F., Cano, J., and Cabanes, F. J. Taxonomy and significance of black aspergilli. Antonie Van Leeuwenhoek 86(1), 33-49. 2004.
- Abbas, H. K., Mirocha, C. J., Pawlosky, R. J., and Pusch, D. J. Effect of cleaning, milling, and baking on deoxynivalenol in wheat. Appl.Environ.Microbiol. 50(2), 482-486. 1985.
- Abbas, H. K., Shier, W. T., Gronwald, J. W., and Lee, Y. W. Comparison of phytotoxicity and mammalian cytotoxicity of nontrichothecene mycotoxins. J.Nat.Toxins. 11(3), 173-186. 2002a.
- Abbas, H. K., Williams, W. P., Windham, G. L., Pringle, H. C., III, Xie, W., and Shier, W. T. Aflatoxin and fumonisin contamination of commercial corn (Zea mays) hybrids in Mississippi. J.Agric.Food Chem. 50(18), 5246-5254. 8-28-2002b.
- Abdel-Wahhab, M. A., Hassan, A. M., Amer, H. A., and Naguib, K. M. Prevention of fumonisin-induced maternal and developmental toxicity in rats by certain plant extracts. J.Appl.Toxicol. 24(6), 469-474. 2004.
- Abdelhamid, A. M., el Ayouty, S. A., and el Saadany, H. H. The influence of contamination with separate mycotoxins (aflatoxins, ochratoxin A, citrinin, patulin, penicillic acid or sterigmatocystin) on the in vitro dry matter and organic matter digestibilities of some roughages (berseem hay and wheat straw). Arch.Tierernahr. 42(2), 179-185. 1992.
- Abdelhamid, A. M., el Shawaf, I., el Ayoty, S. A., Ali, M. M., and Gamil, T. Effect of low level of dietary aflatoxins on baladi rabbits. Arch.Tierernahr. 40(5-6), 517-537. 1990.
- Abramson, D., Mills, J. T., and Sinha, R. N. Mycotoxin production in amber durum wheat stored at 15 and 19% moisture content. Food Addit.Contam 7(5), 617-627. 1990.
- Abramson, D., Richter, W., Rintelen, J., Sinha, R. N., and Schuster, M. Ochratoxin A production in Bavarian cereal grains stored at 15 and 19% moisture content. Arch.Environ.Contam Toxicol. 23(2), 259-265. 1992.
- Abramson, D., Sinha, R. N., and Mills, J. T. Mycotoxin formation in moist 2-row and 6-row barley during granary storage. Mycopathologia 97(3), 179-185. 1987.
- Atroshi, F., Rizzo, A., Westermarck, T., and Ali-Vehmas, T. Effects of tamoxifen, melatonin, coenzyme Q10, and L-carnitine supplementation on bacterial growth in the presence of mycotoxins. Pharmacol.Res. 38(4), 289-295. 1998.
- Atroshi, F., Rizzo, A., Westermarck, T., and Ali-Vehmas, T. Antioxidant nutrients and mycotoxins. Toxicology 180(2), 151-167. 11-15-2002.
- Bayman, P., Baker, J. L., and Mahoney, N. E. Aspergillus on tree nuts: incidence and associations. Mycopathologia 155(3), 161-169. 2002.
- Becker, B. A., Pace, L., Rottinghaus, G. E., Shelby, R., Misfeldt, M., and Ross, P. F. Effects of feeding fumonisin B1 in lactating sows and their suckling pigs. Am.J.Vet.Res. 56(9), 1253-1258. 1995.

Bennett, J. W. and Klich, M. Mycotoxins. Clin.Microbiol.Rev. 16(3), 497-516. 2003.

- Beretta, B., De Domenico, R., Gaiaschi, A., Ballabio, C., Galli, C. L., Gigliotti, C., and Restani, P. Ochratoxin A in cereal-based baby foods: occurrence and safety evaluation. Food Addit.Contam 19(1), 70-75. 2002.
- Beretta, B., Gaiaschi, A., Galli, C. L., and Restani, P. Patulin in apple-based foods: occurrence and safety evaluation. Food Addit.Contam 17(5), 399-406. 2000.
- Biffi, R., Munari, M., Dioguardi, L., Ballabio, C., Cattaneo, A., Galli, C. L., and Restani, P. Ochratoxin A in conventional and organic cereal derivatives: a survey of the Italian market, 2001-02. Food Addit.Contam 21(6), 586-591. 2004a.
- Biffi, R., Munari, M., Dioguardi, L., Ballabio, C., Cattaneo, A., Galli, C. L., and Restani, P. Ochratoxin A in conventional and organic cereal derivatives: a survey of the Italian market, 2001-02. Food Addit.Contam 21(6), 586-591. 2004b.
- Birzele, B., Meier, A., Hindorf, H., Kramer, J., and Dehne, H. W. Epidemiology of *Fusarium* Infection and Deoxynivalenol Content in Winter Wheat in the Rhineland, Germany. European Journal of Plant Pathology 108, 667-673. 2002.
- Birzele, B., Prange, A., and Kramer, J. Deoxynivalenol and ochratoxin A in German wheat and changes of level in relation to storage parameters. Food Addit.Contam 17(12), 1027-1035. 2000.
- Brandt, K. and Leifert, C. Healthier Organic Crop Porducts -- Improving the Nutritional Quality and Avoiding Mycotoxin Contamination. QLIF News May 2005, Number 2. 2005.
- Bunger, J., Westphal, G., Monnich, A., Hinnendahl, B., Hallier, E., and Muller, M. Cytotoxicity of occupationally and environmentally relevant mycotoxins. Toxicology 202(3), 199-211. 10-1-2004.
- Carvajal, M., Rojo, F., Mendez, I., and Bolanos, A. Aflatoxin B1 and its interconverting metabolite aflatoxicol in milk: the situation in Mexico. Food Addit.Contam 20(11), 1077-1086. 2003.
- Cirillo, T., Ritieni, A., Visone, M., and Cocchieri, R. A. Evaluation of conventional and organic italian foodstuffs for deoxynivalenol and fumonisins B(1) and B(2). J.Agric.Food Chem. 51(27), 8128-8131. 12-31-2003.
- Clements, M. J., Campbell, K. W., Maragos, C. M., Pilcher, C., Headrick, J. M., Pataky, J. K., and White, D. G. Influence of Cry1Ab Protein and Hybrid Genotype on Fumonisin Contamination and Fusarium Ear Rot of Corn. Crop Science 43, 1283-1293. 2003.
- Creppy, E. E. Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicol.Lett. 127(1-3), 19-28. 2-28-2002.
- D'Mello, J. P., MacDonald, A. M. C., Postel, D., Dijksma, W. T. P., Dujardin, A., and Placinta, C. M. Pesticide Use and Mycotoxin Production in *Fusarium* and *Aspergillus* Phytopathogens. European Journal of Plant Pathology 104, 741-751. 1998.
- Diekman, M. A. and Green, M. L. Mycotoxins and reproduction in domestic livestock. J.Anim Sci. 70(5), 1615-1627. 1992.

- Doll, S., Valenta, H., Danicke, S., and Flachowsky, G. *Fusarium* Mycotoxins in Conventionally and Organically Grown Grain from Thuringia/Germany. Landbauforschung Volkenrode 52, 91-96. 2002.
- Drusch, S. and Ragab, W. Mycotoxins in fruits, fruit juices, and dried fruits. J.Food Prot. 66(8), 1514-1527. 2003.
- Edwards, S. G. Influence of agricultural practices on fusarium infection of cereals and subsequent contamination of grain by trichothecene mycotoxins. Toxicol.Lett. 153(1), 29-35. 10-10-2004a.
- Edwards, S. G. Influence of agricultural practices on fusarium infection of cereals and subsequent contamination of grain by trichothecene mycotoxins. Toxicol.Lett. 153(1), 29-35. 10-10-2004b.
- Edwards, S. G., Pirgozliev, S. R., Hare, M. C., and Jenkinson, P. Quantification of trichotheceneproducing Fusarium species in harvested grain by competitive PCR to determine efficacies of fungicides against Fusarium head blight of winter wheat. Appl.Environ.Microbiol. 67(4), 1575-1580. 2001.
- Eskola, M., Parikka, P., and Rizzo, A. Trichothecenes, ochratoxin A and zearalenone contamination and fusarium infection in Finnish cereal samples in 1998. Food Addit.Contam 18(8), 707-718. 2001.
- Finamore, A., Britti, M. S., Roselli, M., Bellovino, D., Gaetani, S., and Mengheri, E. Novel approach for food safety evaluation. Results of a pilot experiment to evaluate organic and conventional foods. J.Agric.Food Chem. 52(24), 7425-7431. 12-1-2004.
- Fink-Gremmels, J. Mycotoxins: their implications for human and animal health. Vet.Q. 21(4), 115-120. 1999.
- Food and Agriculture Organization. Worldwide regulations for mycotoxins in food and feed in 2003. FAO Food and Nutrition Paper 81.
- Food and Agriculture Organization. Worldwide regulations for mycotoxins in food and feed in 2003. FAO Food and Nutrition Paper 81. 2004.
- Food Standards Agency. Survey of maize-based retail products for mycotoxins. Food Survey Information Sheet 72/05. 2005.
- Fung, F. and Clark, R. F. Health effects of mycotoxins: a toxicological overview. J.Toxicol.Clin.Toxicol. 42(2), 217-234. 2004.
- Green, M. L., Diekman, M. A., Malayer, J. R., Scheidt, A. B., and Long, G. G. Effect of prepubertal consumption of zearalenone on puberty and subsequent reproduction of gilts. J.Anim Sci. 68(1), 171-178. 1990.
- Gunst, L., Krebs, H., Dubois, D., and Forrer, H. R. The Effect of Farming System, Previous Crop and Fertilization on the Incidence of Ear Diseases of Wheat in the DOK Trial. Pflanzengesundheit . 2001.
- Gunst, L., Krebs, H., Dubois, D., and Forrer, H. R. The Effect of Farming System, Previous Crop and Fertilization on the Incidence of Ear Diseases of Wheat in the DOK Trial. Pflanzengesundheit. 2001

- Hasan, H. A. Action of carbamate biocides on sterols, gibberellin and aflatoxin formation. J.Basic Microbiol. 34(4), 225-230. 1994.
- Hasan, H. A. Alternaria mycotoxins in black rot lesion of tomato fruit: conditions and regulation of their production. Acta Microbiol.Immunol.Hung. 43(2-3), 125-133. 1996.
- Hasan, H. A. Fungal utilization of organophosphate pesticides and their degradation by Aspergillus flavus and A. sydowii in soil. Folia Microbiol.(Praha) 44(1), 77-84. 1999.
- Hollinger, K. and Ekperigin, H. E. Mycotoxicosis in food producing animals. Vet.Clin.North Am.Food Anim Pract. 15(1), 133-65, x. 1999.
- Hope, R. and Magan, N. Two-dimensional environmental profiles of growth, deoxynivalenol and nivalenol production by Fusarium culmorum on a wheat-based substrate. Lett.Appl.Microbiol. 37(1), 70-74. 2003.
- Hsia, C. C., Wu, J. L., Lu, X. Q., and Li, Y. S. Natural occurrence and clastogenic effects of nivalenol, deoxynivalenol, 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol, and zearalenone in corn from a high-risk area of esophageal cancer. Cancer Detect.Prev. 13(2), 79-86. 1988.
- Hsia, C. C., Wu, Z. Y., Li, Y. S., Zhang, F., and Sun, Z. T. Nivalenol, a main Fusarium toxin in dietary foods from high-risk areas of cancer of esophagus and gastric cardia in China, induced benign and malignant tumors in mice. Oncol.Rep. 12(2), 449-456. 2004.
- Jennings, P., Turner, J. A., and Nicholson, P. Overview of *Fusarium* Ear Blight in the U.K. -- Effect of Fungicide Treatment on Disease Control and Mycotoxin Production. Proceedings of the Brighton Crop Protection Conference, 2000, 707-712. 2000.
- Jestoi, M., Somma, M. C., Kouva, M., Veijalainen, P., Rizzo, A., Ritieni, A., and Peltonen, K. Levels of mycotoxins and sample cytotoxicity of selected organic and conventional grain-based products purchased from Finnish and Italian markets. Mol.Nutr.Food Res. 48(4), 299-307. 2004.
- Key, T. J., Thorogood, M., Appleby, P. N., and Burr, M. L. Dietary habits and mortality in 11,000 vegetarians and health conscious people: results of a 17 year follow up. BMJ 313(7060), 775-779. 9-28-1996.
- Krebs, H., Dubois, D., and Kulling, C. Effects of Preceeding Crop and Tillage on the Incidence of *Fusarium* spp. and Mycotoxin Deoxynivalenol Content in Winter Wheat Grain. AGRARForschung 7(6), 264-268. 2000.
- Leblanc, J. C., Malmauret, L., Delobel, D., and Verger, P. Simulation of the exposure to deoxynivalenol of French consumers of organic and conventional foodstuffs. Regul. Toxicol. Pharmacol. 36(2), 149-154. 2002.
- Li, F. Q., Li, Y. W., Luo, X. Y., and Yoshizawa, T. Fusarium toxins in wheat from an area in Henan Province, PR China, with a previous human red mould intoxication episode. Food Addit.Contam 19(2), 163-167. 2002.

- Li, F. Q., Yoshizawa, T., Kawamura, O., Luo, X. Y., and Li, Y. W. Aflatoxins and fumonisins in corn from the high-incidence area for human hepatocellular carcinoma in Guangxi, China. J.Agric.Food Chem. 49(8), 4122-4126. 2001.
- Lo Curto, R., Pellicano, T., Vilasi, F., Munafo, P., and Dugo, G. Ochratoxin A occurence in experimental wines in relationship with different pesticide treatments on grapes. Food Chemistry 84, 71-75. 2004.
- Long, G. G., Diekman, M. A., Tuite, J. F., Shannon, G. M., and Vesonder, R. F. Effect of Fusarium roseum (Gibberella zea) on pregnancy and the estrous cycle in gilts fed molded corn on days 7-17 post-estrus. Vet.Res.Commun. 6(3), 199-204. 1983.
- Lutz, M. P., Feichtinger, G., Defago, G., and Duffy, B. Mycotoxigenic Fusarium and deoxynivalenol production repress chitinase gene expression in the biocontrol agent Trichoderma atroviride P1. Appl.Environ.Microbiol. 69(6), 3077-3084. 2003a.
- Lutz, M. P., Feichtinger, G., Defago, G., and Duffy, B. Mycotoxigenic Fusarium and deoxynivalenol production repress chitinase gene expression in the biocontrol agent Trichoderma atroviride P1. Appl.Environ.Microbiol. 69(6), 3077-3084. 2003b.
- Malmauret, L., Parent-Massin, D., Hardy, J. L., and Verger, P. Contaminants in organic and conventional foodstuffs in France. Food Addit.Contam 19(6), 524-532. 2002.
- Marquardt, R. R. and Frohlich, A. A. A review of recent advances in understanding ochratoxicosis. J.Anim Sci. 70(12), 3968-3988. 1992.
- Mazzola, M. Mechanisms of natural soil suppressiveness to soilborne diseases. Antonie Van Leeuwenhoek 81(1-4), 557-564. 2002.
- Menniti, A. M., Pancaldi, D., Maccaferri, M., and Casalini, L. Effect of Fungicides on *Fusarium* Head Blight and Deoxynivalenol Content in Durum Wheat. European Journal of Plant Pathology 109, 109-115. 2003.
- Miraglia, M., de Dominicis, A., Brera, C., Corneli, S., Cava, E., Menghetti, E., and Miraglia, E. Ochratoxin A levels in human milk and related food samples: an exposure assessment. Nat.Toxins. 3(6), 436-444. 1995.
- Moss, M. O. Mycotoxin Review 2. Fusarium. Mycologist 16, Part 4. 2002.
- Munkvold, G. P. Cultural and genetic approaches to managing mycotoxins in maize. Annu.Rev.Phytopathol. 41, 99-116. 2003.
- Paulsen, H. M. and WeiBmann, F. Relevance of Mycotoxins to Product Quality and Animal Health in Organic Farming. Proceedings of the 14th IFOAM World Congress. 2002.
- Pieters, M. N., Bakker, M., and Slob, W. Reduced intake of deoxynivalenol in The Netherlands: a risk assessment update. Toxicol.Lett. 153(1), 145-153. 10-10-2004.
- Pieters, M. N., Freijer, J., Baars, B. J., Fiolet, D. C., van Klaveren, J., and Slob, W. Risk assessment of deoxynivalenol in food: concentration limits, exposure and effects. Adv.Exp.Med.Biol. 504, 235-248. 2002.

- Poapolathep, A., Sugita-Konishi, Y., Phitsanu, T., Doi, K., and Kumagai, S. Placental and milk transmission of trichothecene mycotoxins, nivalenol and fusarenon-X, in mice. Toxicon 44(1), 111-113. 2004a.
- Poapolathep, A., Sugita-Konishi, Y., Phitsanu, T., Doi, K., and Kumagai, S. Placental and milk transmission of trichothecene mycotoxins, nivalenol and fusarenon-X, in mice. Toxicon 44(1), 111-113. 2004b.
- Ritieni, A. Patulin in Italian commercial apple products. J.Agric.Food Chem. 51(20), 6086-6090. 9-24-2003.
- Rizzo, A. F., Atroshi, F., Ahotupa, M., Sankari, S., and Elovaara, E. Protective effect of antioxidants against free radical-mediated lipid peroxidation induced by DON or T-2 toxin. Zentralbl.Veterinarmed.A 41(2), 81-90. 1994.
- Scalera, G. Effects of conditioned food aversions on nutritional behavior in humans. Nutr.Neurosci. 5(3), 159-188. 2002.
- Schisler, D. A., Khan, N. I., and Boehm, M. J. Biological control of Fusarium head blight of wheat and deoxynivalenol levels in grain via use of microbial antagonists. Adv.Exp.Med.Biol. 504, 53-69. 2002.
- Schollenberger, M., Drochner, W., Rufle, M., Suchy, S., Terry-Jara, H., and Muller, H. M. Trichothecene toxins in different groups of conventional and organic bread of the German market. Journal of Food Composition and Analysis . 2003.
- Schollenberger, M., Jara, H. T., Suchy, S., Drochner, W., and Muller, H. M. Fusarium toxins in wheat flour collected in an area in southwest Germany. Int.J.Food Microbiol. 72(1-2), 85-89. 1-30-2002.
- Schollenberger, M., Muller, H. M., Rufle, M., Suchy, S., Planck, S., and Drochner, W. Survey of Fusarium toxins in foodstuffs of plant origin marketed in Germany. Int.J.Food Microbiol. 97(3), 317-326. 1-1-2005.
- Schollenberger, M., Suchy, S., Jara, H. T., Drochner, W., and Muller, H. M. A survey of Fusarium toxins in cereal-based foods marketed in an area of southwest Germany. Mycopathologia 147(1), 49-57. 1999.
- Schothorst, R. C. and van Egmond, H. P. Report from SCOOP task 3.2.10 "collection of occurrence data of Fusarium toxins in food and assessment of dietary intake by the population of EU member states". Subtask: trichothecenes. Toxicol.Lett. 153(1), 133-143. 10-10-2004.
- Skaug, M. A. Analysis of Norwegian milk and infant formulas for ochratoxin A. Food Addit.Contam 16(2), 75-78. 1999a.
- Skaug, M. A. Analysis of Norwegian milk and infant formulas for ochratoxin A. Food Addit.Contam 16(2), 75-78. 1999b.
- Skaug, M. A., Helland, I., Solvoll, K., and Saugstad, O. D. Presence of ochratoxin A in human milk in relation to dietary intake. Food Addit.Contam 18(4), 321-327. 2001.

- Skaug, M. A., Stormer, F. C., and Saugstad, O. D. Ochratoxin A: a naturally occurring mycotoxin found in human milk samples from Norway. Acta Paediatr. 87(12), 1275-1278. 1998.
- Steyn, P. S. Mycotoxins, general view, chemistry and structure. Toxicol.Lett. 82-83, 843-851. 1995.
- Tafuri.A., Ferracane, R., and Ritieni, A. Ochratoxin A in Italian marketed cocoa products. Food Chemistry 88, 487-494. 2004.
- Theunissen, I. Food for thought. Science in Africa, 1-4. 2002.
- Trucksess, M. W. and Tang, Y. Solid phase extraction method for patulin in apple juice and unfiltered apple juice. Methods Mol.Biol. 157, 205-213. 2001.
- Turconi, G., Guarcello, M., Livieri, C., Comizzoli, S., Maccarini, L., Castellazzi, A. M., Pietri, A., Piva, G., and Roggi, C. Evaluation of xenobiotics in human milk and ingestion by the newborn--an epidemiological survey in Lombardy (Northern Italy). Eur.J.Nutr. 43(4), 191-197. 2004.
- Vardon, C., McLaughlin, C., and Nardinelli, C. Potential Economic Costs of Mycotoxins in the United States. Council for Agricultural Science and Technology Task Force Report No. 139. 2003.
- Wangikar, P. B., Dwivedi, P., Sharma, A. K., and Sinha, N. Effect in rats of simultaneous prenatal exposure to ochratoxin A and aflatoxin B1. II. Histopathological features of teratological anomalies induced in fetuses. Birth Defects Res.B Dev.Reprod.Toxicol. 71(6), 352-358. 2004a.
- Wangikar, P. B., Dwivedi, P., and Sinha, N. Effect in rats of simultaneous prenatal exposure to ochratoxin A and aflatoxin B1. I. Maternal toxicity and fetal malformations. Birth Defects Res.B Dev.Reprod.Toxicol. 71(6), 343-351. 2004b.
- Wangikar, P. B., Dwivedi, P., Sinha, N., Sharma, A. K., and Telang, A. G. Effects of aflatoxin B1 on embryo fetal development in rabbits. Food Chem.Toxicol. 43(4), 607-615. 2005.
- Wild, C. P. and Hall, A. J. Primary prevention of hepatocellular carcinoma in developing countries. Mutat.Res. 462(2-3), 381-393. 2000.
- Wu, F. Mycotoxin risk assessment for the purpose of setting international regulatory standards. Environ.Sci.Technol. 38(15), 4049-4055. 8-1-2004.