

## Cottonseed sampling / testing. Preventing aflatoxin contamination of milk and animal feed.

According to the U.S. Food and Drug Administration (FDA): "Aflatoxicosis is poisoning that results from ingestion to aflatoxins in contaminated food or feed. The aflatoxins are a group of structurally related toxic compounds produced by certain strains of the fungi *Aspergillus flavus* and *A. parasiticus*. Under favorable conditions of temperature and humidity, these fungi grow on certain foods and feeds, resulting in the production of aflatoxins. The most pronounced contamination has been encountered in tree nuts, peanuts, and other oilseeds, including corn and cottonseed. Aflatoxins produce acute necrosis, cirrhosis, and carcinoma of the liver in a number of animal species; no animal species is resistant to the acute toxic effects of aflatoxins; hence it is logical to assume that humans may be similarly affected."

Quoting from Wikipedia, the online encyclopedia: "The toxin (aflatoxin) can also be found in the milk of animals which are fed contaminated feed."

The name aflatoxin comes from A (*Aspergillus*) + FLA (*flavus*) + toxin.

In 1986 the Aflatoxin Certification Program was established to protect the Arizona cottonseed industry, dairies and ultimately consumers of milk. Several major events led to the establishment of this Program:

- Modern research into aflatoxin in 1961 looked into what caused the deaths of 100,000 young turkey poults in England. The research traced the poison to contaminated Brazilian peanut meal that had been used as feed.
- As a result of cottonseed testing for aflatoxin by the FDA in Arizona, 130,000 gallons of raw milk for pasteurization, and 10,000 gallons of finished product were dumped between August, 1978 and January 1979.
- In 1980 cottonseed from Texas contaminated large quantities of milk in Arizona which had to be dumped.
- In 1982 the Arizona State Chemist Office surveyed aflatoxin levels in Arizona cottonseed state-wide showing high levels of aflatoxin. As a result the ammoniation process was approved to reduce aflatoxin levels below 20ppb.

Since Arizona agriculture is a major producer of cottonseed for animal feed, the Arizona Department of Agriculture, Environmental Services Division, has an ongoing program for the sampling and testing of cottonseed for aflatoxin. There are three approved methods of sampling: Corkscrew Trier, Probe-A-Vac, and Stream Sample.



The **Corkscrew Trier** is a mechanical screw device, approximately 50 inches long. Each probe by the trier can hold at least a three pound sample. Normally, a minimum of ten probes or not less than thirty pounds of cottonseed is obtained per sample taken from each pile.





The **Probe-A-Vac** uses a vacuum suction device to pull the cottonseed sample into a hollow tube, that is then emptied into a sealable container.



The container used for **Stream Sampling** is approximately 8" x 5" x 5.5" and is attached to a pole that passes through the falling stream of cottonseed.

Detailed, documented sampling procedures are followed by inspection staff, to assure that representative samples are delivered to State Agricultural Laboratory for testing. The Administrative Code Web site shown below contains the applicable Rule:

[http://www.azsos.gov/public\\_services/Title\\_03/3-03.htm](http://www.azsos.gov/public_services/Title_03/3-03.htm)

Scroll to Rule R3-3-913.

The FDA has established the following aflatoxin action limits which are applied to cottonseed meal in Arizona.

- 20 ppb for humans, immature animals (including poultry) and all dairy animals.
- 100 ppb for breeding beef cattle, swine and mature poultry.
- 200 ppb for finishing swine (100 pounds and up).
- 300 ppb for feeder cattle.

ppb = parts per billion

Initially, the samples are delivered to the Chandler Analytical Laboratory where the cottonseed is run through a decorticator and ground to separate the hulls and meats. During the decorticating process a sub-sample is taken which is then sent to the State Agriculture Laboratory for analysis.



Upon receiving the cottonseed samples, the State Agricultural Laboratory completes the appropriate paperwork, ensuring that the Chain of Custody and seals are proper before logging the samples into the LIMS (Laboratory Information Management System). The samples are passed through a 20 micron sieve. Any cottonseed material not passing through the sieve is further ground to meet this size requirement. The ground material is then mixed to obtain uniformity before a 50g subsample

The samples are then extracted for 30 minutes with a mixture of acetone and water. The sample extracts then undergo a series of clean-up steps to further eliminate other chemicals inherent in the cottonseed that might interfere with the analysis of the aflatoxin content. Once purified, the sample extracts are evaporated in order to increase the concentration of the aflatoxins and improve the sensitivity and accuracy of the analysis.

Determination of the amount of aflatoxins present in the extract is done by a process called Thin Layer Chromatography (TLC). A very small volume of the sample extract is precisely spotted onto a glass plate covered with silica gel (aka TLC plate). On the same plate, known amounts of pure aflatoxins (called reference standards) are similarly spotted next to the sample extracts. The plate is then developed in a tank partially filled with solvent. The aflatoxins separate from other chemicals present in the sample as well as separating from each other on the TLC plate. There are four aflatoxins which are commonly detected in grain materials. They are Aflatoxin B1, B2, G1 and G2; however, for cottonseed grown in Arizona, typically only B1 and B2 are present.

Once the plate is developed and dried, the plate is examined in a cabinet using Ultraviolet (UV) light. One of the natural properties of the aflatoxins is their fluorescence when exposed to UV light. In essence, they glow in the dark when exposed to UV light. By visually comparing the amount of fluorescence of the aflatoxins in the sample extract and in the known reference standard, the level of aflatoxin in sample extract is calculated and mathematically converted to represent the amount of aflatoxin present in the original sample.

Any cottonseed that exceeds the FDA guidelines could be considered adulterated for feed purposes. The Department has the authority to penalize a feed manufacturer or dealer found distributing feed contaminated with aflatoxin. The penalties could be in an amount equal to twice the selling price of the feed, plus the consumer is entitled to a refund of the purchase price.

