Mycotoxin levels in Distillers Grains:

Managing your Risk in a Changing Environment

May 2013
Angela Carlson
Mycotoxins

- What are mycotoxins?
- Environmental factors in their production
- Stages for monitoring in the ethanol production process
  - Pre-harvest
  - Inbound grain
  - Co-product
- Test methods
- Looking back at 2012 and ahead at 2013
What Is A Mycotoxin?

- Toxic secondary metabolite produced by fungi
- Over 300 known mycotoxins, but only a few considered significant risks to feed and food safety
- Exposure to mycotoxin contaminated feed/food leads to mycotoxicosis
  - Consumption of
  - Physical contact
  - Inhalation
- Mycotoxicosis
  - Liver and kidney toxicity
  - Central nervous system effects
  - Estrogenic effects
# Mould Species That Produce Mycotoxins

## Table 1 - Moulds and mycotoxins of world-wide importance

<table>
<thead>
<tr>
<th>Mould species</th>
<th>Mycotoxins produced</th>
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</thead>
<tbody>
<tr>
<td><em>Aspergillus parasiticus</em></td>
<td>Aflatoxins B₁, B₂, G₁, G₂</td>
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<tr>
<td><em>Aspergillus flavus</em></td>
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<tr>
<td><em>Fusarium sporotrichioides</em></td>
<td>T-2 toxin</td>
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<td><em>Fusarium graminearum</em></td>
<td>Deoxynivalenol (or nivalenol)</td>
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<td><em>Fusarium moniliforme (F. verticillioides)</em></td>
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<td><em>Penicillium verrucosum</em></td>
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<td><em>Aspergillus ochraceus</em></td>
<td>Ochratoxin A</td>
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Primary contribution to mould growth

- Temperature
- Moisture
- Pests

Mould may have a optimal growth conditions but...

- production of mycotoxins can be increased in stressed conditions
- Visible mould not an indicator or mycotoxin potential (or vice versa)
Temperature

- Growth range from 10 - 40 C (50-104 F)
  - Many fungi can grow as low as 4 C (typical refrigerator temperature) or less

- Survival of mould by spores at extreme conditions
Moisture

- **Equilibrium Moisture content (EMC):** the point the product is not gaining nor losing moisture
  - This is dynamic, dependant on temperature and RH

- **Relative Humidity:** the ratio of the partial pressure of water vapor in the mixture to the saturated vapor pressure of water at a given temperature.
  - Relative humidity is expressed as a percentage.

- **Water Activity** ($a_\omega$) = RH/100, indicator of the moisture available to microbes
  - Typical mould growth is at 0.7 - 0.99 water activity
### Corn Equilibrium Moisture Content Table

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University of Arkansas, Division of Agriculture

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**DDGS EMC**

Sorption isotherm of corn distillers dried grains with solubles (DDGS) and its prediction using chemical composition; Kingsly, Ileliji 2009
Pests

- Insects directly damage seed through feeding

- Indirect damage is from the insects’ respiration
  - Respiration increases temperature in area
  - Storage fungi will closely follow insect damage

- Insects also spread fungal spores
6 Primary Mycotoxin Classes

- Aflatoxins
- Zearalenone
- Trichothecenes
- Fumonisins
- Ochratoxin A
- Ergot alkaloids

<table>
<thead>
<tr>
<th>COMMODITIES</th>
<th>MYCOTOXIN ANALYTICAL PRIORITY GUIDANCE*</th>
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<tbody>
<tr>
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<td>Priority 1</td>
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<tr>
<td>Barley, Oats, Wheat and Rye and their Products</td>
<td>Vomitory</td>
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<tr>
<td>Corn and Corn Products</td>
<td>Aflatoxins</td>
</tr>
<tr>
<td>Corn and Corn Products</td>
<td>Aflatoxins</td>
</tr>
<tr>
<td>Cottonseed, Peanuts, and Sorghum (milo) and their Products</td>
<td>Aflatoxins</td>
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<td>Rice and Rice Products</td>
<td>Zearalenone</td>
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<td>Soybean and Soybean Products</td>
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<td>Horse and Rabbit Feed</td>
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<td>Swine Feed</td>
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<td>Cattle and Poultry Feed</td>
<td>Aflatoxins</td>
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<tr>
<td>Dog and Cat Food</td>
<td>Vomitory</td>
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</table>

*The mycotoxin(s) mentioned in the Priority 1 column for a particular commodity should be analyzed for in almost every sample. The mycotoxin(s) mentioned in the Priority 2 column for a particular commodity should be analyzed for in most samples. The mycotoxin(s) mentioned in the Priority 3 column for a particular commodity should be analyzed for occasionally. The mycotoxin(s) mentioned in the Priority 4 column for a particular commodity should only be analyzed for on rare occasions.
Pre-Harvest

- Communicate with your local suppliers
- Offer testing to local suppliers prior to harvest with onsite test kits
In-Bound Grain

- Set your acceptance limits based on grain supply and annual fluctuations.
  - What market will you be targeting for sale of your product
  - Remember that In-bound → Final co-product = 3-4X concentration

- Probe trucks according to GIPSA standards (www.gipsa.usda.gov)

- Course grind as large of subplot as possible from probed sample
  - Further divide after course grind to 500g subsample and grind further for onsite testing (20mesh particle size)
Uncertainty in sampling and testing
<table>
<thead>
<tr>
<th>Sample (kg)</th>
<th>Sub-sample (gr)</th>
<th>aflatoxin (ppb)</th>
<th>95% confidence interval aflatoxin/corn</th>
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<td>20</td>
<td>50</td>
<td>20</td>
<td>2</td>
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</table>
In-Bound Grain

- New Crop
  - Minimum Daily
  - High incidence - every truckload

- High incidence – continue to check every truckload or other designated level
  - If on-site testing not available send weekly composite

- Low incidence – move to weekly composite after multiple loads tested per supplier
Final Co-Product

- Collect at dryer, allow to cool, test using onsite kits
  - Aflatoxin and Vomitoxin
  - Zearalenone, Fumonisin

- High Incidence - sample every 2hrs, test daily composite
  - No onsite test kit – send weekly composite to lab
  - Composite monthly for HPLC testing methodology for full scan of mycotoxins

- Low incidence – test weekly composite
  - Composite monthly for HPLC testing methodology for full scan of mycotoxins
Testing Methods

- Lateral Flow Strips (LFS)
  - Inexpensive screening method
  - Works for most ranges of mycotoxins
  - Can be done onsite without special equipment

- GIPSA approved kits are listed on their website (updated 4/26/13)

Onsite Testing kits

- Coarsely grind as large of initial sample as feasible
- Understand the range of the test kit
- Follow the directions!!
  - Ask for training
Testing Methods

- **ELISA testing**
  - Inexpensive screening method
  - Robust, works for most ranges of mycotoxins
  - 1 day turn around time

- Place extract antibody coated ELISA plate with corresponding enzyme conjugate
  - Mycotoxins in sample compete with enzymes conjugates for antibody binding sites

- Add color solution which reacts with bound enzyme conjugate creating blue color
  - *Dark Color= less mycotoxins*
Testing Methods

- High Performance Liquid Chromatography (HPLC) with Ultraviolet (UV), Fluorescent (FLD), detection Mass Spectrometry
  - precise and accurate
  - Can achieve wider range of toxins
  - 4-10 days turn around time

- Thin Layer Chromatography (TLC), Gas Chromatography (GC)
2012

- Aflatoxin
  - Drought leading to high levels in regional areas
    - SD, NE, IN, IL, MO, KS, IA
  - Trade impacts:
    - US 2012 38.9% global supply vs 52% in 2011
    - Former Soviet Union 2012 16.8% vs 8% in 2011
  - Domestically decreases in cattle, pork production

- Vomitoxin
  - Still present in regional areas

2013

- Weather Outlook
Questions?

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www.sgs.com/us-feed