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   Agronomic & Regional Services
   Bureau of Plant Industry
   ebubb@pa.gov
   717-772-5215

   Dr. Dave Griswold; Assistant Director
   Bureau of Animal Health and Diagnostic Services
   dgriswold@pa.gov
   717-772-2852
Procedures for the Management of Mycotoxin Contaminated Grain

I. Introduction

Deoxynivalenol (DON), commonly referred to as vomitoxin, is one of several mycotoxins produced by certain Fusarium species that frequently infect corn, wheat, oats, barley, rice, and other grains in the field or during storage. It is a toxic byproduct of Fusarium head scab in grains and feeds.

In general, ingestion of DON by animals can result in decreased feed consumption, vomiting, and reduced growth. F. graminearum can also produce the mycotoxin zearalenone and T-2 toxin, which may also cause health problems. Therefore, suspect grain should be tested for these mycotoxins by chemical analysis before being fed to animals.

Potential human exposure to DON may occur from ingestion of products made from contaminated grains. There also is a risk of occupational exposure by inhalation of pathogenic species of filamentous fungi and mycotoxins among farmers engaged in grain threshing and other individuals who have to handle the contaminated grain.

Human exposures to DON-contaminated grains have been reported to cause acute temporary nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and fever. In human food, DON levels at 1 ppm and below should not pose a threat to public health among the general population.

II. Background

Extremely wet growing conditions in late summer and fall of 2011 were favorable for disease development throughout Pennsylvania. Gibberella Ear Rot is a disease of corn caused by the fungus Fusarium graminearum and may produce several mycotoxins, including, Deoxynivalenol (DON) in corn. For the disease to occur, three factors need to be in place: the pathogen Fusarium which may be found in grains even under normal weather conditions, susceptible hybrids, and favorable weather conditions.

III. Regulatory Status

Infested grains testing 10 ppm or less for DON may be used in animal feed according to the Food & Drug Administration’s (FDA) guidance level based on the species, portion of the diet and level of DON. FDA does not recommend the use of grain with levels of DON that exceed 10 ppm in animal feed. (See page 5)

Affected crop that cannot be used as feed or food would be considered an agricultural waste (residual waste) generated through normal farming operations.

Toxins can increase in storage if conditions are favorable for the fungus to develop. The fungus prefers warm and moist conditions, which can occur in grain that is not dried well. These will cause pockets of mold development and potentially mycotoxin formation. Cool temperatures, air circulation and low moisture conditions
will minimize fungal growth and toxin production. These will not reduce the levels of toxin formed in the grain prior to storage.

Beware of products intended to be used for or promoted to bind mycotoxins and other harmful toxins. They must be the subject of an approved Food Additive Petition (FAP) from the FDA or Generally Recognized as Safe (GRAS) for use in food or feed if they are sold or intended to be used for this purpose.

Activated charcoal is not an approved food/feed additive and is not GRAS. Food or feed containing activated charcoal is considered adulterated under the Federal Food, Drug and Cosmetic Act.

Sodium aluminosilicate and hydrated sodium calcium aluminosilicate are GRAS when used as anticaking agents in animal feed at a level not exceeding 2 percent in accordance with good manufacturing or feeding practices. However, FDA's Center for Veterinary Medicine (CVM) has consistently maintained that the use of sodium aluminosilicate or hydrated sodium calcium aluminosilicate as binders for mycotoxins is not GRAS and approved FAPs must be obtained before these products may be used or claims may be made regarding their utility as mycotoxin binders. Products which are not the subject of an approved FAP may be subject to regulatory action.

CVM is concerned that all mycotoxins are not uniformly bound by anticaking agents and that similar anticaking agents do not bind mycotoxins to the same degree.

Furthermore, any mycotoxins which are bound might not remain bound when the feed is consumed and exposed to the acid environment of the gut. If this were to occur, the animal could be exposed to unknown and potentially unsafe levels of mycotoxin which could result in mycotoxin residues in meat, milk, or eggs.

Crop producers that are covered under crop insurance policies subsidized by Federal Crop Insurance must meet standards established by USDA’s Risk Management Agency (RMA). For insured crop that has Zero-Market Value Production (DON levels greater than 10 ppm), RMA loss procedures require destruction of the crop.
FDA has established the following guidance levels for DON in animal feed and food.

<table>
<thead>
<tr>
<th>Class of Animal</th>
<th>Feed Ingredients &amp; Portion of Diet</th>
<th>DON Levels in Grains &amp; Grain By-products and (Finished Feed)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ruminating beef and feedlot cattle older than 4 months</strong></td>
<td>Grain and grain by-products not to exceed 50% of the diet</td>
<td><strong>10ppm (10ppm in beef) (5ppm in dairy)</strong></td>
</tr>
<tr>
<td><strong>Chicken</strong></td>
<td>Grain and grain by-products not to exceed 50% of the diet</td>
<td>10ppm (5ppm)</td>
</tr>
<tr>
<td><strong>Swine</strong></td>
<td>Grain and grain by-products not to exceed 20% of the diet</td>
<td>5ppm(1ppm)</td>
</tr>
<tr>
<td>All other animals</td>
<td>Grain and grain by-products not to exceed 40% of the diet</td>
<td>5ppm (2ppm)</td>
</tr>
<tr>
<td><strong>Humans</strong></td>
<td>Finished wheat products</td>
<td>1ppm</td>
</tr>
</tbody>
</table>

Infested grains testing 10 ppm or less for DON may be used in animal feed according to the advisory based on the species, portion of the diet and level of DON. FDA does not recommend the use of grain with levels of DON that exceed 10 ppm in animal feed.

**FDA has updated the advisory levels of DON in distiller’s grains, brewer’s grains, gluten and gluten meal. Ruminating beef cattle and dairy cattle over 4 months of age- 30ppm not to exceed 50% of the diet and not to exceed 10ppm in beef cattle finished feed and not to exceed 5ppm in dairy cattle finished feed.**

Mycotoxins in corn are concentrated about three fold in distillers dry grains, i.e. during ethanol production, removing the starch from corn (the content of which can average about 60 percent) concentrates levels of these mycotoxins. Mycotoxins are likely to concentrate in a number of other grain by-products.
IV. Testing

The Pennsylvania Department of Agriculture will be conducting a sampling survey in collaboration with the FDA Philadelphia District Office of Pennsylvania feed manufacturers statewide to determine the DON levels in finished animal feed. The laboratory resources at the Department are primarily for regulatory enforcement and consumer complaints.

Other grain and grain by-products (distillers dried grains, wheat, wheat middlings, and other corn by-products, etc) that may be used to produce a finished animal feed may also contribute to the level of DON in the final product. It is important to monitor all ingredients used to formulate an animal feed. Mycotoxin test kits and service laboratories should be used for routine private testing purposes.

The Department is requesting your assistance by alerting the Department of any elevated levels of DON with incoming ingredients, as well as any reported adverse animal effects. Your assistance will help us monitor any emerging feed safety issue by better indicating the areas that may be at risk.

Please contact Erin Bubb, Chief, Division of Agronomic and Regional Services at 717-772-5215 or ebubb@pa.gov with any information or questions.

A. Sampling Procedure

Mycotoxin-contaminated grain is not distributed uniformly throughout the bin. In most cases the toxin level varies from one kernel to another. Moreover, it is possible for highly contaminated kernels to be next to kernels in which the toxin cannot be detected. A single highly contaminated kernel within a 1-pound sample can be a source of significant contamination. Because of this uneven distribution, it is very difficult to obtain representative samples to analyze. In addition, reliability of the methods for mycotoxin analysis is greatly influenced by the sampling procedure and sample size.

To achieve a more accurate estimate of the degree of mycotoxin contamination, it is critical that the collected grain sample actually represent the entire bin or truckload of grain. Follow these tips on how to collect a random sample.

1. Obtain a minimum of 12 samples from different locations in the bin or truck, and bulk all into one. The total weight of the composite sample should be approximately 10 lbs.

2. Hand-mix the sample. Subdivide the 10-pound sample into two 5-pound sub samples. The sample size required varies from one laboratory to another. Studies have shown that the chances of detecting parts per million (ppm) or parts per billion (ppb) levels of toxins in samples are greatly reduced with a smaller sample size. Choose one 5-pound sample to be sent to the laboratory for analysis.

3. Make sure that the moisture content of the sample does not exceed 13 percent. Samples high in moisture cannot be processed immediately. This results in delays and may allow mycotoxin levels to increase during shipment.
4. Pack the sample in a paper bag (not plastic) and place it in a container with a note stating the type of services you wish the laboratory to perform.

5. Information pertaining to the type of analysis, types of mycotoxins that can be analyzed, cost of analysis, size of the sample and time required to report results are provided in the Table below.

6. In a few special cases there are some advantages of collecting a biased sample. For instance, if there is evidence of moisture damage due to a leak in the bin, it may be more appropriate to collect a sample from the moistened spot and another from the dry area.

Contact the laboratory for shipping instructions and other additional information prior to sending samples. Some laboratories offer a 24-hour emergency service at higher cost, discounts on volume analysis, and a wide variety of other services.

Laboratories on this list are those that have given us their addresses and/or answered our questionnaire. Inclusion on this list does not imply a recommendation of their service. Exclusion of a laboratory from the list does not imply that it is not to be used. (Ohio State Extension Article – Where to Send Grain Samples For Mycotoxin Analysis, PE Lipps & Dennis Mills - reference)

<table>
<thead>
<tr>
<th>Laboratory Address Phone and Fax</th>
<th>Types of Mycotoxins Analyzed</th>
<th>Methods Used</th>
<th>Required Sample Size</th>
<th>Time to Report Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>East Indiana Grain Inspection  (designated by USDA) 7020 N Walnut St Muncie, IN 47303 765-289-1206 voice &amp; fax</td>
<td>Vomitoxin Aflatoxin Fumonisnin</td>
<td>ELISA Quantitive</td>
<td>3-5 lb</td>
<td>1 day</td>
</tr>
<tr>
<td>Holmes Laboratory  Box 204 3559 US Rt. 62 Winesburg, OH 44654 800-344-1101 330-893-3094 fax <a href="http://www.holmeslab.com">www.holmeslab.com</a></td>
<td>Alfatoxin Vomitoxin Zearalenone Fumonisnin Ochratoxin T-2</td>
<td>ELISA</td>
<td>1 qt</td>
<td>2-4 days</td>
</tr>
<tr>
<td>Laboratory Address</td>
<td>Types of Mycotoxins Analyzed</td>
<td>Methods Used</td>
<td>Required Sample Size</td>
<td>Time to Report Results</td>
</tr>
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</tr>
<tr>
<td><strong>Illinois Dept. of Agriculture Animal Disease Laboratory</strong>&lt;br&gt;9732 Shattuc Rd.&lt;br&gt;Centralia, IL 62804&lt;br&gt;618-532-6701&lt;br&gt;618-532-1195 fax&lt;br&gt;Steve Kasten&lt;br&gt;<a href="mailto:skasten@agr.state.il.us">skasten@agr.state.il.us</a></td>
<td>Alfatoxin&lt;br&gt;Zearalenone&lt;br&gt;Deoxynivalenol&lt;br&gt;T-2&lt;br&gt;Fumonisin B1</td>
<td>TLC, HPLC&lt;br&gt;TLC&lt;br&gt;TLC, HPLC&lt;br&gt;TLC</td>
<td>3-5 lb</td>
<td>2 days</td>
</tr>
<tr>
<td><strong>Iowa State University Veterinary Diagnostic Lab</strong>&lt;br&gt;Ames, IA 50011&lt;br&gt;515-294-1950&lt;br&gt;515-294-3564 fax&lt;br&gt;Thomas L. Carson DVM, PhD&lt;br/www.vdpam.iastate.edu</td>
<td>Alfatoxin&lt;br&gt;Zearalenone&lt;br&gt;Deoxynivalenol&lt;br&gt;T-2&lt;br&gt;Ochratoxin&lt;br&gt;Panel includes all the above</td>
<td>TLC, HPLC&lt;br&gt;TLC, HPLC&lt;br&gt;TLC, GC&lt;br&gt;TLC, GC&lt;br&gt;TLC, HPLC</td>
<td>0.5 lb</td>
<td>3 days</td>
</tr>
<tr>
<td><strong>Minnesota Valley Testing Laboratories, Inc</strong>&lt;br&gt;P.O. Box 249&lt;br&gt;1126 North Front St.&lt;br&gt;New Ulm, MN 56073-0249&lt;br&gt;800-782-3557&lt;br&gt;507-359-2890 fax&lt;br&gt;Kathy Jones</td>
<td>Alfatoxin</td>
<td>ELISA&lt;br&gt;HPLC</td>
<td>0.25 lb</td>
<td>7-10 days</td>
</tr>
<tr>
<td><strong>Trilogy Analytical Laboratory</strong>&lt;br&gt;111 West 4th St.&lt;br&gt;Washington, MO 63090&lt;br&gt;636-239-1521&lt;br&gt;636-239-1531 fax&lt;br/www.trilogylab.com</td>
<td>Alfatoxin&lt;br&gt;Deoxynivalenol&lt;br&gt;Ochratoxin&lt;br&gt;T-2&lt;br&gt;Zearalenone&lt;br&gt;Fumonisin</td>
<td>HPLC and TLC</td>
<td>1 lb</td>
<td>1-5 days</td>
</tr>
<tr>
<td><strong>University of Missouri Veterinary Medical Diagnostic Laboratory</strong>&lt;br&gt;P.O. Box 6023&lt;br&gt;Columbia, MO 65205&lt;br&gt;573-882-6811&lt;br&gt;573-882-1411 fax&lt;br&gt;George Rottinghaus</td>
<td>Alfatoxin&lt;br&gt;Deoxynivalenol&lt;br&gt;Zearalenone&lt;br&gt;T-2&lt;br&gt;Vomitoxin&lt;br&gt;Ochratoxin&lt;br&gt;Fumonisin</td>
<td>TLC&lt;br&gt;TLC&lt;br&gt;TLC&lt;br&gt;TLC&lt;br&gt;TLC&lt;br&gt;TLC&lt;br&gt;TLC</td>
<td>0.5 lb</td>
<td>2-3 days</td>
</tr>
<tr>
<td>Laboratory Address</td>
<td>Types of Mycotoxins Analyzed</td>
<td>Methods Used</td>
<td>Required Sample Size</td>
<td>Time to Report Results</td>
</tr>
<tr>
<td>--------------------</td>
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<td>------------------------</td>
</tr>
</tbody>
</table>
| Veterinary Diagnostic Center  
University of Nebraska-Lincoln  
Fair St. and East Campus Loop  
Lincoln, NE 68583  
402-472-1434  
402-472-3094 fax  
Michael P. Carlson  
www.veterinarycenter.unl.edu | Mycotoxin screen includes: Alfatoxin Zearalenone Deoxynivalenol T-2 Ochratoxin A | TLC | 5 lb | 3-5 days |
| Waters Agricultural Laboratories  
P.O. Box 382  
257 Newton Highway  
Camilla, GA 31730  
229-336-7216  
229-336-7967 fax  
Keith Dominey  
www.watersag.com | Alfatoxin T-2 Vomitoxin Zearalenone | Immunoassay | 1 lb | 24 - 48 hours |
| Woodsen-Tenent Laboratories Inc  
313 E Helena St.  
P.O. Box 164  
Dayton, OH 45404  
937-222-4179  
937-222-7401 fax  
Dr. Bill Hirt  
www.woodsnetenent.com | Alfatoxin T-2 Zearalenone Deoxynivalenol Fumonisn Ochratoxin | TLC, ELISA | 0.1 lb | 5-7 days |
| Agri Analysis, Inc.  
280 Newport Road  
PO Box 483  
Leola, PA 17540  
717-656-9326  
info@agrianalysis.com  
www.agrianalysis.com | Alfatoxin Vomitoxin Zearalenone Fumonisn T-2 Panel includes all the above | TLC, HPLC | 50 gram | 5-6 days |
| New Jersey Feed Laboratory  
1686 5th Street  
Trenton, NJ 08638-3037  
609-882-6800  
www.njfl.com | Alfatoxin Deoxynivalenol Ochratoxin T-2 Zearalenone Fumonisn Panel includes all the above | ELISA | 50 gram minimum | 2-3 days |
| Skyview Laboratories  
PO Box 273  
Jennerstown, PA 15547  
814-629-5441  
www.skyviewlab.com | Alfatoxin Deoxynivalenol Ochratoxin T-2 Zearalenone Fumonisn | TLC | 1 lb | 3 days |
B. Mycotoxin Test Kit Suppliers

MEDTOX, Inc.
1238 Anthony Road
Burlington, NC 27215
Telephone: 800-334-1116
Fax: 336-229-4471
www.medtox.com/agri.html

NEOGEN Corp.
620 Lesher Place
Lansing, MI 48912
Telephone: 800-234-5333, 517-372-9200
Fax: 517-372-2006
e-mail: neogen-info@neogen.com
www.neogen.com

ROMER LABS Inc.
1301 Stylemaster Drive
Union, MO 63084
Telephone: 800-769-1380, 314-583-8600
Fax: 314-583-6553
e-mail: marketing@romerlabs.com
www.romerlabs.com

VICAM LP
313 Pleasant Street
Watertown, MA 02172
Telephone: 800-338-4381, 617-926-7045
Fax: 617-923-8055
e-mail: vicam@vicam.com

Charm Sciences, Inc.
659 Andover Street
Lawrence, MA 01843-1032
Telephone: 978-687-9200
Fax: 978-687-9216
e-mail: Info@charm.com

(Kansas State – Mycotoxins in Feed Grains and Ingredients)

V. Handling Recommendations

- Use of respiratory protection N95 dust masks by anyone that may come in direct contact with the waste material (contaminated grain, flour, etc.).

- Waste material (contaminated grain, flour, etc.) must be packaged in a bladder bag, supersac or equivalent packaging technology which can completely enclose the material for acceptance at intended disposal facility/location.
Transport vehicles transporting the enclosed waste material should also be covered (tarped) to prevent air dispersal of waste material (contaminated grain, flour, etc.) and/or mycotoxins.

For landfill disposal, the waste material (contaminated grain, flour, etc.) should be covered immediately after deposition at the landfill working face.

For disposal at a waste-to-energy facility, the waste material (contaminated grain, flour, etc.) should be fed directly into the burning chamber and not placed into the waste pit.

VI. Disposal Options

1. For contaminated and useable grain/processed grain products/feed
   - Growers, grain distributors and mills can recondition grains that exceed the advisory levels for DON by cleaning and removing dockage. The efficiency of cleaning will depend on the proportion of the total DON present in the shriveled, lighter weight, severely infected kernels with those of normal weight and size. (*Mycotoxins in Feed Grains and Ingredients* by Tim Herrman, Kansas State)
   - Fines, middlings, screenings and other by-products from the cleaning and milling processes must be monitored closely because toxin levels may concentrate in these fractions.
   - Grains that exceed the advisory level may be used as seed. The Fusarium fungi that cause head scab disease can reduce germination in seed. The fungi can be controlled on seed by cleaning and using seed treatment fungicides. The Department’s Seed Laboratory will test seed grains for germination and purity for a fee.

     For more information about seed testing:
     Johnny Zook, Seed Program Supervisor
     PDA Seed Lab
     2301 Cameron St.
     Harrisburg PA 17110
     717-787-4894  jzook@pa.gov

   - Physical mixing of contaminated (greater than 10 ppm DON) grain with uncontaminated grain for animal feed is not an acceptable practice. Blending of “clean” grain with adulterated grain is generally not permitted and the final product resulting from blending is unlawful, regardless of the level of the contaminant.

2. On-farm disposal:
   At the present time, there are no recommended on-farm disposal options and the land application of mycotoxin contaminated grain is not a recommended agronomic practice even if the grain is incorporated into the soil.
3. Biofuels production:
The contaminated grain may be used in biofuels production provided it meets the quality specifications of the production facility and the facility is permitted under General Permit WMGR109 [http://www.depweb.state.pa.us/landrecwaste/lib/landrecwaste/residual_waste/gp/wmgr109.pdf](http://www.depweb.state.pa.us/landrecwaste/lib/landrecwaste/residual_waste/gp/wmgr109.pdf), other DEP permit authorization, or under a co-product determination. In addition, the contamination levels of the distiller’s grain must be evaluated before it is allowed to be used as animal feed.

4. Boiler fuel:
The waste material (contaminated grain, etc.) may be used as fuel (co-product for energy recovery under Chapter 287.1 of the residual waste regulations) [http://www.pacode.com/secure/data/025/chapter287/s287.1.html](http://www.pacode.com/secure/data/025/chapter287/s287.1.html) in an industrial boiler provided permit approval is granted by the DEP’s Air Quality Program and the waste material has at least 5,000 BTUs/lb.

5. Waste-to-energy facility:
The waste material (contaminated grain, etc.) may be processed at a waste-to-energy facility as a residual waste (agricultural waste) if accomplished in accordance with these procedures, the waste-to-energy facility operator has received "Form U - Request to Process or Dispose of Residual Waste” approval, and the waste-to-energy facility operator has determined the waste material will not adversely effect the facility operations and has received Air Quality Program approval. No chemical analysis is required in accordance with the Chemical Analysis Waiver provisions under Section D4. of the Form U provided the chemical analysis waiver does not conflict with the waste-to-energy facility’s approved waste acceptance plan, see [http://www.elibrary.dep.state.pa.us/dsweb/View/Collection-9584](http://www.elibrary.dep.state.pa.us/dsweb/View/Collection-9584). In addition, the waste material should be fed directly into the burning chamber and not placed into the waste pit.

6. Landfill Disposal:
It has been determined that the waste material (contaminated grain, etc.) may be disposed in Pennsylvania landfills as a residual waste (agricultural waste) if accomplished in accordance with these procedures and if the landfill operator has received “Form U - Request to Process or Dispose of Residual Waste” approval. No chemical analysis is required in accordance with the Chemical Analysis Waiver provisions under Section D4. of the Form U provided the chemical analysis waiver does not conflict with the landfill’s approved waste acceptance plan, see [http://www.elibrary.dep.state.pa.us/dsweb/View/Collection-9584](http://www.elibrary.dep.state.pa.us/dsweb/View/Collection-9584). However, the landfill operator must still determine that the waste material will not adversely effect the landfill operations, liner and/or leachate collection and treatment capabilities.

VII. DEP Regional Offices

- The following DEP regional offices may be contacted for information on permitting, Form U approval requests, and other disposal options listed above:

I. Bucks, Chester, Delaware, Montgomery, Philadelphia.

Northeast Regional Office
2 Public Square
Wilkes-Barre, PA 18711-0790
Phone: (570) 826 – 2516

III. Adams, Bedford, Berks, Blair, Cumberland, Dauphin, Franklin, Fulton, Huntingdon, Juniata, Lancaster, Lebanon, Mifflin, Perry, York.

Southcentral Regional Office
909 Elmerton Avenue
Harrisburg, PA 17110-8200
Phone: (717) 705 – 4706

IV. Bradford, Cameron, Centre, Clearfield, Clinton, Columbia, Lycoming, Montour, Northumberland, Potter, Snyder, Sullivan, Tioga, Union.

Northcentral Regional Office
208 West 3rd Street – Suite 101
Williamsport, PA 17701
Phone: (570) 327 – 3653


Southwest Regional Office
400 Waterfront Drive
Pittsburgh, PA 15222-4745
Phone: (412) 442 – 4000

VI. Butler, Clarion, Crawford, Elk, Erie, Forest, Jefferson, Lawrence, McKean, Mercer, Venango, Warren.

Northwest Regional Office
230 Chestnut Street
Meadville, PA 16335-3481
Phone: 814-332 – 6848

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