Mycotoxins & Food Safety

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VICAM, A Waters Business
Mycotoxins

• **Mykes**: Greek for fungus/mold
• **Toxicum**: Latin for poison/toxin

- **Mycotoxins** are metabolic products of food spoilage fungi that induce toxic responses when consumed by animals or people.

- **Hundreds** of mycotoxins have been identified; They will fall into many different chemical classes, and induce a wide variety of toxic responses.
HISTORY OF MYCOTOXINS

- Ergotism (Saint Anthony’s Fire) in the Middle Ages
  - Vasoconstriction, gangrene
  - Medical uses: Parkinsons, post-labor and migraines
- Alimentary Toxic Aleukia in Russia during World War 2 (T-2)
  - 10% mortality rate
  - Possible chemical warfare agent
- “Turkey X” Syndrome in England in 1960 (aflatoxin)
  - 100,000 turkey poultts (2-20 weeks old)
  - Discovery of aflatoxin
- Kenyan aflatoxin Poisoning (since) 2004
  - Aflatoxin levels up to 8,000 parts per billion (ppb)
  - 500 Acute Illnesses, 200 Fatalities
- Dog food aflatoxin outbreak 2006
  - 76 Companion Animals Lost
<table>
<thead>
<tr>
<th>Mycotoxins</th>
<th>AFLATOXINS B1, B2, G1, G2, M1</th>
<th>DEOXYNIVALENOL</th>
<th>FUMONISINS B1, B2, B3</th>
<th>OCHRATOXIN A</th>
<th>T-2/HT-2</th>
<th>ZEARALENONE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Selected Molds That Produce Toxins</strong></td>
<td><strong>Aspergillus flavus, Aspergillus parasiticus</strong></td>
<td><strong>Fusarium graminearum</strong></td>
<td><strong>Fusarium verticillioides</strong></td>
<td><strong>Aspergillus ochraceus Penicillium verrucosum</strong></td>
<td><strong>Fusarium and other mold species</strong></td>
<td><strong>Fusarium graminearum</strong></td>
</tr>
<tr>
<td><strong>Foods Susceptible to Contamination</strong></td>
<td><strong>Maize, groundnuts, nuts, cottonseed, copra, spices, milk, wheat, oats, barley, and rice</strong></td>
<td><strong>Maize, wheat, barley, malted barley, and oats</strong></td>
<td><strong>Maize and other cereal grains</strong></td>
<td><strong>Maize, wheat, barley, beer, oats, sorghum, dried vine fruits, wine, coffee, and cocoa</strong></td>
<td><strong>Maize, wheat, barley, oats, rice, sorghum, and other cereal grains</strong></td>
<td><strong>Maize, wheat, barley, grain, and sorghum</strong></td>
</tr>
<tr>
<td><strong>Health Effects</strong></td>
<td><strong>Liver cancer and damage</strong></td>
<td><strong>Damage to digestive tract, bone marrow, spleen, reproductive organs</strong></td>
<td><strong>Cancer in rats</strong></td>
<td><strong>Kidney damage and cancer</strong></td>
<td><strong>Skin and oral lesions in livestock and humans</strong></td>
<td><strong>Negatively impacts reproduction, fetal development, and the health of newborns</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Immunosuppression</strong></td>
<td><strong>Decreased milk and egg production</strong></td>
<td><strong>Brain decay in horses</strong></td>
<td><strong>Immunosuppression</strong></td>
<td><strong>Alimentary toxic aleukia in humans</strong></td>
<td><strong>Causes feminization in animals at 1 ppm</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Weight loss, vomiting, and feed refusal</strong></td>
<td><strong>Lung congestion in pigs</strong></td>
<td><strong>Cancer in rats</strong></td>
<td><strong>Immunosuppression</strong></td>
<td><strong>Human Esophageal Cancer</strong></td>
<td><strong>Considered 10x more toxic than DON</strong></td>
</tr>
</tbody>
</table>
Food & Agricultural Products Affected by Mycotoxin Contamination

- Tree Nuts
- Peanuts
- Grain
- Wine
- Coffee
- Flour Milling
- Cereals
- Feed
- Oats
- Ethanol
- Dairy
- Rice
- Botanicals
- Spices
- Snack Foods
- Pet Food
Ecological parameters affecting mycotoxin production

- Moisture
- Spore Load
- Temperature
- Competing Microflora
- Substrate
- CO$_2$/O$_2$
- Severe Weather
- Time
- Mechanical Damage
- Insect Damage
Which Sample Contains Mycotoxins?

300+ ppb

0 ppb

A

B
Colorado Agriculture

Colorado Ag Facts:

- CO Ranks #1 in Millet Production
- 34,200 Farms
- $7.1 Billion in Ag Receipts
- 60% is from Livestock Farming

<table>
<thead>
<tr>
<th>Rank</th>
<th>Commodity</th>
<th>Revenue</th>
<th>% of State</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cattle &amp; Calves</td>
<td>$3.663 billion</td>
<td>48.0%</td>
</tr>
<tr>
<td>2</td>
<td>Corn</td>
<td>$939,395,000</td>
<td>12.3%</td>
</tr>
<tr>
<td>3</td>
<td>Dairy Products</td>
<td>$593,526,000</td>
<td>7.8%</td>
</tr>
<tr>
<td>4</td>
<td>Wheat</td>
<td>$532,398,000</td>
<td>7.0%</td>
</tr>
<tr>
<td>5</td>
<td>Hay</td>
<td>$461,743,000</td>
<td>6.0%</td>
</tr>
</tbody>
</table>

Source: ers.usda.gov
Cannabis Industry - Edibles

- Edibles account for nearly 50% of cannabis sales
- Baked goods account for 10% of cannabis sales
- Potential Sources of Mycotoxin Contamination:
  - Baking Ingredients
  - Extracts/Plant Tissue
Chemistry of Mycotoxins

- 500+ mycotoxins identified
- Low molecular weight
- Secondary metabolites
- Diverse structures & toxicity
- Difficult or impossible to remove once established

Source: https://www.researchgate.net/figure/264635821_fig4_Fig-4-Chemical-structure-of-some-of-the-important-mycotoxins
Biological Activity of Mycotoxins

- Range of activity across animal species, demographics
  - Mutagenic (aflatoxins, patulin)
  - Carcinogenic (aflatoxins, ochratoxin A)
  - Neurotoxic (fumonisins, T-2)
  - Nephrotoxic (ochratoxin, citrinin)
  - Teratogenic (aflatoxin, ochratoxin, citrinin)
  - Immunotoxic (aflatoxins, ochratoxin A)
  - Disrupt Protein Synthesis
  - Gastrointestinal distress
  - Reproductive dysfunction
  - Hepatotoxin (aflatoxins)
  - Equine Leukoencephalomalacia (fumonisins)
  - .....and more
Factors in Mycotoxin Exposure

• Type of mycotoxin
• Amount and duration of exposure
• Age
• Sex
• Health
• Animal species
• Synergistic effects
  - genetics, diet, other toxic insults

** Mycotoxins; Clinical Microbiology Reviews J. W. Bennett1,* and M. Klich2;.. July 2003, vol. 16 no.3
Aflatoxins

Aflatoxin B1

Aflatoxin B2

Aflatoxin M1

Aflatoxin G2

Aflatoxin G1
Aflatoxins

• Produced by *Aspergillus flavus* and *A. parasiticus* molds

• Four key aflatoxins: B1, B2, G1, G2

• Found in many commodities
  • *Grow in soil and decaying vegetation*

• Severe liver damage

• IARC -class 1 human carcinogen
  • *One of the most carcinogenic substances known to man*
Aflatoxins

- Can be ingested, inhaled, and $B_1$ can even permeate the skin\(^1\)
- Stunted growth, delayed development, liver damage, immune suppression\(^2\), and cancer – *form DNA adducts within liver cells.*


Deoxynivalenol (DON)

- Cool, wet weather encourages vomitoxin (DON) production
- Fusarium graminearum, F. culmorum
- Fusarium Head Blight, Scab
- Swine, companion animals are most sensitive
- Immediate gastrointestinal distress; bone marrow, spleen and reproductive organs
- Weight loss, vomiting and feed refusal (most pronounced in swine)
Fumonisins (B1, B2 & B3)

- One of about 40 compounds produced by Fusarium molds (F. moniliforme and F. proliferatum)
- Horse (Equine Leukoencephalomalacia)
- Swine - Porcine Pulmonary Edema
- Neural Tube Defects and Esophageal Cancer Human Risk
Zearalenone

- Produced by Fusarium and Gibberella species
- Corn, wheat, oats, wheat, rice and sorghum
- Estrogenic Toxin – disrupts normal reproductive processes
- $\alpha$-zearalenol and $\beta$-zearalenol are metabolites formed during metabolism in the liver. $\alpha$-zearalenol is 4x more toxic than zearalenone.
- Human Health Risks Under Review
Ochratoxin A

- Produced by Aspergillus & Penicillium molds
- Corn, wheat, coffee, cocoa, dried fruit, wine, beer, etc.
- Long half-life in humans, easily detected in serum
- Kidneys: produces renal tumors, DNA Adducts and chromosomal aberrations in kidney cells
- Contributes to endemic nephrotoxicity and carcinogenicity
- IARC classifies OTA as a Group 2B Possible Human Carcinogen
Other Mycotoxins and Dynamics

• T-2, HT-2 in grains/animal feed

• Patulin in apple juice

• Sterigmatocystin – produced within aflatoxin metabolic pathway

• Masked Mycotoxins – modified by host plant; may not be detectable by traditional methods

• Multiple Mycotoxins – modern laboratories use this approach

• Research continues…
Relative Toxicity of Major Mycotoxins by Animal Species

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Poultry</th>
<th>Swine</th>
<th>Ruminant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxins</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Ochratoxins</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>T2 toxin</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Fumonisin</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

### 2012 Global Mycotoxin Survey of Cereal Grains, Milling Coproducts & Fodder Samples

- **804 Samples Analyzed**
- **89% contained at least one mycotoxin**
- **53% contained more than one mycotoxin**

<table>
<thead>
<tr>
<th>Geographic Region</th>
<th>Mycotoxin presence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Afla</td>
</tr>
<tr>
<td>North America</td>
<td>38</td>
</tr>
<tr>
<td>Central America</td>
<td>100</td>
</tr>
<tr>
<td>South America</td>
<td>17</td>
</tr>
<tr>
<td>Northern Europe</td>
<td>100</td>
</tr>
<tr>
<td>Central Europe</td>
<td>0</td>
</tr>
<tr>
<td>Southern Europe</td>
<td>54</td>
</tr>
<tr>
<td>Middle East</td>
<td>29</td>
</tr>
<tr>
<td>Africa</td>
<td>0</td>
</tr>
<tr>
<td>North Asia</td>
<td>22</td>
</tr>
<tr>
<td>South-East Asia</td>
<td>8</td>
</tr>
<tr>
<td>South Asia</td>
<td>86</td>
</tr>
<tr>
<td>Oceania</td>
<td>9</td>
</tr>
<tr>
<td><strong>Average positives</strong></td>
<td><strong>31</strong></td>
</tr>
</tbody>
</table>

Economic Impact(s) of Mycotoxins

- Yield Losses
- Reduced Crop Value
- Losses in Animal Production
- Human health

A few examples:
- Annual Cost to U.S. from Cereal Crop Losses: $932M USD
- Aflatoxin, fumonisin and deoxynivalenol (DON)
- 1998 Hungarian Wheat contamination: 100M EUR
- Turkey X Disease - UK 1960: 1-5M EUR
Global Implications

• 25% of Global Crops Affected

• Smallholder Farms

• Limits or Prevents Export/Trade

• Local Consumption

• Peanuts, Maize/Corn
Mycotoxin Control in Raw Commodities

• Mechanical Cleaning
  - Removes broken pieces/fines

• Sorting
  - Optical ID of off-color nuts/grains

• Chemical Treatment
  - Ammoniation
  - Enzyme

• Segregation of Contaminated Lots
  - Market contaminated grains and milling byproducts to alternative markets (feed, industrial use)
  - Blending ‘clean’ with contaminated crops is not allowed.

• Biological control
  - Gene editing
  - Innoculation of farm fields with fungal strains that do not produce target mycotoxin, “AflaSafe”
Worldwide regulation of Mycotoxins

Figure 8.1. Counties known to regulate mycotoxins in food and feed (yellow), those where it is unknown whether regulations exist (green), and nations known to have no specific regulations (red) (Food and Agriculture Organization 1997).

(CAST report, 2003)
## FDA Regulatory Limits – Aflatoxin

<table>
<thead>
<tr>
<th>Action Level</th>
<th>Commodity</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 ppb {Aflatoxin M1}</td>
<td>Milk</td>
<td>Humans</td>
</tr>
<tr>
<td>20 ppb</td>
<td>Any Food</td>
<td>Humans</td>
</tr>
<tr>
<td>20 ppb</td>
<td>Feed</td>
<td>All species of animals</td>
</tr>
</tbody>
</table>

**Exceptions:**

<table>
<thead>
<tr>
<th>Action Level</th>
<th>Commodity</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 ppb</td>
<td>Corn</td>
<td>Breeding Cattle, breeding swine, and mature poultry</td>
</tr>
<tr>
<td>200 ppb</td>
<td>Corn</td>
<td>Swine</td>
</tr>
<tr>
<td>300 ppb</td>
<td>Corn</td>
<td>Beef Cattle</td>
</tr>
<tr>
<td>300 ppb</td>
<td>Cottonseed meal used in feed</td>
<td>All species of animals</td>
</tr>
</tbody>
</table>
## FDA Advisory Levels – Other Mycotoxins

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Levels</th>
<th>Commodity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fumonisin (B1 + B2 + B3)</td>
<td>2,000 ppb (2.0 ppm)</td>
<td>Degermed, dry milled corn products (flaking grits, corn grits, corn meal and corn flour with fat content ≤ 2.25 %)</td>
</tr>
<tr>
<td></td>
<td>3,000 ppb (3.0 ppm)</td>
<td>Popcorn</td>
</tr>
<tr>
<td></td>
<td>4,000 (4.0 ppm)</td>
<td>Dry milled corn bran, whole or partially degermed dry milled products (flaking grits, corn grits, corn meal and corn flour w/ fat content ≥ 2.25%)</td>
</tr>
<tr>
<td>Patulin</td>
<td>50 ppb</td>
<td>Apple Juice, apple juice concentrate, apple components in processed foods</td>
</tr>
<tr>
<td>Deoxynivalenol (DON)</td>
<td>1,000 ppb (1.0 ppm)</td>
<td>Finished wheat products</td>
</tr>
<tr>
<td>Zearalenone &amp; Ochratoxin A</td>
<td>No Active Regulatory Limits or Guidance</td>
<td>Corn, wheat, barley, rice, millet, finished feed or pet food</td>
</tr>
</tbody>
</table>
## EU Regulatory Limits – Aflatoxin (ppb)

<table>
<thead>
<tr>
<th>Nuts</th>
<th>Aflatoxin B1</th>
<th>Total Aflatoxins ({B1, B2, G1 &amp; G2})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peanuts</strong></td>
<td>Ready to Eat: 2 ppb&lt;br&gt;Further Processing: 8 ppb</td>
<td>Ready to Eat: 8 ppb&lt;br&gt;Further Processing: 15 ppb</td>
</tr>
<tr>
<td><strong>Almonds, Pistachios</strong></td>
<td>Ready to Eat: 8 ppb&lt;br&gt;Further Processing: 12 ppb</td>
<td>Ready to Eat: 10 ppb&lt;br&gt;Further Processing: 15 ppb</td>
</tr>
<tr>
<td><strong>Hazelnuts, Brazil Nuts</strong></td>
<td>Ready to Eat: 5 ppb&lt;br&gt;Further Processing: 8 ppb</td>
<td>Ready to Eat: 10 ppb&lt;br&gt;Further Processing: 15 ppb</td>
</tr>
<tr>
<td><strong>Other Tree Nuts (Walnuts, etc.)</strong></td>
<td>Ready to Eat: 2 ppb&lt;br&gt;Further Processing: 5 ppb</td>
<td>Ready to Eat: 4 ppb&lt;br&gt;Further Processing: 10 ppb</td>
</tr>
<tr>
<td><strong>Corn</strong></td>
<td>Ready to Eat: 2 ppb&lt;br&gt;Further Processing: 5 ppb</td>
<td>Ready to Eat: 4 ppb&lt;br&gt;Further Processing: 10 ppb</td>
</tr>
<tr>
<td><strong>Rice</strong></td>
<td>Ready to Eat: 2 ppb&lt;br&gt;Further Processing: 5 ppb</td>
<td>Ready to Eat: 4 ppb&lt;br&gt;Further Processing: 10 ppb</td>
</tr>
</tbody>
</table>
### EU Regulatory Limits – Aflatoxins

<table>
<thead>
<tr>
<th>Food Category</th>
<th>Aflatoxin B1</th>
<th>Total Aflatoxins {B1, B2, G1 &amp; G2}</th>
<th>Aflatoxin M1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spices</strong> {Chillies, chilli powder, cayenne &amp; paprika, turmeric, nutmeg, etc}</td>
<td>5 ppb</td>
<td>10 ppb</td>
<td></td>
</tr>
<tr>
<td><strong>Dried Figs</strong></td>
<td>6 ppb</td>
<td>10 ppb</td>
<td></td>
</tr>
<tr>
<td><strong>Raw milk, heat-treated milk and further manufacturing of milk-based products</strong></td>
<td></td>
<td></td>
<td>0.05 ppb</td>
</tr>
<tr>
<td><strong>Processed cereal based foods and infant foods</strong></td>
<td>0.1 ppb</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Infant formula</strong></td>
<td></td>
<td></td>
<td>0.025 ppb</td>
</tr>
<tr>
<td><strong>Dietary foods for special medical purposes (infants)</strong></td>
<td>0.1 ppb</td>
<td></td>
<td>0.025 ppb</td>
</tr>
</tbody>
</table>
## EU Regulatory Limits – Deoxynivalenol (DON)

<table>
<thead>
<tr>
<th>Category</th>
<th>Limit</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unprocessed cereals (other than durum wheat, oats and maize)</td>
<td>1,250 ppb</td>
<td>1.25 ppm</td>
</tr>
<tr>
<td>Unprocessed Durum Wheat &amp; Oats</td>
<td>1,750 ppb</td>
<td>1.75 ppm</td>
</tr>
<tr>
<td>Unprocessed maize, with exception of u.m. intended for wet milling process</td>
<td>1,750 ppb</td>
<td>1.75 ppm</td>
</tr>
<tr>
<td>Cereals intended for direct human consumption (cereal flour, bran and germ)</td>
<td>750 ppb</td>
<td>0.75 ppm</td>
</tr>
<tr>
<td>Pasta (dry)</td>
<td>750 ppb</td>
<td>0.75 ppm</td>
</tr>
<tr>
<td>Bread, pastries, biscuits, cereal snacks, etc.</td>
<td>500 ppb</td>
<td>0.5 ppm</td>
</tr>
<tr>
<td>Processed, cereal based foods and baby foods for infants and children</td>
<td>200 ppb</td>
<td>0.200 ppm</td>
</tr>
<tr>
<td>Product</td>
<td>Ochratoxin A</td>
<td></td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>--------------</td>
<td></td>
</tr>
<tr>
<td>Unprocessed cereals</td>
<td>5 ppb</td>
<td></td>
</tr>
<tr>
<td>Processed cereals, including for direct human consumption</td>
<td>3 ppb</td>
<td></td>
</tr>
<tr>
<td>Dried Vine Fruit (currants, raisins, sultanas)</td>
<td>10 ppb</td>
<td></td>
</tr>
<tr>
<td>Roasted coffee beans, ground roasted coffee</td>
<td>5 ppb</td>
<td></td>
</tr>
<tr>
<td>Soluble coffee</td>
<td>10 ppb</td>
<td></td>
</tr>
<tr>
<td>Wine, grape juice</td>
<td>2 ppb</td>
<td></td>
</tr>
<tr>
<td>Processed cereal products for infants and young children</td>
<td>0.5 ppb</td>
<td></td>
</tr>
<tr>
<td>Spices</td>
<td>15 ppb</td>
<td></td>
</tr>
<tr>
<td>Liquorice root, for herbal infusion</td>
<td>20 ppb</td>
<td></td>
</tr>
<tr>
<td>Liquorice Extract</td>
<td>80 ppb</td>
<td></td>
</tr>
<tr>
<td>Wheat gluten (not for direct consumption)</td>
<td>8 ppb</td>
<td></td>
</tr>
</tbody>
</table>
World AFM1 Thresholds

Target Dairy Exporters!
How Can Exporters Verify Regulatory Limits

Worldwide Mycotoxin Regulations Tool

www.commodityregs.com
Accessing Regulations by Geographic Region

Regions

- USA/Canada
- Latin America
- Europe
- Africa & Middle East
- Asia/Australia/NZ
Visual Guide To Target Commodities
Food Safety Modernization Act (FSMA)

• Potential Hazard Identification

• Preventive Controls

• For Mycotoxin Risks/Threats
  - Which Mycotoxins Are Likely to Occur?
  - Crop Year Re-Evaluation
  - Storage & Processing Conditions
  - Supplier Screening

• On the farm testing?

• Awareness of Potential for Multiple Mycotoxins in One Product
Monitoring for Prevention & Compliance

Harvest
- Screening
- Confirmation

Storage
- Segregation
- QC Before Processing

Finished Products
- Laboratory QC
- Prior to Export or Import

Lower Complexity/Cost

Higher Complexity/Cost
Technologies Commonly Used to Verify Compliance:

Raw Commodities
Buying Points
Peanut Shellers
Grain & Feed

Finished Product Testing
Import/Export
Research
Independent Laboratory

Lower Complexity/Cost

Qualitative Strip tests
Quantitative Strip tests or Immunoaffinity columns with Fluorometer

Higher Complexity/Cost

HPLC or UPLC
LC-MS UPLC-MS-MS
VICAM Technologies

Immunooaffinity Column Sample Concentration

Fluorometer

Qualitative Screening

LC and LC/MS/MS

Quantitative, Eco-Friendly Rapid Detection
Typical Testing Procedure

Ground sample (5g)

Extract with water/AQUA solution or methanol/water (1-2 min)

Filter (1-3 min)

Mix well with diluent

Transfer 100μl to Strip

Develop for 3-5 min

Read result (20s)

(Total testing time is about 10 min)
Thank you!