Food Borne Toxicants and Prevention in Toxicology

Thomas W. Kensler, PhD
Bloomberg School of Public Health
Mycotoxins—What Are They?
“The Poison in the Corn”—Aflatoxin
Aflatoxin—Acute and Chronic Toxicity
Aflatoxin—A Human Carcinogen
Prevention of Aflatoxin Hepatocarcinogenesis
Section A

Mycotoxins—What Are They?
Mycotoxin: A Toxin Produced by a Fungus

- The Food and Agriculture Organization estimates that 25% of the world’s food crops are affected by mycotoxins.
- Water stress, high-temperature stress, and insect damage are major determining factors in mold infestation.
Mycotoxin: A Toxin Produced by a Fungus

- The economic impacts associated with mycotoxins include the following:
  - Lower yields of crops, livestock, and dairy products for the producer
  - Less nutritious foods and adverse effects for the consumer
  - And regulatory efforts for the surveillance, assay, and enforcement—all yielding higher consumer costs
# Mycotoxin and Contaminated Food

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Contaminated Food</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxins</td>
<td>corn, peanuts, cottonseed, tree nuts</td>
</tr>
<tr>
<td>Altenuene</td>
<td>tomato</td>
</tr>
<tr>
<td>Citrinin</td>
<td>barley, oats</td>
</tr>
<tr>
<td>Cyclopiazonic acid</td>
<td>peanuts</td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>corn, wheat, barley</td>
</tr>
<tr>
<td>Ergot alkaloids</td>
<td>rye, wheat</td>
</tr>
<tr>
<td>Ochratoxin</td>
<td>barley, oats, corn, sorghum, wheat, rice, green coffee</td>
</tr>
<tr>
<td>Patulin</td>
<td>apples</td>
</tr>
<tr>
<td>Penicillic acid</td>
<td>corn, dried beans</td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>barley, corn, sorghum</td>
</tr>
<tr>
<td>Trichotheclin</td>
<td>wine</td>
</tr>
</tbody>
</table>
Human Diseases Caused by Moldy Foods

<table>
<thead>
<tr>
<th>Disease</th>
<th>Molds Involved</th>
<th>Toxins Implicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxicosis</td>
<td><em>Aspergillus flavus A. parasiticus</em></td>
<td>Aflatoxins</td>
</tr>
<tr>
<td>Ergotism</td>
<td><em>Claviceps purpurea, C. paspali, C. fusiformis</em></td>
<td>Ergot alkaloids</td>
</tr>
<tr>
<td>Alimentary toxic aleukia</td>
<td><em>Fusarium sporotrichiodes</em></td>
<td>T-2 toxin</td>
</tr>
<tr>
<td>Yellow rice (cardiac beri-beri)</td>
<td><em>Penicillium citreoviride, P. citrinum, P. islandicum</em></td>
<td>Luteoskyrin, Islanditoxin</td>
</tr>
</tbody>
</table>
Structures of Selected Mycotoxins

Ochratoxin A

Zearalenone

Ergotamine

Aflatoxin B₁

Fumonisins

A₁: R¹ = COCH₂CH₂(CO₂H)CH₂CO₂H; R² = OH; R³ = C₂H₅OC₂H₅
A₂: R¹ = COCH₂CH₂(CO₂H)CH₂CO₂H; R² = H; R³ = COCH₃
B₁: R¹ = COCH₂CH₂(CO₂H)CH₂CO₂H; R² = OH; R³ = H
B₂: R¹ = COCH₂CH₂(CO₂H)CH₂CO₂H; R² = R³ = H
Fumonisins

- Produced by *F. moniliforme*, fumonisin B1 (FB1), B2, B3, B4, A1, and A2. {equine leukoencephalomalacia, porcine pulmonary edema, and human esophageal cancer}
- Levels of FB1 vary yearly, but are consistently in the 0.5 to 2-ppm range in U.S. cornmeal, and have been reported as high as 150 ppm in corn destined for human consumption in South Africa
Ochratoxins

- Excessive exposure plays a role in Balken Endemic Nephropathy (BEN)
- Most potent kidney carcinogen found in NTP bioassay over 35 years
- Evidence of an extremely high incidence of urinary tract tumors in the endemic areas of BEN.
- In the 1980’s, 75-85% of blood samples analyzed in Germany were positive for OA exposure.
Other Mycotoxins

- **Zearalenone**, in corn, has estrogenic activity and binds to estrogen receptors.
- **Tricothecenes**, over 150 characterized (Yellow Rain)
- **Vomitoxin**, from fusarium molds contaminating wheat.
- **Patulin**, is produced by molds that grow on fruit, grains and cheese and occurs in apple juice, apples and pears with brown rot.
Ergotism

- Assyrian tablet [600 BC] alluded to “noxious pustule in the ear of grain”
- Parsees [400 to 300 BC]: “Among the evil things created by Angro Maynes are the noxious grasses that cause pregnant women to drop the womb and die in childbirth”
Ergotism

- Middle Ages: St. Anthony’s fire—epidemics characterized by gangrene of the feet, legs, hands, and arms (limbs were said to be consumed by the Holy Fire and blackened like charcoal)

- Loncier [1582]: Ergot known as obstetrical herb—used by midwives
Ergotism

- Hosack [1824]: Medical Society of NY inquiry on stillbirths—“The ergot has been called . . . palvis ad partum; as it regards the child, it may, with almost equal truth be denominated the pulvis ad mortem”
- There have been outbreaks of ergotism in recent times in Africa and India
St. Anthony’s Fire

- Ergotism
- *Claviceps purpurea* contaminating wheat and other grains
- Burning sensation, hallucinations, LSD-like symptoms
- Salem Witch Trials
Section B

“The Poison in the Corn”—Aflatoxin
The Poison in the Corn

Last summer’s drought left a legacy of poison that threatens the country’s food supply. Dry weather spurred the spread of the fungus that makes aflatoxin, a highly potent inducer of liver cancer. Dangerous amounts of aflatoxin contami-
### Foods Frequently Contaminated by Aflatoxins

<table>
<thead>
<tr>
<th>Peanuts</th>
<th>Barley</th>
<th>Oats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coconut</td>
<td>Rice</td>
<td>Wheat</td>
</tr>
<tr>
<td>Sunflower</td>
<td>Cottonseed</td>
<td>Millet</td>
</tr>
<tr>
<td>Linseed</td>
<td>Soybean</td>
<td>Sorghum</td>
</tr>
<tr>
<td>Rye</td>
<td>Cocoa</td>
<td>Corn</td>
</tr>
</tbody>
</table>

For images of aflatoxin-contaminated corn, please visit [http://aes.missouri.edu/delta/croppest/aflacorn.stm](http://aes.missouri.edu/delta/croppest/aflacorn.stm)
Aflatoxin Contamination in the United States

- Pre-harvest mold growth and production of aflatoxins in peanuts and corn are favored by warm ambient temperatures and prolonged drought conditions typical of many parts of the world, including southwestern United States
Aflatoxin Contamination in the United States

- Post-harvest production of aflatoxins on corn and peanuts is favored by warm temperatures and high humidity, which is also typical in the southern United States.
- The greatest problems with contamination by aflatoxins have occurred in corn and peanuts in the southeastern area and cottonseed in the southwestern area of the United States.
Frequency Distribution of Total Aflatoxins in Shelled Maize Produced in NC 1977–83
Frequency Distribution of Total Aflatoxins Levels in Raw Shelled Peanuts Produced in the US, 1973–84
Aflatoxin Varieties

AFB₁

AFG₁

AFB₂

AFG₂
Aflatoxin-Contaminated Peanut Butter (21 ppb)
Peanut Butter Factoids (Circa 1985)

- Peanut butter celebrated its 100th birthday as a sandwich spread in 1985
- It is consumed by approximately 40 million Americans every day
- Americans prefer creamy peanut butter to the crunchy variety, 60% to 40%
- Elvis Presley’s favorite sandwich was grilled peanut butter with bananas

Source: USA Today
Peanut Butter Factoids (Circa 1985)

- The average child will eat 1,500 peanut butter sandwiches by the time he or she graduates from high school
- 85% of Americans have a jar of peanut butter in their homes
- Each year, Americans consume enough peanut butter to coat the entire floor of the Grand Canyon

Source: USA Today
<table>
<thead>
<tr>
<th>Action level (ppb)</th>
<th>Commodity</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 <em>(aflatoxin ( M_1 )</em></td>
<td>milk</td>
<td>humans</td>
</tr>
<tr>
<td>20.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>300.0</td>
<td>cottonseed meal used in feed</td>
<td>all species</td>
</tr>
<tr>
<td>300.0</td>
<td>corn</td>
<td>finishing beef cattle</td>
</tr>
<tr>
<td>200.0</td>
<td>corn</td>
<td>finishing swine (( &gt;100) #)</td>
</tr>
<tr>
<td>100.0</td>
<td>corn</td>
<td>breeding cattle, breeding swine, and mature poultry</td>
</tr>
</tbody>
</table>

* Specifically for aflatoxin \( M_1 \), a toxic metabolite of AFB\(_1\) that occurs in milk
Section C

Aflatoxin—Acute and Chronic Toxicology
Aflatoxicoses

- Acute lethal toxicity
- Immune suppression
- Hemorrhagic anemia syndrome
- Hepatotoxicity
- Teratogenicity
- Carcinogenicity
### LD₅₀ Values for Different Species

*Single Oral Doses of Aflatoxin B₁*

<table>
<thead>
<tr>
<th>Animal Species</th>
<th>LD₅₀ (mg per kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duckling</td>
<td>0.3–0.6</td>
</tr>
<tr>
<td>Pig</td>
<td>0.6</td>
</tr>
<tr>
<td>Trout</td>
<td>0.8</td>
</tr>
<tr>
<td>Dog</td>
<td>1.0</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>1.4–2.0</td>
</tr>
<tr>
<td>Sheep</td>
<td>2.0</td>
</tr>
<tr>
<td>Monkey</td>
<td>2.2</td>
</tr>
<tr>
<td>Rat</td>
<td>5.5–17.9</td>
</tr>
<tr>
<td>Chicken</td>
<td>6.3</td>
</tr>
<tr>
<td>Mouse</td>
<td>9.0</td>
</tr>
</tbody>
</table>
Effect of Age on AFB₁ Toxicity in F344 Rats

LD₅₀ (mg AFB₁/kg, i.p.)

AGE (days)
Effects of Aflatoxins on Immunity

- Effects on cellular responses
  - Phagocytosis by macrophages reduced
  - Delayed cutaneous hypersensitivity reduced
  - Lymphoblastogenesis reduced (response to mitogens)
  - Graft versus host response reduced

Continued
Effects of Aflatoxins on Immunity

- Effects on humoral factors
  - Immunoglobulins (IgG and IgA) concentrations in serum may be reduced
  - Complement activity reduced
  - Bacteriocidal activity of serum reduced
Reduction in Phagocytosis of Macrophages with Increased Doses of Aflatoxins in Rabbits

Source: Pier, et. al. Medical Mycology, 1980
Iraq's Biological Weapons
The Past as Future?

- Between 1985 and April 1991, Iraq developed anthrax, botulinum toxin, and aflatoxin for biological warfare; 200 bombs and 25 ballistic missiles laden with biological agents were deployed by the time Operation Desert Storm occurred. ... Despite the Gulf War defeat, the Iraqi biological warfare threat has not been extinguished. ... 

- JAMA. 1997;278:418-424 (R.A. Zilinskas)
Acute Aflatoxicosis in Humans

- Syndrome characterized by vomiting, abdominal pain, pulmonary edema, and fatty infiltration and necrosis of the liver
- Western India (1974): Unseasonal rain and scarce food prompted consumption of heavily molded corn [6-16 mg AFB1/kg (ppm)] by people in over 200 villages—at least 97 fatalities

Continued
Acute Aflatoxicosis in Humans

- Acute aflatoxicoses also reported in Uganda, Kenya, Thailand, and Taiwan
Mutagenicity Of Aflatoxin B\textsubscript{1}

- *Salmonella typhimurium*
- *E. coli*
- *Bacillus subtilis*
- Algae
- Neurospora
- Rodent cells (V79, C3H)
- Human cells (lymphoblasts)
Carcinogenicity of Aflatoxin B$_1$ in Vertebrates

- Guppy
- Sockeye salmon
- Rainbow trout
- Duck
- Syrian hamster
- Mouse
- Rat
- Ferret
- Tree shrew
- Marmoset
- Monkey
- Man
Dose-response Characteristics of Aflatoxin B₁ Carcinogenesis in Male F344 Rats

<table>
<thead>
<tr>
<th>Dietary Aflatoxin Level (ppb)</th>
<th>Duration of Feeding (weeks)</th>
<th>Liver Carcinoma Incidence</th>
<th>Preneoplastic Liver Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>74–109</td>
<td>0/18</td>
<td>0/18</td>
</tr>
<tr>
<td>2</td>
<td>79–105</td>
<td>2/22</td>
<td>7/22</td>
</tr>
<tr>
<td>5</td>
<td>65–93</td>
<td>1/22</td>
<td>5/22</td>
</tr>
<tr>
<td>15</td>
<td>69–96</td>
<td>4/22</td>
<td>13/22</td>
</tr>
<tr>
<td>50</td>
<td>71–97</td>
<td>20/25</td>
<td>5/25</td>
</tr>
<tr>
<td>100</td>
<td>54–88</td>
<td>28/28</td>
<td>—</td>
</tr>
</tbody>
</table>
Section D

Aflatoxin—A Human Carcinogen
Incidence of Liver Cancer (HCC)

- 589,000 deaths in 1999 (WHO)
- HCC is the third leading cause of cancer death worldwide
- >80% of HCC occurs in the developing world
- >400 million HBV carriers worldwide
### AFLATOXIN INGESTION AND LIVER CANCER INCIDENCE IN HUMANS

<table>
<thead>
<tr>
<th>Population</th>
<th>Dietary Aflatoxin Intake (ng/kg of Body Weight/d)</th>
<th>Cases of Liver Cancer in Adults (&gt; 15 Years Old)</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No./100,000 Population/Yr</td>
<td>Incidence</td>
<td>No./100,000 Population/Yr</td>
</tr>
<tr>
<td><strong>Kenya</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High altitude</td>
<td>3–5</td>
<td>1</td>
<td>3.1</td>
<td>0</td>
</tr>
<tr>
<td>Medium altitude</td>
<td>6–8</td>
<td>13</td>
<td>10.8</td>
<td>6</td>
</tr>
<tr>
<td>Low altitude</td>
<td>10–15</td>
<td>16</td>
<td>12.9</td>
<td>9</td>
</tr>
<tr>
<td><strong>Swaziland</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highveld</td>
<td>5–9</td>
<td>9</td>
<td>7.0</td>
<td>2</td>
</tr>
<tr>
<td>Middleveld</td>
<td>9–14</td>
<td>24</td>
<td>14.8</td>
<td>5</td>
</tr>
<tr>
<td>Lebombo</td>
<td>15–20</td>
<td>4</td>
<td>18.7</td>
<td>0</td>
</tr>
<tr>
<td>Lowveld</td>
<td>43–53</td>
<td>35</td>
<td>26.7</td>
<td>7</td>
</tr>
<tr>
<td><strong>Thailand</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Songkhala</td>
<td>5–8</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ratburi</td>
<td>45–77</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Mozambique</strong></td>
<td>222</td>
<td>—</td>
<td>35.0</td>
<td>—</td>
</tr>
</tbody>
</table>

Data were compiled for one year in Thailand, three years in Mozambique, and four years in Kenya and Swaziland.

*a* Statistics for the total population were cases per 100,000 with an incidence of 6.0. In Ratburi, Thailand, statistics for the total population were two cases per 100,000 population per year with an incidence of 2.0.

*b* Incidence for the total population was 25.4 cases per 100,000 population per year.
Aflatoxin Metabolism

**Phase 1**
- P-450 1A2
- P-450 1A2, 3A4
- AFB₁-8,9-Epoxide

**Phase 2**
- GSH Transferase
- AFB₁-Diol
- Albumin Adducts
- AFB₁-Mercapturic Acid

Other Metabolites
Aflatoxin B$_1$ binds to DNA at the guanine base in liver cells, corrupting the genetic code that regulates cell growth, thereby leading to formation of tumors.
Excretion of DNA Damage Products Into Urine

Base is damaged → Specific DNA Glycosylase removes base

Excision: Exonuclease removes stretch of DNA

Damaged Deoxynucleoside → To Urine

Damaged Base → To Urine
Immunoaffinity-hplc Analysis of Human Urine

Graph showing the analysis of human urine with peaks labeled AFB-N7-Guanine, Aflatoxin P1, and Aflatoxin M1. The graph is measured at 0.0001 AU at 340 nm.
Aflatoxin Biomarkers for Molecular Epidemiology

Aflatoxin B₁ - 8,9 - Oxide

AFB₁-N⁷-Guanine Adduct (Urine)

AFB₁-Lysine Adduct (Serum)
AFB-N\textsuperscript{7}-gua Adduct Excretion in Urine

Association between Total AFB-N\textsuperscript{7}-gua Adduct Excretion in Urine and Total Dietary Aflatoxin Exposure in Guangxi, P.R.C.

$r = 0.80$

$p < 0.01$

Adapted by CTLT from Groopman, et. al., Cancer Research 52: 45–52, 1992.
Aflatoxin-Albumin and Aflatoxin Intake

Relationship between Levels of Aflatoxin-Albumin Adducts in Serum and Mean Daily Aflatoxin Food Intake in the Gambia, West Africa

Adapted by CTLT from Groopman, et. al., Cancer Research 52: 45–52, 1992.
Major Risk Factors for Liver Cancer

- Hepatitis viruses (HBV, HCV)
- Aflatoxins
- Alcohol
- Oral contraceptives
- Iron overload
- Vinyl chloride
- Nutritional imbalances
Nested Case-Control Study of Liver Cancer Risk Factors

- Nested case-control study of liver cancer risk factors in Shanghai
  - 18,244 urine samples collected from healthy males (ages 45–64) during 1986–1989
  - 50 liver cancers developed in this group and cases were age matched to 267 controls
- IAC/HPLC analysis was done on the coded samples for aflatoxin urinary biomarkers
- Hepatitis virus B antigen status was determined
## HBsAg and Urinary Aflatoxin Biomarker

Combined Effect of HBsAg and Presence of Urinary Aflatoxin Biomarker on Risk of Hepatocellular Carcinoma in Shanghai

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Relative Risk</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Aflatoxin biomarker</td>
<td>3.4</td>
<td>(1.1–10.0)</td>
</tr>
<tr>
<td>HBsAg ⊕</td>
<td>7.3</td>
<td>(2.4–24.4)</td>
</tr>
<tr>
<td>Aflatoxin biomarker and HBsAg ⊕</td>
<td><strong>59.4</strong></td>
<td>(16.6–212)</td>
</tr>
</tbody>
</table>
INCIDENCE RATES OF LIVER CANCER IN QIDONG (1972-2001)
LIVER CANCER SURVIVAL RATES IN QIDONG, P.R.C.

One Year Survival is < 10%

TRENDS IN CANCER INCIDENCE RATES
(USA 1992-2000)

ANNUAL PERCENT CHANGE IN CANCER INCIDENCE

-4 -3 -2 -1 0 1 2 3 4

Liver
Thyroid
Melanoma
Kidney
Breast
Lung (female)
Hodgkin's
All CA except lung
Stomach
Leukemia
Lung (males)
Cervix
Prostate

JNCI 95:776, 2003
Dietary Staples in Qidong Consistently Contaminated with Aflatoxins

- Soy Sauce
- Rice
- Peanuts
- Maize
Aflatoxin-Albumin Adducts in Residents of Daxin Township, PRC
P53 Mutational Spectrum in Hepatocellular Carcinoma