

CHAPTER 3

SAMPLE PREPARATION

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### 3.1 GENERAL INFORMATION

The manner in which samples are obtained and processed is an important consideration when testing for aflatoxin. To ensure that the test results accurately reflect the aflatoxin concentration present in a lot, samples must be representative of the lot and of sufficient size to compensate for the uneven distribution of the contaminant.

Corn samples that contain an excessive amount of moisture content (above 20%) are problematic to the aflatoxin grinding and testing procedures. High moisture corn does not grind to a suitable particle size therefore affecting the accuracy of the test results. Therefore, official personnel must ensure that high moisture corn samples are allowed to **naturally dry** to a moisture level of **20% or less before grinding and testing.**

### 3.2 SAMPLE SIZE

Obtain samples according to the instruction in the Grain Inspection Handbook, Book I, "Grain Sampling."

The minimum sample size is based on the type of lot. Applicants may request a sample size larger than the minimum sample size.

<b>Lot Type</b>	<b>Minimum Sample Size (lbs.)/ grams</b>
Trucks	2 pounds / approximately 908 grams
Railcars	3 pounds / approximately 1,362 grams
Barges/Sublots	10 pounds / approximately 4,540 grams
Submitted Samples	10 pounds / approximately 4,540 grams ( <b>recommended</b> )

**NOTE: A minimum sample size of 10 pounds is required for composite type samples (e.g., a single sample representing multiple carriers). A 10-pound sample size is also recommended, but not required, for submitted samples.**

**Testing locations that receive submitted samples that contain less than the recommended 10-pound sample size must grind the entire sample as submitted. For submitted samples that are 10-pounds or more, a minimum of 10-pounds must be ground for testing purposes.**

### 3.3 WORK RECORDS

Each testing laboratory must maintain work records for each test that include the name of the applicant, date of service, sample or carrier identification, test results, initials of official personnel performing the test, and any other information deemed necessary to properly certificate the test results and bill the applicant. As practical, use existing forms, such as FGIS-992, "Services Performed Report;" FGIS-920, "Grain Sample Ticket;" or FGIS-921, "Inspection Log," to record laboratory results.

Any sample sent to TSD (including the Board of Appeals and Review) for aflatoxin testing or monitoring purposes must include the necessary information to facilitate sample processing and testing.

### 3.4 SAMPLE PORTIONS

#### a. Subportions.

Grind the entire sample obtained for aflatoxin testing and prepare two 500-gram sub-portions from the ground sample. Prepare a 500-gram work portion for original testing services and a 500-gram file sample portion for review testing. For submitted samples, retain as large a sample as possible.

For subplot testing of corn at export locations, save an additional 500-gram file (three 500-gram sub-portions total) for Food and Drug Administration (FDA) analysis, when applicable, for "actionable lots".

From the 500-gram work portion, obtain a 50-gram test portion and weigh on an FGIS-approved type scale with a minimum division size of 0.1 gram, using one of the following options.

- (1) Collect the 500-gram sample and divide (using an approved divider) out a 50-gram test portion for analysis. Maintain the balance as a file sample.

Or

- (2) Collect the 500-gram sample in a clean container and stir/mix the sample with a spatula or spoon for about 30 seconds ensuring a homogeneous blend (low to high). Using a spatula or spoon dip out a 50-gram test portion for analysis. Maintain the balance as a file sample.

b. Saving File Samples.

Maintain file samples (including the FDA file sample when applicable) for all lots/samples that:

do not meet the contractual specification of the applicant for service;

are required for the aflatoxin monitoring program; or

exceed FDA action limits of 20 ppb.

When applicable, maintain a representative file sample for each lot, subplot, composite, or submitted sample tested. For submitted samples that are less than 500 grams, retain as large a sample as possible. For information concerning file sample retention periods refer to FGIS Directive 9170.13, "Uniform File Sample Retention System".

c. Storing File Samples.

If file samples are required, store each sample in a manner that will maintain the representativeness of the sample and prevent possible manipulation or substitution. Place the sample in paper bags or envelopes and label each file sample with the test date and identification. Take precautions to ensure that file sample containers are strong enough to prevent loss of sample integrity when storing samples. Do not store samples near heat, windows, or in direct sunlight. (Store samples in cold storage if available.)

d. Disposition of File Samples.

At the end of the retention period, label the file samples as follows: "FOR LABORATORY USE ONLY - NOT FOR USE AS FOOD OR FEEDSTUFF," and discard the file samples in a dumpster or landfill disposal site.

e. Shipping Samples.

When it is necessary to send samples to other laboratory locations, take precautions to maintain sample integrity by securely packaging the samples. Label the shipping container "NOT FOR HUMAN CONSUMPTION".

### 3.5 OPERATION OF GRINDERS

Samples must be ground to a fine particle size that is sufficiently fine enough to obtain a homogeneous blend. Avoid over-grinding or pulverizing a sample because it produces an excessively powdery mix that will slow down the filtration process.

Grinding must be performed in an area separate from the testing area. Use the Romer Mill - Model 2A, Bunn Grinder, or equivalent to grind the sample.

FGIS employees must follow the manufacturer's safety procedures for operating the grinder and must wear protective equipment (i.e., labcoat, mask, gloves, and hairnet) when grinding samples.

a. Romer Mill.

1. General Operating Instructions.

The Romer Mill simultaneously grinds and sub-samples of corn at the rate of approximately 1 pound per minute. An adjustable restrictor door located above the collection chute varies the amount of ground sample allowed into the collection chute. Official personnel must adjust the grinder to obtain the required testing and file portions from the sample.

**Note: DO NOT dip out the 500 gram portion used for work and file samples.**

Adjust the grinder by locating the first line (far left) etched on the restrictor door. Position the door approximately 1/3 of the way between the first and second line. For a 10-pound sample, approximately 500-grams will be collected through the collection chute.

Once the grinder is adjusted to obtain the 500-gram sample, mark the location of the setting. To increase the sample size, move the restrictor door to the left.

If a composite sample is required in addition to the subplot-by-subplot analysis, adjust portion sizes as needed to obtain an adequate size composite and still maintain individual file samples. Obtain the composite sample from the ground subplot samples.

2. Grinding the Sample.

Grind the entire 10-pound sample with the grind lever set at the finest range.

If the grinder is experiencing difficulty (e.g., over-heating, bogging down) at the fine setting, change the grind lever to the coarsest setting. After grinding the remainder of the sample at the coarsest setting, switch the setting back to fine. Collect the entire 10-pound portion and regrind at the fine setting.

**Note: If the grinder motor overheats the breaker switch will release and turn off the power to the grinder. If this occurs, allow the motor to cool and then set the grind lever to the coarse setting. Do not grind samples with moisture content of 20 percent or more.**

b. Bunn Grinder.

(1) General Operating Instructions.

The Bunn-O-Matic grinds corn at a rate of approximately 2 pounds per minute and has a holding capacity of approximately 3 to 4 pounds when fully closed. Official personnel must grind the entire sample (see section 3.2) and cut it down (using an FGIS-approved divider) to obtain the required 500-gram work and file portions from the official sample.

**Note: DO NOT dip out the 500 gram portion used for work and file samples.**

(2) Grinding Samples.

Grind the entire 10-pound sample with the grind lever set at the fine selection. Add 3 to 4 pounds at a time into the hopper until all 10 pounds are ground. If the grinder is experiencing difficulty (e.g., over-heating, bogging down) at the fine setting, change the grind lever to the coarse setting.

After grinding the remainder of the sample at the coarse setting, switch the setting back to fine. Collect the entire 10-pound portion and regrind at the fine setting.

**Note: If the grinder motor overheats the breaker switch will release and turn off the power to the grinder. If this occurs, allow the motor to cool and then set the grind lever to the coarse setting. Do not grind samples with moisture content of 20 percent or more.**

Obtain the composite sample from the ground subplot samples. Official personnel must use an approved divider to reduce the size of the ground portion to the stated 500-gram work and file samples.

### 3.6 CLEANING GRINDERS

A small amount of ground sample will remain in the grinder after the total sample has been ground. To prevent the contamination of subsequent samples, clean the grinder using one of the following cleaning procedures:

a. If a Vacuum Cleaner is Available.

After a sample has been ground and collected, with the unit turned on, use a vacuum cleaner with an attachment that will fit over the mouth of the chute. Place the attachment at the bottom of each chute for about 30 seconds. After all the chutes have been cleaned, turn the power off and prepare for the next sample.

b. If a Vacuum Cleaner is Not Available.

Clear the grinder by discarding a small portion (first 10 to 15 grams) of the next sample to be tested.

- (1) Pour the sample into the grinder and turn it on long enough to collect the first 10 to 15 grams.
- (2) Turn the power off, and discard the 10-15 grams ground sample.
- (3) Turn the power back on and finish grinding the sample to collect the remaining subsample for analysis.



### 3.7 CHECKING PARTICLE SIZE

a. Procedures for Checking the Performance of the Grinder.

For locations that perform mycotoxin testing on coarse (e.g., corn) and small grains, perform the check using a 100-gram sample portion of corn using the following procedures.

- (1) Grind a sample portion of approximately 100-grams of corn having a moisture content of 14.0 percent or less.
- (2) Weigh the entire portion that was ground.
- (3) Sieve the portion across a standard No. 20 wire woven sieve.
- (4) Weigh the portion that passed through the sieve.
- (5) Determine the percent of fine material, by weight, as follows:

$$\text{Fines} = \text{weight from step (4) divided by the weight from step (2) X 100.}$$

b. Optimum Particle Size.

The optimum range for particles of coarse and small grain passing through the No. 20 sieve is between 60 and 75 percent. Whenever the ground particles appear to be too coarse, or the results of a grinder check indicate that less than 50 percent of the ground portion passes through the No. 20 sieve, the grinder should be adjusted or repaired to meet the optimum range requirements.

Grinding apparatuses must be checked periodically to determine whether they are producing a final product that meets the particle size requirements as listed above. Official personnel shall determine the frequency of the checks based on a number of items that include visual observation of the ground product, number of samples ground since last check, and time (number of days) since the last check was performed. Record all particle check results in a convenient location for future reference purposes.