

CHAPTER 07 - MOLECULAR BIOLOGY AND NATURAL TOXINS

SUBJECT: MYCOTOXINS IN DOMESTIC AND IMPORTED FOODS FY 07/08 This program has completed a Good Guidance Practices clearance by CFSAN's ORP and OC/DFP/CPB in December 2006.	IMPLEMENTATION DATE January 22, 2007
	COMPLETION DATE September 30, 2008
DATA REPORTING	
PRODUCT CODES	PRODUCT/ASSIGNMENT CODES
See Attachment A	07001 All domestic and import sample collections and analysis

NOTE: Material that is not releasable under the Freedom of Information Act (FOIA) has been redacted/deleted from this electronic version of the program. Deletions are marked as follow: (#) denotes one or more words were deleted; (&) denotes one or more paragraphs were deleted; and (%) denotes an entire attachment was deleted.

FIELD Reporting Requirements

1. Report all collections/analyses of domestic and imported **food** samples for aflatoxin, patulin, deoxynivalenol, fumonisin, or ochratoxin A contamination against the above PAC even though samples were collected during operations conducted under other compliance programs.
2. The analyzing district will report analytical results into FACTS using PAF = "MYC" and ensure that the correct Mycotoxin Code **for the mycotoxins analyzed for** is selected from the FACTS drop-down box.
3. When entering information into FACTS:
 - use operation code 31 to report domestic sample collection and operation code 41 to report domestic sample analysis.
 - use operation code 33 to report import sample collection and operation code 43 to report import sample analysis.
4. Refer to Part IV for sample classification instructions.

PART I - BACKGROUND**A. General**

Mycotoxins are toxic metabolites produced by certain fungi that can infect and proliferate on various agricultural commodities in the field and/or during storage. The occurrence of these toxins on grains, nuts and other commodities susceptible to mold infestation is influenced by environmental factors such as temperature, humidity, and extent of rainfall during the pre-harvesting, harvesting, and post-harvesting periods. Mycotoxins may exhibit various toxicological manifestations; some are teratogenic, mutagenic and /or carcinogenic in susceptible animal species and are associated with various diseases in domestic animals, livestock, and humans in many parts of the world.

The occurrence of mycotoxins in foods and feeds is not entirely avoidable; therefore small amounts of these toxins may be in foods and feeds. Strategies used by the Food and Drug Administration (FDA) to minimize mycotoxins in the United States (U.S.) food supply include establishing guidelines (e.g., limits established in compliance policy guides (CPG)), by monitoring the food supply by collecting and analyzing domestic and import foods. The data obtained over the years from FDA's monitoring programs are used to provide: (a) estimates of the incidence and levels of contamination by various mycotoxins in affected areas in the country, (b) dietary exposure data (estimates) for use in making risk assessments for specific mycotoxins, (c) background data for use in considering the establishment of guidelines for specific mycotoxins, (d) an estimate of the economic impact of the enforcement of regulatory guidelines on foods and feeds during a given crop year, (e) information needed to prepare answers to questions including congressional inquiries, and (f) basic information needed to support the position and recommendations of U.S. delegates participating in international meetings. The monitoring data also serve as a database describing the background distribution of various mycotoxins in domestic grains and their products in the U.S. as a function of geographic area and environmental conditions.

B. Specific Mycotoxins to be included in this program

1. **Aflatoxins**, metabolic products of the molds *Aspergillus flavus* and *A. parasiticus*, may occur in food as a result of mold growth in a number of susceptible commodities, including peanuts and corn. Other domestic nuts and grains are susceptible but less prone to contamination with aflatoxins. Because aflatoxins are known carcinogens to laboratory animals, and presumably to man, the presence of aflatoxins in foods should be restricted to the minimum practical levels attainable using modern processing techniques. The current CPG limits for aflatoxins can be found in the appropriate section of the CPG. See Part V for CPG references. Historically, aflatoxin levels in peanuts and corn have been highest in the Southeastern states. Corn from anywhere in the U.S. may be affected, however, depending on the growth, harvesting and storage conditions involved, as was the case in the Mid-west in 1988 and in Texas in 1987.
2. **Patulin** is a toxic substance produced by *Penicillium*, *Aspergillus*, and *Byssoschlamys* molds that may grow on apples. Since Patulin is

not destroyed by heat processing, and can occur at high levels in apple juice, including pasteurized apple juice, if rotten, moldy or damaged apples are used to make juice, both pasteurized and non-pasteurized single strength juice and concentrated juices are to be collected. Animal feeding studies have demonstrated that high levels of patulin in apple juice could pose a health risk if the juice is consumed over an extended period of time. In 2001, FDA established a limit for patulin in apple juice and in the apple juice component of a food that contains apple juice as an ingredient. The CPG limit for patulin can be found at Section 510.150. See Part V for CPG references. The limit is based upon the patulin level in single strength apple juice, reconstituted single strength apple juice (if the food is an apple juice concentrate), or the single strength apple juice component of the food (if the food contains apple juice as an ingredient). Single strength juice is 100 percent juice that is unconcentrated (see 21 CFR 101.30(h)). Under the juice Hazard Analysis Critical Control Point (HACCP) regulations that are presently in effect, some apple juice processors may need to establish control measures such as using only tree picked fruit, and culling their apples to be used for juice production to remove rotten and damaged fruit.

3. **Deoxynivalenol (DON)**, commonly called vomitoxin, is a natural toxin produced by several molds of the genus *Fusarium*, especially *F. graminearum*, which is a common contaminant of several grains, including wheat, corn, barley, and rye. DON has been associated with a number of adverse health effects in humans and animals. Several adverse weather related DON contamination episodes in the U.S. have caused the FDA to issue call-in levels for food (wheat) and feed in 1982 and updated levels in 1993. (See Part VI, Additional References, #7.) FDA is continuing to study the scope and toxicological significance of the DON problem to determine if further regulatory measures are needed to control DON in food and feed products.
4. **Fumonisin** (Fumonisin FB₁, Fumonisin FB₂, and Fumonisin FB₃) are natural toxins produced by *Fusarium verticillioides* (previously known as *F. moniliforme*), and other *Fusarium* species; these molds are common natural contaminants of corn. Fumonisins have been linked to fatalities in horses and swine. Recent studies have demonstrated the presence of fumonisins in human foods, including corn meal and breakfast cereals. Epidemiological investigations demonstrating a possible association of *F. verticillioides* with esophageal cancer and recent animal studies indicating the carcinogenicity of fumonisin FB₁ have highlighted the need to ensure that foods do not contain excessive amounts of fumonisins. Dry milling of whole corn kernels generally results in the production of fractions called bran, flaking grits, grits, meal, and flour. Because fumonisins are concentrated in the germ and the hull of the whole corn kernel, dry milling results in fractions with different concentrations of fumonisins. For example, dry milled fractions (except for the bran fraction) obtained from degermed corn contain lower levels of fumonisins than dry milled fractions obtained from non-degermed or partially-degermed corn. Industry information indicates that dry milling results in fumonisin-containing fractions in descending order of highest to lowest fumonisin levels: bran, flour, meal, grits, and flaking grits.

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5. **Ochratoxin A** is a naturally occurring nephrotoxic fungal metabolite produced by certain species of the genera *Aspergillus* and *Penicillium*. It is mainly a contaminant of cereals (corn, barley, wheat and oats), and has been found in edible animal tissues as well as in human blood sera and milk. Studies indicate that this toxin is carcinogenic in mice and rats. It is not completely destroyed during the processing and cooking of food, therefore the implication of risk to human health and safety must be considered. FDA needs up to date information on the incidence and levels of occurrence of this toxin in the U.S. for use in considering any necessary regulatory control measures for this substance.

PART II - IMPLEMENTATION

OBJECTIVES

To collect and analyze domestic and import samples of various food products to determine the occurrence and levels of aflatoxins, patulin, fumonisins, deoxynivalenol, and ochratoxin A;

To collect monitoring and incidence data to support establishment of future limits for fumonisins, deoxynivalenol, and ochratoxin A in foods.

PROGRAM MANAGEMENT INSTRUCTIONS

Federal/State Relations

State officials are valuable sources of information on current and potential aflatoxin problems in foods. In the past, a number of states have participated in an aflatoxin data exchange program with FDA. Districts should encourage state participation in this data exchange program and should coordinate aflatoxin program activities with State officials to prevent duplication of efforts in both food and feed sampling. Information on this data exchange program can be obtained from the Division of Federal-State Relations, HFC-150, (301) 827-6906.

PART III - INSPECTIONAL

A. Inspectional

This program does not direct inspections. However, districts will conduct follow-up inspections if levels of aflatoxin that exceed CPG limits are detected in compliance (not surveillance) samples.

Inspectional instructions for mycotoxin inspections are contained in Section 8 of the DFI Guide to Inspections of Manufacturers of Miscellaneous Food Products Volume II. This guide is available online at:

http://www.fda.gov/ora/inspect_ref/igs/foodsp2.htm

B. Sampling

General Instructions:

NOTE: Duplicate 702(b) reserve sample portions are required for all domestic products.

Refer to the current ORA workplan and to Attachment A of this compliance program for all sample obligations, i.e., number of samples to be collected for each mycotoxin and the products to sample for each mycotoxin.

It is imperative that the products specified in Attachment A, for each analyte, be collected and analyzed as a unique sample; therefore, a sample from a single lot of a product is **not** to be collected for multiple mycotoxin analyses. Specifically, a product collected for aflatoxin, fumonisin or ochratoxin analysis is not to be analyzed for more than one mycotoxin. In the past, with CFSAN's concurrence, samples were bundled, i.e., a single sample was collected for multiple mycotoxin analysis. This approach did not yield the requested analyte/product combinations. The collection report must explicitly state the specific mycotoxin that is being tested for so that the lab does not need to contact the investigator before beginning the analysis.

Mycotoxin contamination can occur in localized pockets at high concentrations in foods such as unprocessed grains and nuts. For sampling bulk products, representative samples should be obtained by using a trier or other device that will provide representative portions from all sections of the container sampled. Commodities such as fruit juice, other fluid items, and mixed preparations (paste, spreads, butters) are generally homogenous and do not require any special devices for sampling.

Collect subsamples randomly so as to be representative of the lot.

Sample only the foods listed in Attachment A. For subsample/sample size follow instructions in this compliance program. If none are provided, follow the instructions in the IOM Chapter 4, Sample Schedule Chart 6.

1. Aflatoxin

- a. Sample only the foods listed in Attachment A, Section 1.

If the District wishes to sample another product, **BEFORE** sampling consult the Domestic and Import Mycotoxin Monitor, Monali Yajnik, at (301) 436-1616 or via email at Monali.Yajnik@fda.hhs.gov. If she cannot be reached and an immediate answer is needed, contact Brenda Aloï at (301) 436-2065 or via email at Brenda.Aloï@fda.hhs.gov.

If the product collection is pre-authorized by CFSAN, the investigator must note this in the remarks section of the Collection Report. Otherwise, the laboratory will need to consult with CFSAN prior to beginning analyses.

If not referenced below, additional sample sizes are referenced in the Investigations Operations Manual (IOM), Chapter 4, Sample Schedule Chart 6. IOM Sample Schedule Chart 6 provides instructions on sample sizes for surveillance and follow-up sampling for aflatoxins. An initial sample (surveillance) is a sample collected in reduced amounts (to lower costs) to determine if there is an aflatoxin problem with the product. This sample is not large enough to be considered representative of the lot, and, therefore a follow-up sample must be collected for regulatory purposes. See Sample Reporting section below for additional instructions.

Do not collect more than 2 samples of any specific commodity at any firm, unless there is a need to collect more samples of that commodity for compliance purposes.

- b. Corn and Corn Products

Do not sample unpopped popcorn for aflatoxin unless there is reason to believe that aflatoxin contamination may be present due to late harvest or adverse environmental conditions. The characteristics of the cultivar of corn used for popping make it unlikely to be aflatoxin contaminated.

Aflatoxin levels, in food products made from corn (grits, meal, flour, snack foods or cereals), are likely to be higher in "full fat" than in degerminated products, since the highest levels of aflatoxin in the kernel are associated with the germ.

Do not sample sweet corn for aflatoxin analysis. FDA has analyzed samples of fresh, frozen and/or canned sweet corn for the presence of aflatoxins and no violative samples were found in the past.

Samples of shelled corn (designated for human use), corn meal, corn based snacks, and corn based breakfast cereals (corn flakes, grits) will be collected and analyzed for the presence of aflatoxins.

Various corn-based foods such as tacos, chips, cereals and snack foods are acceptable for sampling under this program for aflatoxin testing only. However, it is preferable to sample the corn ingredient that will be used in manufacturing these products.

Consider using ultra-violet light (blacklighting) as a screening technique, prior to sampling shelled corn, if many lots are available for sampling at one location. In some cases, corn contaminated by molds will fluoresce a bright yellowish green color. See IOM 427.04g; Blacklight Test Screening Procedure for Aflatoxins in Corn, for instructions on procedure.

c. Peanuts

The testing for aflatoxin in roasted in-shell and shelled peanuts, as well as processed peanut products for consumer use, is the responsibility of FDA.

In general, the varieties, grade, and geographical growing area for peanuts used for roasting have resulted in low aflatoxin levels in roasted-in-shell peanuts. However, when there are shortages of the usual peanut varieties used for roasting, a variety of "Runner" peanut grown in northern Florida may be substituted. Peanuts of this variety and from this area have consistently had a relatively high incidence and level of aflatoxin contamination.

Do not sample in-shell peanuts (except the "Runner" variety of peanut when roasted in-shell) or nut meats destined for processing that is intended to remove aflatoxin contaminated nuts.

Do not sample raw peanuts (shelled or in-shell) for aflatoxins. USDA will collect and analyze samples of domestic and imported raw peanuts (shelled or in-shell) to determine if aflatoxin is less than 20 ppb. USDA will conduct all testing of raw peanuts, domestic and imports, for aflatoxins in accordance with FDA Memorandum of Understanding (MOU) with the United States Department of Agriculture (USDA), Agricultural Marketing Service (AMS) - MOU with USDA/AMS Concerning Aflatoxins in Peanuts and Peanut products (FDA-225-96-2001), formerly CPG 7155a.14. (See Sec.570.375 Aflatoxins in Peanuts and Peanut Products (CPG 7112.02), http://www.fda.gov/ora/compliance_ref/cpg/cpgfod/cpg570-375.html

d. Almonds, macadamia, pecans, pistachios, walnuts, and hazelnuts are susceptible to aflatoxin contamination, but samples of these nuts have been largely in compliance (less than 1% adverse) for several years. FDA surveillance of these crops, however, is necessary to assure that industry-implemented quality control procedures continue to effectively prevent the marketing of aflatoxin contaminated nuts.

e. Domestic Milk Products Only

When dairy animals consume feed containing high levels of aflatoxins, one of the metabolized aflatoxins (aflatoxin B₁) may be secreted into the animals' milk as aflatoxin M₁. Cattle consuming feed that contains less than 20 ppb of total aflatoxins, however, should produce milk that complies with FDA's limit that is listed in the CPG for aflatoxin M₁ in milk.

Sample milk for aflatoxin M₁ if state coverage is inadequate in areas where the potential for aflatoxin in dairy rations exists. Use the results of the District's sampling of feed under Center for Vet. Medicine directives and the results of State feed analyses as indications of suspect dairy rations.

Do not sample dairy products such as cheese or yogurt, unless there is reason to suspect they were made from milk containing levels of aflatoxin M₁ that exceed CPG limits.

f. Import Products Only

Increased Surveillance of Imported Peanut Butter as a result of previous problems (e.g. recall of peanut butter from Argentina) and recent market information indicating an increase in the importation of peanut butter or imported peanuts shipped to another country (e.g., Canada), (where they are further processed into peanut butter and subsequently exported to the U.S.). Refer to the current Workplan for the numbers of samples to be collected.

In addition, nut pastes and similar ethnic foods containing nuts susceptible to contamination may be sampled.

The USDA only tests unprocessed agricultural commodities. For Brazil nuts, only in-shell un-roasted nuts are USDA Tested (See MOU 225-96-2002). For pistachios, only unroasted pistachios are USDA tested (See MOU 225-96-2003). Roasted pistachios and shelled or roasted Brazil nuts are not covered by the MOU's because they are processed. They are suitable for collection for aflatoxin analyses.

Special Surveillance Products to be Collected

See Attachment A, Section 1a for a list of these products.

Refer to the IOM, Chapter 4, Sample Schedule Chart 6 for a list of sample sizes. Collect samples randomly so as to be representative of the lot.

Cashews should no longer be collected for surveillance sampling for aflatoxin.

2. Patulin

- a. See Attachment A, Section 2. Collect **only** apple juice and/or concentrated apple juice. Since patulin is not destroyed by heat processing, and can occur at high levels in pasteurized apple juice, if rotten, moldy or damaged apples are known to have been used to make the juice, then both pasteurized and non-pasteurized single strength juice and concentrated juices are to be collected.

- b. Sample size is dependent on product form:
- | | |
|-----------------------------------|--|
| Frozen | Collect six subsamples with a minimum |
| Concentrate: | volume of 400 mL (approximately 12 fluid ounces) per subsample; |
| Single Strength (ready to drink): | Collect six subsamples with a minimum volume of 500 mL (approximately 16 fluid ounces) per subsample |

If necessary, collect additional units to make up minimum volume requirements.

- c. Domestic Products Only

If samples are collected in conjunction with the inspection of an apple juice processor under the Juice HACCP Inspection Program (7303.847) consider collecting samples if the processor does not cull apples to be used to produce juice (including stored apples) to remove rotten apples and visibly damaged apples, i.e., bruising, breaks in the skin, apples with holes, visible mold, hail damage, bird pecks. Consider collecting samples also if it is established that the processor uses drops, i.e., apples that have fallen off the tree and are harvested from the ground (also known as grounders or ground fruit) to produce juice. The Juice HACCP Inspection Program can be found at #

3. Deoxynivalenol (DON)

- a. See Attachment A, Section 3 for products to be collected.
- b. Dry milled wheat products (whole wheat flour, white flour and bran) will be collected.
- c. Samples will consist of # 450 gram (approximately 1 pound) subsamples to be collected from a single lot of product.
- d. Domestic Products Only

Some samples of bran that may be used as a component of bran cereal, but not the cereal itself, may be collected at cereal manufacturers.

- e. **Do not collect samples of unfinished wheat commodities (i.e. products that will be further processed) for either domestic or imported products.**

4. Fumonisin FB₁ and FB₂

- a. See Attachment A, Section 4 for products to be collected.

Samples of whole, partially degermed, and degermed dry-milled corn products (flour, meal, grits, flaking grits, bran) and cleaned corn intended for masa production and for popcorn (unpopped) will be collected and analyzed for the presence of fumonisins.

- b. Collect # 454-gram (one pound) subsamples per sample.

- c. Domestic Products Only

For program needs, the Center requests approximately half of the samples to be degermed, i.e., low fat. Therefore, each district should try to collect half of their samples as whole or partially degermed dry-milled corn products (fat content greater than or equal to 2.25%, dry weight basis) **AND** for the other half, degermed dry-milled corn products (fat content less than 2.25%, dry weight basis) of corn flour, corn meal, corn grits and corn flaking grits. Because of this requirement, these products should be collected at a mill if possible; however, major processors using these products will have fat content as one of the specifications and if the investigator can determine the approximate fat content, samples can be collected from processors. State on the collection report, in the remarks section that the product is either whole, partially degermed or degermed and state the approximate fat content obtained from either the miller or processor.

5. Ochratoxin A

- a. See Attachment A, Section 5 for products to be collected.
- b. Rye flour, wheat flour, barley (cereals), oats (whole/cereals) dried beans, corn meal, raisins, coffee, soya flour and soya based baby foods will be collected and analyzed for ochratoxin A.

The corn meal and corn based cereal products listed in 4a (above) will also be collected and analyzed for ochratoxin A.

- c. For cereals and soya based baby food products, collect # 200 gram (Approximately 8 ounces) subsamples per sample, for all other products collect # 450 gram (approximately one pound) subsamples per sample.

C. Sample Reporting

When collecting an additional sample as follow up to a positive surveillance sample for aflatoxin, record the sample number and ppb findings for the surveillance sample in the "Remarks" section of the new collection report.

D. Sample Handling

DO NOT pack samples (other than milk or fluid items) in plastic bags or other moisture-proof containers as this may cause sweating and result in an unstable sample.

E. Sample Submission

Consult the current ORA Field Work Plan, Part 1, Appendix 3 to determine the servicing laboratory for all samples collected under this program.

PART IV - ANALYTICAL**I. Mycotoxins addressed by Compliance Policy Guides****A. Aflatoxin Analyses**

Prior to beginning analyses review the collection report (C/R) to verify that collection of a product not listed on Attachment A, Section 1 has been pre-authorized by CFSAN. If not, contact the general assignment contact for instructions.

See ORA Workplan - Part I, Appendix III for the current listings of Servicing Laboratories.

SAFETY: Be aware of the potential hazards in the preparation of aflatoxin samples. See Section 49.2.01 (AOAC Method 977.16), 17th Edition of the Official Methods of Analysis of the AOAC.

1. Follow the procedures and methods in the 17th Edition (or, as updated) of the Official Methods of Analysis of the AOAC:
 - a. Chapter 49 - General precautions
 - b. Section 49.2.01 (AOAC Method 977.16) - Sampling and preparation of sample and safety precautions.
 - c. Sections 49.2.02 (AOAC Method 970.44) and 49.2.03 (AOAC Method 971.22) - Preparation of standards
 - d. Section 49.2.08 (AOAC Method 968.22) - Method I (CB Method) for peanuts/peanut products (applicable to all nut products in this program)
 - e. Section 49.2.09A (AOAC Method 998.03) - (Alternative BF Method) - Aflatoxins in peanuts and peanut butter
 - f. Section 49.2.11 (AOAC Method 971.23) - Aflatoxins in cocoa beans
 - g. Section 49.2.12 (AOAC Method 971.24) Aflatoxins in coconut, copra, and copra meal
 - h. Section 49.2.15 (AOAC Method 993.17) - Aflatoxins in corn and peanuts
 - i. Section 49.2.17 (AOAC Method 990.33) - Corn and peanut butter
 - j. Section 49.2.18 (AOAC Method 991.31) - Corn, raw peanuts and peanut butter.
 - k. Section 49.2.19A (AOAC Method 994.08) - Corn, almonds, Brazil nuts, peanuts, and pistachio nuts.
 - l. Section 49.2.26 (AOAC Method 975.37) - Identification of the aflatoxin B₁ by derivative formation on TLC plate.
 - m. Section 49.2.27 (AOAC Method 985.17) - Identification of aflatoxin B₁, confirmation method

- n. Section 49.2.29 (AOAC Method 999.07) - Aflatoxins in peanut butter, pistachio paste, fig paste, and paprika - immunoaffinity column/LC with post column derivatization.
- o. Section 49.3.01 (AOAC Method 974.17) - Aflatoxins M₁ in dairy products
- p. Section 49.3.02 (AOAC Method 980.21) - Aflatoxin M₁ in milk and cheese; confirmation of identity by derivative formation on TLC plate
- q. Section 49.3.06 (AOAC Method 986.16) - Aflatoxin M₁ and M₂ in fluid milk
- r. Section 49.2.30 (AOAC Method 2000.16)- Aflatoxin in baby food
- s. Section 49.3.07 (AOAC 2000.18)- Aflatoxin M₁ in liquid milk

The chart below may be used to facilitate calculations for nut samples.

<u>Nuts</u>	<u>Meat, % by Wt.</u>
Almonds	40
Peanuts	70
Pecans	50
Pistachios	50
Macadamia	29
Pumpkin seeds	74
Walnuts	50
Brazil nuts	50

2. In accordance with Sec. 555.400 - Foods Adulteration with Aflatoxin (CPG 7120.26), http://www.fda.gov/ora/compliance_ref/cpg/cpgfood/cpg555-400.html, when original analyses show levels above 100 ppb, contact the Compliance Branch of the collecting district for instructions.
3. Confirmation:

Refer to Compliance Policy Guides (See Part V) for required confirmation of identity procedures for compliance samples. References #3 and #4 in Part VI of the program also pertain to this method.

NRL is responsible for confirmation of aflatoxin identity by negative chemical ionization mass spectrometry, as required. However, any originating laboratory may, at its discretion, perform the confirmation analysis instead of submitting it to NRL.

Mass spectral confirmation of aflatoxin identity is not required of domestic peanuts/peanut products, corn/corn meal, pistachio nuts, pumpkin seeds, cottonseed/meal, coconut meal, or copra.

When shipping samples for confirmatory analyses, follow the instructions below:

- wrap the vial containing the aflatoxin B1 in foil to protect the contents against light and moisture;
- after notifying the confirmation lab of its transmittal, send the vial by a one-day delivery service to
NRL(HFR-NE580); 158-15 Liberty Avenue, Jamaica, NY
11433;(718) 622-5439).

4. Reporting:

The analyzing district will report analytical results into FACTS using PAF = "MYC" and the correct Mycotoxin Code **for the mycotoxins analyzed for** selected from the FACTS drop-down box.

For follow-up compliance samples, report the sample number and ppb findings for the surveillance samples in the narrative field of the FDA 2196(b).

Do not conduct a check analysis on surveillance samples.
If any level of aflatoxin is found in a surveillance sample, immediately arrange for collection of an official sample which is representative of the lot.

B. Patulin Analyses

1. General: Samples of frozen concentrate should be diluted either as per recommendation for dilution or to a Brix value of 11.5 (single strength) before analysis (Federal Register 56 No. 127, pp30452-30466, 1991).

2. Methods:

Follow the procedures and methods in the 17th Edition (or, as updated) of the Official Methods of Analysis of the AOAC:

Section 49.7.02 (AOAC Method 995.10) - Patulin in apple juice, liquid chromatographic method, AOAC-IUPAC-IFJU Method. AOAC International adopted this method in 1995. The method is published in JAOAC 79(2): 451-455, 1996.

Section 49.7.03 (AOAC Method 2000.02)- Patulin in clear and cloudy apple juices and apple puree

3. Confirmation of Identity of Patulin:

For compliance samples, confirmation is to be performed by mass spectrometry using the most convenient techniques. Examples of two acceptable procedures are:

Rupp, H.S., Turnipseed, S.B. "Confirmation of patulin and 5-hydroxymethylfurfural in apple juice by gas chromatography/mass spectrometry", J. AOAC Int. 83: 612-626, 2000)

Roach, J.A.G., White, K.D., Trucksess, M.W., and Thomas, F.S.,

"Capillary gas chromatography/mass spectrometry with chemical ionization and negative ion detection for confirmation of identity of patulin in apple juice", J. AOAC Int. 104-112, 2000)

4. Reporting

The analyzing district will report analytical results into FACTS using PAF = "MYC" and the correct Mycotoxin Code for the mycotoxins analyzed for selected from the FACTS drop-down box.

II. Mycotoxins Not Addressed By Compliance Policy Guides

A. Deoxynivalenol Analyses (DON)

1. Method of analysis:

Trucksess, M.W., Ready, D.E., Pender, M.K., Ligmond, C.A., Wood, G.E., Page, S.W. "Determination and survey of deoxynivalenol in white flour, whole wheat flour and bran", *J.AOAC Int.* 883-887(1996).

2. Confirmation of identity of deoxynivalenol:

For compliance samples, confirmation is to be performed by mass spectrometry using the most convenient techniques. An example of an acceptable procedure is:

Plattner, R.D., and Maragos, C.M. (2003) Detection of deoxynivalenol and nivalenol in corn and wheat by HPLC with electrospray MS. *Journal of AOAC International* 86(1):61-65.

3. Reporting:

The analyzing district will report analytical results into FACTS using PAF = "MYC" and the correct Mycotoxin Code for the mycotoxins analyzed for selected from the FACTS drop-down box.

Lab findings above the level of 1 ppm for DON should be referred to the mycotoxin program monitor via email for evaluation. Complete details on the product type, form and intended use must be provided in order for CFSAN to adequately evaluate the significance of these findings. Lab findings at or below 1 ppm require no further action.

B. Fumonisin Analyses

Follow the procedures and methods in the 17th Edition (or, as updated) of the Official Methods of Analysis of the AOAC:

1. Method of analysis:

Section 49.5.01 (AOAC Method 995.15) - Fumonisin FB₁, FB₂, and FB₃ in corn, liquid chromatographic method. The method was developed specifically for corn, therefore for products other than corn, it is recommended that recovery studies be done on such products before the final analysis.

Section 49.5.02 (AOAC Method 2001.04) - Determination of fumonisin FB₁ and FB₂ in flour and corn flakes by LC with immunoaffinity column cleanup (J. AOAC Int., 84(6):1828-1837, 2001)

2. Confirmation of identity of fumonisin:

For compliance samples, confirmation is to be performed by mass spectrometry using the most convenient technique. An example of an acceptable procedure is:

Musser, S.M., Eppley, R.M., Trucksess, M.W. (2002) "Electrospray Mass Spectrometry for Fumonisin Detection and Method Validation" in *Mycotoxins and Food Safety (Advances in Experimental Medicine and Biology, volume 504)*, Devries, J.W., Trucksess, M.W., Jackson, L.S. (eds), Kluwer Academic/Plenum Publishers, New York, pg. 95-105.

3. Reporting:

The analyzing district will report analytical results into FACTS using PAF = "MYC" and the correct Mycotoxin Code for the mycotoxins analyzed for selected from the FACTS drop-down box.

When reporting results for fumonisin, lab findings in the original analysis above the levels noted below for fumonisin require that the laboratory performs a check analysis and confirmation. If the findings are confirmed, the complete analytical packet should then be forwarded to the district compliance branch in order for the home district compliance branch to prepare a regulatory recommendation.

The following levels for fumonisins (FB₁+FB₂+FB₃) in foods have been established.

Levels for Fumonisin in Foods	Total Fumonisin (FB ₁ +FB ₂ +FB ₃)
Degermed dry milled corn product	2 ppm
Whole/partly degermed dry milled corn product	4 ppm
Dry milled corn bran	4 ppm
Cleaned corn intended for popcorn	3 ppm
Cleaned corn for masa production	4 ppm

Note: These levels are more commonly referred to as micrograms per gram but because limitations in electronic transmission cause the symbol for microgram to be

distorted or omitted, the ppm unit of measurement is being used.

C. Ochratoxin A Analyses

1. Method of analysis:

Section 49.6.03 (AOAC Method 991.44)- Ochratoxin A in Corn and Barley. The method was published in JAOAC 79:1102 1996. This method was modified and a copy of the modified method was supplied to the PRL-NW and to the KAN-DO lab.

Section 49.6.04(AOAC method 2000.03)- Ochratoxin A in barley, immunoaffinity column/LC method.

2. Confirmation of identity of ochratoxin A:

For compliance samples, confirmation is to be performed by mass spectrometry using the most convenient techniques. Examples of two acceptable approaches are:

Section 49.6.03 (AOAC Method 991.44) part J. Lau, B.P.-Y., Scott, P.M., Lewis, D.A. & Kanhere, S.R. (1999)"Quantitative determination of ochratoxin A by liquid chromatography/electrospray tandem mass spectrometry', J.Mass. Spectrom. **34**, 23-32.

Lombaert, G.A., Pellaers, P., Chettiar, M., Lavalee, D., Scott, P.M. & Lau, B.P.-Y (2002)"Survey of Canadian retail coffees for ochratoxin A', Food Addit. Contamin. **19**, 869-877.

3. Reporting:

The analyzing district will report analytical results into FACTS using PAF = "MYC" and the correct Mycotoxin Code **for the mycotoxins analyzed for** selected from the FACTS drop-down box.

Lab findings at or below 20 ppb require no further action. Lab findings above 20 ppb for ochratoxin A should be sent to the mycotoxin program monitor via email for evaluation. Complete details on the product type, form and intended use must be provided in order for CFSAN to adequately evaluate the significance of these findings.

PART V - REGULATORY/ADMINISTRATIVE STRATEGY

In general, refer to the Compliance Policy Guides (CPG's), (http://www.fda.gov/ora/compliance_ref/cpg/cpgfod/default.htm) and Import Alerts (<http://alpha.ora.fda.gov/fiars/>), which provide specific call-in on criteria for regulatory action. In addition to those resources, criteria and actions relevant to this compliance programs are listed below.

A. Aflatoxin

IOM Sample Schedule Chart 6 provides instructions on sample sizes for Initial/surveillance and follow-up sampling for aflatoxins. A Surveillance sample is a sample collected in reduced amounts (to lower costs) to determine if there is an aflatoxin problem with the product. This sample is not large enough to be considered representative of the lot, and, therefore a follow-up sample must be collected for regulatory purposes. See Sample Reporting section below for additional instructions.

The following Compliance Policy Guides (available online at http://www.fda.gov/ora/compliance_ref/cpg/default.htm) are applicable when recommending legal actions against products collected under this program:

- Section 527.400 **Whole Milk, Low Fat Milk, Skim Milk - Aflatoxin M₁** (CPG 7106.10)
http://www.fda.gov/ora/compliance_ref/cpg/cpgfod/cpg527-400.html.
- Section 570.200 **Brazil Nuts - Adulteration with Aflatoxin** (CPG 7112.07)
http://www.fda.gov/ora/compliance_ref/cpg/cpgfod/cpg570-200.html.
- Section 570.375 **Aflatoxin in Peanuts and Peanut Products** (CPG 7112.02)
http://www.fda.gov/ora/compliance_ref/cpg/cpgfod/cpg570-375.html.
- Section 570.500 **Pistachio Nuts - Aflatoxin Adulteration** (CPG 7112.08)
http://www.fda.gov/ora/compliance_ref/cpg/cpgfod/cpg570-500.html.
- Section 555.400 **Foods, Adulteration with Aflatoxin** (CPG 7120.26)

The following MOUs with USDA are in effect:

Peanuts and Peanut Products:	225-96-2001
Brazil Nuts:	225-96-2002
Pistachio Nuts:	225-96-2003

Complete copies of the MOUs can be obtained by contacting the Division of Compliance Policy, Office of Enforcement, HFC-230, at (240) 632-6860.

Immediately notify the Office of International Affairs, HFG-1, at (301) 827-4480, when informed of export lots of corn identified by USDA as appearing to be actionable, so that appropriate follow-up can be initiated.

Import Products Only

In accordance with CPG 7120.26, when original analyses show total aflatoxin levels at or above 100 ppb, lots of food products may be held or detained pending check and confirmatory analyses.

Domestic Products Only

The Home District must report analytical results on compliance samples that exceed CPG limits to the responsible firm and to cooperating State officials within their Districts.

When milk samples exceed the CPG limits for aflatoxin M₁, dairy feed should be sampled under the appropriate Center for Veterinary Medicine (CVM) programs to determine the source of the contamination.

Initiate appropriate followup action consistent with CPG 527.400, for dairy products under this compliance program, and for dairy feed using the instructions contained in the appropriate CVM compliance program #.

If the district has a situation in which an aflatoxin reconditioning is required, please contact Chief of the Domestic Branch in the Office of Compliance, Division of Enforcement, CFSAN, at 301-436-1611.

Districts with recurrent aflatoxin problems within their boundaries should consider conducting aflatoxin control workshops. Assistance (workshop materials, speakers on selected topics, etc.) in developing such programs is available through CFSAN Industry Activities Staff, HFS-565, (301) 436-2600.

B. Patulin

Consult the following Compliance Policy Guide when recommending followup actions for products collected under this program:

Section 510.150: Apple Juice, Apple Juice Concentrates, and Apple Juice Products - Adulteration with Patulin

Identity of patulin must be confirmed by gas chromatography/mass spectrometry for all domestic and import samples.

Mycotoxins Not Addressed in Compliance Policy Guides

C. Fumonisin, Deoxynivalenol, Ochratoxin A

When reporting results for fumonisin, lab findings in the original analysis above the level noted in part IV of the program for fumonisin require that the laboratory perform a check analysis and confirmation. If the findings are confirmed, the complete analytical packet should then be forwarded to the district compliance branch in order for the home district compliance branch to prepare a regulatory recommendation for submission to the Division of Enforcement, Office of Compliance at CFSAN, through the Center's Case Management System. For imported products, districts should prepare a recommendation for Detention Without Physical Examination for future shipments of the product and submit to ORA/Division of Import Operations for Review.

Lab findings above the levels noted in the program for DON and ochratoxin should be sent to the mycotoxin program monitor via email for evaluation. The mycotoxin program monitor will provide the information to the Office of Plant and Dairy Foods and the Division of Enforcement who will jointly determine whether the results warrant a check and confirmatory analysis to support a potential follow-up action. Lab findings at or below levels stated in the compliance program require no further action.

D. Imports

If the district encounters problem importers, refer to Chapter 9 of the Regulatory Procedures Manual (RPM) that contains a section on Priority Enforcement Strategy for Problem Importers (http://www.fda.gov/ora/compliance_ref/rpm_new2/ch9strat.html). The Center intends to focus enforcement efforts on problem importers to assure they assume appropriate responsibility for the commodities they import. The Center will routinely review import data to identify problem importers that may warrant increased observation and firm-based enforcement. CFSAN will consider field assignments to conduct additional sampling and analyses to meet detention criteria for Detention Without Physical Examination (DWPE) actions. See the RPM (http://www.fda.gov/ora/compliance_ref/rpm/) for DWPE criteria and procedures.

The field import compliance or inspection branches should contact CFSAN's Office of Compliance, Division of Enforcement when they encounter such situations in their district.

PART VI - ATTACHMENTS, REFERENCES, AND PROGRAM CONTACTS**ATTACHMENTS**

Attachment A - Mycotoxin Products for Sampling and Acceptable Product Codes

PROGRAM CONTACTS

Center Compliance Program Inquiries - Monali Yajnik, Office of Compliance, Division of Field Programs, Compliance Programs Branch, HFS-636, (301) 436-1616 FAX (301) 436-2657. In her absence, contact Brenda Aloji, Office of Compliance, Division of Field Programs, Compliance Programs Branch, HFS-636, (301) 436-2065 FAX (301) 426-2657.

Center Scientific Inquiries - Dr. Garnett Wood, Office of Plant and Dairy Foods, Division of Plant Product Safety, Implementation and Compliance Branch, HFS-305, (301) 436-1942 FAX (301) 436-2632

Center Analytical Inquiries - Henry Njapau, Office of Plant and Dairy Foods, Division of Natural Products, HFS-346, (301) 436-2043, FAX (301) 436-2644

Center Domestic Regulatory Inquiries - Dr. Donald Greaves, Office of Compliance, Division of Enforcement, Domestic Branch, HFS-607, (301) 436-2361

Center Import Regulatory Inquiries - Sandra Purnell, Office of Compliance, Division of Enforcement, Imports Branch, HFS-606, (301) 436-1613, FAX (301) 436-2657

ORA Import Investigation Inquiries - Doug Randes, Office of Regulatory Affairs, Division of Import Operations and Policy, HFC-171, (301) 594-3854

ORA Investigations Inquiries - Barbara Marcelletti, ORA, Division of Field Investigations, HFC-132, (301) 827-5635

ORA Analytical Inquiries - Division of Field Science, George Salem, HFC-141, (301) 827-1031

ADDITIONAL REFERENCES

1. U.S. Food and Drug Administration, Compliance Policy Guide Sections 675.300, Moisture Damaged Grain, (CPG 7126.10) (http://www.fda.gov/ora/compliance_ref/cpg/cpgvet/cpg675-300.html), 675.400, Rendered Animal Feed Ingredients, (CPG 7126.24) (http://www.fda.gov/ora/compliance_ref/cpg/cpgvet/cpg675-400.html), and 683.100, Action Levels for Aflatoxins in Animal Feeds, (CPG 7126.33) (http://www.fda.gov/ora/compliance_ref/cpg/cpgvet/cpg683-100.html)
2. Stoloff, L., and Dalrymple, B., Aflatoxin and Zearalenone Occurrence in Dry Milled Corn Products. JAOAC 60:579-582 (1977).
3. Brumley, W.C., et. al., "Negative Ion Chemical Ionization Mass Spectrometry of Aflatoxins and Related Mycotoxins", Anal. Chem. 53:2003, 1981.
4. Nesheim, S., and Brumley, W.C., "Confirmation of Identity of Aflatoxins", JAOCS 58: No. 12, 945a-949a, 1981.

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5. LMS Method - Code 050 (For Milk only).
 6. DEIO Guide to Inspections of Manufacturers of Miscellaneous Food Products, Volume II, issued September 1996.
 7. Letter to State Agricultural Directors, State Feed Control Officials and Food, Feed and Grain Trade Organizations from R. G. Chesemore, Associate Commissioner for Regulatory Affairs, FDA, dated September 16, 1993.

PART VII - CENTER RESPONSIBILITIES

PROGRAM EVALUATIONS

The Director, Office of Plant and Dairy Foods (OPDF) has the responsibility to prepare periodic formal evaluations of this compliance program. &

MYCOTOXIN PRODUCTS FOR SAMPLING AND ACCEPTABLE PRODUCT CODESFOODS FOR HUMAN USE ONLYDomestic and Import Products**Section 1 -- Aflatoxins**

<u>PRODUCT</u>	<u>PRODUCT CODES</u>
Corn, shelled, whole kernel	02A[][]01
Corn Meal or Flour(including Hominy Grits)	02B[][]01 to 99
Cereals, Corn (including Ready to Eat, Quick Cook, Instant, and Grits)	05A or B[][]01
Hominy	24A[][]61
Snack Foods ¹	07A or B[][]02
Peanut Butter	23C[][]07
Peanut Products, imitation	23N[][]01
Peanuts, in shell, roasted	23A[]H07
Peanuts, shelled roasted	23B[]H07
Peanuts, toppings	23F[][]07
Peanuts, in shell, raw (follow-up samples only)	23A[]B07
Peanuts, shelled, raw (follow-up samples only)	23B[]B07
Almonds	23A to F[][]01
Macadamia	23B to F[][]18
Pecans	23A to F[][]08
Walnuts	23A to F[][]12
Pistachios	23A to F[][]11
Coconut meal	23A,Y[][]99
Cotton seed	23Y[][]99
Cotton seed meal	23Y[][]99
Nut and Edible seeds n.e.c	23A,Y()()99
Pumpkin seeds	23K()()01
Brazil Nuts	23A to F()()02
Pine nuts/pinon	23A to F ()()09
Sunflower seeds	23K ()()04
Melon seeds	23K ()()05
Ginger	28A to B ()()19
Hazelnut	23A to F ()()06
Rice, cultivate, whole grain	02A[]05
Rice, wild, whole grain	02A[]06

¹ Miscellaneous corn-based foods such as tacos, chips, cereals and similar products that are primarily corn are acceptable for collection. It is, however, preferable to collect the corn ingredients that will be used in these foods.

NOTE: Nut pastes and similar ethnic foods containing nuts are susceptible to aflatoxin contamination and may be sampled.

Refer to PART III, section B (f) before collecting milk or milk products.

<u>PRODUCT</u>	<u>PRODUCT CODES</u>
Milk, fluid	09C to E[][]01, 07, 09 to 11
Milk, concentrated	09C to E[][]03
Milk, nonfat dried	09C to E[][]16
Yogurt	09C to E[][]15
Cheese, natural	12A[][]01 to 61
Cheese products	12B[][]01 to 13, and 99
Frozen milk products	13A to G and Y[][]01 to 06, and 99

Section 1a - "SPECIAL SURVEILLANCE" PRODUCTS FOR AFLATOXIN ANALYSIS

<u>PRODUCT</u>	<u>PRODUCT CODE</u>
Sesame Seed	23K()()02
Nutmeg	28A or B()()30
Figs	21S or T()()03

Cashews should no longer be collected for surveillance sampling for aflatoxin.

Section 2 -- Patulin

<u>PRODUCT</u>	<u>PRODUCT CODES</u>
Apple Juice and Apple Juice Concentrate	20S()() 01

Section 3 -- Deoxynivalenol

<u>PRODUCT</u>	<u>PRODUCT CODES</u>
Whole wheat or white flour	02E[]() 01
Wheat Bran <u>for human consumption</u>	02F()() 01

Section 4 -- Fumonisin

PRODUCT	PRODUCT CODES
Popcorn (unpopped)	02A()() 04
Cleaned corn intended for masa production	02A()() 04
Dry milled corn bran	02B()() 99

Refer to Part III, 4.c. regarding the need to determine the fat content before collecting these products:

Corn flour	02B()() 01
Corn meal	02B()() 01 thru 99
Corn grits	02B()() 06
Flaking grits	02B()()()06

Section 5 -- Ochratoxin A

PRODUCT	PRODUCT CODES
Barley (whole)	02A()() 02
Barley Malt	02J()() 01
Baby Cereals (barley, corn, oat, rice, wheat)	40B()() 01 to 06
Cereals (barley)	05A or B()() 99
Cereals, (corn) (including Ready to Eat, Quick Cook, Instant, and Grits)	05A or B[[]]01
Cereals (oat)	05A or B()() 02
Cereals (wheat)	05A or B()() 04
Cereals (rice)	05A or B()()03
Coffee Beans	31A()()01
Corn Meal (including hominy grits)	02B()() 01 thru 99
Dried Beans/Peas	24B()() 02 thru 99
Oats (whole)	02A()() 03
Raisins	20B()() 10
Rye Flour	02G()() 10
Soya Based Baby Food Products	40A or B ()() 99, 40C()() 25, 40Y()() 99
Wheat kernels (whole)	02A()() 09
Whole wheat or white flour	02E()() 01