

SOP FOR SAMPLING AND TESTING FOR AFLATOXIN

MAIZE GRAINS

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1. Purpose

This document establishes procedures for determining aflatoxin in grain, and certifying the results.

2. Background

General

Aflatoxin is a naturally occurring mycotoxin produced by two types of mold: *Aspergillus flavus* and *Aspergillus parasiticus*. *Aspergillus flavus* is common and widespread in nature and is most often found when certain grains are grown under stressful conditions such as drought. The mold occurs in soil, decaying vegetation, hay, and grains undergoing microbiological deterioration and invades all types of organic substrates whenever and wherever the conditions are favorable for its growth. Favorable conditions include high moisture content and high temperature. At least 13 different types of aflatoxin are produced in nature with aflatoxin B1 considered as the most toxic. While the presence of *Aspergillus flavus* does not always indicate harmful levels of aflatoxin, it does mean that the potential for aflatoxin production is present.

Sub-Saharan Africa

There is ample evidence that the inhabitants of Sub-Saharan Africa are experiencing heavy dietary exposure to foodborne mycotoxins particularly aflatoxins and fumonisins. Diseases caused by mycotoxins lead to reduced life expectancy in Africa where the need to eat outweighs other considerations such as safety. The tropical climate together with an all year round high ambient temperature and relative humidity provide optimal conditions for growth of toxigenic moulds in most countries of sub-Saharan Africa. Given such conditions, it is essential that food is carefully and properly stored or processed in order to avoid contamination by these fungi and their toxins.

Consequences

In young children aflatoxins is associated with underweight and stunting, neurological impairment, immune suppression and child mortality

The outbreak of acute aflatoxicosis which occurred in Kenya from May to September 2004 touched 341 people and caused 123 deaths.

Prevention

- Promotion of rapid methods for drying (ideally within 48h after harvest), by sun drying (on a tarpaulin, or concrete clean space) or mechanical drying.
- Evaluation and adaptation of storage technologies to local conditions, for example, grain warehouses with dryers and rapid grain moisture testers.
- Promotion of good traditional practices such as sorting (i.e. sorting and elimination of mouldy cobs or grains).
- The use of sieving and density segregation to reduce mycotoxin contamination

Official limits

- In 2003, 14 African countries had standards for aflatoxins.
- US FDA has established action levels for aflatoxin present in food to 20 ppb.
- European Union standard are 4 ppb in cereals and cereal products (e.g. MML)
- WFP general specifications: 20 ppb max.

3. Sampling

The most important step in the process of identifying aflatoxin is sampling due to the skewing of distribution of aflatoxins in foods which often results in high sampling error.

3.1. Reference

Reference: *ISO 24333 (First edition: 2009-12-15) Cereals and cereal products — Sampling*

3.2. Scope

This International Standard specifies requirements for the dynamic or static sampling, by manual or mechanical means, of cereals and cereal products, for assessment of their quality and condition.

It is applicable to sampling for the determination of heterogeneously distributed contaminants, undesirable substances, and parameters usually homogeneously distributed like those used to assess quality or compliance with specification.

3.3. Definitions

- **Aggregate sample:** means a quantity of material taken that is fully representative of a lot or sub-lot;
- **Incremental sample:** means quantity of material taken in one action (e.g. by spear or container)
- **Laboratory sample:** means a sample intended for the laboratory analyses.
- **Retention sample:** means a sample kept for counter analysis (e.g. for litigation issues).
- **Lot:** means an identifiable quantity of a food commodity delivered at one time and determined by the official to have common characteristics, such as origin, variety, type of packing, packer, consignor or markings
- **Sub-lot:** means a designated part of a large lot in order to apply the sampling method on that designated part; each sub-lot must be physically separate and identifiable

For aflatoxin, the lab sample MUST be at least of 10kg and retention sample of 10 kg, total quantity to sample MUST be 20 kg.

3.4. Flowing bulk grain sampling

For instance, for moving grains, on conveyor belt, auger, discharging silo, etc.

- A sample will be taken for each sub-lot of 500MT
- To take at least 100 increments.
- Each increment should be of 220g minimum.
- The timing of sampling is linked to the speed of the flowing grain, e.g.

T (number of increment/hour) = $i / b \times FR$

b. sub-lot [500 MT]

i. increment [100 increments]

FR. Flow Rate in MT of grain/hour

For example: $T = 100 / 500 \times 2.5 \text{ MT/h} = 0.5 \text{ increment/h}$ or 1 increment every 2 hours.

For each 500MT, 100 increments are required; the timing of taking them is related to the flow of discharge / loading – see formula above.

3.5. For bagged grain sampling:

For each sub-lot of 500MT

$F(n) = [m(b) \times m(i)] / [m(a) \times m(p)]$. $F(n) = [500,000 \times 0.1] / [10 \times 50]$

b. sub-lot

i. increment

a. lab sample

p. bag

Increment will be taken every 100 bags.

If 20kg are needed – each increment should be of 220g minimum

4. Lab sample

4.1. References

WFP's intention is to build a lab sample of **10 kg** as recommended by *ISO 24333: 2009*¹ and *European Commission Regulation (EC) No 401/2006*

4.2. Homogenization

The aggregate sample (i.e. all increment samples) shall be thoroughly homogenized prior to any division procedure intended to obtain the laboratory sample and the retention sample.

4.3. Division

Either use of a sample divider on a flat surface, or follow the coning or quartering method as described hereunder:

- a) Thoroughly mix the aggregate sample by repeating operations 2 and 3 at least twice before dividing as described in operations 3 and 4. Work on a clean, non-absorbent surface.
- b) Gather the grains together into a cone-shaped pile.
- c) Flatten out the surface of the pile and then divide the pile into quarters, A, B, C, and D.
- d) Discard two diagonally opposed quarters (B and C) and mix the two remaining quarters (A and D).
- e) Repeat the whole process until the laboratory sample of the required size is obtained.

4.4. Packaging of samples

Laboratory samples shall be placed in clean containers. The containers shall be suitable for the 10kg of the laboratory samples. The containers shall also preserve the initial characteristics of laboratory samples. These containers shall be completely full and shall be sealed to avoid any change in their contents. Seals shall be tamper-proof and identifiable.

4.5. Labels for samples

The information listed below shall be marked indelibly and legibly. The information on the labels on the laboratory sample shall include the instructions required under the terms of the contract, for example:

- a) Maize grains;
- b) 10kg;
- c) the lot identifier;
- d) the contract number;
- e) the sampling date;
- f) the location and point of sampling;
- g) the name of the person who carried out the sampling.

4.6. Shipment of samples

Samples should be sent to the laboratory as quickly as possible. The samples should be stored and transported in conditions appropriate to the preservation of their integrity.

4.7. Sampling report

The sampling report may contain some or all of the following information:

- a) the date of sampling;
- h) the name and signature of the persons authorized to carry out sampling;
- i) if necessary:
 - the name and signature of the seller,
 - the name and signature of the buyer,
 - the name and signature of the deliverer;
- j) the description of the product, including:
 - sample reference,
 - sample mass,
 - lot size,

¹ Cereal and cereal products – sampling.

- sample origin (e.g. vertical silo, lorry);
- k) the description of the sampling operation, including:
 - the location and point of sampling,
 - the number of increments per lot,
 - the number of laboratory samples per lot,
 - the sampling procedure used (equipment, static/flowing, etc.),
 - the destination of the sample, e.g. the name and address to which the samples are to be shipped,
 - comments if any;
- l) the transportation and storage conditions.

5. Testing

5.1. Reference

The **European Commission Regulation (EC) No 401/2006** must be followed to analyse the 10 kg sample. The method implies to grind the 10 kg, mix the flour thoroughly and then, only then, to extract the quantity needed for the analysis.

The method is available on line and the link should be provided to the laboratory.

<http://www.icc.or.at/task/EC401-2006.pdf>

5.2. Existing methods for detection of mycotoxins in foods

- Thin Layer Chromatography (TLC),
- High Performance Liquid Chromatography (HPLC),
- Minicolumns, Immunoassays such as Enzyme Linked Immunosorbent Assay (ELISA)
- Immunoaffinity Columns (IAC).

QUALITATIVE Test kits, such as Aflacard T20, AgraStrip, AgriScreen, QuickTox, ReVeal, Rosa-P/N, Diachemix FPA, MycoAflatox, ReVealSQ can be used

QUANTITATIVE Test kits, such as Aflatest, Fluoroquant, Myco, RidascreenFastAflatoxin, Rosa-Quantitative, or VeratoxAST can be used for maize, sorghum, and maize meal.

6. References:

- Health and Nutrition impacts of Aflatoxins in foods. Report of an Expert Group Meeting Held from 24 to 27 May 2005 by the **World Health Organisation** Regional Office for Africa, Brazzaville, Republic of Congo
- **International Standard** ISO 24333: 2009: Cereals and cereal products – Sampling.
- **European Commission Regulation (EC) No 401/2006** laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs
- Aflatoxin Handbook. **US Department of Agriculture**, Grain Inspection, Packers and Stockyards Administration, Federal Grain Inspection Service. May 2009.