

CHAPTER 07 - MOLECULAR BIOLOGY AND NATURAL TOXINS

<b>SUBJECT:</b>  MYCOTOXINS IN DOMESTIC FOODS FY 02/03/04	<b>IMPLEMENTATION DATE</b>  October 1, 2001
	<b>COMPLETION DATE</b>  September 30, 2004
<b>DATA REPORTING</b>	
<b>PRODUCT CODES</b>	<b>PRODUCT/ASSIGNMENT CODES</b>
See Attachment A	07001 All sample collections and analysis

**FIELD REPORTS TO HEADQUARTERS**

1. Report all collections/analyses of domestic **food** samples for \*aflatoxin, patulin, deoxynivalenol, fumonisin, or ochratoxin A\* contamination against this program even though the samples were collected during inspections scheduled under other compliance programs.
2. The analyzing district will report analytical results into FACTS using the PAF = "MYC" and insure that the correct Mycotoxin Code **for the mycotoxins analyzed for** is used.
3. \* When entering information into FACTS, use operation code 31 to report domestic sample collection and operation code 41 to report domestic sample analysis. \*

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. Under section 402(a)1 of the Federal Food, Drug and Cosmetic Act, a food is deemed adulterated if it contains poisonous or deleterious substance, such as mycotoxins, which may render it injurious to health. Mycotoxins can be considered added poisonous or deleterious substances because their presence in human food and/or animal feeds can be avoided in part by good agronomic and manufacturing practices. Strategies used by the Food and Drug Administration (FDA) to minimize mycotoxins in the U.S. food supply include establishing guidelines (e.g., action levels, guidance levels), monitoring the food supply, through formal compliance programs (domestic and import) and **taking regulatory action against product that exceeds action levels, where action levels have been established**. The monitoring data obtained over the years from FDA's monitoring programs is 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 governmental 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 serves 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 action level for total aflatoxins in human food is 20 micrograms per kilogram (20 ppb); the action level for aflatoxin M<sub>1</sub> in milk is 0.5 micrograms per kilogram (0.5

- ppb).
2. **Patulin** is a toxic substance produced by *Penicillium*, *Aspergillus*, and *Byssochylamys* 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 an action level of 50 micrograms per kilogram (50 ppb) for patulin in apple juice and in the apple juice component of a food that contains apple juice as an ingredient. The action level 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 set to take effect from 2002-2004, 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 motivated the FDA to issue guidance 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. The FDA has established a guidance level of 1 microgram per gram (1 ppm) for deoxynivalenol in finished wheat products that may be consumed by humans. No guidance level has been established for raw wheat intended for milling into human food products.
  4. **Fumonisin** (Fumonisin FB<sub>1</sub> and Fumonisin FB<sub>2</sub>) are natural toxins produced by *Fusarium verticillioides* (previously known as *F. moniliforme*), and other *Fusarium* species; these molds are common natural contaminants of corn. Fumonisin have been linked to fatalities in horses and swine. Recent studies have demonstrated the presence of fumonisin 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 FB<sub>1</sub> have highlighted the need to ensure that foods do not contain excessive amounts of fumonisin. Dry milling of whole corn kernel generally results in the production of fractions called bran, flaking grits, grits, meal, and flour. Because fumonisin are concentrated in the germ and the hull of the whole corn kernel, dry milling results in fractions with different concentrations of fumonisin. 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.

The FDA has established the following guidance levels for fumonisins (FB<sub>1</sub>+FB<sub>2</sub>+FB<sub>3</sub>) in foods.

Guidance Levels for Fumonisins in Foods	<u>Total Fumonisins</u> (FB <sub>1</sub> +FB <sub>2</sub> +FB <sub>3</sub> )
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.

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 current 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. The recommended analytical method that will be used has a lower limit of quantitation than methods employed in previous field assignments.

**C. Products that are to be sampled include:**

1. **\*Apple Juice and Apple Juice Concentrate**

Apple juice and apple juice concentrate are to be collected for patulin analysis. If samples are collected in conjunction with the inspection of an apple juice processor, 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, 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 from the tree and are harvested from the ground, (also known as grounders, windfalls or ground fruit) to produce juice. \*

2. **Corn and Corn Products**

Historically, aflatoxin levels in 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.

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.

FDA samples of fresh, frozen and/or canned sweet corn have been analyzed for the presence of aflatoxin and no violative samples found in the past. Therefore, this program does not call for sampling of sweet corn for aflatoxin analysis.

\*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.

The corn meal and corn based cereal products listed above will also be collected and analyzed for ochratoxin A.

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. \*

### 3. **Milk**

When dairy animals consume feed containing high levels of aflatoxin, one of the metabolized aflatoxins (aflatoxin B<sub>1</sub>) may be secreted into the animals' milk as aflatoxin M<sub>1</sub>. Cattle consuming feed that contains less than 20 ppb of total aflatoxins, however, should produce milk that complies with FDA's guideline of 0.5 ppb for aflatoxin M<sub>1</sub> in milk.

### 4. **Peanuts**

**Raw:** \* Testing of raw peanuts, domestic and imports, for aflatoxins is conducted by USDA in accordance with the FDA/USDA MOU (See Sec. 570.375 CPG 7112.02). \*

**Processed:** 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 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.

### 5. **Other Nuts**

Almonds, macadamia pecans, pistachios, and walnuts are susceptible to aflatoxin contamination, but samples of these domestic 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.

6. **Wheat Products**

Milled wheat products (whole wheat flour, white flour and bran) will be collected at wheat product manufacturers. 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. All of these samples will be analyzed for deoxynivalenol.

7. **Other Products**

Rye flour, wheat flour, barley (cereals), oats (whole/cereals) dried beans, soya flour and soya based baby foods will be collected and analyzed for ochratoxin A.

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PART II - IMPLEMENTATION

OBJECTIVES

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

To remove from interstate commerce those foods that contain aflatoxins \*or patulin\* at levels judged to be of regulatory significance;

\*To provide a database to support FDA's positions in international activities concerning mycotoxins. \*

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, (303) 443-3360.

PART III - INSPECTIONAL

## A. Inspectional

This program does not direct inspections. However, time is allocated for follow-up inspections if violative levels of aflatoxin \*or patulin\* are detected in regulatory (not \*surveillance\*) samples.

Inspectional guidance for mycotoxin inspections is contained in Section 8 of DEIO Guide to Inspections of Manufactures of Miscellaneous Food Products Volume II.

## B. Sampling

General Guidance:

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

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 end up yielding the requested analyte/product combinations. \*

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.

If not referenced below, additional sample sizes are referenced in the Investigations Operations Manual (IOM), Chapter 4, Sample Schedule Chart 6. Collect samples randomly so as to be representative of the lot. Sample only the foods listed in Attachment A. Except for patulin analysis and except for the number of surveillance subsamples and size of surveillance subsamples (as listed in sections 3, 4 and 5 below) the information in Sample Schedule Chart 6 should be followed.

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 Mycotoxin Monitor, Robyn Jones, at (301) 436-2575. If she cannot be reached and an immediate answer is needed, contact Dr. Garnett Wood at (301) 436-1942, and notify Robyn Jones by e-mail. \*

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 427.4g; Blacklight Test Screening Procedure for Aflatoxins in Corn, for guidance on procedure.

- b. 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.
- c. 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.
- d. Do not sample raw peanuts except as a follow-up to violative levels of aflatoxins in a finished product or when raw peanuts are offered directly to consumers. This is in accordance with the FDA/USDA MOU. (Sec. 570.375 CPG 7112.02).
- e. Do not sample popcorn 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.
- f. Sample milk for aflatoxin M<sub>1</sub> 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 violative levels of aflatoxin M<sub>1</sub>.

- g. When collecting a follow-up regulatory sample to a positive \*surveillance\* aflatoxin sample, record the sample number and ppb findings for the \*surveillance\* in the "Remarks" section of the new C/R.

## 2. Patulin

- a. See Attachment A, Section 2. **Only** collect apple juice and/or concentrated apple juice. 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 the juice, both pasteurized and non-pasteurized single strength juice

and concentrated juices are to be collected.

If samples are collected in conjunction with the inspection of an apple juice processor, 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, 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.

- b. Surveillance sample size is dependent on product form.

Frozen Concentrate: Collect six subsamples with a minimum 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. When collecting a regulatory sample as a follow-up to a positive surveillance patulin sample, record the sample number and ppb findings for the surveillance in the "Remarks" section of the new C/R.

- d. Regulatory sample size is dependent on product form

Frozen Concentrate: Collect six subsamples with a minimum 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.

If retail packaged samples are collected, then separate 702(b) samples must also be collected.

3. Surveillance Sampling for **Deoxynivalenol (DON)**

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

Surveillance samples will consist of four (4) 450 gram (approximately 1 pound) subsamples to be collected from a single lot of product.

4. Surveillance Sampling for **Fumonisin FB<sub>1</sub> and FB<sub>2</sub>**

See Attachment A, Section 3 for products to be collected.

\* For data base requirements, CFSAN needs approximately half of the samples to be degermed, i.e., low fat. Therefore, the investigator should try to collect half of samples of 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. \*

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

5. Surveillance Sampling for **Ochratoxin A**

See Attachment A, Section 5 for products to be collected. For cereals and soya based baby food products, collect 4 (four) 200 gram (Approximately 8 ounces) subsamples per sample, for all other products collect \*4 (four)\* 450 gram (approximately one pound) subsamples per sample.

C. 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. Refer to IOM, Chapter 4.

D. Sample Submission

Each FY, consult the ORA Field Work Plan, Part 1, Appendix 3 to determine where all mycotoxin samples should be sent. At the time this program is being written, the following analyzing laboratories are correct; however, if there is a conflict after the work plan is issued, the workplan is the lead document.

1. All regions should submit samples for \*surveillance and regulatory\* **afatoxin analysis** to SRL at the following address:

U. S. Food and Drug Administration  
Southeast Regional Laboratory (HFR-SE600)  
60 Eighth Street, NE  
Atlanta, GA 30309

2. All regions should submit samples for **fumonisin analysis** to the KAN-DO Lab at the following address:

U. S. Food and Drug Administration  
Kansas City Laboratory (HFR-SW360)  
11630 West 80th Street  
Lenexa, KS 66214-3338

3. All regions should submit samples for **deoxynivalenol, ochratoxin, and patulin analysis** as per the following table:

Region	Deoxynivalenol laboratory	ochratoxin laboratory	patulin laboratory
Northeast	NRL	NRL	NRL
Southeast	SRL	KAN	SRL
Central (BLT, CIN, NWJ, PHI)	NRL	NRL	NRL
Central (CHI, DET, MIN)	SRL	KAN	SRL
Southwest	KAN	KAN	KAN
Pacific	PRL-NW	PRL-NW	PRL-NW

PART IV - ANALYTICALA. Analyzing Laboratories:

Each FY, consult the current year ORA Field Work Plan, Part 1, Appendix 3 to determine where aflatoxin, fumonisin, deoxynivalenol, ochratoxin A and patulin surveillance samples will be analyzed. At the time this document is being written, the following information is current. If when this information differs from that published in the ORA Field Work Plan, the Field Work Plan is the lead document.

Laboratory	Analyzes for:
Kansas City District Lab (KAN)	Fumonisin, DON, Ochratoxin, Patulin
Northeast Regional Lab (NRL)	Aflatoxin, DON, Ochratoxin, Patulin
Pacific Regional Lab-NW (PRL-NW)	DON, Ochratoxin, Patulin
Southeast Regional Lab (SRL)	Aflatoxin, DON, Ochratoxin, Patulin

B. Aflatoxin Analyses

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

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

1. Follow the procedures and methods in the 17<sup>th</sup> Edition 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 49.2.04 (AOAC Method 975.35) - Identification of aflatoxins by TLC - Alternative Developing Solvents
  - e. Section 49.2.08 (AOAC Method 968.22) - Method I (CB Method) for peanuts/peanut products (applicable to all nut products in this program)
  - f. Section 49.2.09 (AOAC Method 970.45) - (BF Method) - Aflatoxins in peanuts and peanut butter
  - g. Section 49.2.10 (AOAC Method 991.45) - Total aflatoxin levels in peanut butter
  - h. Section 49.2.11 (AOAC Method 971.23) - Aflatoxins in cocoa

beans

- i. Section 49.2.12 (AOAC Method 971.24) Aflatoxins in coconut, copra, and copra meal
- j. Section 49.2.15 (AOAC Method 993.17) - Aflatoxins in corn and peanuts
- k. Section 49.2.17 (AOAC Method 990.33) - Corn and peanut butter
- l. Section 49.2.18 (AOAC Method 991.31) - Corn, raw peanuts and peanut butter.
- m. Section 49.2.19 (AOAC Method 980.20) - Aflatoxins in cottonseed products
- n. Section 49.2.19A (AOAC Method 994.08) - Corn, almonds, Brazil nuts, peanuts, and pistachio nuts.
- o. Section 49.2.26 (AOAC Method 975.37) - Identification of the aflatoxin B<sub>1</sub> by derivative formation on TLC plate.
- p. Section 49.2.27 (AOAC Method 985.17) - Identification of aflatoxin B<sub>1</sub>, confirmation method
- q. \*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. \*
- r. Section 49.3.01 (AOAC Method 974.17) - Aflatoxins M<sub>1</sub> in dairy products
- s. Section 49.3.02 (AOAC Method 980.21) - Aflatoxin M<sub>1</sub> in milk and cheese; confirmation of identity by derivative formation on TLC plate

\*AOAC methods that are not in the 17<sup>th</sup> edition of Edition of the Official Methods of Analysis of the AOAC:

- t. AOAC Method 2000.16 Aflatoxin in baby food, immunoaffinity column cleanup and LC (J.AOAC Int. 2001 in press)
- u. AOAC 2000.18 Aflatoxin M<sub>1</sub> in liquid milk, immunoaffinity column/LC (Dragacci, S. Grosso, F., and Gilbert, J., "Immunoaffinity column cleanup with liquid chromatography for determination of aflatoxin M<sub>1</sub> in liquid milk: collaborative study", J. AOAC Int. 84: 437-443, 2001) \*

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

Do not conduct a check analysis on \*surveillance\* samples.

If any \*surveillance\* sample is positive (any level), use lab class "2" and immediately arrange for collection of a regulatory sample which is representative of the lot. \*Use class "1" for a surveillance sample only if \* no aflatoxin is detected.

## 2 Confirmation

Refer to Compliance Policy Guides for required confirmation of identity procedures for regulatory samples. 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 aflatoxin levels exceed the guideline in any other product:

- 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 either to NRL (HFR-NE580; 158-15 Liberty Avenue, Jamaica, NY 11433;(718) 622-5439).
- Note: At the option of the lab doing the initial analysis, that lab may also do the confirmation analysis instead of sending it to NRL. \*

Confirmation of identity will be by negative ion chemical ionization mass spectrometry as per B.1.s.above. In Part VI, references #3 and #4 pertain to this method.

## 3. Reporting

The analyzing district will report analytical results into FACTS using the LMS codes in the "LMS Code Manual". It is particularly important that all analysis be entered using the flag code "MYC" and that the correct Mycotoxin Code for the mycotoxins analyzed for be used.

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

C. Patulin Analyses

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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 17<sup>th</sup> Edition 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.

AOAC method that is not in the 17<sup>th</sup> edition of Edition of the Official Methods of Analysis of the AOAC:

AOAC method 2000.02 Patulin in clear and cloudy apple juices and apple puree (McDonald, S., Long, M., and Gilbert, J., "Liquid chromatographic method for determination of patulin in clear and cloudy apple juices and apple puree: collaborative study", J.AOAC Int. 83:1387-1394)

3. Confirmation of Identity of Patulin:

For regulatory samples, confirmation is to be done by mass spec. 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 LMS codes from the "LMS Code Manual". It is particularly important that all analysis be entered using the flag code "MYC" and that the correct Mycotoxin Code for the mycotoxins analyzed for be used.

D. Deoxynivalenol Analyses (DON)

The method of analysis is published in J AOAC Intl. 79(4):883-887, 1996. This is the same method used in previous deoxynivalenol field assignments.

The analyzing district will report analytical results into FACTS using LMS codes from the "LMS Code Manual". It is particularly important that all analysis be entered using the flag code "MYC" and that the

correct Mycotoxin Code for the mycotoxins analyzed for be entered into field position 25 as per Appendix I.

If DON is not found, the sample should be assigned a Lab Class "1". If it is found at any level, it should be assigned a Lab Class "2".

\*Notify the Domestic Mycotoxin Monitor, Robyn Jones by e-mail when the Deoxynivalenol guidance level of 1 ppm is exceeded. \*

E. Fumonisin Analyses

\*Follow the procedures and methods in the 17<sup>th</sup> Edition of the Official Methods of Analysis of the AOAC:

Section 49.5.01 \* AOAC Method 995.15 - Fumonisin FB<sub>1</sub>, FB<sub>2</sub>, and FB<sub>3</sub> in corn, liquid chromatographic method, AOAC-IUPAC method. This method was adopted by AOAC International in 1995, and is published in the J. AOAC Intl. 79(3): 688-696, 1996. 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 analyses.

\*AOAC method that is not in the 17<sup>th</sup> edition of Edition of the Official Methods of Analysis of the AOAC:

AOAC Method 2001.04 Determination of fumonisin FB<sub>1</sub> and FB<sub>2</sub> in flour and corn flakes by LC with immunoaffinity column cleanup(J. AOAC Int., in press 2001)\*

The analyzing district will report analytical results into FACTS using LMS codes from the "LMS Code Manual". It is particularly important that all analysis be entered using the flag code "MYC" and that the correct Mycotoxin Code for the mycotoxins analyzed for be used.

If Fumonisin is not found, the sample should be assigned a Lab Class "1". If it is found at any level, it should be assigned a Lab Class "2".

\*Notify the Domestic Mycotoxin Monitor, Robyn Jones by e-mail when the guidance levels (listed in Part I.B.4) for total fumonisins (FB<sub>1</sub> + FB<sub>2</sub> + FB<sub>3</sub>) are exceeded. \*

F. Ochratoxin A Analyses

The method of analysis is the 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 KAN, and SEA.

\* (AOAC method not in the 17<sup>th</sup> edition of Edition of the Official Methods of Analysis of the AOAC) AOAC method 2000.03 Ochratoxin A in barley, immunoaffinity column/LC (Entwisle, A.C., Williams, A.C., Mann, P.J., and Slack, P.T., "Liquid chromatographic method with immunoaffinity column cleanup for determination of ochratoxin A in barley: collaborative study", J. AOAC Int. 83:1376-1383, 2000) \*

The analyzing district will report analytical results into FACTS using LMS codes from the "LMS Code Manual". It is particularly important that all analysis be entered using the flag code "MYC" and that the correct Mycotoxin Code for the mycotoxins analyzed for be used.

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If ochratoxin is not found, the sample should be assigned a Lab Class "1". If it is found at any level, it should be assigned a Lab Class "2".

\*Notify the Domestic Mycotoxin Monitor, Robyn Jones by e-mail when an Ochratoxin A level greater than **4 ppb** is found in any commodity. A maximum level of 5 ppb has been proposed as an international standard by CODEX for ochratoxin A in wheat, barley, rye, and their derived products; therefore data is needed from this Compliance Program to support the U.S. position on this issue. \*

PART V - REGULATORY/ADMINISTRATIVE STRATEGY

## A. Aflatoxin

The following sections of the Compliance Policy Guides are applicable when recommending legal actions against products collected under this program:

- Section 527.400 **Whole Milk, Low Fat Milk, Skim Milk - Aflatoxin M<sub>1</sub>** (CPG 7106.10)  
Section 570.200 **Brazil Nuts - Adulteration with Aflatoxin** (CPG 7112.07)  
Section 570.375 **Aflatoxin in Peanuts and Peanut Products** (CPG 7112.02)  
Section 570.500 **Pistachio Nuts - Aflatoxin Adulteration** (CPG 7112.08)  
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 (301) 827-0420.\*

Mass spectral confirmation of aflatoxin identity is required for all domestic foods except peanuts/peanut products, corn/corn meal, pistachio nuts, pumpkin seeds, cottonseed/meal, coconut meal, and copra. Aflatoxin identity of all samples should be confirmed using AOAC method 975.37

The Home District must report analytical results on regulatory samples that are "out of compliance" (i.e., judged to be of regulatory significance) to the responsible firm and to cooperating State officials within their Districts.

When milk samples contain more than 0.5 ppb of aflatoxin M<sub>1</sub>, dairy feed should be sampled under the appropriate Center for Veterinary Medicine (CVM) programs to determine the source of the contamination. Initiate appropriate legal action against dairy products under this program, and against feed under CVM's instructions.

FDA has agreed that aflatoxin contaminated peanuts may be processed into oil since normal refining effectively removes aflatoxins present in the crude oil. The residual meal cannot be used for domestic food or feed unless shown by analysis to be at a level for aflatoxins that is not of regulatory significance.

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.

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 Industry Activities Staff, HFS-565, (202) 205-5251.

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B. Patulin

\*The following Compliance Policy Guide is applicable when recommending legal actions against products collected under this program:

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

Mass spectral confirmation of patulin identity is required for all domestic and import samples.

C. **Fumonisin, Deoxynivalenol, Ochratoxin A**

CFSAN will use the data on the incidence and level of these mycotoxins in various commodities in conjunction with toxicological data:

- to access the need for guidance and/or enforcement levels;
- to conduct risk assessment;
- to address international concerns;
- to initiate discussions with industry;
- and to conduct any follow up action necessary for Public Health Protection. \*

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PART VI - ATTACHMENTS, REFERENCES, AND PROGRAM CONTACTS

ATTACHMENTS

Attachment A - Mycotoxin Products for Sampling and Acceptable Product Codes

PROGRAM CONTACTS

Center Compliance Program Inquiries - Robyn Jones, Office of Compliance, Division of Field Programs, Compliance Programs Branch, HFS-636, (301) 436-2575 FAX (301) 436-2657

Center Scientific Inquiries - Dr. Garnett Wood, Office of Plant and Dairy Foods and Beverages, Division of Risk Assessment, Exposure Assessment Branch, HFS-308, (301) 436-1942 FAX (301) 436-2632

\*Center Analytical Inquiries - Dr. Mary W. Trucksess, Office of Plant and Dairy Foods and Beverages, Division of Natural Products, HFS-346, (301) 436-1957, FAX (301) 436-2644

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

ORA Investigations Inquiries - Barbara Marcelletti, DEIO, Investigations Branch, 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, 675.400, and 683.100 - formerly CPG Sections 7126.10, 7126.25, and 7126.33.
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.
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. Chesmore, Associate Commissioner to Regulatory Affairs, FDA dated September 16, 1993.

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PART VII - CENTER RESPONSIBILITIES

Program Evaluation: During the course of this program, but no later than thirty (30) days after the expected date of final data receipt, the Director, Office of Plant and Dairy Foods and Beverages will identify any deficiencies in the conduct of field operations or program quality to the Division of Enforcement and Programs, HFS-605, so that any necessary corrective action may be initiated.

The Director, Office of Plant and Dairy Foods and Beverages will forward an evaluation of this program to the Division of Enforcement and Programs, HFS-605, within 12 months of program completion.

FACTS data concerning aflatoxins in foods and in feeds will be evaluated in conjunction with this Compliance Program. The Director, Office of Plant and Dairy Foods and Beverages will consult with the Division of Voluntary Compliance and Hearings, Programs and Hearings Branch of the Center for Veterinary Medicine (HFV-242) during the evaluation process.

MYCOTOXIN PRODUCTS FOR SAMPLING AND ACCEPTABLE PRODUCT CODESFOODS FOR HUMAN USE ONLY**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 <sup>1</sup>	07A or B[[]]02
Peanut Butter	23C[[]]07
Peanut Products, imitation	23N[[]]01
Peanuts, in shell, roasted	23A[ ]G07 and H07
Peanuts, shelled roasted	23B[ ]G07 and H07
Peanuts, toppings	23F[[]]07
Peanuts, in shell, raw (follow-up samples only)	23A[ ]A07 and B07
Peanuts, shelled, raw (follow-up samples only)	23B[ ]A07 and B07
Almonds	3A to F[[]]01
Macadamia	18A to F[[]]99
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
Pumpkin seeds	23K[[]]01

<sup>1</sup> 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.

Refer to PART III, B, paragraph 6 before collecting milk or milk products.

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 2 -- Patulin**

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

MYCOTOXIN PRODUCTS FOR SAMPLING AND ACCEPTABLE PRODUCT CODESFOODS FOR HUMAN USE ONLY**Section 3 -- Fumonisin**

<u>PRODUCT</u>	<u>PRODUCT CODES</u>
Popcorn (unpopped) see Part III, 1,e	02A()() 04
*Cleansed corn intended for masa production	02A()() 04
Dry milled corn bran	02B()() 99

Refer to Part III, B, 4 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()() 01 *

**Section 4 -- 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 5 -- Ochratoxin A**

<u>PRODUCT</u>	<u>PRODUCT CODES</u>
Barley (whole)	02A()() 02
Barley Malt	02J()() 01
Baby Cereals (barley, corn, oat, 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
Corn Meal (including hominy grits)	02B()() 01 thru 99
Dried Beans	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