Annex 1

SAMPLING PLAN FOR TOTAL AFLATOXINS IN PEANUTS INTENDED FOR FURTHER PROCESSING

INTRODUCTION

1. The sampling plan calls for a single 20 kg laboratory sample of shelled peanuts (27 kg of unshelled peanuts) to be taken from a peanut lot (sub-lot) and tested against a maximum level of 15 μg/kg total aflatoxins.

2. This sampling plan has been designed for enforcement and controls concerning total aflatoxins in bulk consignments of peanuts traded in the export market. To assist member countries in implementing the sampling plan, sample selection methods, sample preparation methods and analytical methods required, to quantify aflatoxin in bulk peanut lots are described in this document.

A. DEFINITIONS

Lot	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.
Sublot	Designated part of a large lot in order to apply the sampling method on that designated part. Each sublot must be physically separate and identifiable.
Sampling plan	It is defined by an aflatoxin test procedure and an accept/reject limit. An aflatoxin test procedure consists of three steps: sample selection, sample preparation and aflatoxin quantification. The accept/reject limit is a tolerance usually equal to the Codex maximum level.
Incremental sample	A quantity of material taken from a single random place in the lot or sublot.
Aggregate sample	The combined total of all the incremental samples taken from the lot or sublot. The aggregate sample has to be at least as large as the 20 kg laboratory sample.
Laboratory sample	The smallest quantity of peanuts comminuted in a mill. The laboratory sample may be a portion of or the entire aggregate sample. If the aggregate sample is larger than 20 kg, a 20 kg laboratory sample should be removed in a random manner from the aggregate sample. The sample should be finely ground and mixed thoroughly using a process that approaches as complete a homogenisation as possible.
Test portion	A portion of the comminuted laboratory sample. The entire 20 kg laboratory sample should be comminuted in a mill. A portion of the comminuted 20 kg sample is randomly removed for the extraction of the aflatoxin for chemical analysis. Based upon grinder capacity, the 20 kg aggregate sample can be divided into several equal sized samples, if all results are averaged.

B. SAMPLING

Material to be sampled

3. Each lot, which is to be examined, must be sampled separately. Large lots should be subdivided into sublots to be sampled separately. The subdivision can be done following provisions laid down in Table 1 below.

4. Taking into account that the weight of the lot is not always an exact multiple of the weight of the sublots, the weight of the sublot may exceed the mentioned weight by a maximum of 20%.

Table 1. Subdivision of large lots into sublots for sampling

Commodity	Lot weight – tonne (T)	Weight or number of sublots	Number of incremental samples	Laboratory sample weight (kg)
Peanuts	≥ 500	100 tonnes	100	20
	> 100 and < 500	5 sublots	100	20
	≥ 25 and ≤ 100	25 tonnes	100	20
	> 15 and <= 25	--1 sublot	100	20

Number of incremental samples for lots of less than 15 tonnes

5. The number of incremental samples to be taken depends on the weight of the lot, with a minimum of 10 and a maximum of 100. The figures in the following Table 2 may be used to determine the number of incremental samples to be taken. It is necessary that the total sample weight of 20 kg is achieved.

Table 2. Number of incremental samples to be taken depending on the weight of the lot

Lot weight tonnes – (T)	N° of incremental samples
$T \leq 1$	10
$1 < T \leq 5$	40
$5 < T \leq 10$	60
$10 < T < 15$	80

Incremental sample selection

6. Procedures used to take incremental samples from a peanut lot are extremely important. Every individual peanut in the lot should have an equal chance of being chosen. Biases will be introduced by the sample selection methods if equipment and procedures used to select the incremental samples prohibit or reduce the chances of any item in the lot from being chosen.

7. Since there is no way to know if the contaminated peanut kernels are uniformly dispersed throughout the lot, it is essential that the aggregate sample be the accumulation of many small portions or increments of the product selected from different locations throughout the lot. If the aggregate sample is larger than desired, it should be blended and subdivided until the desired laboratory sample size is achieved.

Static lots

8. A static lot can be defined as a large mass of peanuts contained either in a single large container such as a wagon, truck, or railcar or in many small containers such as sacks or boxes and the peanuts are stationary at the time a sample is selected. Selecting a truly random sample from a static lot can be difficult because the container may not allow access to all peanuts.

9. Taking a aggregate sample from a static lot usually requires the use of probing devices to select product from the lot. The probing devices used should be specially designed for the type of container. The probe should (1) be long enough to reach all product, (2) not restrict any item in the lot from being selected, and (3) not alter the items in the lot. As mentioned above, the aggregate sample should be a composite from many small increments of product taken from many different locations throughout the lot.

10. For lots traded in individual packages, the sampling frequency (SF), or number of packages that incremental samples are taken from, is a function of the lot weight (LT), incremental sample weight (IS), aggregate sample weight (AS) and the individual packing weight (IP), as follows:

    Equation 1: $SF = (LT \times IS) / (AS \times IP)$

    The sampling frequency (SF) is the number of packages sampled. All weights should be in the same mass units such as kg.

Dynamic lots

11. True random sampling can be more nearly achieved when selecting an aggregate sample from a moving stream of peanuts as, the lot is transferred, for example, by a conveyor belt from one location to another. When sampling from a moving stream, take small increments of product from the entire length of the moving stream; composite the peanuts to obtain an aggregate sample; if the aggregate sample is larger than the required laboratory sample, then blend and subdivide the aggregate sample to obtain the desired size laboratory sample.

12. Automatic sampling equipment such as cross-cut samplers are commercially available with timers that automatically pass a diverter cup through the moving stream at predetermined and uniform intervals. When automatic equipment is not available, a person can be assigned to manually pass a cup though the stream at periodic intervals to collect incremental samples. Whether using automatic or manual methods, small increments of peanuts should be collected and composited at frequent and uniform intervals throughout the entire time peanuts flow past the sampling point.

13. Cross-cut samplers should be installed in the following manner: (1) the plane of the opening of the diverter cup should be perpendicular to the direction of flow; (2) the diverter cup should pass through the entire cross sectional area of the stream; and (3) the opening of the diverter cup should be wide enough to accept all items of interest in the lot. As a general rule, the width of the diverter cup opening should be about three times the largest dimensions of the items in the lot.

14. The size of the aggregate sample (S) in kg, taken from a lot by a cross cut sampler is:

    Equation 2: $S = (D \times LT) / (T \times V)$

    D is the width of the diverter cup opening (in cm), LT is the lot size (in kg), T is interval or time between cup movement through the stream (in seconds), and V is cup velocity (in cm/sec).

15. If the mass flow rate of the moving stream, MR (kg/sec), is known, then the sampling frequency (SF), or number of cuts made by the automatic sampler cup is:

    Equation 3: $SF = (S \times V) / (D \times MR)$

16. Equation 2 can also be used to compute other terms of interest such as the time between cuts (T). For example, the required time (T) between cuts of the diverter cup to obtain a 20 kg aggregate sample from a 30 000 kg lot where the diverter cup width is 5.08 cm (2 inches), and the cup velocity through the stream 30 cm/sec. Solving for T in Equation 2.

    $T = (5.08 \text{ cm} \times 30\,000 \text{ kg}) / (20 \text{ kg} \times 30 \text{ cm/sec}) = 254 \text{ sec}$

17. If the lot is moving at 500 kg per minute, the entire lot will pass through the sampler in 60 minutes and only 14 cuts (14 incremental samples) will be made by the cup through the lot. This may be considered too infrequent in that too much product passes through the sampler between the time the cup cuts through the stream.

Weight of the incremental sample

18. The weight of the incremental sample should be approximately 200 g or greater, depending on the total number of increments, to obtain an aggregate sample of 20 kg.

Packaging and transmission of samples

19. Each laboratory sample shall be placed in a clean, inert container offering adequate protection from contamination and against damage in transit. All necessary precautions shall be taken to avoid any change in composition of the laboratory sample which might arise during transportation or storage.

Sealing and labelling of samples

20. Each laboratory sample taken for official use shall be sealed at the place of sampling and identified. A record must be kept of each sampling, permitting each lot to be identified unambiguously and giving the date and place of sampling together with any additional information likely to be of assistance to the analyst.

C. SAMPLE PREPARATION

Precautions

21. Daylight should be excluded as much as possible during the procedure, since aflatoxin gradually breaks down under the influence of ultra-violet light.

Homogenisation – Grinding

22. As the distribution of aflatoxin is extremely non-homogeneous, samples should be prepared - and especially homogenised - with extreme care. All laboratory sample obtained from aggregate sample is to be used for the homogenisation/grinding of the sample.

23. The sample should be finely ground and mixed thoroughly using a process that approaches as complete a homogenisation as possible.

24. The use of a hammer mill with a #14 screen (3.1 mm diameter hole in the screen) has been proven to represent a compromise in terms of cost and precision. A better homogenisation (finer grind – slurry) can be obtained by more sophisticated equipment, resulting in a lower sample preparation variance.

Test portion

25. A minimum test portion size of 100 g taken from the laboratory sample.

D. ANALYTICAL METHODS

Background

26. A criteria-based approach, whereby a set of performance criteria is established with which the analytical method used should comply, is appropriate. The criteria-based approach has the advantage that, by avoiding setting down specific details of the method used, developments in methodology can be exploited without having to reconsider or modify the specified method. The performance criteria established for methods should include all the parameters that need to be addressed by each laboratory such as the detection limit, repeatability coefficient of variation, reproducibility coefficient of variation, and the percent recovery necessary for various statutory limits. Utilising this approach, laboratories would be free to use the analytical method most appropriate for their facilities. Analytical methods that are accepted by chemists internationally (such as AOAC) may be used. These methods are regularly monitored and improved depending upon technology.

Performance criteria for methods of analysis

Table 3. Specific requirements with which methods of analysis should comply

Criterion	Concentration Range	Recommended Value	Maximum Permitted Value
Blanks	All	Negligible	-
Recovery-Aflatoxins Total	1 – 15 μg/kg	70 to 110%	
	> 15 μg/kg	80 to 110%	
Precision RSD_R	All	As derived from Horwitz Equation	2 x value derived from Horwitz Equation
Precision RSD_r may be calculated as 0.66 times Precision RSD_R at the concentration of interest			

- The detection limits of the methods used are not stated as the precision values are given at the concentrations of interest;
- The precision values are calculated from the Horwitz equation, i.e.:

$$RSD_R = 2^{(1-0.5\log C)}$$

where:

  - RSD_R is the relative standard deviation calculated from results generated under reproducibility conditions $[(S_r / \bar{x}) \times 100]$
  - C is the concentration ratio (i.e. 1 = 100 g/100 g, 0.001 = 1 000 mg/kg)

27. This is a generalised precision equation, which has been found to be independent of analyte and matrix but solely dependent on concentration for most routine methods of analysis.