

## **LABORATORY APPROVAL PROGRAM - MYCOTOXINS**

### **1. Purpose**

The purpose of this document is to outline the requirements of the Laboratory Approval Program for Analysis of Mycotoxins (LAP-Mycotoxins) by the Laboratory Approval Service (LAS). The document describes the technical competency and quality management requirements a laboratory must demonstrate to be a USDA-approved laboratory.

LAPs are developed at the request of industry, other Federal agencies, or foreign governments in support of domestic and international trade. LAS approves, or accredits, laboratories to perform testing services to verify that the analysis of food and agricultural products meet country or customer specified requirements and that the testing of products marketed is conducted by qualified and approved laboratories.

LAS is part of the Agricultural Marketing Service ([AMS](#)), Science and Technology ([S&T](#)) Program, Laboratory Approval and Testing Division ([LATD](#)).

### **2. Scope**

The provisions of this document apply to a laboratory seeking to obtain and maintain its status as a USDA-approved laboratory for the analysis of mycotoxins in almonds, pistachios, and/or peanuts based on the stipulations of the final market destination: U.S. domestic, U.S. import, and export. This Program verifies technical and quality control competencies of a laboratory for the analysis of mycotoxins. All aspects of a laboratory's quality management system (business processes relative to the scope of approval) are applicable and are critical for ensuring the defensibility of the analytical results produced under the LAP.

- 2.1. Almonds for aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>) (AFL) for export to the European Union (EU) for the Almond Board of California ([ABC](#)) Pre-Export Certification program ([PEC](#)).
- 2.2. Almonds for aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>) (AFL) for export to Japan in accordance with Protocol for the Control of Aflatoxin in U.S. Almonds Exported to Japan between the U.S. Department of Agriculture (USDA) and the Ministry of Health, Labour and Welfare (MHLW).
- 2.3. Peanuts for aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>) marketed domestically for human consumption, including imports, in accordance with [7 CFR 996](#).
- 2.4. Peanuts for aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>) for export to the EU for the American Peanut Council ([APC](#)) in accordance with the [APC Sheller MOU and Peanut Export Program \(PEP\)](#).
- 2.5. Pistachios for aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>) for domestic and import markets in accordance with [7 CFR 983](#) and [7 CFR §999.600](#), respectively.

- 2.6. Pistachios for aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>) for export to the EU for the Administrative Committee for Pistachios ([ACP](#)) Pistachio Export Aflatoxin Reporting ([PEAR](#)) program.
- 2.7. Pistachios for ochratoxin A (OTA) for export to the EU for the [ACP PEAR](#) program.

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Table 2. Performance criteria for ochratoxin A. Criteria applies to all concentrations\* ..... 12

### 4. **Glossary of Terms**

ABC                      Almond Board of California



ACP	Administrative Committee for Pistachios
AFL	Aflatoxins (B <sub>1</sub> , B <sub>2</sub> , G <sub>1</sub> , G <sub>2</sub> )
AMS	Agricultural Marketing Service
AOAC	Association of Official Analytical Collaboration International
AOCS	American Oil Chemists' Society
APC	American Peanut Council
CFR	Code of Federal Regulations
CCV	Continuing Calibration Verification
CV	Coefficient of Variation
EC	European Commission
EU	European Union
FAPAS	Food Analysis Performance Assessment Scheme
FDA	Food and Drug Administration
FLD	Fluorescence Detector
HPLC	High Performance Liquid Chromatography
HS	Harmonized System
IAC	Immunoaffinity Column
ICV	Independent Calibration Verification
ISO/IEC	International Organization for Standardization/International Electrotechnical Commission
LAP	Laboratory Approval Program
LAP-Mycotoxins	LAP for Analysis of Mycotoxins
LAS	Laboratory Approval Service
LATD	Laboratory Approval and Testing Division
LC	Liquid Chromatography (e.g., HPLC, UPLC)
LOQ	Limit of Quantification
MHLW	Ministry of Health, Labour, and Welfare, Japan
ML	Maximum Level
MOU	Memorandum of Understanding
MU	Measurement Uncertainty
OTA	Ochratoxin A
PEAR	Pistachio Export Aflatoxin Reporting Program
PEC	Pre-Export Certification
PEP	Peanut Export Program
PHRED	Photochemical Reactor Enhanced Detection
PM	Program Manager
PT	Proficiency Testing
S&T	Science & Technology Program
RSD <sub>r</sub>	Repeatability Relative Standard Deviation
RSD <sub>WR</sub>	Within-laboratory Reproducibility Standard Deviation
TLC	Thin Layer Chromatography
UPLC	Ultra (High) Performance Liquid Chromatography
USDA	United States Department of Agriculture

VCM

Vertical Cutter Mill

## **5. References**

- 5.1. The following are referenced in this document. Dated references apply to the edition cited and undated references apply to the latest edition published (including any amendments).
- 5.2. Laboratory Approval Program:
- a) [LAP-PR.05](#), Laboratory Approval Program – General Policies and Procedures.
  - b) [LAP-PR.06](#), Laboratory Approval Program – Fees.
  - c) USDA AMS LATD LAS Website, <https://www.ams.usda.gov/services/lab-testing/lab-approval>.
- 5.3. Quality Assurance Standards:
- a) ISO/IEC 17025:2017. General requirements for the competence of testing and calibration laboratories.
  - b) USDA AMS Laboratory Standards of Practice.
- 5.4. USDA Requirements:
- a) [7 CFR Part 981](#) – Almonds grown in California.
  - b) [7 CFR Part 983](#) – Pistachios grown in California, Arizona, and New Mexico.
  - c) [7 CFR Part 996](#) – Minimum quality and handling standards for domestic and imported peanuts marketed in the United States.
  - d) [7 CFR Part 999.600](#) – Specialty Crops; Import Regulations. Regulation governing the importation of pistachios.
  - e) [Milled Peanuts, Inspection Instructions](#). USDA MRP AMS SCP SCID.
  - f) [Pistachio Nuts In the Shell Shipping Point and Market Inspection Instructions](#). USDA AMS, February 2005.
  - g) [Pistachio Nuts In the Shell Shipping Point and Market Inspection Instructions, Patch # 045, Testing Samples For Aflatoxin](#). USDA AMS, April 15, 2019.
  - h) [Pistachio Nuts In the Shell Shipping Point and Market Inspection Instructions, Patch #066, Aflatoxin Sampling and Testing for Imported Raw and Blanched Pistachios](#). USDA AMS, April 28, 2021.
- 5.5. FDA Requirements:
- a) [MOU 225-19-031](#). Memorandum of Understanding between AMS and FDA in inspecting, sampling, and testing peanuts, Brazil nuts, and pistachio nuts for aflatoxins.
  - b) [FDA Compliance Policy Guides, Section 570.375](#) Aflatoxin in Peanuts and Peanut Products.
  - c) [FDA Compliance Policy Guides, Section 570.500](#) Pistachio Nuts - Aflatoxin Adulteration.

- d) [FDA Compliance Program Guidance Manual, 7307.001, Chapter 07](#) – Molecular Biology and Natural Toxins. Mycotoxins in domestic and imported foods.
- e) [Guidelines for the Validation of Chemical Methods in Food, Feed, Cosmetics, and Veterinary Products](#), 3rd Edition. US FDA. FDA Foods Program, October 2019.

5.6. Industry Requirements:

- a) [Administrative Committee for Pistachios: Handler's Guide](#).
- b) [Almond Board of California, Pre-Export Checks \(PEC\) Program Manual](#).
- c) [American Peanut Council Sheller MOU](#).
- d) [Peanut Export Program Manual](#) (PEP)
- e) [Pistachio Export Aflatoxin Reporting \(PEAR\) Program](#).

5.7. Methodology:

- a) [AOAC International, Official Methods 977.16](#), Sampling for Aflatoxins - Preparation for Sample Procedure.
- b) [AOAC International, Official Methods 991.31](#), Aflatoxins in Corn, Raw Peanuts, and Peanut Butter - Immunoaffinity Column (Aflatest) Method.
- c) [AOAC International, Official Methods 998.03](#), Aflatoxins in Peanuts - Alternative BF Method.
- d) [AOAC International, Official Methods 999.07](#), Aflatoxins B<sub>1</sub> and Total Aflatoxins in Peanut Butter, Pistachio Paste, Fig Paste, and Paprika Powder – Immunoaffinity Column Liquid Chromatography with Post-Column Derivatization.
- e) [AOAC International, Official Methods 2005.08](#), Aflatoxins in Corn, Raw Peanuts, and Peanut Butter - Liquid Chromatography with Post-Column Photochemical Derivatization.
- f) Francis Jr., O. J. 1979. Sample Preparation of Some Shelled Tree Nuts and Peanuts in a Vertical Cutter-Mixer for Mycotoxin Analysis. [JAOAC. 62\(5\):1182-5](#).

5.8. Relevant Country Regulations and Agreements:

- a) [CXS 193-1995](#). CODEX Alimentarius, International Food Standards, General Standards for Contaminants and Toxins in Food and Feed.
- b) [EU 2015/949](#). Commission Implementing Regulation (EU) No. 2015/949 of 19 June 2015 approving the pre-export checks carried out on certain food by certain third countries as regards the presence of certain mycotoxins.
- c) [EU 2023/915](#). Commission Regulation EU 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation 1881/2006.
- d) [EU 2023/2782](#). Commission Implementing Regulation EU 2023/2782 of 14 December 2023 laying down the methods of sampling and analysis for the control of the levels of mycotoxins in food and repealing Regulation EC 401/2006.

- e) Japan Protocol. Protocol for Control of Aflatoxin in U.S. Almonds Exported to Japan Between the U.S. Department of Agriculture (USDA) and the Ministry of Health, Labour and Welfare (MHLW).

5.9. Additional Resources:

- a) [Eurachem Guides](#).
- b) Good Laboratory and Clinical Practices, Techniques for the Quality Assurance Professional, edited by P.A. Carson and N.J. Dent, 1990.

## **GENERAL REQUIREMENTS**

### **6. Laboratory Approval Program Administrative Policy**

- 6.1. A laboratory seeking admission to the LAP must fulfill the requirements and follow the process described in the program procedure, [LAP-PR.05](#), *Laboratory Approval Program - General Policies and Procedures*. This procedure describes the process for application, assessment audits, acceptance, maintaining program status, suspension, withdrawal, dismissal, and appeals.
- 6.2. This program is administered on an annual, calendar year, basis.
- 6.3. The program procedure, [LAP-PR.06](#), *Laboratory Approval Program – Fees*, explains the fees for services.
- 6.4. The administrative procedures above are available on the [LAS website](#), or by contacting the LAS Mycotoxin Program Manager (PM) for the current version of the procedure.

### **7. Summary of General Program Requirements**

- 7.1. The laboratory must comply with all requirements set forth in this document to be compliant with the LAP. For a laboratory to maintain good standing, each year it must:
  - a) meet all program requirements relevant to the scope of approval;
  - b) comply with mandatory laboratory practices based on the ISO/IEC 17025 standard (see §8);
  - c) use test method(s) approved by AMS (see §9);
  - d) validate analytical and sample preparation methods prior to use and ensure the validation data package is available for review upon request (see §10);
  - e) evaluate analyst competency and maintain satisfactory status (see §11);
  - f) participate in proficiency testing (PT) programs and maintain satisfactory status (see §11);
  - g) submit sieve testing results to PM (see §14.3.h));
  - h) communicate regularly with the PM to share vital information regarding the laboratory and make all information relevant to the LAP available to PM upon request (see §5.2.a));
  - i) notify the PM within 30 days of significant changes relevant to the laboratory's approval status and/or ability to meet the Program's requirements including but not limited to: legal, organizational, or ownership status; laboratory policies, procedures, and resources; change in key managerial personnel, contact persons, and signatories; and verification of adequate method performance after a change in location, equipment, facilities, and working environment. It is at the discretion of LAS whether an onsite visit/audit is required to evaluate any significant change that a laboratory undergoes (see §5.2.a));

- j) be available for a biennial (every other year) re-assessment audit, have actual sample(s) ready to demonstrate its competency of performing the test method, and comply with requests for documents and records before and during the audit (see §5.2.a));
- k) respond to each nonconformance found with a record of an investigation, root cause analysis, and correction or, if warranted due to time, a corrective action plan, within 30 calendar days of being reported (see §5.2.a));
- l) resolve each nonconformance in a timely manner, whether identified by LAS auditor, another organization, or internally (see §5.2.a));
- m) pay all program fees by the due date on the billing invoice (see §5.2.b)).

## **8. Mandatory Quality Assurance Practices**

- 8.1. Implement quality assurance and quality control procedures to ensure a validated and qualified analysis, prove competence, and ensure defensible data. This Program does not require ISO 17025 accreditation; however, it is a common practice for testing laboratories to become ISO 17025 accredited.
- 8.2. LAS uses the ISO 17025 standard to evaluate all laboratory quality systems regardless of accreditation status. A nonconformance identified during a LAS assessment audit may be cited to the ISO 17025 standard (see §5.2.a)).
- 8.3. Maintain records for at least three years (see §5.2.a)).

## **9. Method Selection**

- 9.1. Use analytical testing methods approved by AMS.
- 9.2. Use method fit for purpose based upon the needs of the customer and consistent with specified requirements.
- 9.3. Use methods as validated; for the purposes of this Program, consider methods, with collaborative studies, specifically those published by USDA, FDA, AOAC and AOCS, as validated methods (see §5.7).
- 9.4. Refer to sections below for approved methods and other technical requirements:
  - a) Almonds – AFL – Export to EU: See §18.
  - b) Almonds – AFL – Export to Japan: See §24.
  - c) Peanuts – AFL – U.S. Domestic & U.S. Imports: See §19.
  - d) Peanuts – AFL – Export to EU: See §20.
  - e) Pistachios – AFL – U.S. Domestic & U.S. Imports: See §21.
  - f) Pistachios – AFL – Export to EU: See §22.



g) Pistachios – OTA – Export to EU:

See §23.

#### **10. Demonstration of Method Performance by Validation and Verification Evaluation**

- 10.1. Demonstrate the sample test method(s) is competently and proficiently performed prior to using it for testing and reporting results by using a validation / verification process, where:
  - a) Validation is the process of demonstrating that a method is suitable for its intended purpose,
  - b) Verification is the process of demonstrating a validated method can be performed to the same level of performance determined during the validation, and
  - c) It is recognized that there are multiple ways to demonstrate performance; therefore, where the Program does not specify a specific protocol, consult with the PM to determine a suitable validation / verification plan using U.S. and internationally recognized protocols.
- 10.2. Demonstrate that variations or modifications to a validated method (e.g., different matrix, different analyte, etc.) are fit-for-purpose.
- 10.3. Prepare a Method Validation Data Package to record the procedure has been demonstrated as fit for use.
  - a) Consolidate pertinent information into an integrated and auditable data package to support the objective's conclusion that includes at least the following: cover statement containing the conclusion of the validation / verification process, test method procedure, relevant statistics (i.e., linearity, accuracy, precision, measurement uncertainty (MU), and limit of quantification (LOQ)), and traceable data (raw and summarized).
  - b) Send validation / verification package to the PM for review and approval.
  - c) Ensure validation / verification data package is readily available at the laboratory for as long as the method is utilized, plus three years after the last reported results.
  - d) Ensure official record of approval for method is maintained and readily available.
- 10.4. Validate the sample preparation procedure for grinding samples to achieve an adequate particle size and homogenous grind for each analyte/matrix.
  - a) Determine optimal parameters to achieve a homogeneous grind, including type of grinder, type of blade (e.g., smooth-edge, serrated, notch-edge), and minimum time to grind.
  - b) Select an unground sample with either a known incurred natural mycotoxins contamination or spike the nuts (ideally in one location).
  - c) Grind sample using optimized parameters.
  - d) Analyze at least 10 representative subsamples to determine if the resulting grind is homogeneous.
  - e) Evaluate results from the subsamples. Acceptance criteria for a homogeneous grind is percent recovery as found in Table 1; and Coefficient of Variation (CV)  $\leq 20\%$ .

- f) Establish sieve test acceptance criteria for maintaining vertical cutter mill equipment by performing a particle size tests using subsamples of the grind passed through a No. 20 sieve (see §14.3).
  - g) Document, in a procedure, the final parameters validated including type of grinder, type of blade, minimum grind time, and sieve test acceptance criteria.
  - h) For significant changes made to the grinding procedure perform validation to demonstrate the changes result in a homogeneous grind and re-establish the sieve test acceptance criteria.
  - i) An exception or variation to AOAC 977.16 method may be considered by submitting a request and validation data that demonstrates the variation provides the same or better performance than the original procedure.
- 10.5. Demonstrate the sample testing method(s) can be competently and proficiently performed prior to using it for testing and reporting results.
- a) Demonstrate performance criteria of the reference method (e.g., AOAC) are met or exceeded (e.g., linearity, accuracy, precision, MU, LOQ).
  - b) Demonstrate performance criteria outlined in [EU 2023/2782](#) Annex II, Part 4, as relevant to scope of approval including export to EU (e.g., parts 4.2, 4.3, and 4.4) (see §5.8.d)).

## **11. Analyst Competency and Proficiency Testing**

### **11.1. Analyst Competency:**

- a) Ensure new analyst responsible for performing test method(s) demonstrates competency and provide record of training and competence evaluation to the PM.
- b) Ensure every analyst responsible for performing the method(s) participates in the PT program at least once in a two-year span.

### **11.2. Laboratory Analytical Competency:**

- a) Participate in a PT program on a quarterly basis.
- b) Use a PT program relevant to the approved method in terms of analyte, concentration range, and matrix on the scope of approval—administered by an external third-party program such as AOCS, FAPAS, or similar (with preference to ISO 17043 accredited provider).
- c) Participate in the FAPAS PT program when scope of approval includes export to EU.
- d) Participate in PT Program and at the frequency specified by the PM when a quarterly PT program is unavailable.
- e) Review PT report and initiate the corrective action process when unsatisfactory results are observed. Unsatisfactory PT results and action to take are defined as follows:

$ z  \geq 3$	Initiate immediate corrective action investigation on the part of the laboratory to establish root cause.
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$2 \leq |z| \leq 3$

Evaluate in the context of other scores obtained in the same test and other PTs over time. Investigate to determine the root cause and take action as needed.

11.3. Submit to the PM:

- a) Record of new analyst training and demonstration of competency.
- b) Copy of the PT report with analyst name, and PT identifier, within 30 days of receipt.
- c) Record of corrective action response for unsatisfactory results, within 30 days of receiving the PT report.

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**The following sections include requirements organized by scope; and are in addition to the requirements outlined above.**

## TECHNICAL REQUIREMENTS (GENERAL)

### 12. Performance Criteria

Table 1. Performance criteria for aflatoxins ( $B_1$ ,  $B_2$ ,  $G_1$ ,  $G_2$ ), for both the sum and the individual toxins. Criteria applies to all concentrations and all individual toxins\*.

Average Recovery* %	Precision*		LOQ* ug/kg	MU**
	RSD <sub>r</sub> , %	RSD <sub>WR</sub> , %		
70 to 120	≤ 20	≤ 20	≤ 1	Certainty of 95%

\* [EU 2023/2782](#), Annex II, 4.2.1.1

\*\* EU 2023/2782, Annex II, 4.3

Table 2. Performance criteria for ochratoxin A. Criteria applies to all concentrations\*.

Average Recovery* %	Precision*		LOQ* ug/kg	MU**
	RSD <sub>r</sub> , %	RSD <sub>WR</sub> , %		
70 to 120	≤ 20	≤ 20	Shall be ≤ (0.5)*(ML); and should be ≤ (0.2)*(ML)	Certainty of 95%

\* [EU 2023/2782](#), Annex II, 4.2.1.1

\*\* EU 2023/2782, Annex II, 4.3

#### 12.1. Apply these definitions to calculate method performance:

- Average Recovery is the average value from replicates obtained during validation when determining the precision parameters RSD<sub>r</sub> and RSD<sub>WR</sub> (see §5.8.d), Annex II, 4.2.1.1).
- RSD<sub>r</sub> (repeatability relative standard deviation) means the relative standard deviation (%) calculated from results generated under repeatability conditions (repeatability precision): using the same method on the same sample material in one laboratory by the same operator, with the same instrument, within a short interval of time (1 day or 1 sequence) (see §5.8.d), Article 1).
- RSD<sub>WR</sub> (within-laboratory reproducibility relative standard deviation) means the relative standard deviation (%) calculated from results generated under within-laboratory reproducibility conditions (intermediate precision): using the same method on the same sample material in one laboratory but different days (preferably a longer time interval), and may include other conditions, such as involving different operators and/or different (equivalent) instruments (see §5.8.d), Article 1).

Note:  $RSD_{WR}$  guarantees compliance with the  $RSD_r$  criterion; therefore, there is no need to provide separate evidence for  $RSD_r$  (see §5.8.d), Annex II, 4.2.1.1).

- d) LOQ (limit of quantification) means the lowest content of the analyte which can be measured with reasonable statistical certainty. In the context of [EU 2023/2782](#) this means the lowest successfully validated level: the lowest test concentration of the analyte in a sample material, for which it has been demonstrated that the criteria for recovery, precision, and identification are met (see §5.8.d), Article 1).
- e) When a specific requirement is set, the method shall have an LOQ at or below the value set ((see §5.8.d), Annex II 4.2.1.1)
- f) ML (maximum level) are set for certain mycotoxins in [EU 2023/915](#) (see §5.8.d).
- g) Expanded MU (measurement uncertainty) may be reported as 50%, as a default, provided that all precision requirements are met (see §5.8.d), Annex II, 4.3.1.b).

### **13. Quality Controls**

- 13.1. Use controls, daily with use, to demonstrate testing is performed correctly and factors that could negatively impact the results are mitigated.
- 13.2. Take immediate action prior to continuing testing and/or reporting results when any quality control does not perform as expected.
- 13.3. Include minimum quality controls:
  - a) Matrix spike, matrix blank and/or reagent blank, calibration standard, and
  - b) For LC-FLD, or similar method, continued calibration verification (CCV).
- 13.4. LAS' interpretation of each quality control and its purpose is defined below. It is not a requirement for the laboratory to use the same term for each type of control as long as the correct control is used for the correct purpose.
  - a) Matrix Blank: Substance that closely matches the samples being analyzed with regard to matrix components. Ideally, the matrix blank does not contain the analyte(s) of interest but is subjected to all sample processing operations including all reagents used to test the samples. Use to determine the absence of significant interference due to matrix, reagents, and equipment used in the analysis.
  - b) Matrix Spike: Aliquot of a sample prepared by adding a known amount of analyte(s) to a specified amount of matrix and subjected to the entire procedure. Use to establish if the method is appropriate for the analysis of a specific analyte(s) in a particular matrix.
  - c) Incurred Sample: Sample that contains the analyte(s) of interest, which were not derived from laboratory fortification.
  - d) Reagent Blank: Sample containing only the reagents taken through the entire procedure. Optional if a matrix blank is used.

- e) Calibration Standard: Solution containing known amount or concentration of analyte. Without further indication, calibration standard(s) is usually prepared by adding the analyte(s) of interest in neat solvent/solution. A “matrix-matched calibration standard(s)” is prepared by adding analyte(s) of interest in matrix blank. Use to establish a reference for identification and/or quantification for measuring/detection system.
- f) Continuing Calibration Verification (CCV): Solution of known concentration, typically at or near the midpoint of the calibration curve. Use to evaluate instrument stability throughout the sequence and repeatability.
- g) Independent Calibration Verification (ICV): Solution of known concentration that is from a different manufacturer, or same manufacturer but different lot, or a separate preparation from the solutions used to calibrate. Use to evaluate the accuracy of reference material and/or accuracy of preparation techniques. Optional; however, inclusion is a best practice.

#### **14. Quality Measures**

- 14.1. Evaluate quality controls to identify acceptability of data, trends, and potential problems.
- 14.2. Take immediate action prior to continuing testing and/or reporting results when any quality measure not performing as expected.
- 14.3. Vertical Cutter Mill (VCM) Sieve Test: Use to monitor VCM equipment (i.e., blade sharpness). Perform at a minimum frequency of weekly or with use.
  - a) Use a No. 20 sieve.
  - b) Place ~50 - 100g of ground sample in sieve that has been weighed.
  - c) Work the sample through the sieve with warm water (tap) being careful not to lose sample, as needed.
  - d) Dry the sieve and remaining material in an oven at ~100 – 105°C or air-dry until there is no more change in weight. Avoid cooking or burning the material.
  - e) Weigh and calculate % pass through.
  - f) Record result and evaluate against acceptance criteria defined in sample preparation procedure.
  - g) Monitor trending decrease of % pass through to facilitate maintaining acceptable VCM performance.
  - h) Submit sieve test data to the PM quarterly.
- 14.4. Calibration Curve: Use to quantify concentration of unknown samples.
  - a) Use the same type of curve used during method validation.
  - b) Ensure a minimum of 4 points is used for LC.
  - c) Ensure  $R^2$  value for each curve must be  $\geq 0.995$ .
  - d) Ensure regression equation is not forced through zero.

- e) Ensure calibration range brackets the range of result reported and includes the LOQ for the method. Results outside of the calibration range may be diluted and re-analyzed so the result falls within the range. It is not acceptable to extrapolate the concentration.

14.5. Continuing Calibration Verification (CCV): Use to demonstrate stability of the instrument/method (e.g., calibration curve).

- a) Collect CCV data points at a minimum, at the beginning of a batch, the end of the batch, and throughout the batch with a recommended frequency of every 10 ( $\pm 2$ ) injections.
- b) Calculate percent recovery of CCV data point (see §14.7).
- c) Evaluate against the acceptance criteria, where recovered concentration must be within 15% of expected concentration.

14.6. Coefficient of Variation (CV): Use to demonstrate the repeatability.

- a) Calculate coefficient of variation (CV) for a group of data points using equation:

$$\% CV = \frac{\text{Standard Deviation}}{\text{Average}} * 100$$

- b) Evaluate % CV during validation of sample preparation procedure (see §10.4); and during validation of sample testing method (see §10.5).

14.7. Percent recovery: Use to compare recovered concentration to actual expected concentration to evaluate method performance.

- a) Calculate percent recovery using equation:

$$\% Recovery = \frac{[\text{recovered}]}{[\text{expected}]} * 100$$

- b) Evaluate recovery of a matrix spike for aflatoxins against performance criteria defined in (see §12).
- c) Evaluate recovery of a matrix spike for ochratoxin A against performance criteria defined in (see §12).

14.8. Control Charting: Use to track performance over time and serve as an indicator if something in the analytical process is out of control and needs investigation or correction.

- a) Plot percent recovery results of each matrix spike analyzed.
- b) Plot for the method regardless of analyst or instrument used to evaluate overall performance of the method.
- c) Evaluate whether results are acceptable using acceptance criteria, (AFL) or (OTA). All data points must be included.
- d) Additional, optional, control charting methods can be used to evaluate other variables of the testing procedure. For example, plotting by analyst or by instrument can show individual performance when more than one is used.

## **15. Data Analysis**

- 15.1. Significant Figures: Collect data to the appropriate significant figures for the methodology (see §18-24). Additionally, when two or more data points are calculated, the final value must not have more significant figures than the original data points.
- 15.2. Rounding Rule: If 4 and under, round down; and if 5 and over, round up. For example:
  - a) a result of 9.4 ppb would be rounded to 9 ppb, whereas a result of 9.5 ppb would be rounded to 10 ppb for the final report; and
  - b) a result of 4.24 ppb would be rounded to 4.2 ppb, whereas a result of 4.25 ppb would be rounded to 4.3 ppb.
- 15.3. Correction for recovery (export only, see §5.8.d)):
  - a) Correct the analytical result for recovery where appropriate and relevant, and when corrected it shall be stated. The correction for recovery is not necessary in the case the percent recovery is between 90-110%.
  - b) Calculate recovery correction, if applicable, for each of the individual toxins (e.g., aflatoxins) before summation of the concentrations. Applying a lower bound approach, use zero for the individual toxins when <LOQ for calculation of the sum.
- 15.4. Calculate and report results on a kernel basis.

## **16. Critical Equipment & Reagents**

- 16.1. Calibrate or verify equipment and instrumentation considered critical to the analytical method before putting into service and thereafter calibrate / verify regularly while in service.
  - a) Calibration refers to checking the measurements of a device and adjusting the device if corrections are needed.
  - b) Verification refers to checking that a device's measurement remains within a determined acceptable range, adjustments should not be needed.
- 16.2. Investigate and correct issue before using when a device is found to be outside tolerance at verification.
- 16.3. Maintain records of calibration, intermediate checks, performance verification, maintenance, and significant repair.
- 16.4. Grinding Equipment: Keep in good working order. Monitor performance using VCM sieve test (see §14.3). Record blade sharpening.
- 16.5. Balances & Scales:
  - a) Daily when in use: Verify with working weights that fall within the weight range used by the laboratory.



- b) Annual calibration: Performed by an accredited organization or internally with the same requirements being met.
- 16.6. Reference & Working Weights:
  - a) Reference weights: Verify every 5 years.
  - b) Working weights: Verify against reference weights annually.
- 16.7. Analytical Instruments: Keep in good working order.
- 16.8. Volumetric Devices:
  - a) Mechanical pipets, micropipettors, mechanical burets, and bottle-top dispensers: Verify at least every 6 months for accuracy and precision using a gravimetric or colorimetric method.
  - b) Positive displacement syringes: Verify for accuracy upon receipt (a manufacturer's certificate of accuracy may be accepted).
  - c) Plastic graduated cylinders: Verify for accuracy and precision upon receipt (prior to use) and every five years for accuracy using a gravimetric method. Additional verification should occur if damage is visible.
  - d) Volumetric non-class A glassware: Verify for accuracy and precision using a gravimetric method upon receipt (prior to use).
- 16.9. Critical reagents and consumables: Store according to the manufacturer's instructions and do not use past the designated expiration date.

## **17. Official Certificate of Analysis/Report**

- 17.1. Meet the ISO 17025 standard for reporting results.
- 17.2. Meet commodity specific requirements for reporting results.
- 17.3. Meet customer's requirements for reporting results.

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**The following sections include requirements organized by scope; and are in addition to the requirements outlined above.**

## TECHNICAL REQUIREMENTS (SCOPE SPECIFIC)

### **18. Almond – AFL – Export to EU**

18.1. Applies to analysis for aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>).

18.2. Sample Preparation Method:

- a) Protect sample from daylight (see §5.8.d), Annex II 1.1).
- b) Ensure sample is representative of the incoming sample prior to grinding. Mixing may occur at 1) the processing facility as part of the sampling process (See §5.6.b), Product Sample Selection and Analysis); or 2) at the laboratory (see §5.8.d), Annex I D.3, D.4). When the incoming sample is split prior to grinding at the laboratory, ensure the sub-samples are representative of the incoming sample.
- c) Grind sample to achieve particle size reduction and a well-mixed sample (based on AOAC 977.16).

18.3. Test Method:

- a) IAC with LC-FLD, Iodine: AOAC 991.31, A-F, H
- b) IAC with LC-FLD, Kobra Cell: AOAC 991.31, A-F, H / AOAC 999.07
- c) IAC with LC-FLD, PHRED: AOAC 991.31, A-F, H / AOAC 2005.08
- d) LC-MS/MS AOAC 991.31

18.4. Data Analysis:

- a) Analyze to the nearest tenth ppb (i.e. 0.1 ppb), where ppb = 1 ng/g.
- b) Calculate and report shell/kernel ratio of whole nuts (see §5.8.d), Annex II 1.2), if applicable.

18.5. Official Certificate of Analysis / Test Report:

18.5.1. Include all the following information:

- a) Sample lot number.
- b) Analytical method used (see §5.8.d)).
- c) Limit of quantification, for the individual toxins and/or the sum (see §5.8.d)).
- d) Percent recovery for B1 and total aflatoxins (see §5.8.d)).
- e) Analytical result, for the individual toxins and the sum (see §5.8.d)).
- f) A statement about correction for recovery (see §5.8.d)).
- g) Measurement uncertainty presented as the result  $\pm U$  (i.e.,  $10.4 \pm 2.1 \mu\text{g/kg}$ ), where U is the expanded MU, using a coverage factor of 2 which gives a level of confidence of approximately 95% (see §5.8.d)).

18.5.2. Use the following type of statement if claiming compliance with the [EU 2023/2782](#):

“Sample analysis was conducted in compliance with Annex II of Regulation (EU) No. 2023/2782 for aflatoxins in almonds.”

18.5.3. Use the following type of statement if reporting results for in shell nuts:

“In shell almonds are reported on a kernel basis for a [insert X/Y] shell/kernel ratio.”

18.5.4. Use the following type of statement if including an interpretation in view of acceptance or rejection of a lot for the maximum limits set by Regulation [EU 2023/915](#):

- a) “Result exceeds the limit of 12.0 µg/kg aflatoxin B<sub>1</sub> and/or 15.0 µg/kg sum of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> for almonds to be subjected to sorting or other physical treatment before placing on the market for the final consumer or use as an ingredient in food (§1.1.8 of Annex I Regulation EU 2023/915).”
- b) “Result exceeds the limit of 8.0 µg/kg aflatoxin B<sub>1</sub> and/or 10.0 µg/kg sum of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> for almonds placed on the market for the final consumer or use as an ingredient in food (§1.1.9 of Annex I Regulation EU 2023/915).”

Note: Interpretation of results by the laboratory does not generally occur. The commodity’s destination is often decided based on the test results. It is the responsibility of the handler (processor) not the laboratory, to determine which limits apply to their product.

**19. Peanut – AFL – U.S. Domestic & U.S. Imports**

19.1. Applies to analysis for aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>).

19.2. Sample Preparation Method:

- a) Visually inspect to verify incoming sample bag (unground) is marked with weight on the positive lot identification (PLI) tag (see §5.4.e), Sample Weight) and it is not less than 48lbs.
- b) Ensure sample is representative of the incoming sample prior to grinding.

Note: Samples are mixed prior to grinding at the processing facility as part of the USDA Federal/State Inspection Service sampling process (see §5.4.e), Sampling).

- c) Grind sample to achieve particle size reduction and a well-mixed sample (based on AOAC 977.16).

19.3. Test Method:

- a) IAC with direct fluorometry: AOAC 991.31, A-G
- b) TLC: AOAC 998.03
- c) IAC with LC-FLD, Iodine: AOAC 991.31, A-F, H
- d) IAC with LC-FLD, Kobra Cell: AOAC 991.31, A-F, H / AOAC 999.07
- e) IAC with LC-FLD, PHRED: AOAC 991.31, A-F, H / AOAC 2005.08
- f) LC-MS/MS AOAC 991.31

19.4. Data Analysis:

19.4.1. Analyze to the nearest:

- a) IAC with direct fluorometry: whole integer
- b) TLC: whole integer
- c) IAC with LC-FLD: tenth ppb (i.e. 0.1 ppb), where ppb = 1 ng/g

19.5. Official Certificate of Analysis / Test Report:

19.5.1. Include the statement:

“The designation of aflatoxins negative is defined as the average analytical result of 15 parts per billion (ppb) or less aflatoxins and applies to product distributed within the United States under 7 CFR Part 996. Results are reported as whole integers; therefore, further calculations must be rounded to the nearest whole integer for proper interpretation.”

19.5.2. Include the statement:

“USDA or USDA-approved laboratory to test for total aflatoxins content in samples for domestic and imported peanuts marketed in the United States.”

19.5.3. Include the following type of statement for the approved methodology used, to include AOAC method number or internal procedure number, where applicable:

- a) Immunoaffinity column with direct fluorometry method of analysis (AOAC 991.31).
- b) Water slurry method with thin-layer chromatography (TLC) analysis designated as the alternative Best Foods (BF) method of analysis (AOAC 998.03).
- c) Immunoaffinity column cleanup with high performance liquid chromatography (LC) method.

19.6. Reporting to USDA:

- a) Submit records of sample analysis to AMS Specialty Crops Inspection Division ([sciinspectionoperations@usda.gov](mailto:sciinspectionoperations@usda.gov)) in the format requested.
- b) Submit records of sample analysis to AMS Specialty Crops Market Development Division ([8Eimports@usda.gov](mailto:8Eimports@usda.gov) and [complianceinfo@usda.gov](mailto:complianceinfo@usda.gov)) in the format requested.

**20. Peanut – AFL – Export to EU**

20.1. Applies to analysis for aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>).

20.2. Sample Preparation Method:

- a) Visually inspect to verify incoming unground sample has the weight recorded on the positive lot identification (PLI) tag and it is not less than 48lbs (see §5.4.e), Sample Weight).
- b) Protect sample from daylight (see §5.8.d), Annex II 1.1).
- c) Ensure the sub-samples are representative of the incoming sample when the incoming sample is split prior to grinding at the laboratory.

Note: Samples are mixed prior to grinding at the processing facility as part of the USDA Federal/State Inspection Service sampling process (see §5.4.e), Sampling).

- d) Grind sample to achieve particle size reduction and a well-mixed sample (based on AOAC 977.16).

20.3. Test Method:

- a) IAC with LC-FLD, Iodine: AOAC 991.31, A-F, H
- b) IAC with LC-FLD, Kobra Cell: AOAC 991.31, A-F, H / AOAC 999.07
- c) IAC with LC-FLD, PHRED: AOAC 991.31, A-F, H / AOAC 2005.08
- d) LC-MS/MS AOAC 991.31

20.4. Data Analysis:

- a) Analyze to the nearest tenth ppb (i.e. 0.1 ppb), where ppb = 1 ng/g.
- b) Calculate and report shell/kernel ratio of whole nuts (see §5.8.d), Annex II 1.2), if applicable.

20.5. Official Certificate of Analysis / Test Report:

20.5.1. Include all the following information:

- a) Sample lot number (positive lot identification, PLI).
- b) Analytical method used (see §5.8.d)).
- c) Limit of quantification, for the individual toxins and/or the sum (see §5.8.d)).
- d) Percent recovery for B<sub>1</sub> and total aflatoxins (see §5.8.d)).
- e) Analytical result, for the individual toxins and the sum (see §5.8.d)).
- f) A statement about correction for recovery (see §5.8.d)).
- g) Measurement uncertainty presented as the result ± U (i.e., 10.4 ± 2.1 µg/kg), where U is the expanded MU, using a coverage factor of 2 which gives a level of confidence of approximately 95% (see §5.8.d))
- h) State whether the sheller is a signatory of the APC MOU.

20.5.2. Use the following type of statement if claiming compliance with the [EU 2023/2782](#):

“Sample analysis was conducted in compliance with Annex II of Regulation EU No. 2023/2782 for aflatoxins in peanuts.”

20.5.3. Use the following type of statement if reporting in shell nuts:

“In shell peanut are reported on a kernel basis for a [insert X/Y] shell/kernel ratio.”

20.5.4. Use the following type of statement if including an interpretation in view of acceptance or rejection of a lot for the maximum limits set by Regulation [EU 2023/915](#):

- a) “Result exceeds the limit of 8.0 µg/kg aflatoxin B<sub>1</sub> and/or 15.0 µg/kg sum of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> for peanuts to be subjected to sorting or other physical treatment before placing on the market for the final consumer or use as an ingredient in food with the exception of peanuts for crushing for refined vegetable oil production (§1.1.4 of Annex I Regulation EU 2023/915).”
- b) “Result exceeds the limit of 2.0 µg/kg aflatoxin B<sub>1</sub> and/or 4.0 µg/kg sum of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> for peanuts used as only ingredient or processed products from peanuts placed on the market for the final consumer or use as an ingredient in food with the exception of crude vegetable oils destined for refining and refined vegetable oils (§1.1.5 of Annex I Regulation EU 2023/915).”

Note: Interpretation of results by the laboratory does not generally occur. The commodity’s destination is often decided based on the test results. It is the responsibility of the handler (processor) not the laboratory, to determine which limits apply to their product.

**21. Pistachio – AFL – U.S. Domestic & U.S. Imports**

21.1. Applies to analysis for aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>).

21.2. Sample Preparation Method:

- a) Ensure sample is representative of the incoming sample prior to grinding. Mixing may occur at 1) the processing facility as part of the sampling process (see §5.4.g) and 5.4.h)); or 2) at the laboratory.
- b) Grind sample to achieve particle size reduction and a well-mixed sample (based on AOAC 977.16).

21.3. Test Method:

- a) IAC with direct fluorometry: AOAC 991.31, A-G
- b) IAC with LC-FLD, Iodine: AOAC 991.31, A-F, H
- c) IAC with LC-FLD, Kobra Cell: AOAC 991.31, A-F, H / AOAC 999.07
- d) IAC with LC-FLD, PHRED: AOAC 991.31, A-F, H / AOAC 2005.08
- e) LC-MS/MS AOAC 991.31

21.4. Data Analysis:

- a) IAC with direct fluorometry: whole integer
- b) IAC with LC-FLD: nearest tenth ppb (i.e. 0.1 ppb), where ppb = 1 ng/g.

21.5. Official Certificate of Analysis / Test Report:

21.5.1. Include the statement:

“USDA or USDA-approved laboratory to test for total aflatoxins content in samples for domestic and imported pistachios marketed in the United States.”

21.5.2. Include the following type of statement for the approved methodology used, to include AOAC method number or internal procedure number, where applicable:

- a) Immunoaffinity column with direct fluorometry method of analysis (AOAC 991.31).
- b) Immunoaffinity column cleanup with high performance liquid chromatography (LC) method.

21.5.3. Use the following type of statement if including an interpretation in view of acceptance or rejection of a lot for the maximum limit:

“Result exceeds the limit of 15 ppb total aflatoxins for domestic human consumption (7 CFR 983.150).”

Note: Interpretation of results by the laboratory does not generally occur. The commodity’s destination is often decided based on the test results. It is the responsibility of the handler (processor) not the laboratory, to determine which limits apply to their product.



21.6. Reporting to USDA:

- a) Submit records of sample analysis to AMS Specialty Crops Inspection Division ([sciinspectionoperations@usda.gov](mailto:sciinspectionoperations@usda.gov)) in the format requested.
- b) Submit records of sample analysis to AMS Specialty Crops Market Development Division ([8Eimports@usda.gov](mailto:8Eimports@usda.gov) and [complianceinfo@usda.gov](mailto:complianceinfo@usda.gov)) in the format requested.

## **22. Pistachio – AFL – Export to EU**

22.1. Applies to analysis for aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>).

22.2. Sample Preparation Method:

- a) Protect sample from daylight (see §5.8.d), Annex II 1.1).
- b) Ensure sample is representative of the incoming sample prior to grinding. Mixing may occur at 1) the processing facility as part of the sampling process (See §5.6.e), Creating and Sampling the Lot); or 2) at the laboratory (see §5.8.d), Annex I D. 3, D.4). When the incoming sample is split prior to grinding at the laboratory, ensure the sub-samples are representative of the incoming sample.
- c) Grind sample to achieve particle size reduction and a well-mixed sample (based on AOAC 977.16).

22.3. Test Method:

- a) IAC with LC-FLD, Iodine: AOAC 991.31, A-F, H
- b) IAC with LC-FLD, Kobra Cell: AOAC 991.31, A-F, H / AOAC 999.07
- c) IAC with LC-FLD, PHRED: AOAC 991.31, A-F, H / AOAC 2005.08
- d) LC-MS/MS AOAC 991.31

22.4. Data Analysis:

- a) Analyze to the nearest tenth ppb (i.e. 0.1 ppb), where ppb = 1 ng/g.
- b) Calculate and report shell/kernel ratio of whole nuts (see §5.8.d), Annex II 1.2), if applicable, where the in shell pistachio ratio is accepted as 50/50 (see §5.5.d)).

22.5. Official Certificate of Analysis / Test Report:

22.5.1. Include all the following information:

- a) Sample lot number.
- b) Analytical method used (see §5.8.d)).
- c) Limit of quantification, for the individual toxins and/or the sum (see §5.8.d)).
- d) Percent recovery for B<sub>1</sub> and total aflatoxins (see §5.8.d)).
- e) Analytical result, for the individual toxins and the sum (see §5.8.d)).
- f) A statement about correction for recovery (see §5.8.d)).
- g) Measurement uncertainty presented as the result ± U (i.e., 10.4 ± 2.1 µg/kg), where U is the expanded MU, using a coverage factor of 2 which gives a level of confidence of approximately 95% (see §5.8.d)).

22.5.2. Use the following type of statement if claiming compliance with the [EU 2023/2782](#):

“Sample analysis was conducted in compliance with Annex II of Regulation EU No.2023/2782 for aflatoxins in pistachio.”

22.5.3. Use the following type of statement if reporting in shell nuts:

“In shell pistachio results are reported on a kernel basis for a 50/50 shell/kernel ratio.”

22.5.4. Use the following type of statement if including an interpretation in view of acceptance or rejection of a lot for the maximum limits set by Regulation [EU 2023/915](#):

- a) “Result exceeds the limit of 12.0 µg/kg aflatoxin B<sub>1</sub> and/or aflatoxins 15.0 µg/kg sum of B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> for pistachios to be subjected to sorting or other physical treatment before placing on the market for the final consumer or use as an ingredient in food (§1.1.8 of Annex I Regulation EU 2023/915).”
- b) “Result exceeds the limit of 8.0 µg/kg aflatoxin B<sub>1</sub> and/or 10.0 µg/kg sum of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> for pistachios placed on the market for the final consumer or use as an ingredient in food (§1.1.9 of Annex I Regulation EU 2023/915).”

Note: Interpretation of results by the laboratory does not generally occur. The commodity’s destination is often decided based on the test results. It is the responsibility of the handler (processor) not the laboratory, to determine which limits apply to their product.

### **23. Pistachio – OTA – Export to EU**

23.1. Applies to analysis of ochratoxin A.

23.2. Sample Preparation Method:

- a) Protect sample from daylight (see §5.8.d), Annex II 1.1).
- b) Ensure sample is representative of the incoming sample prior to grinding. Mixing may occur at 1) the processing facility as part of the sampling process (See §5.6.e), Creating and Sampling the Lot); or 2) at the laboratory (see §5.8.d), Annex I D.3, D.4). When the incoming sample is split prior to grinding at the laboratory, ensure the sub-samples are representative of the incoming sample.
- c) Grind sample to achieve particle size reduction and a well-mixed sample (based on AOAC 977.16).

23.3. Test Method:

- a) Use method fit for purpose and that meets the validation / verification requirements outlined in §10.

23.4. Data Analysis:

- a) Analyze to the nearest tenth ppb (i.e. 0.1 ppb), where ppb = 1 ng/g.
- b) Calculate and report shell/kernel ratio of whole nuts (see §5.8.d)), if applicable; where the in shell pistachio ratio is accepted as 50/50 (see §5.5.d)).

23.5. Official Certificate of Analysis / Test Report:

23.5.1. Include all the following information:

- a) Sample lot number.
- b) Analytical method used (see §5.8.d)).
- c) Limit of quantification (see §5.8.d)).
- d) Percent recovery for OTA (see §5.8.d)).
- e) Analytical result (see §5.8.d)).
- f) A statement about correction for recovery (see §5.8.d)).
- g) Measurement uncertainty presented as the result  $\pm$  U (i.e.,  $10.4 \pm 2.1$  µg/kg), where U is the expanded MU, using a coverage factor of 2 which gives a level of confidence of approximately 95% (see §5.8.d).

23.5.2. Use the following type of statement if claiming compliance with the [EU 2023/2782](#):

“Sample analysis was conducted in compliance with Annex II of Regulation (EU) No. 2023/2782 for ochratoxin in pistachio.”

23.5.3. Use the following type of statement if reporting in shell nuts:

“In shell pistachio results are reported on a kernel basis for a 50/50 shell/kernel ratio.”

23.5.4. Use the following type of statement if including an interpretation in view of acceptance or rejection of a lot for the maximum limits set by Regulation [EU 2023/915](#):

- a) “Result exceeds the limit of 10 µg/kg ochratoxin for pistachios to be subjected to sorting or other physical treatment before placing on the market for final consumer use or use as an ingredient in food (§1.2.3 Annex I Regulation EU 2023/915).”
- b) “Result exceeds the limit of 5 µg/kg ochratoxin for pistachios placed on the market for final consumer or use as an ingredient in foodstuffs (§1.2.4 Annex I Regulation EU 2023/915).”

Note: Interpretation of results by the laboratory does not generally occur. The commodity’s destination is often decided based on the test results. It is the responsibility of the handler (processor) not the laboratory, to determine which limits apply to their product.

**24. Almonds – AFL – Export to Japan**

- 24.1. Applies to analysis for aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>) for unprocessed consignments of U.S. almond kernel (Harmonized System – HS 0802.12) and inshell almond (HS 0802.11) shipped directly from the U.S. to Japan (see §5.8.e)).
- 24.2. Sample Preparation Method:
- a) Protect sample from daylight (see §5.8.a), Annex II, 34).
  - b) Ensure sample is representative of the incoming sample prior to grinding. Mixing may occur at 1) the processing facility as part of the sampling process (see §5.8.a), Annex II, 25; or 2) at the laboratory. When the incoming sample is split prior to grinding at the laboratory, ensure the sub-samples are representative of the incoming sample.
  - c) Grind 1-10 kg sample from the aggregate 20 kg sample received (see §5.8.e)).
  - d) Grind sample to achieve particle size reduction and a well-mixed sample (based on AOAC 977.16).
- 24.3. Test Method:
- a) IAC with LC-FLD, Iodine: AOAC 991.31, A-F, H
  - b) IAC with LC-FLD, Kobra Cell: AOAC 991.31, A-F, H / AOAC 999.07
  - c) IAC with LC-FLD, PHRED: AOAC 991.31, A-F, H / AOAC 2005.08
  - d) LC-MS/MS AOAC 991.31
- 24.4. Data Analysis:
- a) Analyze to the nearest tenth ppb (i.e. 0.1 ppb), where ppb = 1 ng/g.
  - b) Calculate and report shell/kernel ratio of whole nuts, if applicable.
- 24.5. Official Certificate of Analysis / Test Report:
- 24.5.1. Include all the following information:
- a) Sample lot number.
  - b) Analytical method used.
  - c) Limit of quantification, for the individual toxins and/or the sum.
  - d) Percent recovery for total aflatoxins.
  - e) Analytical result, for total aflatoxins.
  - f) A statement about correction for recovery.
  - c) Measurement uncertainty presented as the result  $\pm$  U (i.e., 10.4  $\pm$  2.1  $\mu$ g/kg), where U is the expanded MU, using a coverage factor of 2 which gives a level of confidence of approximately 95%.

24.5.2. Use the following statement to claim compliance with the Japan protocol when results comply with Japan's regulatory action limit of 10 ppb total aflatoxin (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>):

"The laboratory is USDA-approved and in good standing with the USDA/AMS Laboratory Approval Program for the Analysis of Mycotoxins (<https://www.ams.usda.gov/services/lab-testing/mycotoxins>). The lot referenced has been tested per the *Protocol for the Control of Aflatoxin in U.S. Almonds Exported to Japan*."

24.5.3. Use the following type of statement if reporting results for in shell nuts:

"In shell almonds are reported on a kernel basis for a [insert X/Y] shell/kernel ratio."

## **25. Explanations for Technical Requirements**

25.1. For sample grinding:

- a) Particle size is a performance control measurement of homogeneity (see §5.7.f)).
- b) Adequate grind is described in AOAC 977.16 and summarized as: "Aim at maximum practical size reduction and thoroughness of mixing to achieve effective distribution of contaminated portions...To achieve this degree of size reduction, nut must be ground to pass No. 20 sieve.

## **26. Revision History**

- 08/12/22      Original. This document is the product of rebranding LAP-Aflatoxins to incorporate additional mycotoxins into the Program's scope.      Prepared by Grace Vaillant, Branch Chief and Heath McClure, Program Manager
- 02/08/23      Added §20.5.1.h) "State whether the sheller is a signatory of the APC MOU" because the requirement was added to the APC MOU for 2023.      Prepared by Grace Vaillant, Branch Chief
- 06/01/24      Revisions were triggered by Regulation EC 401/2006 being repealed and replaced with EU 2023/2782; 1: Reorganized content to align with LAP-PR.05 and LAP-PR.06; §2.3: Added mention of APC and the MOU; §2.5: Added abbreviation for APC; §2.6: Applied APC abbreviation; §4: Added ML, MU, RSD<sub>r</sub>, RSD<sub>WR</sub>, Changed 'quantitation' to 'quantification' to align with EU 2023/2782; §5.6: Added reference for APC Sheller MOU; §5.7: Added doi.org links where available; §5.8: Added reference for EU 2023/2782 and EU 2023/915, removed EC 401/2006 and 1881/2006; §10.5: updated citation; §12 Table 1 & 2: Updated with new performance criteria; §12.1. Added definitions from EU 2023/2782 for how to perform the statistical calculations for evaluating method performance to ensure that all participants calculate in the same way; §14.5.c: edited for clarity; §14.7: edited for clarity and to make applicable to all mycotoxins; §14.8.c: edited for clarity; §15.a: edited to reflect updated language in EU 2023/2782; §15.b: added new requirement from EU 2023/2782 about calculating recovery for sum-ML; §15.4: added for clarity and auditability; §16.2: Edited sentence to begin with action; §18.5.1.c, 20.5.1.c, 22.5.1.c: updated for new LOQ requirement of EU 2023/2782; §18.5.1.e, 20.5.1.c, 22.5.1.c: updated for new sum-ML requirement of EU 2023/2782; §18.5.1.g, 20.5.1.g, 22.5.1.g, 23.5.1.g: updated with new MU requirement of EU 2023/2782; §18.5.2, 20.5.2, 22.5.2, 23.5.2: Changed allow for 'type of statement' and updated citation; §18.5.3, 19.5.3, 20.5.3, 21.5.3, 22.5.3, 23.5.3: Changed to allow for 'type of statement'; §18.5.4, 20.5.4, 22.5.4, 23.5.4: Changed to allow for 'type of statement', rearranged sentence to begin with action, updated citations to regulation and sections within, updated language to align with regulation, moved note to be after the statement options to improve readability. Prepared by Grace Vaillant, Branch Chief.
- 03/07/2025      Expanded Program scope to include Almonds to Japan and added LC-MS/MS as an acceptable detection method. Requirements for Almonds to Japan are based on the requirements for Almonds to EU and adjusted as needed to meet Japan's requirements to maintain consistency across the program and reduce burden for participants that participate in

elements of the Program's scope. §2.1: Edited for sentence structure. §2.3: Added citation to Peanut Export Program; §4: Added HS, MHLW and PEP; §5.6.d: Added reference for Peanut Export Program document; §5.8: Added references for Codex and Japan protocol documents; §9.4: Added Almonds to Japan; §18.2.c, 19.2.c, 20.2.d, 21.2.b, 22.2.c, 23.2.c: Edited for clarity and to focus on the grind resulting in a homogeneous and well mix sample, and made consistent across sections, §18.3.d, 19.3.f, 20.3.d, 21.3.e: Added LC-MS/MS as an acceptable methodology because first lab was approved in 2024 and review of CFR does not show conflict; §18.5.1.c, 20.5.1.c, 22.5.1.c: Edited to 'and/or' instead of 'and' based on lessons learned auditing the laboratory's implementation; §24: New section. Prepared by Branch Chief and Program Manager.

## **27. Review / Approvals**

Heath McClure  
Program Manager  
Reviewer

Grace Vaillant  
Branch Chief  
Approver