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Japan's Aflatoxin Testing Protocol

Report Categories:

Sanitary/Phytosanitary/Food Safety

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Report Highlights:

Japan's testing protocol for aflatoxin differs from the protocol in the United States. In addition to a more stringent action level of 10 parts per billion (ppb), compared to the U.S. 20ppb, Japan does not average the test results from the same shipment, allow reworking, or a retest. While concentrations of aflatoxin are naturally highly variable (heterogeneous), Japan's protocol increases the likelihood of detecting isolated concentrations and the risk of violating Japan's aflatoxin action level.

Keywords: JA8061

General Information:

Aflatoxin are a family of toxins produced by certain fungi and associated with increased risk to health. As such, many countries, including the United States, regulate the action level of aflatoxin concentration in domestic and imported foods. Japan revised its protocol for testing aflatoxin in 2011, in particular to increase the sample size from 1 kilogram (kg) to 5kg. While the revision brought the Japanese protocol closer to the protocol in the United States, there remain a number of significant differences that can contribute to disparate test results.

Note: Although Japan also regulates aflatoxin M1 in milk, this paper focuses on plant and plant-processed products.

The Ministry of Health, Labour and Welfare (MHLW) is Japan's regulator for domestic and imported foods. Each year, MHLW publishes a monitoring plan for imported foods on March 31 for the upcoming Japan fiscal year (April – March) (see the [section 2 and 4 of MHLW's Imported Foods Inspection Services Home Page](#) for further details) [available in English]. The 2018 plan targets foods for aflatoxin monitoring in:

1. Vegetables
2. Fruit
3. Grains (including minimum-access imported rice and tariff rice), beans and nuts
4. Tea

MHLW focuses mycotoxin monitoring in commodities that are susceptible to infestation by *Aspergillus flavus* (a fungus releasing aflatoxin). MHLW revises the target commodities each fiscal year based on the number of aflatoxin violations detected in the previous fiscal year. Generally, MHLW focuses on cereals, nuts, and dried fruits.

Separate from MHLW's monitoring plan, commodities may be subject to aflatoxin testing due to an inspection order. MHLW issues an inspection order following two violations by two parties from the same country in a single 12-month period. When subject to an inspection order, all shipments from that country are held at the port until a test confirms its compliance with Japan's standards (a action level of 10 parts per billion (ppb)). As of August 1, 2018, MHLW has issued an inspection order for the following U.S. commodities (including any processed product that contains 10 percent or more in volume of the following, in alphabetical order): almonds, brazil nuts, chili peppers, corn (including flour), dried dates, dried figs, giant corn, Job's tears, nutmeg, pistachio nuts, peanuts, red peppers, and walnuts. For further details, see [section 3 of MHLW's Imported Foods Inspection Services Home Page](#) (available in English).

MHLW requires 300 clean shipments of the commodity under an inspection order in a period longer than one year, or the passage of two years with no violations to lift an inspection order.

On March 31, 2011, MHLW published the official sampling protocol for aflatoxin testing in "Notice Syoku-An No. 0331-6". According to the notice, MHLW determines the sampling volumes by the weight of a single unit of the import commodity (grain, seed, kernel, etc...), and the number of sub-lots per shipment or import declaration. *Note: a sub-lot is defined here as a form of packaging (e.g.*

packaged, canned, carton, or bulk) as appropriate per commodity and shipment.

When the weight of a single unit of commodity is 0.1 gram or less:

Representative commodities for this category are sesame seeds, cottonseeds, rice (including wild rice), and sliced or shredded foods.

Case 1 - Products in bags that each exceed 20 kg (bulk shipment)

Number of sub-lots per lot	Number of sub-lots sampled for inspection	Quantity collected	Number of Laboratory Samples
Less than 280	32	1 kg	1
281 - 500	50	1 kg	1
501-1,200	80	1 kg	1
1,201 – 3,200	130 - (65 x 2 sets)	2 kg - (1 kg x 2 sets)	2
More than 3,201	210 - (70 x 3 sets)	3 kg - (1 kg x 3 sets)	3

Case 2 - Products in cans or cartons that exceeds 4.5 kg

Number of sub-lots per lot	Number of sub-lots sampled for inspection	Quantity collected	Number of Laboratory Samples
Less than 50	2	1 kg	1
51 - 500	4 (2 x 2 sets)	2 kg - (0.5 kg x 2) x 2 sets	2
More than 501	6 (2 x 3 sets)	3 kg - (0.5 kg x 2) x 3 sets	3

Case 3 - Products other than in cases 1 and 2 above (typically in small packages)

Number of sub-lots per lot	Number of sub-lots sampled for inspection	Quantity collected	Number of Laboratory Samples
Less than 50	2	The minimum laboratory sample shall be 150 grams. The inspector shall draw from additional sub-lots to collect a minimum of 150 grams.	1
51 - 500	3		1
501 – 3,200	6 (3 x 2 sets)		2
More than 3,201	9 (3 x 3 sets)		3

When the weight of a single unit of commodity is greater than 0.1 gram:

Commodities in this category include (but are not limited to) almonds, dried figs, cacao beans, coffee beans, turmeric, soybeans, chili peppers, corn, pistachios, hazel nuts, and peanuts.

Case 1 - Products in bags that exceed 20 kg (bulk shipment)

Number of sub-lots per lot	Number of sub-lots sampled for inspection	Quantity collected	Number of Laboratory Samples
Less than 280	32	5 kg	1
281 - 500	50	5 kg	1
501-1,200	80	5 kg	1
1,201 – 3,200	130 - (65 x 2 sets)	10 kg - (5 kg x 2 sets)	2
More than 3,201	210 - (70 x 3 sets)	15 kg - (5 kg x 3 sets)	3

Case 2 - Products in cans or cartons that exceed 4.5 kg

Number of sub-lots per lot	Number of sub-lots sampled for inspection	Quantity collected	Number of Laboratory Samples
Less than 50	2	5 kg	1
51 - 500	4 (2 x 2 sets)	10 kg - (2.5 kg x 2) x 2 sets	2
More than 501	6 (2 x 3 sets)	15 kg - (2.5 kg x 2) x 3 sets	3

Case 3 - Products other than in cases 1 and 2 above (typically in small packages)

Number of sub-lots per lot	Number of sub-lots sampled for inspection	Quantity collected	Number of Laboratory Samples
Less than 50	2	The minimum laboratory sample shall be 150 grams. The inspector shall draw from additional sub-lots to collect a minimum of 150 grams.	1
51 - 500	3		1
501 – 3,200	6 (3 x 2 sets)		2
More than 3,201	9 (3 x 3 sets)		3

On August 16, 2011, MHLW published its aflatoxin testing protocols in “Notice Syoku-An No. 0816-2”. In the notification, MHLW introduced two methods for aflatoxin testing. One is a method using a

multifunctional column and the other is with use of an immune-affinity column. Whereas the former column is the default method for enrichment/purification of samples for aflatoxin testing, MHLW suggests the use of the latter column for coffee beans, roasted cereals, dried figs, spices, processed products and others in which the former column is unlikely to purify sufficiently for aflatoxin analysis. These samples are then analyzed by HPLC-FL (High Pressure Liquid Chromatography equipped with Fluorometry), LC-MS (Liquid Chromatography equipped with Mass Spectrometer), or LC-MS/MS (Liquid Chromatography equipped with tandem Mass Spectrometer). For complete details of these testing protocols, please see [“Related to the Method of Testing Aggregate Aflatoxin”](#) (in Japanese only).

Japanese officials collect test sample(s) based on the above-described tables and the information contained in the import request form. Each test sample is crushed and homogenized, from which three sets of fifty grams are taken for aflatoxin analysis by HPLC. Japan treats these as independent analyses and does not average the values of these test results (in case multiple test samples are collected). Therefore, the shipment (as described in the import request form) is rejected if one of these three test results exceeds the action level allowed for aflatoxin of 10 ppb.

MHLW does not allow retesting for a valid aflatoxin analysis. MHLW validates aflatoxin testing based on the parameters in the table below. For complete details of validity assessment, please see page 10 of [“Related to the Method of Testing Aggregate Aflatoxin”](#) (in Japanese only).

Target	Number of Trials	Accuracy (%)	Repeatability/ Intra-Assay Precision (RSD %)*	Intermediate Precision (RSD %)*
Aflatoxin B ₁	5	70 - 110	Less than 20%	Less than 30%
Aflatoxin B ₂	5	70 - 110	Less than 20%	Less than 30%
Aflatoxin G ₁	5	70 - 110	Less than 20%	Less than 30%
Aflatoxin G ₂	5	70 - 110	Less than 20%	Less than 30%

* RSD – Relative Standard Deviation

On March 31, 2011, MHLW notified modifications to its aflatoxin regulations in “Notice Syoku-An No. 0331-5”. The modification revises the definition of total aflatoxin as the sum of aflatoxin B₁, B₂, G₁ and G₂. As a result, the current ML of aflatoxin in Japan is 10 ppb of the total aflatoxin for all food items. For any test with multiple laboratory samples, i.e. a shipment with a large number of sub-lots, any result found to exceed 10 ppb of total aflatoxin results in a violation for the entire lot. Furthermore, though retesting is not precluded by the regulation, MHLW does not grant requests to retest.

Comparison of the U.S. and Japan aflatoxin regulations

There are several technical differences between the U.S. and Japanese aflatoxin protocols and these are summarized in the table below. The following summarizes the substantial differences between the each protocol.

Homogenization of tests samples

Japan treats each laboratory result as an independent inspection. The United States homogenizes all samples into a single laboratory sample for testing.

The action level of aflatoxin concentration allowed

The action level of aflatoxin concentration permitted in the United States is 20 ppb for all products including nuts and grains. Japan's action level is 10 ppb.

Averaging test results (if multiple tests)

The United States averages the results of multiple tests. Japan treats each test result as independent.

Statistical variation

The United States takes into account the statistical variation of the test results (when multiple tests are conducted). Japan does not consider statistical variation – for the three sample results that are averaged into a single laboratory result, or for the three laboratory results.

Reworking

The U.S. system provides options to rework shipments/lots that exceed the action level. However, as Japan's system does not provide a reworking option, shipments/lots that exceed Japan's action level must be disposed or diverted for non-human consumption.

Retesting

Japan does not allow retesting under any circumstance. The United States does allow retesting if reworking has been conducted.