Sampling and Testing For Aflatoxin in Corn

Aflatoxins are a group of chemicals produced by certain mold fungi. These fungi, *Aspergillus flavus* and *Aspergillus parasiticus*, can be recognized by yellow-green or gray-green, respectively, on corn kernels, either in the field or in storage Although aflatoxins are not automatically produced whenever grain becomes moldy, the risk of aflatoxin contamination is greater in damaged, moldy corn than in corn with little mold. Aflatoxins are harmful and can be fatal to livestock. Aflatoxins are carcinogenic (cancer-causing) to animals and humans. In the Midwest, aflatoxin levels are highest during hot, dry summers. The prime conditions for the fungus to produce toxin are warm August nights in a period of drought. Certain portions of the Corn Belt experienced these conditions in 2005, along with continued warm weather into October.

In high-risk years, aflatoxin screening may be done at the elevator or where the corn is marketed. It may also be necessary to estimate aflatoxin levels for insurance or future animal feeding reasons. Rapid, on-site tests, such as test strips or the black light, can determine the **possible** presence of aflatoxin. However, these tests do not provide definitive or quantitative results. The toxins are produced inside the corn kernels and their presence can be determined only by analytical tests. Because aflatoxin levels vary greatly from kernel to kernel, sampling is the most critical step in determining actual levels of aflatoxin. An excellent and comprehensive discussion of aflatoxin and aflatoxin testing has been published by the USDA, Grain Inspection Packers and Stockyards Administration (USDA-GIPSA) at http://www.gipsa.usda.gov/pubs/mycobook.pdf. The publication PM-1800, Aflatoxins in Corn, http://www.extension.iastate.edu/Publications/PM1800.pdf summarizes experiences with aflatoxin from other problem years (1983, 1988, 2002).

How to sample corn for aflatoxin testing

Because aflatoxin does not occur uniformly through a field or lot of grain, the best approach is to make a composite sample consisting of subsamples from every part of a load, bin, or other unit of corn. Sampling recommendations vary by the type of lot to be sampled. In general, the smaller the sample size, the higher the variability. The USDA handbook cited above reports sampling errors of about 40% of the value for 10 lb samples, increasing to about 100-125% of the value for 1 lb samples.

Grain in Bins

The recommended procedure is to sample periodically from each load as the grain is being placed in the bin, combining these samples to obtain a composite corn sample of 10 lb or more. As a bin is being filled, take a 1 lb cut with a pan or coffee can from each load. Sample at about the middle of unloading. Combine the load samples, and take the entire composite sample to the laboratory that will perform the test. The lab will have the correct equipment for reducing sample size before grinding. Do not climb on the wagon or into the combine grain tank to collect samples; this is not safe. Sampling at bin filling is the best way to collect information for future feeding or marketing decisions.

An alternative is to sample with a power probe through a storage unit (five perimeter samples and one center sample for each 6 feet of bin height). Again take the entire composite sample to the laboratory. **The objective in either case is to get about 1 lb of sample per 500 bushels.** This is essentially the same sampling frequency used by USDA GIPSA for Official aflatoxin measurements of flowing grain as it is being loaded on ships for export. Most elevators loading railcars, barges and ships are equipped with diverter samplers, which take a 1 lb cut approximately every 500 bushels of grain.

Grain in Trucks or Wagons

Grain in trucks or wagons can be probed to collect at least 5 lb of sample per load. Take 5-7 probes per load (depending on the amount of grain per probe). The GIPSA sampling pattern for trucks is shown below. Larger containers, such as railcars or barges, have expanded probe sampling patterns; see the GIPSA mycotoxin handbook, *http://151.121.3.117/pubs/mycobook.pdf* for probe sampling patterns to use in various situations.

Recognize that even a 5 lb sample is a loss of accuracy compared to a 10 lb sample. Smaller samples are a compromise for practicality reasons. However, a single truckload is far less grain than is in an entire bin; the error of analysis affects less grain and can be counterbalanced by the testing of multiple loads.

Since all sampling and testing procedures are subject to error, it is wise to identify for each load received (and accepted) the storage bin in which it was first placed. Maintain a composite sample for each bin by collecting a divided 1-lb (approximate) subsamples of each load in large container (eg 5 gallon bucket). When a bin is full, mix its composite and submit for laboratory analysis. Large bins may have several composites, representing layers. This procedure will provide a backup to screening at delivery and a more accurate inventory picture of the corn as it is in storage for future sale. Retention of this composite is the same as collecting the composite when filling farm bins.

Grain in the Field

In the field, sample individual fields or parts of fields separately. Fields that vary in cropping history, tillage practices, planting date, soil type, or hybrid can differ greatly in aflatoxin vulnerability. Sample a minimum of 10 to 30 locations within each field. To reach the same sampling frequency as testing grain in trucks, collect one sample (5-10 lb) for about every 5 acres. This would be about 2 ears per acre sampled. Use a GPS-based grid if the field is mapped.

Immediately dry ear samples to 12–14 percent moisture to prevent aflatoxin development during transit or storage. High-moisture samples should be frozen and delivered to the laboratory in the frozen state. Dried samples maintain their quality best if shipped in cloth bags (as opposed to plastic bags). Do not use paper bags; there is too much chance of breakage. If the laboratory cannot shell the ears themselves, do not have them shelled more than a day in advance of delivering to the lab.

The USDA Risk Management Agency offers strip sampling as an alternative to random collection of ears. One strip is to be left per 40 acres with a minimum of three strips for fields under 10 acres and a minimum of four strips for the first 40 acres of larger fields. This will give approximately 1 lb of sample per 600 bushels of corn, at 150 bu/acre yields, more in smaller fields. The best method to sample strips would be to sample the grain from each strip after it is harvested, rather than attempting to identify a representative location within the strip to take an ear sample.

Field sampling is inherently much more variable than sampling of flowing grain because the geographic variation is added to the variation within a load or flowing stream. A field sample is best used to identify presence or absence of aflatoxin, as opposed to measuring specific levels. The most accurate way to estimate aflatoxin levels of a field is to sample every load of the grain as it is being delivered to an elevator or placed in a bin.

How to test corn for aflatoxin

Currently, two types of screening tests are available: blacklight tests and commercial ELISA-based test kits. The blacklight (also called ultraviolet light) test is a visual inspection for the presence of a greenish gold fluorescence under light at a wavelength of 365 nm (nanometers). The greenish gold fluorescence looks like a firefly glow. It is necessary to use a color standard (www.seedburo.com) to interpret black-light results because there are several types of fluorescence in grain. More than four glowing particles per 5-pound sample (before grinding) indicate a likelihood of a +20 ppb (parts per billion) level of aflatoxins. However, remember that this test is an initial screening for the presence of aflatoxin and the results should be verified by laboratory analysis. If there are less than four glowing particles per 5 lb sample, this does not guarantee that the sample is free of aflatoxins. In previous aflatoxin outbreaks, very few samples with over 20ppb of aflatoxin failed to have at least one glowing particle, but 30-50% of samples with 1,2 or 3 glowing particles tested negative for aflatoxin in a laboratory.

Commercial test kits with immunoassay or ELISA techniques are available for on-site tests for aflatoxin. Immunoassay analysis is based on the detection of specific proteins found in aflatoxins using antibodies to identify these proteins. The tests are very specific for aflatoxin with operator training and practice. Some tests determine only the presence or absence of aflatoxin; others can quantify, the amount of aflatoxin present. If a lot of corn is rejected based on the results of an immunoassay test kit, the results also should be confirmed by laboratory analysis. Test kits take a very small amount of ground grain; the **entire sample** should be ground before removing the subsample for the test kit. Do not subdivide 5-10 lb samples with a whole grain divider; this will cause frequent underestimation of aflatoxin levels and occasionally an alarmingly high test result.

GIPSA has evaluated several new qualitative quick test based on "lateral flow strip technology" (similar to pregnancy testing kits). These are immunoassay tests, but are much simpler (and faster) and can be used with minimal training. These are qualitative tests, but were very reliable in the GIPSA testing. The GIPSA evaluation program focuses on these as screening tests with very low incidence of false negatives (20 ppb

cutoff). If a positive is detected, the sample should be evaluated using a quantitative kit or laboratory reference method. The use of these kits is described in:

http://151.121.3.117/reference-library/directives/9181-2.pdf

The aflatoxin kits approved by USDA-GIPSA (including the lateral flow strip kits) are listed at:

http://151.121.3.117/tech-servsup/metheqp/testkit.htm

Storage and receiving decisions based on either black light or test kit screening will contain some error. Composite samples of bins or shipments should be retained to determine actual levels and future risk exposure. For farm bins, use the procedure given above. For commercial grain handling, keep composite samples of 1 lb per load received according to the bin the corn was assigned to. For larger bins (more than 25,000 bu), consider keeping the composites in 25,000 bu increments with by date of receiving so that the order of filling is known. The more information available, the greater will be the management and handling options should a problem develop.

Laboratory (quantitative) aflatoxin testing services

Analytical laboratories use one of several procedures such as thin-layer chromatography, mini columns, or gas chromatography to determine aflatoxin levels. These procedures are highly accurate and quantitative. The laboratory will grind the entire sample of corn and mix before taking subsamples for analysis. Some labs may use gas chromatography when very accurate results are needed for aflatoxins, but most currently use high-performance liquid chromatography.

Corn samples can be tested by official USDA–GIPSA or private laboratories. The Iowa State University Veterinary Diagnostic Laboratory analyzes samples submitted through a veterinarian. The site www.iowagrain.org at the aflatoxin link contains a list of public and private laboratories equipped for aflatoxin analysis. Grain trade or other governmental needs (eg crop insurance) may require an Official aflatoxin test, done by a designated agency of USDA-GIPSA.

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