Significance of Mycotoxins to Food Safety and Human Health

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ABSTRACT

Mycotoxins are toxic substances produced by molds, which cause disease in animals or man. Acute diseases caused by mycotoxins are called mycotoxicoses. History has recorded several human disease outbreaks and numerous animal poisonings thought to be mycotoxicoses. The outbreak of Turkey X disease in England in 1960 culminated in the discovery of aflatoxins and the realization that low levels of mold metabolites in foods and feed could cause disease in man and animals. This gave great impetus to the study of mycotoxins. Mycotoxin-producing molds are quite ubiquitous and frequently contaminate food and agricultural commodities. Fortunately, the mere presence of a toxic mold in food does not automatically mean the presence of mycotoxins. Mycotoxins currently receiving the most attention as potential hazards to human and animal health include aflatoxins, ochratoxin A, sterigmatocystin, patulin, penicillic acid, citrinin, zearalenone and the toxic trichothecenes. These compounds all cause some degree of acute toxicity when given in high amounts. In addition, aflatoxins, sterigmatocystin, patulin and penicillic acid are potential carcinogens.

The significance of mycotoxins as causes of human diseases is difficult to determine because there is no direct evidence of such involvement in terms of controlled experiments with man. Human cases of ergotism and alimentary toxic aleukia are known to be of fungal origin. Recent reports have linked aflatoxins to acute poisonings of humans in Africa, southeast Asia and India. Epidemiological studies have correlated aflatoxin contamination of foodstuffs with high incidences of liver cancer and other liver disease in certain regions of the world. It has been suggested that ochratoxin A may be involved in a fatal kidney disease of humans known as Balkan Endemic Nephropathy. Ochratoxin A has been found in foodstuffs from the endemic areas of this disease.

Mycotoxins may enter the food supply by direct contamination, resulting from mold growth on the food, or by indirect contamination through the use of contaminated ingredients in processed foods. Indirect exposure to mycotoxins can also result from consumption of animal products, such as milk, which contain mycotoxin residues, caused by feeding moldy feed to the food-producing animal. Commodities susceptible to direct contamination with mycotoxins include nuts, oilseeds, grains and to a limited extent, certain fruits. Residues of aflatoxin have been found in animal products such as fluid milk, nonfat dry milk, cottage cheese and imported cheeses. In feeding experiments with aflatoxins, the toxins were found in livers, kidneys and certain tissues of pigs and broiler chickens, and in eggs from laying hens fed aflatoxin. Residues of ochratoxin A have been found in livers, kidneys, muscle and adipose tissues of bacon pigs and poultry.

Refrigerated foods, such as cheeses, cured meats and certain flour-based products, subject to mold growth during storage, have been shown to be contaminated with a variety of potential mycotoxin-producing molds. Experimental evidence indicates that certain mycotoxins could be produced on refrigerated foods under certain conditions. Aflatoxin production is favored by temperatures of 20 to 25 C, but has been reported to occur as low as 7 to 12 C. Toxins produced by Penicillium species can be produced at temperatures as low as 5 C; however, patulin and penicillic acid do not appear to be produced to any extent on substrates such as cheeses and cured meats. Aflatoxins and ochratoxins appear to be relatively stable in most foods, whereas patulin and penicillic acid are not stable in proteinaceous foods such as cheeses and meats. Stability data on other mycotoxins are lacking for most foods. In general, mycotoxins are most stable in grains, nuts and olives. The current tolerance level for aflatoxins in foods is 20 ppb, which will probably be lowered to 15 ppb in the near future. Recently, an action level of 0.5 ppb for aflatoxin in milk and milk products was announced which is essentially a tolerance level for these products.

Mycotoxin is a general term used to describe compounds or metabolites, which are toxic or have other biological effects in living organisms (primarily animals and/or man), and which are produced by molds. The term is derived from the Greek words “mykes” meaning fungus and “toxicum” meaning poison or toxin (60). Thus, the term literally means fungus poison or fungus toxin. A number of the compounds which are today classed as mycotoxins were actually first studied as potential antibiotics in the 1930’s and 1940’s, only to be discarded as being too toxic to higher life forms to be of value in treating disease. At that time the potential health problems that these compounds might pose as contaminants of the food supply was not recognized.

The acute diseases caused by mycotoxins are referred to as mycotoxicoses. History has recorded a number of outbreaks of human mycotoxicoses. In addition, the scientific literature contains many more references to disease outbreaks among domestic animals that were
either proven or suspected of being caused by mycotoxins. Outbreaks of ergotism have been recorded in Europe as far back as the Middle Ages (60). This disease, also known as “St. Anthony’s Fire,” killed thousands of people in France in 943 A.D. It wasn’t until the 19th century that the cause of this disease was recognized as a group of alkaloid compounds produced by the fungus *Claviceps purpurea*, commonly known as ergot, which parasitizes rye and other grains. A human disease known as Alimentary Toxic Aleukia (ATA) occurred in the Orenburg Province of Russia during World War II (77). The disease, caused by consumption of overwinted moldy grain, was manifested by severe dermal necroses, hemorrhaging, leucopenia (abnormal decrease in leucocytes) and bone marrow degeneration. Mortality rates were as high as 60% in some instances, with up to 10% of the population being affected. Several molds were subsequently shown to be involved in the etiology of the disease, including *Fusarium poae*, *Fusarium sporotrichoides* and several *Cladosporium* species. About this same time, a disease of horses known as Stachybotryotoxosis also occurred in Russia (54). This disease was caused by the feeding of moldy hay containing the mold *Stachybotrys alternans* (atra). Also, about this time in history, the Japanese recorded a condition known as “yellowed rice” which caused serious liver damage when ingested by animals (140). Subsequently, several compounds known as yellow rice toxins were isolated. It was found that a number of *Penicillium* species, notably *Penicillium citreo-viride*, *Penicillium citrinum*, *Penicillium islandicum* and *Penicillium rugulosum* were involved. Much of the Japanese’s detailed work was published in the early 1950’s, yet the field of mycotoxins received very little attention until 1960. In that year a severe toxic outbreak occurred in England, which became known as “Turkey X Disease” because of the involvement of large numbers of turkey poults (10). In addition, ducklings and other young farm animals were also affected (5). The cause of the disease was traced to a feed component, peanut meal, which was heavily infested with the common storage mold *Aspergillus flavus*. Analysis of the feed led to discovery of a series of fluorescent compounds which were named aflatoxins, for *A. flavus* toxins. At about the same time, an outbreak of trout hepatoma was observed in the U.S. (180). This was later related to aflatoxin-contaminated cottonseed meal used in the diet of the trout (65).

Further study of the effects of aflatoxins in animals revealed that these compounds were toxic to a wide range of animals and that the effects observed varied, depending among other things on the dosage given (1.2.178,179). In large doses aflatoxins were found to be acutely toxic, causing gross liver damage with intestinal and peritoneal hemorrhaging, resulting in death of the animal. Sub-lethal doses resulted in a number of moderate to severe histopathological changes in the liver, such as necrosis, hemorrhage, chronic fibrosis, bile duct hyperplasia and fatty degeneration. Chronic exposure of rats to low levels of aflatoxins revealed that the compounds were carcinogenic and were capable of inducing liver tumors (95,178,179). These studies showed aflatoxin B1 to be the most potent carcinogen known, when it was found that microgram quantities were capable of producing tumors in a high percentage of test animals. This led to the first realization that chronic exposure to low levels of mold metabolites could cause disease in man and animals.

The early work with aflatoxin gave great impetus to the study of mold metabolites as possible disease agents and potentially harmful contaminants in food and feed supplies. To date, studies have shown that a number of additional mycotoxins also exhibit the properties of acute, sub-acute and chronic effects in animals with some also being carcinogenic. In addition, some mycotoxins are also now known to be mutagenic, capable of causing mutations in susceptible organisms (which may also suggest carcinogenicity) and teratogenic, capable of causing deformities in developing embryos.

**MYCOTOXIN-PRODUCING MOLDS IN FOODS**

Many of the molds capable of producing mycotoxins are also frequent contaminants of food and agricultural commodities. Molds which are of importance in foods because of potential mycotoxin production include members of the genera *Aspergillus, Penicillium, Fusarium, Alternaria, Trichotheceum, Cladosporium, Bysschlamys and Sclerotinia*. These organisms are capable of growth on a variety of substrates and under a diversity of conditions of moisture, pH and temperature. Thus, most foods are susceptible to fungal invasion during some stage of production, processing, transport or storage. If mold growth occurs, there is always the concomitant possibility of mycotoxin production. However, the presence of toxigenic molds in a food product does not automatically mean the presence of mycotoxins, especially if growth has not occurred, but rather that a potential for mycotoxin contamination exists. On the other hand, the absence of toxigenic molds does not guarantee that the commodity is free of mycotoxins, since the toxins may persist long after the molds have disappeared.

Numerous studies have reported the incidence and types of toxigenic molds on various food and agricultural commodities (Table 1). These studies indicate the ubiquitous distribution of potential mycotoxin-producing molds. The types of molds present in a commodity are affected by such factors as the substrate, moisture and storage conditions. The mycoflora of cereal grains, for example, can be divided into three groups: (a) field fungi, which invade the grain in the field before harvest, and include species of *Alternaria, Fusarium, Helminthosporium* and *Cladosporium*; (b) storage fungi, which predominate in grains during storage after harvest and consist primarily of species of *Aspergillus* and *Penicillium*; (c) advanced decay fungi, such as *Fusarium* and *Chaetomium*, which grow after considerable damage.
TABLE 1. Summary of some selected reports of isolations of potentially toxic molds from various food or agricultural commodities.

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Potentially toxic genera/species found</th>
<th>Potential mycotoxins</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour, bread, cornmeal, popcorn</td>
<td>Aspergillus flavus, ochraceus versicolor</td>
<td>Aflatoxin, ochratoxin, sterigmatocystin, patulin, penicillic acid</td>
<td>16, 22, 25, 63</td>
</tr>
<tr>
<td>Peanut, in-shell pecans</td>
<td>Cladosporium, Fusarium</td>
<td>Aflatoxins, ochratoxin, patulin, sterigmatocystin</td>
<td>5, 49, 101, 141</td>
</tr>
<tr>
<td>Apples and apple products</td>
<td>Penicillium expansum</td>
<td>Patulin</td>
<td>12, 155</td>
</tr>
<tr>
<td>Meat pies, cooked meats, cocoa powder, hops, cheese</td>
<td>Aspergillus flavus, ochraceus versicolor Penicillium scoparium</td>
<td>Aflotoxins, ochratoxin, patulin, penicillic acid</td>
<td>17, 18, 19</td>
</tr>
<tr>
<td>Aged salami and sausage, country cured ham, moldy meats</td>
<td>Penicillium flavus ochraceus versicolor</td>
<td>Aflatoxins, ochratoxin, patulin, sterigmatocystin</td>
<td>20, 21, 52, 96, 162, 163, 164</td>
</tr>
<tr>
<td>Black and red pepper, macaroni</td>
<td>Penicillium species</td>
<td>Aflatoxins, ochratoxin</td>
<td>29, 30, 32</td>
</tr>
<tr>
<td>Dry beans, soybeans</td>
<td>Penicillium expansum</td>
<td>Patulin, citrinin, penicillic acid, griseofulvin</td>
<td>110, 111</td>
</tr>
<tr>
<td>Refrigerated and frozen pastries</td>
<td>Penicillium flavus ochraceus versicolor</td>
<td>Aflatoxins, sterigmatocystin, ochratoxin, citrinin, patulin, penicillic acid</td>
<td>92, 93</td>
</tr>
<tr>
<td>Moldy supermarket foods</td>
<td>Penicillium cyclopium</td>
<td>Penicillic acid, T-2, possibly other Penicillium toxins</td>
<td>45</td>
</tr>
<tr>
<td>Foods stored in homes, both refrigerated and non-refrigerated</td>
<td>Penicillium species, Aspergillus species</td>
<td>Aflatoxin, kojic acid, ochratoxin A, patulin, penicillic acid</td>
<td>166</td>
</tr>
</tbody>
</table>

From other microorganisms has occurred (29,31). Field fungi and advanced decay fungi require moisture levels of 20 to 25% to grow, whereas storage fungi can grow at moisture levels of 13 to 18%. Recent evidence indicates that A. flavus may invade grains, particularly corn, in the field when the grain has suffered insect or hail damage. Most mycotoxic fungi associated with grains and grain products as well as most other foods are species of Aspergillus, Penicillium and Fusarium (74).

Mycotoxins

Many toxic compounds have been isolated from mold cultures. However, not all of these have been shown to have a role in human or animal diseases. The literature concerning mycotoxins has been extensively reviewed in detail in numerous volumes and review articles over the past several years (37,40,59,67,78,79,103,118,129,130, 136,137,158,170,186,187). These reviews discuss the known mycotoxins in some depth. For purposes of this
discussion, attention will be directed toward those toxins which may be considered to pose the greatest potential hazard to human health as food contaminants. These toxins include aflatoxins, ochratoxin A, sterigmatocystin, patulin, penicillic acid, citrinin, zearalenone and the toxic trichothecenes. Some of the chemical and physical properties of these mycotoxins are summarized in Table 2, and toxicological and biological properties are summarized in Table 3.

Aflatoxins

Aflatoxins are produced primarily by some strains of *A. flavus* and most, if not all, strains of *Aspergillus parasiticus* (43). Aflatoxins are a group of closely related heterocyclic compounds of which six are most common (Fig. 1). There are four main aflatoxins, B₁, B₂, G₁ and G₂. Of these, B₁ and G₁ occur most frequently and in largest amounts. Under long-wave ultraviolet light, aflatoxins B₁ and B₂ fluoresce blue and aflatoxins G₁ and G₂ fluoresce green (Table 2). The B and G designations of the toxins refer to the color of fluorescence. The subscripts 1 and 2 refer to the separation pattern of these compounds on thin-layer chromatography (TLC) plates, with B₁ having the highest Rf value followed by B₂ then G₁ and G₂ in most solvent systems. In addition to these four aflatoxins, two additional toxins are of significance; these are aflatoxins M₁ and M₂. The M toxins were first isolated from the milk of lactating animals fed aflatoxin preparations; hence, the M designation (73). The subscripts again refer to separation patterns on TLC plates (Table 2). The M toxins also fluoresce blue when exposed to long-wave U.V. light, but separate at a lower Rf value on TLC plates than the B and G toxins. Chemically, the aflatoxins are difuranocoumarin derivatives structurally related to coumarin (45).

Aflatoxins are potent hepatotoxins and also potent carcinogens. Aflatoxin B₁ is the most toxic of the group (79). Effects of aflatoxins in vivo vary with dose, duration of exposure, animal species, breed and diet or nutritional status of the animal affected. As mentioned earlier, these toxins may be acutely toxic when given in large doses; sub-lethal doses produce a chronic toxicity and low levels of chronic exposure result in carcinogenic responses in a number of animals. The oral 7-day LD₉⁰ values for aflatoxins in ducklings are shown in Table 3. LD₉⁰ values for other animals range from 0.5 to 10 mg/kg of body weight (179). Many animal species are affected by aflatoxins. In general, young animals of any species are more susceptible to the acute toxic effects of aflatoxins than are older animals of the same species. Susceptibility also varies between species. With poultry, ducklings are most susceptible followed by pregnant sows, fattening pigs, mature cattle and sheep (1,2). Trout and dogs are also susceptible to the effects of aflatoxins.

The clinical signs of acute aflatoxicosis in most species include lack of appetite, weight loss, unthriftiness, neurological abnormalities, jaundice of mucous membranes, convulsions and death (67). Gross liver damage is also evident, livers being pale or discolored with necrosis and fat accumulation. There may also be fluid accumulation in the body cavity and hemorrhaging of the kidneys and intestinal tract. Sub-lethal, chronic exposure to aflatoxins results in jaundice of the carcass and cirrhosis of the liver, with bile duct proliferation and fibrosis. Prolonged exposure to low levels of aflatoxins results in liver tumors in a number of species including trout, ducklings and rats. Trout are most susceptible to the carcinogenic effects of aflatoxins and develop liver tumors when exposed to only a few parts per billion of aflatoxins.

Aflatoxins have been found in a number of products, particularly peanuts and peanut products, cottonseed and corn. Besides these products, other commodities considered most likely to be contaminated with aflatoxins include copra, Brazil nuts, pistachio nuts, almonds, pecans and walnuts (156).

Sterigmatocystin

Sterigmatocystin is produced by *Aspergillus versicolor*. *A. flavus*, *A. nidulans*, *A. rugulosus*, *Penicillium luteum* and a *Bipolaris* species (43,46). Structurally, sterigmatocystin resembles the aflatoxins and is basically a xanthone nucleus attached to a bifuran ring (Fig. 2). Several related compounds also exist, but sterigmatocystin appears to be the most important. It is thought to be a precursor in the biosynthesis of aflatoxin. The acute toxicity of sterigmatocystin is low with an oral LD₅₀ in mice of 800 mg/kg (102). The main concern with sterigmatocystin is that it is carcinogenic when given orally to rats, resulting in liver cancer (132,133). Sterigmatocystin is about one-tenth as potent a carcinogen as aflatoxins, but certain cultures of *A. versicolor* are capable of producing large amounts of the compound. Sterigmatocystin has been detected in low levels in green coffee and moldy wheat (131,144).

Ochratoxins

Ochratoxins are a group of related compounds that are produced by *Aspergillus ochraceus* and related species, as well as *Penicillium viridicatum* and certain other *Penicillium* species (33,67,117,186). The main toxin in this group is ochratoxin A (Fig. 3). Chemically, ochratoxin A is a dihydroyisocoumarin linked to L-β-phenylalanine (67,186). Ochratoxin A is a potent mycotoxin that causes kidney damage in rats, dogs and swine (Table 3). Ochratoxin is thought to be involved in a disease of swine in Denmark known as porcine nephropathy, which has been associated with the feeding of moldy barley (86,88,90). The LD₅₀ values for ochratoxin A (Table 3) in chicks, swine and trout range from 2.1 to 4.67 mg/kg (117,186). The main pathological effects of acute ochratoxicosis are necrosis of the renal tubular epithelium of the kidney and perportal liver cells with accompanying enteritis (67). Ochratoxin has also been reported to be teratogenic to mice, rats and chicken embryos (117). Ochratoxin has been detected in
TABLE 2. Some chemical and physical properties of several mycotoxins (28, 36, 186).

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Mol. Wt.</th>
<th>Approx. Rf</th>
<th>Solvent system</th>
<th>Solvent ratio</th>
<th>Fluorescent color in U.V. light (365 nm)</th>
<th>Maxima (nm)b</th>
<th>E</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>312</td>
<td>0.56</td>
<td>C:M</td>
<td>97:3</td>
<td>Blue</td>
<td>223; 265; 362</td>
<td>25,600; 13,400; 21,800</td>
<td>Fig. 1</td>
</tr>
<tr>
<td>B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>314</td>
<td>0.53</td>
<td>C:M</td>
<td>97:3</td>
<td>Blue</td>
<td>265; 263</td>
<td>11,700; 23,400</td>
<td>Fig. 1</td>
</tr>
<tr>
<td>G&lt;sub&gt;1&lt;/sub&gt;</td>
<td>328</td>
<td>0.48</td>
<td>C:M</td>
<td>97:3</td>
<td>Green</td>
<td>243; 257; 264; 362</td>
<td>11,500; 9,900; 10,000; 16,100</td>
<td>Fig. 1</td>
</tr>
<tr>
<td>G&lt;sub&gt;2&lt;/sub&gt;</td>
<td>332</td>
<td>0.46</td>
<td>C:M</td>
<td>97:3</td>
<td>Blue</td>
<td>265; 263</td>
<td>9,700; 21,000</td>
<td>Fig. 1</td>
</tr>
<tr>
<td>M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>328</td>
<td>0.40</td>
<td>C:M</td>
<td>97:3</td>
<td>Blue</td>
<td>223; 265; 357</td>
<td>23,100; 11,600; 19,000</td>
<td>Fig. 1</td>
</tr>
<tr>
<td>M&lt;sub&gt;2&lt;/sub&gt;</td>
<td>330</td>
<td>0.30</td>
<td>C:M</td>
<td>97:3</td>
<td>Red-brown</td>
<td>221; 264; 357</td>
<td>20,000; 10,900; 21,000</td>
<td>Fig. 1</td>
</tr>
<tr>
<td>Sterigmatocystin</td>
<td>324</td>
<td>0.85</td>
<td>T:E:F</td>
<td>6:3:1</td>
<td>(Brick red)</td>
<td>250; 326 (CHCl&lt;sub&gt;3&lt;/sub&gt;)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>403</td>
<td>0.55</td>
<td>T:E:F</td>
<td>6:3:1</td>
<td>Greenish-blue</td>
<td>213; 332</td>
<td>36,800; 6,400</td>
<td>Fig. 3</td>
</tr>
<tr>
<td>Citrinin</td>
<td>250</td>
<td>0.16-.48</td>
<td>T:E:F</td>
<td>6:3:1</td>
<td>Bright blue</td>
<td>222; 253; 319</td>
<td>22,280; 8,279; 4,710</td>
<td>Fig. 4</td>
</tr>
<tr>
<td>Patulin</td>
<td>154</td>
<td>0.41</td>
<td>T:E:F</td>
<td>6:3:1</td>
<td>Pale blue</td>
<td>276</td>
<td>14,540</td>
<td>Fig. 5</td>
</tr>
<tr>
<td>Penicillic acid</td>
<td>170</td>
<td>0.47</td>
<td>T:E:F</td>
<td>6:3:1</td>
<td>Bright blue</td>
<td>220</td>
<td>10,500</td>
<td>Fig. 6</td>
</tr>
<tr>
<td>Zearealenone</td>
<td>318</td>
<td>0.78</td>
<td>T:E:F</td>
<td>6:3:1</td>
<td>Faint blue</td>
<td>236; 274; 316</td>
<td>29,700; 13,909; 6,020</td>
<td>Fig. 7</td>
</tr>
<tr>
<td>T-2 Toxin</td>
<td>466</td>
<td>0.36</td>
<td>T:E:F</td>
<td>6:3:1</td>
<td>Gray-pink</td>
<td>220</td>
<td>10,500</td>
<td>Fig. 8</td>
</tr>
</tbody>
</table>

<sup>a</sup>C = chloroform; E = ethyl acetate; F = formic acid (90%); T = toluene.
<sup>b</sup>In ethanol unless otherwise noted.
<sup>c</sup>After exposure to ammonia fumes.
<sup>d</sup>In short wave U.V. light, zearealenone fluoresces green and is brighter.
<sup>e</sup>After spray with anisaldehyde.

TABLE 3. Some biological and toxicological properties of several mycotoxins (28, 36, 186, 187).

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Producing organism</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt; (mg/kg)</th>
<th>Acute toxicity</th>
<th>Animals affected</th>
<th>Effects in animals</th>
<th>Pathological effects</th>
<th>Commodity found contaminated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin</td>
<td></td>
<td>5-10</td>
<td>Several Duckling</td>
<td>oral</td>
<td>Birds</td>
<td>Hepatotoxicity</td>
<td>Peanuts</td>
</tr>
<tr>
<td>B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Aspergillus flavus parasiticus</td>
<td>0.36</td>
<td>Duckling</td>
<td>oral</td>
<td>Liver damage</td>
<td>Corn</td>
<td>Wheat</td>
</tr>
<tr>
<td>B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Same</td>
<td>1.7</td>
<td>Duckling</td>
<td>oral</td>
<td>Hemorrhage</td>
<td>Rice</td>
<td>Cottonseed</td>
</tr>
<tr>
<td>G&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Same</td>
<td>0.78</td>
<td>Duckling</td>
<td>oral</td>
<td>Intestinal tract</td>
<td>Bile duct</td>
<td>Copra</td>
</tr>
<tr>
<td>G&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Same</td>
<td>3.45</td>
<td>Duckling</td>
<td>oral</td>
<td>Kidneys</td>
<td>Hyperplasia</td>
<td>Nuts</td>
</tr>
<tr>
<td>M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Same (Also excreted by animals after consumption)</td>
<td>0.32</td>
<td>Duckling</td>
<td>oral</td>
<td>Mamals</td>
<td>Various foods</td>
<td>Milk</td>
</tr>
<tr>
<td>M&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
<td>1.23</td>
<td>Duckling</td>
<td>oral</td>
<td>Young pigs</td>
<td>Carcinogen</td>
<td>Eggs</td>
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<td></td>
<td></td>
<td></td>
<td>Pregnant sows</td>
<td>Liver tumors</td>
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<td></td>
<td>Dogs</td>
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<td></td>
<td>Calves</td>
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</tr>
<tr>
<td></td>
<td>600 Mouse oral</td>
<td>110 Mouse sc</td>
<td>Data not available but 1-5 mg/kg (ppm) of feed causes physiological responses</td>
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<td>70 Mouse ip</td>
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<td></td>
<td>Guinea pig</td>
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<td></td>
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<td>Moldy hay</td>
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**Significance of Mycotoxins**

- **Penicillium**
  - **Aspergillus**
    - *alliaceus*
    - *mellus*
    - *ochraceus*
    - *ostianus*
    - *quinces*
    - *sclerotiorum*
    - *sulphureus*

- **Penicillium**
  - *aurantio-virens*
  - *baarmense*
  - *commune*
  - *cyclopium*
  - *expansum*
  - *fennellae*
  - *griseofulvum*
  - *janthinellum*
  - *madriti*
  - *martensii*
  - *olivine-viride*
  - *palitans*
  - *puberulum*
  - *roquefortii*
  - *sauvolens*
  - *simplicissimum*
  - *thomii*
  - *viridicatum*

- **Zearalenone (F-2)**
  - *Fusarium*
    - *culmorum*
    - *moniliforme*
    - *oxytroporum*
    - *roseum*
    - *tricinctum*

- **Guppies**
- **Zebra fish larvae**
- **Mouse**
- **Rat**
- **Chicken embryo**
- **Quail**
- **Brine shrimp**

**Physiological Responses**

- Liver damage
- Fatty liver cell necrosis
- Kidney damage
- Digitalis-like action on heart
- Dilates blood vessels
- Antidiuretic
- Edema in rabbit skin
- Carcinogenic
- Antibiotic

- Estrogenic effects
- Swelling and edema of vulva
- Prolapso of vagina
- Enlargement of uterus
- Atrophy of ovaries
- Atrophy of testicles
- Enlargement of mammary glands
- Abortion

- Stored corn
- Cereal grains
- Dried beans
- Moldy tobacco
- Corn
- Moldy hay
- Pelleted commercial feeds

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<tr>
<td>tricium</td>
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* Sc = sub-cutaneous, ip = intraperitoneal, iv = intravenous.
Significance of Mycotoxins

Figure 1. Chemical structures of aflatoxins.

Ochratoxin A

\[
\begin{array}{c}
\text{OCH}_3 \\
\text{CH}_2-\text{CH-NH-C} \\
\text{OH} \\
\text{R} \\
\text{X} \\
\text{Cl}
\end{array}
\]

Ochratoxin B

\[
\begin{array}{c}
\text{OCH}_3 \\
\text{OH} \\
\text{H} \\
\text{H}
\end{array}
\]

Ochratoxin M

\[
\begin{array}{c}
\text{OCH}_3 \\
\text{R} \\
\text{Cl}
\end{array}
\]

Figure 2. Chemical structure of sterigmatocystin.

Citrinin

\[
\begin{array}{c}
\text{HOOC} \\
\text{CH}_3 \\
\text{CH}_3
\end{array}
\]

Patulin

\[
\begin{array}{c}
\text{HOOC} \\
\text{CH}_3 \\
\text{H}
\end{array}
\]

Figure 3. Chemical structure of ochratoxins.

Figure 4. Chemical structure of citrinin.

Figure 5. Chemical structure of patulin.

Commercial corn and barley and in feed grains and mixed feeds of low quality. Ochratoxin has also been found in dried white beans, moldy peanuts, and barley and oats associated with swine nephropathy, and tissues of these swine (67).

Citrinin

Citrinin (Fig. 4) is a yellow cyclic compound with a free carboxylic acid group (67,140). Citrinin is produced by several Penicillium species including P. citrinum, P. viridicatum, P. expansum and P. notatum, as well as Aspergillus species (67,140,186). Like ochratoxin A, citrinin is a kidney toxin (Table 3). Citrinin causes a nephrotoxic response in laboratory animals similar to swine nephropathy (67,170,186). Citrinin may be involved with ochratoxin A in cases of swine nephropathy in Denmark (88). However, the toxicity of citrinin is low compared to ochratoxin (58,88,91), although possible synergistic activity with ochratoxin A cannot be ruled out. Citrinin has been found to occur naturally in feed samples from farms with swine nephropathy in Denmark, moldy grain in Canada and yellowed rice in Japan (67,140,170). In the feed samples and moldy grain, citrinin was found along with ochratoxin A. In feeding trials with rats, citrinin caused renal damage, with pathological examination showing enlarged, turbid, gray-white kidneys and tubular damage to the kidneys (140).

Patulin

Patulin is an unsaturated lactone (Fig. 5) that is similar in structure to certain other carcinogens (39). In early work with patulin, going back to the 1940’s, researchers applied different names to patulin, so several synonyms for this compound exist in the early literature. Other names for patulin are expansin, clavacin, clavatin, claviformin, penicidin, myocin c, gigantic acid, tercinin and leucopin (67,153,158). Patulin is toxic to many biological systems (Table 3), but its role in causing animal and human disease is unclear. Patulin is toxic to many bacterial systems, mammalian cell cultures, higher plants and animals including mice, rats, rabbits, cats, chicken embryos, chickens, quail, guppies, brine shrimp, zebra fish and crustacean Cyclops fuscus (153,158). The oral LD₅₀ for patulin in mice is 35 mg/kg (13). Patulin has also been shown to be carcinogenic when injected intradermally into mice in sub-lethal doses (47).

Patulin is produced by numerous Penicillium and Aspergillus species and Byssochlamys nivea (Table 3). Penicillium expansum, which commonly occurs in apple
rots produces patulin, as does \( P. \ patulum \) ( \textit{urticae} ), \( P. \ claviforme \) and various other \textit{Penicillium} species (158,186). \textit{Aspergillus clavatus}, \textit{A. giganteus} and \textit{A. terrus} also produce patulin (39,158). Patulin is of some public health concern because of its potential carcinogenic properties, and because it has been found in commercial apple juice (143,175,177). Patulin has also been implicated in the deaths of cattle in Japan (189).

The toxic effects of patulin in mice include paralysis of the motor nerves, convulsions and reflex excitement. Pathological findings in experimental cases of patulin toxicosis in rats, mice, rabbits and chickens have been reported to be lung edema, with hemorrhaging, capillary damage in the liver, spleen and kidneys, edema of the brain, and liver damage (67,158).

\textit{Penicillic acid}

Penicillic acid is also an unsaturated lactone-type compound, and in solution exists in two tautomeric forms: as the \( \gamma \)-hydroxy lactone ring and as the \( \gamma \)-keto acid (Fig. 6). It is questionable whether penicillic acid should be classified as a toxin since the oral toxicity of this compound is low (Table 3). The oral LD\(_{50}\) of penicillic acid in mice is 600 mg/kg (39,67). However, the concern about penicillic acid in foods is based on the fact that the compound bears structural similarity to known carcinogenic lactones and has in fact been shown to be carcinogenic to rats when injected subcutaneously (47).

However, the potency of penicillic acid as a carcinogen is much lower than aflatoxins since an injection dose of 1 mg given twice weekly for 64 weeks was required to produce the cancer (47). When given in lethal doses, penicillic acid caused fatty liver degeneration in quail and liver cell necrosis in mice (42). Mixtures of penicillic acid with ochratoxin A have been reported to give a synergistic lethal response in mice (105). Pharmacologically, penicillic acid dilates blood vessels and has antidiuretic effects.

Penicillic acid (Table 3) is produced by strains of \textit{A. ochraceus} and related species and several \textit{Penicilium} species including \textit{P. cyclopium}, \textit{P. martensi}, \textit{P. palitans}, and \textit{P. puberulum}, among others (39,186). Some strains of \textit{A. ochraceus} are capable of producing penicillic acid along with ochratoxin A (6,35). Recently, Olivigni and Bullerman (123,124) reported the simultaneous production of penicillic acid and patulin by an atypical \textit{Penicillium roqueforti}. Penicillic acid has been found in large quantities in stored corn (41,94).

\textit{Zearalenone}

Zearalenone is an estrogeic compound which is also known as F-2 toxin (Fig. 7). It causes vulvovaginitis and estrogeic responses in swine (109). Zearalenone is produced by \textit{Fusarium} species including \textit{F. roseum}, \textit{F. tricinctum}, \textit{F. oxysporum}, \textit{F. culmorum}, and \textit{F. moniliforme} (27,109,116,125). Zearalenone has been found to occur naturally in high moisture corn in late fall and winter, primarily from the growth of \textit{F. roseum} (109). The compound is quite toxic, with 1 to 5 ppm sufficient to cause physiological responses. In addition to swine, rats, turkey pouls and chicks have been shown to be susceptible to the toxic effects of zearalenone (109). Some of the pathological effects of zearalenone in swine include swelling and edema of vulva, prolapse of the vagina, enlargement of the uterus and atrophy of the ovaries in young gilts. Young male swine develop mammary gland hyperplasia and testicular atrophy (109). Zearalenone can be transmitted to piglets in sows' milk and cause estrogenism in the young pigs (67).

\textit{T-2 toxin}

T-2 toxin (Fig. 8) is one of a family of closely related compounds produced by several \textit{Fusarium} species (8). These compounds are derivatives of a ring system referred to as trichothecene, and are characterized as 12-13 epoxytrichothecenes. Their toxicity is attributed to the epoxy group at carbons 12 and 13 and the olefinic bond at carbons 9 and 10 (8). There are more than 20 naturally occurring compounds produced by \textit{Fusarium} species which contain similar structures, including diacetoxyxyscripenal, neosolaniol, nivalenol, diacetylnivalenol, deoxynivalenol, HT-2 toxin and fusarenon X (7).
The chemical and biological properties of these compounds have been reviewed extensively by Bamberg (7) and Bamberg and Strong (8). T-2 toxins and related compounds have been implicated with a disease known as moldy corn toxicosis of swine, symptoms of which include refusal to eat (refusal factor), lack of weight gain, digestive disorders and diarrhea, ultimately leading to death. Pathological findings include hemorrhagic lesions in the stomach, heart, intestines, lungs, bladder and kidneys (154). T-2 toxin is quite toxic to rats, trout and calves with oral LD50 values of 3.8, 6.1 and 0.6 mg/kg, respectively (67). Fatal doses of T-2 toxin in chickens have resulted in severe edema of the body cavity and hemorrhage of the large intestine, along with neurotoxic effects and oral lesions (183,184,185). These effects are similar to those observed with moldy corn toxicosis. T-2 toxin is also thought to be one of the toxins involved in the human disease alimentary toxic aleukia (ATA) and stachybotryotoxicosis of horses (8). Recently, Yagen and Joffe (188) reported that isolates of F. poae and F. sporotrichoides from overwintered cereals associated with ATA in the U.S.S.R. produced T-2 toxins in culture. T-2 toxin also causes severe dermal responses in rabbits, rats and other animals, including humans, when applied to the skin. However, T-2 toxin is not thought to be carcinogenic (8).

**RELATION TO HUMAN HEALTH**

The relationships of mycotoxins to human health as the etiological agents of disease syndromes in man are difficult to determine because there is no direct evidence of such involvement in terms of controlled experiments with man. Certainly the diseases of ergotism and alimentary toxic aleukia can now be attributed to fungal toxins. Evidence also strongly suggests that acute cardiac beriberi, common throughout Asia, may in fact be linked to the so-called yellowed rice toxins (citrinin, luteoskyrin, rugulosin, cyclochlorotine and citr Evelyn) which are produced by numerous Penicillium species isolated from yellowed rice (140). Also, the fact that many different animal species are susceptible to both acute and chronic effects of mycotoxins is strong indirect evidence that man too would most likely be adversely affected in a similar fashion. This is particularly true with those toxins which affect animals which are physiologically similar to man such as primates and swine. While the incidence of mycotoxic disease in animals appears to be higher than in man, this is not surprising and does not mean that animals are necessarily more susceptible to mycotoxins than man. It simply means that animals are more likely to be exposed to mycotoxins than man, because of the quality of feed and in which they are fed, making exposure to moldy feed and possible toxins more frequent. Also, the foraging habits of some animals may expose them to mycotoxins more frequently than man.

In the absence of definitive data to interpret the role of mycotoxins in human disease, one must turn to less direct data such as animal studies, epidemiological data and reports of isolated incidences of human disease, thought to be related to mycotoxins, but for which complete proof is lacking. Based on these considerations, the types of diseases that may be caused in man by mycotoxins can be divided into acute toxicity, chronic toxicity and/or carcinogenicity. The greatest amount of information available in this regard concerns aflatoxins.

**Acute toxicity**

There are reports in the literature that associate aflatoxins with acute poisonings in humans. These reports all involve children and have been reviewed by Moss (112) and Shank (146). In one report, 26 persons in two Taiwan farming villages suffered illness (146). The ill persons were in three families of 10 households. Five households had consumed moldy rice for periods up to 3 weeks and experienced the intoxication. Members of the same families but living in different households which consumed rice that was not moldy were not affected. There were three deaths, all children, ages 4, 5 and 6 years. Symptoms of the disease were edema of the lower extremities, abdominal pain, vomiting, palpable liver but no fever. Samples of the rice were assayed for aflatoxin B1, which was found in two samples at levels of about 200 μg/kg. Another suspected case of fatal aflatoxin poisoning occurred in Uganda and involved a 15-year-old boy (145). Upon admission to a hospital, the boy had a history of abdominal pain, edema of the legs, palpable tender liver, and no fever, symptoms very similar to the Taiwan cases. Pathological findings included pulmonary edema, flabby heart, necrosis of the liver and fatty liver. The diet consisted mainly of cassava which was found to be moldy and contaminated with 1.7 mg of aflatoxin/kg. Extrapolation of data obtained with monkeys, and calculations of the amount of cassava consumed by the boy over a period of several weeks revealed that a fatal dose could easily have been consumed. Two young children in the same family were ill with similar symptoms but recovered. It was postulated that because the young children ate less, they might have been exposed to sub-lethal dose levels. Studies in Thailand show that Reye’s syndrome occurs in epidemic proportions in northeast Thailand (11). Reye’s syndrome occurs in children and is characterized by vomiting, hypoglycemia, convulsions, coma and usually death. Pathological examination reveals severe cerebral edema and fatty degeneration of the liver, kidneys and heart. In one fatal case involving a 3-year-old boy in northeast Thailand, it was found that the boy had eaten only leftover boiled rice for 2 days before becoming ill (11). Examination of the rice revealed that it was moldy and contained more than 10 mg of total aflatoxins/kg. Autopsy of Rey’s syndrome cases in Thailand has shown aflatoxin B1 in human tissue specimens from 22 of 23 cases, and in two cases the toxin levels in the tissues were similar to those in tissues of monkeys that had been given an approximately LD50 dose. Studies of infantile cirrhosis of the liver in India showed that of 16 mothers with children suffering from cirrhosis, four were secreting a
may have a bearing on response to the toxin. This is suggested by the fact that malnutrition, aflatoxins and high incidences of liver disease, including liver cancer, are often all found in the same population. Madhavan et al. (107) showed that reduced protein intake in monkeys significantly increased their susceptibility to aflatoxins. Similar observations have been made with rats by Newberne et al. (119,120) and Madhavan and Gopalan (106). Thus, the greatest threat to human health is likely to be found in those countries least able to reject low quality foods and with the poorest conditions for adequate storage to prevent the development of aflatoxin.

IMPLICATIONS TO FOOD SAFETY

Mycotoxins can enter the food supply in several ways, but these can be grouped into two general routes of contamination, direct or indirect contamination (75). Direct contamination occurs as the result of mold growth on the food material itself. Almost all foods are susceptible to mold growth during some stage of production, processing, storage or transport. Mold growth on foods that are to be consumed directly can result in direct exposure to mycotoxins. Normally, foods in which evidence of mold growth has occurred are rejected by most persons. However, in certain regions, because of shortages, it sometimes becomes necessary to consume food of poor quality to avert starvation. In these situations, exposure to mycotoxins by consumption of food can occur. In some areas of the world, it is common practice to consume moldy foods either because some mold is almost always present, such as in tropical areas, or because of the common use of molds in the fermentation and preparation of foods, such as tempeh, soy sauce and other oriental foods. The molds used in the commercial production of these foods have been examined and shown to be non-mycotoxin producers (71,113,114). However, in these areas of the world, home production of mold-fermented foods is also practiced. It is very possible that these home fermentations may become contaminated with mycotoxin producing molds and thus be a direct source of mycotoxins in the food (71). Direct contamination of and exposure to mycotoxins in food appear to be more of a hazard in tropical areas and regions where food shortages exist and where there is less aversion on the part of the population to consuming moldy food.

Indirect contamination of food occurs as the result of using a food ingredient contaminated with mycotoxins. Processed and prepared foods are the types of foods involved in indirect contamination. Indirect exposure to mycotoxins can also result from consumption of animal products which contain mycotoxin residues where the food producing animal has consumed moldy feed. Indirect routes of contamination are more of a problem.
in those areas of the world where food is more highly processed such as the U.S., Canada and Europe. The recorded incidences of indirect contamination of foods are lower than those of direct contamination.

Nuts and oilseeds

Aflatoxins have been found in a number of nuts, nut products and oilseeds. Peanuts, Brazil nuts, pecans, pistachio nuts and cottonseed have been shown to be susceptible to contamination (156). Contamination of peanuts occurs primarily in the field during harvest, when the seeds are being dried. Mechanical damage, insect damage and excessive rain during the drying period are all contributing factors to invasion by and growth of A. flavus. Since most aflatoxin contamination occurs during the drying period, it is possible to screen nuts for processing and divert contaminated lots from edible use. With peanuts, it is even possible to divert individually contaminated seeds if the lot is not heavily contaminated. Sorting occurs at the farmer-buyer level and again at the sheller-processor level (61,80,156). Studies have shown that peanuts consumed as roasted in-shell nuts in the U.S. are relatively free of aflatoxins at the consumer level. This is due to a variety of reasons, including inspection and sorting that occurs before marketing (156). In addition, processed consumer peanut products in the U.S. have also been found to be relatively free of aflatoxins. Again, this is due to a number of reasons, but inspection, sorting and roasting all contribute to substantial reduction of aflatoxins in processed peanut products at the consumer level (156). Most of the credit for this low level of aflatoxin in consumer peanut products in the U.S. goes to the extensive cooperation between industry and government regulatory agencies in efforts to control the problem and prevent exposure of the consumer to aflatoxin. It has been suggested that because of this awareness and cooperation, the American consumer is now getting safer, higher quality peanuts and peanut products than ever before (61). This is in sharp contrast to conditions in other countries, such as in Southeast Asia and Africa, where studies have shown contamination of peanuts and peanut butter with aflatoxin has ranged from 17 to 97% of samples with levels of 213 to 1530 µg/kg encountered (156). Aflatoxin contamination of Brazil nuts, pistachio nuts and other nut products at the consumer level in the U.S. has similarly been reduced and controlled by inspection at importation ports and programs of education and certification worked out with importers and producing countries (156).

Aflatoxin contamination of cottonseed is also a problem in the U.S. and worldwide. Most of this contamination occurs in the field and can result in contaminated meal being used as feed (156). This may result in significant aflatoxin residues in certain animal products, particularly milk. Treatment with ammonia appears to be a potential means of detoxifying aflatoxins in oilseed meals, making them safe for animal feeds (62). Food oils from peanut, cottonseed and copra may be contaminated with aflatoxin. Normally, the refining process for these oils, as used in the U.S., removes aflatoxin. However, in countries where unrefined oils are used, these can be a source of human exposure to aflatoxins (62,156).

Grains

Grains, especially corn, are also subject to mycotoxin contamination. Contamination may occur in the field and during harvest and storage. Field fungi include Fusarium species, and contamination with these organisms can occur if certain high moisture conditions exist. It is also thought that invasion by A. flavus and aflatoxin production can occur in the field as well as during storage (70). Again, damage to the seed coat from insects, hail or mechanical handling and moisture content are primary factors which permit invasion and growth by A. flavus. It is thought possible that the second generation European corn borer may feed on the kernels and may play a role in damaging the corn and disseminating the fungus (104). Also, Fusarium species can infect corn in the field through the silks and there is experimental evidence that A. flavus can also invade corn in this way (70,157). It is now thought that most aflatoxin contamination of corn in the U.S. occurs before harvest (157).

With newer methods of mechanical harvesting, using picker-shellers at higher moisture contents of corn, there are increased chances for mechanical damage. Since corn harvested by picker-sheller must be mechanically dried, any delay in the drying process can result in mold growth. Such delays can occur as the result of overtaxing of drying equipment during peak harvest time, fuel shortages or attempts to conserve fuel and dry at lower temperatures. In some instances at peak harvest time, drying equipment and storage facilities are overtaxed to the extent that grain may temporarily be stored on the ground. In these situations, conditions favorable to mold growth may occur. Remoistening of grain during storage and transport either accidentally by leaking structures, condensation, or by "sweating" of cold grain may cause moisture levels in dry grain to rise to sufficient levels to permit mold growth. Even if only a small portion of the grain becomes moldy, very high levels of aflatoxin can be produced in a few kernels. These few kernels may contain enough aflatoxin to contaminate an entire lot with unacceptable levels of aflatoxins when the corn is milled into meal or feed.

Aflatoxin contamination of corn is a worldwide problem and appears to be most severe in the Philippines, Thailand and Uganda, where the incidence of aflatoxin contamination ranges from 35 to 97% of the corn supply (157). In the U.S., corn from the southeastern U.S. is more susceptible to contamination (4% incidence level) than corn from the midwest (2.5% incidence level). Aflatoxin levels in corn from the Southeast average 18 µg/kg, whereas contamination levels in the Corn Belt average less than 1 µg/kg (157). In some instances, processing of corn into food or feed
products may reduce the aflatoxin level. For example, alkali processing of corn for preparation of masa used in tortillas substantially reduces aflatoxin content (156,157,169). Wet and dry milling of corn tends to move the aflatoxin concentration to the feed grade and oil fractions (156). Other grains such as wheat, barley, rye, oats, grain sorghum, millet and rice appear to be less susceptible to aflatoxin contamination than corn, provided they are properly stored and handled (157).

Fruits and vegetables

Molds are frequent contaminants of fresh fruits and vegetables. Apples are susceptible to a rot caused by *P. expansum*. This organism produces patulin, and rotted apples have been shown to contain patulin (12,155). Patulin has also been found in commercial apple juice (143,177). Apples used for juice and apple butter are sometimes of poorer quality than apples used for direct consumption (76). If a few such apples contain patulin, entire lots of juice or other products may contain patulin. Sorting of apples before processing to eliminate rotted apples is essential to preventing contamination with patulin.

Low levels of aflatoxin contamination have been detected in figs (14). In surveys of dried dates and raisins, no aflatoxin has been found, though *A. flavus* may be a common contaminant of these commodities (156). Only limited incidences of aflatoxin in wines have been detected, with the average aflatoxin content being lower than 1 μg/l (156). In experimental studies with lettuce, cauliflower and celery and *A. parasiticus*, Raghu et al. (134) found that fungus did not grow well on these substrates and that no detectable levels of aflatoxin were found.

Animal products

Contamination of foods of animal origin with mycotoxins is a possible concern. Aflatoxin M1 has been found in the milk of lactating animals fed aflatoxins. Aflatoxins have been found in commercial fluid milk in the U.S., Germany and South Africa, and in commercial samples of nonfat dry milk and cottage cheese in the U.S., and in various imported cheeses (156,170). Most of the aflatoxin found was in the M1 form and probably came from the feeding of aflatoxin-contaminated feed. Aflatoxins have also been found in livers, kidneys and other tissues of pigs in feeding trials with aflatoxin-contaminated diets (76). Levels were highest in livers and kidneys with only trace amounts occurring in heart, muscle and adipose tissues. Aflatoxins have also been found in the tissues of broiler chickens and eggs of laying hens given experimentally contaminated feed (157). In these animal products, aflatoxins have been found both as B1 and M1. While the levels of aflatoxins in these foods are generally low, these studies indicate that indirect exposure to aflatoxins could occur from consumption of products of animal origin if the animals were fed aflatoxin-contaminated feed.

Residues of ochratoxin A have also been found in animal tissues including liver, kidney, muscle and adipose tissues (76,85). These were tissues of bacon pigs (84) and poultry (51) in Denmark. Ochratoxin has also been found in pork meat in Yugoslavia (89). Thus, human exposure to ochratoxin might also occur as the result of the consumption of animal products from animals fed ochratoxin-contaminated feed.

Refrigerated foods

Mold growth on foods, such as cheeses and cured meats, stored at low temperatures is a common and recurring problem (57). Certain molds are known to be capable of producing mycotoxins at temperatures as low as -2 to 10 C. Many of these molds belong to the genus *Penicillium* which are capable of growth over a wide range of temperatures, including temperatures commonly employed in household refrigerators and supermarket display cases (135). Some psychrotrophic molds may grow at temperatures below 0 C (121). Recently Torrey and Marth (166) reported isolation of potentially toxic molds from home refrigerators and foods stored in home refrigerators.

Kuehn and Gunderson (92) reported that fungi associated with frozen fruit-filled pastries were capable of growing at 0 and 5 C. These workers also found that 50% of the psychrotrophic flora associated with frozen pastry products were members of the genus *Penicillium* (93). Several *Penicillium* species isolated by Kuehn and Gunderson (93) are now known to be potential mycotoxin-producing organisms (Table 1). These organisms include *P. cyclopium, P. expansum, P. frequentans, P. martensi, P. superulum, P. urticae, and P. viridicatum*. Known mycotoxins produced include penicillic acid, patulin and ochratoxin A (39,144,172).

In a more recent study, 82% of the molds found on refrigerated Cheddar cheese belonged to the genus *Penicillium*. 7% were *Aspergillus* species and 1% were *Fusarium* species (26). Toxicological screening of molds isolated from Cheddar cheese indicated that 19.8% of the isolates were toxic to chicken embryos, causing 50% mortality or more. Thin layer chromatographic examination of chloroform extracts of the mold cultures showed the presence of known mycotoxins, including patulin, penicillic acid, ochratoxin A and aflatoxin, in 7.2% of the culture extracts (26). In further studies, most molds found on Swiss cheese were found to be *Penicillium* species which were capable of growing at 5 C (17). Toxicological screening of these isolates showed that 32% of culture extracts of the molds were toxic to chicken embryos. Known mycotoxins found were ochratoxin A, penicillic acid, patulin, citrinin and aflatoxin. In a survey of domestic and imported cheeses, Bullerman (49) reported that the predominant organisms found were *Penicillium* species and that toxigenic species of *P. cyclopium* and *P. viridicatum*, as well as *A. flavus*, were commonly found.

Several workers have studied the formation of mycotoxins in artificially inoculated cheeses and meats. Lie and Marth (98) showed that *A. flavus* and *A. parasiticus* would grow and produce substantial quan-
tities of aflatoxins on Cheddar cheese at room temperature. Oldham et al. (172) working with A. flavus and Cheddar cheese obtained low levels of aflatoxins at 25 C but none at 4.4 or 7.2 C. Kiermeier and Gross (82) obtained aflatoxin production on Tilsit cheese but not on Camembert and Romadur cheeses. Other workers have reported aflatoxin production in Tilsit and Emmental cheeses (55,139). Shih and Marth (152) reported production of aflatoxins on brick cheese at 12.8 C by A. parasiticus after 1 week of incubation, and at 23.9 C by A. parasiticus after 1 week and by A. flavus after 14 weeks of incubation. It is generally believed that A. flavus and A. parasiticus will not grow and produce aflatoxins at temperatures below 13 C (76). However, van Walbeek et al. (171) reported that a strain of A. flavus produced aflatoxin at 7.5 and 10 C in 4 weeks. Strain differences might account for aflatoxin production at temperatures approaching refrigerated storage, but, for the most part, aflatoxins are not considered to be a problem if foods are kept under adequate refrigeration. This is, however, a key point since temperatures in refrigerators and refrigerated display cases may vary considerably. van Walbeek et al. (171) reported in a survey of domestic refrigerators, that the minimum temperatures ranged from 0 to 10 C. More recently, Torrey and Marth (167) reported that mean temperatures ranged from 3.9 to 11.9 C in two home refrigerators with the total range being 1.7 to 20.2 C. In that study an aflatoxigenic strain of A. parasiticus did not grow at 8 C over a period of 21 days; however, Penicillium species grew at 5 C. Recently, Kiermeier and Behringer (81) reported aflatoxin formation in moistened milk powder at temperatures between 1 and 5 C and at 10 C. On the other hand, Lieu and Bullerman (99) reported that A. flavus did not grow on any of several food substrates at 5 or 12 C, but grew extensively and produced aflatoxins at 25 C.

Stott and Bullerman (158) inoculated Cheddar cheese with a patulin-producing strain of P. patulum, and found that the mold grew extensively on the cheese at 5 and 25 C. Patulin production on the cheese was not observed at 5 C and only small variable amounts of patulin were found at 25 C. In the samples that contained patulin, the toxin was localized in the mold mycelia and the first 3-mm layer of cheese. Lieu and Bullerman (99) observed similar results with patulin- and penicillic acid-producing organisms grown on Swiss and Mozarella cheeses. In this study the Penicillium species grew extensively on the cheese at 5, 12 and 25 C; however, no patulin or penicillic acid were detected in the cheese as a result of the mold growth. Olivigni and Bullerman (123) working with an atypical isolate of P. roqueforti obtained patulin and penicillic acid production at 5, 12 and 25 C on laboratory media, but the toxins were not produced on Cheddar or Swiss cheeses.

In addition to refrigerated cheeses and frozen pastries, several workers have reported the incidence of toxic molds in cured and smoked meat products. Bullerman and Ayres (21) screened 66 Aspergillus and Penicillium isolates from country-cured hams and fermented sausages for aflatoxins and found aflatoxin production in one strain of A. flavus (NRRL A16-100). Strzelecki and Badura (162) isolated A. flavus and A. versicolor from dry Cracower sausage in Poland. Four strains of A. flavus out of 36 total isolates were capable of aflatoxin production in yeast-extract sucrose broth. Of 562 molds isolated from country-cured hams by Sutic et al. (164), 403 were Penicillium species and 121 were Aspergillus species including toxigenic strains of A. flavus and A. versicolor. In a study of European dry salami, Ciegler et al. (42) isolated 346 cultures of Penicillium species. About 10% of the Penicillium cultures including six species were capable of producing penicillic acid in liquid media. Leistner and Ayres (96) isolated 307 molds from 40 samples of country cured hams and 27 fermented sausages. Aspergillus and Penicillium species predominated on country-cured hams and Penicillium and Scopulariopsis predominated on fermented sausages. Wu et al. (182) screened 89 cultures of Aspergillus and 54 cultures of Penicillium isolated from aged cured meats for toxicity to chicken embryos and found about 16% of the aspergilli were toxic and about 1% of the penicillia were toxic. Known mycotoxins were not detected. Bullerman (20) isolated potentially toxic strains of P. cyclopium, P. viridicatum and A. flavus from domestic and imported cured meats. In that study Penicillium species were predominantly found.

Experimentally, aflatoxin production on cured meats has been reported at temperatures as low as 15 C. Bullerman et al. (23,24) observed aflatoxin production on bacon, ham and aged salami at 15 C. Tauchman (165) likewise observed aflatoxin production on dry sausage at 15 C. However, Oldham et al. (172) working with cured luncheon meat did not observe aflatoxin production at 4.4 and 7.2 C. Likewise, Lieu and Bullerman (99) did not observe aflatoxin production by A. flavus on bologna or bacon at 5 and 12 C; however, aflatoxin production was evident on these substrates at 25 C.

Wu et al. (181) showed that seven strains of P. viridicatum isolated from country-cured ham were capable of producing citrinin on this type of ham when grown at temperatures from 15 to 30 C. Ochratoxin-producing strains of P. viridicatum have also been isolated from mold fermented sausages (108). Halls and Ayres (64) reported that strains of A. versicolor produced sterigmatocystin on country cured ham at 20 and 28 C. In studies with patulin- and penicillic acid-producing organisms grown on meats, production of these toxins on meats was not observed at 5, 12, or 25 C even though extensive growth of the molds occurred at all temperatures (42,99,123).

It has been suggested that the lack of production of patulin and penicillic acid on cheese and meat substrates may be because these substrates are low in carbohydrate and high in protein (42,99,123,159). This is supported by
the fact that other natural substrates high in protein, such as soybeans, peanuts and cottonseed, likewise do not support penicillic acid production (41). In addition, other studies have shown that laboratory media which are lacking in carbohydrate but high in protein support the production of these toxins in broth substrates containing no carbohydrates, but high amounts of protein (123,159).

Stability of mycotoxins in foods

Once mycotoxins have contaminated and become a part of a food system, it is important to know how long the toxins will persist and retain biological activity. Aflatoxins are generally thought to be stable in most food products. Thermal processing of food, other than roasting of nuts, is not likely to reduce the aflatoxin content, whereas roasting may cause a reduction of 40 to 60% of the aflatoxin present (157). Alkaline treatments and refining of food oils either destroy or remove aflatoxins (157). Aflatoxin levels have been reported to decline in raw peanut butter in storage for up to 6 months (174). Strzelecki (161) reported that recovery of aflatoxin from meat decreased with increasing storage time. Murthy et al. (115) observed a decrease of 98 to 80% in recovery of aflatoxin B1 from fresh beef stored at -18°C for 183 days. Chu et al. (34) found that aflatoxin B1 was partially lost in the mashing and brewing process. Dam et al. (44) reported that fermentation of grains experimentally contaminated with aflatoxin caused a loss of about 60% of the added aflatoxin and that further treatment involved in isolation of distillers protein concentrate destroyed total aflatoxins in excess of 90%. In studies with peanut meal and peanut butter, Bauer (9) reported no significant change in aflatoxin levels when the products were held at 23°C and 50% relative humidity exposed to air for 2 years. Lieu and Bullerman (99) found that aflatoxins B1 and G1 were essentially stable in Swiss cheese, bologna and cooked cornmeal for up to one week at 5°C. Recoveries of aflatoxins ranged from 90 to 100%.

Patulin and penicillic acid appear to be less stable than aflatoxins in certain foods. Scott and Sommers (142) found that patulin and penicillic acid were both stable in grape and apple juice but not in orange juice and flour. They attributed the instability in orange juice and flour to the presence of thiol compounds. Pohland and Allen (127) also reported that patulin was stable in apple juice and dry corn but unstable in wet corn, Durum wheat and sorghum. Hofmann et al. (72) and Ciegler et al. (42) investigated potential production of patulin and penicillic acid in meat and meat products. They concluded that both patulin and penicillic acid at the pH of meat and meat products would react with sulphydryl compounds and amino acids normally occurring in meat and therefore would not be detected by chemical assay procedures. Stott and Bullerman (160) found that patulin became undetectable when added to Cheddar cheese and stored at 5 and 25°C for various periods of time. Further, Lieu and Bullerman (99) found that patulin and penicillic acid became undetectable in bologna after 12 and 48 hours, respectively, when stored at 5°C. With Swiss cheese a low level of patulin and penicillic acid persisted, with 5 to 8% of the added toxins being detected after one week at 5°C. In these same studies, patulin and penicillic acid were stable in cooked cornmeal with about 80% of the toxins recoverable after one week at 5°C. Patulin has also been reported to disappear from apple juice undergoing fermentation by Saccharomyces cerevisiae and Saccharomyces ellipsoideus (68).

The instability of patulin and penicillic acid in foods such as meat and cheese has been attributed to combination of the toxins with amino acids and compounds containing sulphydryl groups (42,72,100,127,142). Binding of patulin and penicillic acid to sulphydryl groups has been shown to reduce the biological activity of these toxins. Hofmann et al. (72) found that patulin bound to glutathione was no longer toxic to chicken embryos, mice and rabbit skin. S-alkylated adducts formed by combination of penicillic acid with cysteine or glutathione were found to be non-toxic to mice and quail by Ciegler et al. (42). In the same study, it was shown by chicken embryo tests that the penicillic acid adduct of glutathione was about 40 to 50% as toxic as penicillic acid and the penicillic acid adduct of cysteine was reported to be as toxic as penicillic acid itself. More recently, Ciegler et al. (38) showed that the adduct of patulin and cysteine was nontoxic to mice and chicken embryos but remained teratogenic to chicken embryos. Lieu and Bullerman (100) studied the toxicity of the adducts of combinations of patulin and penicillic acid with glutathione and cysteine and found them to be non-inhibitory to Bacillus subtilis at 50 μg of toxin equivalent. Patulin and penicillic acid adducts of cysteine at 50 and 150 μg of toxin equivalent were nontoxic to brine shrimp larvae, and the patulin-glutathione adduct and patulin-cysteine adduct both at 100 μg of patulin equivalent were not toxic to 4-day-old chicken embryos. However, the penicillic acid adduct of cysteine at 100 μg equivalent of penicillic acid possessed considerable toxicity to embryos, but the penicillic acid-glutathione adduct in the same amount was less toxic. Simulated peptic digestion of S-alkylated adducts of the two toxins did not result in the regeneration of free toxins. No teratogenic effects were observed in this study.
Ochratoxin appears to be more stable in foods than patulin or penicillic acid but probably somewhat less stable than aflatoxins. In one study, Harwig et al. (66) studied the effect of canning dried white beans contaminated with ochratoxin on stability of ochratoxin A. Thermal processing at 121°C for 1 h reduced the ochratoxin content by 11%, whereas processing at the same temperature for 4 h reduced ochratoxin content by 34%. Soaking and blanching resulted in losses of 21% and 10% of ochratoxin, respectively. In another study, autoclaving of oatmeal and rice cereals for 3 h reduced the amount of added ochratoxin by 60% (168). Simulated coffee roasting conditions, where temperatures of 200°F for 5 min are reached, were found to destroy ochratoxin (97). Ochratoxin and citrinin were also found to be destroyed by the malting and brewing process if moderately contaminated barley was used (34,87). Highly contaminated barley retained 2 to 7% of the original amount of ochratoxin, but barley in that condition was severely deteriorated and would not likely be accepted for brewing use.

From these studies it would appear that of the mycotoxins of concern in foods, aflatoxin would be expected to be the most stable in all foods. Ochratoxin A would also be expected to be stable in many foods, whereas the stability of patulin and penicillic acid would be quite dependent upon the nature of the foodstuffs in which they are found. Stability of patulin and penicillic acid would be expected to be greatest in dry grains and apple products and least in high protein foods such as cured meat and cheese. More studies on the stability of these and other mycotoxins in foods are needed to fully assess the long term stability of all mycotoxins in foods.

Significance of mycotoxins

Control of mycotoxins in foods is a complex and difficult task. Information regarding toxicity, carcinogenicity and teratogenicity to humans, extent of contamination and stability of mycotoxins in foods is lacking for most mycotoxins. Such information is necessary to establish regulatory guidelines, tolerances and seizure policies. In the United States, the U.S. Food, Drug and Cosmetic Act defines food as adulterated if it contains "any poisonous or deleterious substance which may render it injurious to health" (138). The act also gives the Food and Drug Administration (FDA) authority to enforce the Act and remove from interstate commerce any food or feed found to be adulterated with such substances. The FDA treats aflatoxins as poisonous and deleterious substances, and regulates them accordingly. The law distinguishes between poisonous substances that are natural components of foods and those which are added to foods. Aflatoxins, though of natural origin, are not considered natural components of foods and therefore are treated as added components, though as unavoidable contaminants (53,138). Aflatoxins, while they are known carcinogens, are not regulated under the Delaney Amendment since they are considered to be unintentional additives. The Delaney Amendment applies only to those food additives that are intentionally added for some specific purpose of preservation or processing. In actuality there is no tolerance level for aflatoxins in any food (176), since toxicological data for mycotoxins, including aflatoxins, upon which a safe tolerance level might be based are lacking. Thus, a safe tolerance level for any of these compounds has not been established. In the absence of tolerances, FDA has set what it considers to be practical limits for aflatoxins in foods and feeds, based primarily on the limits of detections and measurement of analytical methods and to some extent on the ability of agronomic and technological practices to prevent contamination (176). Practical limits have been set forth by FDA as "working guidelines" for regulatory action and apply to all products known to be susceptible to aflatoxin contamination, and include animal feeds (138). In the U.S., the Food and Drug Administration has a working guideline of 20 ppb (µg/kg) for aflatoxins in susceptible commodities. Currently, FDA is proposing to lower the guideline to 15 ppb for peanut products (138). This change is being proposed because it is clear that present analytical methods can routinely measure aflatoxin levels below 20 ppb. Also, when specific information indicates that contamination of a finished food product could have been controlled or avoided by application of good manufacturing practices, the guideline is not applicable (176). In such situations FDA bases its decision on the specific facts of the case and may, in fact, resort to seizure and compliance action for amounts of aflatoxin less than the established guidelines. Such actions are considered justified and have been taken by FDA in the past (176).

On December 7, 1977, the Food and Drug Administration (53) announced the establishment of an "action level" for aflatoxin in whole milk, skim milk and low fat milk of 0.5 ppb. This means that FDA prohibits the shipment of milk in interstate commerce that contains more than 0.5 ppb of aflatoxin. The action was taken in response to the fact that adverse weather conditions and possible insect damage had resulted in a high degree of aflatoxin contamination of corn in the southeastern U.S. in 1977, and it appeared that aflatoxin-contaminated corn was being fed to dairy animals. The lower level for milk was imposed because milk containing aflatoxins may pose a special risk to infants and young children who may consume large quantities of milk.

Most regulatory considerations to date concerning mycotoxins have involved aflatoxins. However, FDA is currently studying toxicological data and contamination incidences of foods for other mycotoxins including ochratoxin A, patulin, penicillic acid, sterigmatocystin, zearealenone and T-2 toxin. As sufficient toxicological data become available to permit an assessment of the significance of these toxins to human and animal health, guidelines and regulations for these toxins can also be expected (138).
A number of countries have established regulatory programs for aflatoxins in food and feeds. These include Brazil, Canada, Denmark, the United Kingdom, France, Hungary, India, Japan, the Netherlands, South Africa and the Federal Republic of Germany in addition to the U.S. (56, 137). The regulations contain guidelines for aflatoxin contamination of human food in the range of 5-30 ppb (137). The Food and Agriculture Organization (FAO) of the United Nations has sought to assist developing countries in establishing effective control systems and regulations on aflatoxin contamination of foods in these areas of the world (69).

While the efforts of regulatory agencies such as FDA are very important in preventing human and animal exposure to mycotoxins, the responsibility for actual control and prevention of this hazard lies with the agricultural and food industries. It is at this level that control measures to prevent contamination and eliminate contaminated commodities from the food supply must be applied. In addition to FDA, the U.S. Department of Agriculture (USDA) is also involved in mycotoxin control in certain commodities such as peanuts and grains. The USDA has a testing program for peanuts beginning at the farm level and continuing through the marketing system. Also, joint industry-USDA-FDA programs are in effect to control aflatoxin contamination of agricultural commodities such as peanuts, cottonseed, copra, corn and animal feeds (176). These relationships and voluntary programs have resulted in improved industry and government communications and development of food processing and manufacturing practices that have helped to reduce and eliminate mycotoxin contamination of a number of commodities (176).

The control of mycotoxins in foods involves many factors. The best approach to eliminating mycotoxins from foods is to prevent mold growth. However, this is not always as simple and straightforward as it may seem. Mold growth must be prevented at all levels of production, harvesting, transporting and storage of foods. This involves prevention of insect damage and mechanical damage to agricultural commodities as well as moisture control at levels which do not permit mold growth and storing at temperatures and under conditions which minimize mold development. Control of mycotoxins also involves quality control procedures to detect and remove contaminated products from commercial channels before they reach the consumer.

CONCLUSIONS

Our appreciation of the significance of mycotoxins to food safety and human health continues to evolve and increase. While there is no direct evidence, in the form of controlled feeding experiments, for the involvement of mycotoxins in foodborne human disease, the indirect evidence is strong and continues to accumulate. In light of the numerous animal species found to be susceptible to the various effects of mycotoxins, it is difficult to believe that man would not be similarly affected.

Regional studies of acute and chronic disease patterns in humans, and the association of these with mycotoxin contamination of diets and foodstuffs in the same regions, are further evidence of the probable involvement of mycotoxins in foodborne human disease. This is particularly significant when symptoms, and toxin levels in body fluids and tissues, of human victims are found to be similar to those observed in test animals in controlled feeding experiments. It simply remains a matter of time until additional work proves the conclusive involvement of mycotoxins in human disease. Therefore, it is of utmost importance that every attempt be made to keep mycotoxins out of the food and feed supply. Not only must food-producing animals be protected from mycotoxins to maintain production, but also to prevent the occurrence of mycotoxin residues in human foods of animal origin. Foods and commodities must be stored under conditions which prevent mold growth on the farm, in storage facilities, in retail outlets and in homes. Thus, protection of the human food supply from contamination with mycotoxins must occur along the entire food chain, from the point of production on through to the consumers’ own refrigerator and kitchen.

REFERENCES

**SIGNIFICANCE OF MYCOTOXINS**


