General Standard for Contaminants and Toxins in Foods
（CODEX STAN 193-1995）（抜粋）

AFLATOXINS, TOTAL

Reference to JECFA:	31 (1987), 46 (1996), 49 (1997), 68 (2007)
Toxicological guidance:	Carcinogenic potency estimates for aflatoxins B, G, M (1997, Intake should be reduced to levels as low as reasonably possible.)
Residue definition:	Aflatoxins total (B1 +B2 + G1 + G2)
Synonyms:	Abbreviations, AFB, AFG, with numbers, to designate specific compounds
Related Code of Practice:	Code of Practice for the Prevention and Reduction of Aflatoxin Contamination in Peanuts (CAC/RCP 55-2004)
	Code of Practice for the Prevention and Reduction of Aflatoxin Contamination in Tree Nuts (CAC/RCP 59-2005)
	Code of Practice for the Reduction of Aflatoxin B1 in Raw Materials and Supplemental Feedingstuffs for Milk Producing Animals (CAC/RCP 45-1997)
	Code of Practice for the Prevention and Reduction of Aflatoxin Contamination in Dried Figs (CAC/RCP 65-2008)

Commodity/Product Code	Name	Level ug/kg	Suffix	Type	Reference	Notes/Remarks for Codex Alimentarius
SO 0697	Peanut	15		ML		The ML applies to peanuts intended for further processing. For sampling plan, see Annex 1 below.
TN 0660	Almonds	15		ML		The ML applies to almonds intended for further processing For sampling plan, see Annex 2 below
TN 0666	Hazelnuts	15		ML		The ML applies to hazelnuts intended for further processing For sampling plan, see Annex 2 below
TN 0675	Pistachios	15		ML		The ML applies to pistachios intended for further processing For sampling plan, see Annex 2 below
TN 0660	Almonds	10		ML		The ML applies to almonds "ready-to-eat". For sampling plan, see Annex 2
TN 0666	Hazelnuts	10		ML		The ML applies tohazelnuts "ready-to-eat". For sampling plan, see Annex 2
TN 0675	Pistachios	10		ML		The ML applies to pistachios "ready-to-eat". For sampling plan, see Annex 2.

Aflatoxins are a group of highly toxic mycotoxins produced by fungi of the genus Aspergillus. The four main aflatoxins found in contaminated plant products are B1, B2, G1 and G2 and are a group of structurally related difuranocoumarin derivatives that usually occur together in varying ratios, AFB1 usually being the most important one. These compounds pose a substantial hazard to human and animal health. IARC (1992) classified aflatoxin B1 in Group 1 (human carcinogen) and AFM in Group 2B (probable human carcinogen). The liver is the primary target organ.

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 micrograms per kilogram (µg/kg) total aflatoxins.

CODEX STAN 193-1995
Schedule I

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 Codex 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:	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 limit.
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:	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:	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

<u>Number of Incremental Samples for Lots of Less than 15 Tonnes</u>

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≤ 1	10
1 <T ≤ 5	40
5< T ≤ 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 through out 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 x IS)/(AS x 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 x LT) / (T x 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 x V) / (D x 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 cm x 30,000 kg)/(20 kg x 30 cm/sec) = 254 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 grams or greater, depending on the total number of increments, to obtain an aggregate sample of 20kg.

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.

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$_R$ / $\overline{x}$) x 100]

* C is the concentration ratio (i.e. 1 = 100g/100g, 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.

Annex 2

SAMPLING PLANS FOR AFLATOXIN CONTAMINATION IN READY-TO-EAT TREENUTS AND TREENUTS DESTINED FOR FURTHER PROCESSING: ALMONDS, HAZELNUTS AND PISTACHIOS

DEFINITION

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 larger lot in order to apply the sampling method on that designated part. Each sublot must be physically separate and identifiable.

Sampling plan - 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 – the quantity of material taken from a single random place in the lot or sublot.

Aggregate sample - the combined total of all the incremental samples that is taken from the lot or sublot. The aggregate sample has to be at least as large as the laboratory sample or samples combined.

Laboratory sample – the smallest quantity of tree nuts comminuted in a mill. The laboratory sample may be a portion of or the entire aggregate sample. If the aggregate sample is larger than the laboratory sample(s), the laboratory sample(s) should be removed in a random manner from the aggregate sample.

Test portion – a portion of the comminuted laboratory sample. The entire laboratory sample should be comminuted in a mill. A portion of the comminuted laboratory sample is randomly removed for the extraction of the aflatoxin for chemical analysis.

Ready-to-eat treenuts – nuts, which are not intended to undergo an additional processing/treatment that has proven to reduce levels of aflatoxins.

Treenuts destined for further processing – nuts, which are intended to undergo an additional processing/treatment that has proven to reduce levels of aflatoxins before being used as an ingredient in foodstuffs, otherwise processed or offered for human consumption. Processes that have proven to reduce levels of aflatoxins are shelling, blanching followed by color sorting, and sorting by specific gravity and color (damage). There is some evidence that roasting reduces aflatoxins in pistachios but for other nuts the evidence is still to be supplied.

Operating Characteristic (OC) Curve – a plot of the probability of a accepting a lot versus lot concentration when using a specific sampling plan design. The OC curve provides an estimate of good lots rejected (exporter's risk) and bad lots accepted (importer's risk) by a specific aflatoxin sampling plan design.

SAMPLING PLAN DESIGN CONSIDERATIONS

1. Importers may commercially classify treenuts as either "ready-to-eat" (RTE) or "destined for further processing" (DFP). As a result, maximum levels and sampling plans are proposed for both commercial types of treenuts. Maximum levels need to be defined for treenuts destined for further processing and ready-to-eat treenuts before a final decision can be made about a sampling plan design.

2. Treenuts can be marketed either as inshell or shelled nuts. For example, pistachios are predominately marketed as inshell nuts while almonds are predominately marketed as shelled nuts.

3. Sampling statistics, shown in Annex I, are based upon the uncertainty and aflatoxin distribution among laboratory samples of shelled nuts. Because the shelled nut count per kg is different for each of the three treenuts, the laboratory sample size is expressed in number of nuts for statistical purposes. However, the shelled nut count per kg for each treenut, shown in Annex I, can be used to convert laboratory sample size from number of nuts to mass and vice versa.

4. Uncertainty estimates associated with sampling, sample preparation, and analysis, shown in Annex I, and the negative binomial distribution[2] are used to calculate operating characteristic (OC) curves that describe the performance of the proposed aflatoxin-sampling plans (Annex II).

[2] Whitaker, T., Dickens, J., Monroe, R., and Wiser, E. 1972. Comparison of the negative binomial distribution of aflatoxin in shelled peanuts to the negative binomial distribution. J. American Oil Chemists' Society, 49:590-593.

5. In Annex I, the analytical variance reflects a reproducibility relative standard deviation of 22%, which is suggested by Thompson and is based upon Food Analysis Performance Assessment Scheme (FAPAS) data[3]. A relative standard deviation of 22% is considered by FAPAS as an appropriate measure of the best agreement that can be reliably obtained between laboratories. An analytical uncertainty of 22% is larger than the within laboratory variation measured in the sampling studies for the three treenuts. The within laboratory analytical uncertainty for each treenut can be found at the website http://www5.bae.ncsu.edu/usda/www/ResearchActDocs/treenutwg.html.

6. The issue of correcting the analytical test result for recovery is not addressed in this document. However, Table 2 specifies several performance criteria for analytical methods including suggestions for the range of acceptable recovery rates.

AFLATOXIN TEST PROCEDURE AND MAXIMUM LEVELS

7. An aflatoxin-sampling plan is defined by an aflatoxin test procedure and a maximum level. A value for the proposed maximum level and the aflatoxin test procedure are given below in this section.

8. The maximum levels for total aflatoxins in treenuts (almonds, hazelnuts, and pistachios) "ready-to-eat" and "destined for further processing" are 10 and 15 ng/g, respectively.

9. Choice of the number and size of the laboratory sample is a compromise between minimizing risks (false positives and false negatives) and costs related to sampling and restricting trade. For simplicity, it is recommended that the proposed aflatoxin sampling plans use a 20 kg aggregate sample for all three treenuts.

10. The two sampling plans (RTE and DFP) have been designed for enforcement and controls concerning total aflatoxins in bulk consignments (lots) of treenuts traded in the export market.

Treenuts destined for further processing

Maximum level – 15 ng/g total aflatoxins

Number of laboratory samples – 1

Laboratory sample size - 20 kg
   Almonds – shelled nuts
   Hazelnuts – shelled nuts
   Pistachios – inshell nuts (equivalent to about 10kg shelled nuts that is calculated on the basis of the actual edible portion in the sample)

Sample preparation – dry grind with vertical cutter mixer type mill and a 50 g test portion

Analytical method – performance based (see Table 2)

Decision rule – If the aflatoxin test result is less than or equal to 15 ng/g total aflatoxins, then accept the lot. Otherwise, reject the lot.

The operating characteristic curve describing the performance of the sampling plan for the three treenuts destined for further processing is shown in Annex II.

Ready-to-eat treenuts

Maximum level – 10 ng/g total aflatoxins

Number of laboratory samples – 2

Laboratory sample size - 10 kg
   Almonds – shelled nuts
   Hazelnuts – shelled nuts
   Pistachios – inshell nuts (equivalent to about 5 kg shelled nuts per test sample that is calculated on the basis of the actual edible portion in the sample)

Sample preparation – dry grind with vertical cutter mixer type mill and a 50 g test portion

Analytical method – performance based (see Table 2)

Decision rule – If the aflatoxin test result is less than or equal to 10 ng/g total aflatoxin in both test samples, then accept the lot. Otherwise, reject the lot.

[3] Thompson, M. 2000. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing. J. Royal Society of Chemistry, 125:385-386.

The operating characteristic curve describing the performance of the sampling plan for the three ready-to-eat treenuts is shown in Annex II.

11. To assist member countries implement these two Codex sampling plans, sample preparation methods, and analytical methods required to quantify aflatoxin in laboratory samples taken from bulk treenut lots are described in the following sections.

SAMPLE SELECTION

Material to be sampled

12. Each lot, which is to be examined for aflatoxin, must be sampled separately. Lots larger than 25 tonnes should be subdivided into sublots to be sampled separately. If a lot is greater than 25 tonnes, the number of sublots is equal to the lot weight in tonnes divided by 25 tonnes. It is recommended that a lot or a sublot should not exceed 25 tonnes. The minimum lot weight should be 500 kg.

13. Taking into account that the weight of the lot is not always an exact multiple of 25 tonne sublots, the weight of the sublot may exceed the mentioned weight by a maximum of 25%.

14. Samples should be taken from the same lot, i.e. they should have the same batch code or at the very least the same best before date. Any changes which would affect the mycotoxin content, the analytical determination or make the aggregate samples collected unrepresentative should be avoided. For example do not open packaging in adverse weather conditions or expose samples to excessive moisture or sunlight. Avoid cross-contamination from other potentially contaminated consignments nearby.

15. In most cases any truck or container will have to be unloaded to allow representative sampling to be carried out.

Incremental Sample Selection

16. Procedures used to take incremental samples from a treenut lot are extremely important. Every individual nut in the lot should have an equal chance of being chosen. Biases will be introduced by 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.

17. Since there is no way to know if the contaminated treenut kernels are uniformly dispersed throughout the lot, it is essential that the aggregate sample be the accumulation of many small incremental samples of 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.

Number of Incremental Samples
Number of Incremental Samples for Lots of varying weight

18. The number and size of the laboratory sample(s) will not vary with lot (sublot) size. However, the number and size of the incremental samples will vary with lot (sublot) size.

19. The number of incremental samples to be taken from a lot (sublot) depends on the weight of the lot. Table 1 shall be used to determine the number of incremental samples to be taken from lots or sublots of various sizes below 25 tonnes. The number of incremental samples varies from a minimum of 10 and to a maximum of 100.

Table 1. Number and size of incremental samples composited for an aggregate sample of 20 kg[a] as a function of lot (or sublot) weight.

Lot or Sublot Weight[b] (T in Tonnes)	Minimum Number of Incremental Samples	Minimum Incremental Sample Size[c] (g)	Minimum Aggregate Sample Size (kg)
T<1	10	2000	20
1≤T<5	25	800	20
5≤T<10	50	400	20
10≤T<15	75	267	20
15≤T	100	200	20

a/ Minimum aggregate sample size = laboratory sample size of 20 kg

b/ 1 Tonne = 1000 kg
c/ Minimum incremental sample size = laboratory sample size (20 kg)/minimum number of incremental samples.
i.e. for 0.5<T<1 tonne, 2000 g = 2000/10

Weight of the Incremental Sample

20. The suggested minimum weight of the incremental sample should be approximately 200 grams for lots of 25 metric tonnes (25,000 kg). The number and/or size of incremental samples will have to be larger than that suggested in Table 1 for lots sizes below 25,000 kg in order to obtain an aggregate sample greater than or equal to the 20 kg laboratory sample.

Static Lots

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

22. Taking incremental samples from a static lot usually requires the use of probing devices to select product from the lot. The probing devices should be specifically designed for the commodity and type of container. The probe should (1) be long enough to reach all products, (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 incremental samples of product taken from many different locations throughout the lot.

23. 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: $SF=(LT \times IS)/(AS \times IP)$.

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

Dynamic Lots

25. Representative aggregate samples can be more easily produced when selecting incremental samples from a moving stream of treenuts as the lot is transferred from one location to another. When sampling from a moving stream, take small incremental samples of product from the entire length of the moving stream; composite the incremental samples to obtain an aggregate sample; if the aggregate sample is larger than the required laboratory sample(s), then blend and subdivide the aggregate sample to obtain the desired size laboratory sample(s).

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

27. 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 the 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 two to three times the largest dimensions of items in the lot.

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

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

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

29. 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 can be computed from Equation 3 as a function of S, V, D, and MR.

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

30. Equations 2 and 3 can also be used to compute other terms of interest such as the time between cuts (T). For example, the time (T) required between cuts of the diverter cup to obtain a 20 kg aggregate sample from a 20,000 kg lot where the diverter cup width is 5.0 cm and the cup velocity through the stream 30 cm/sec. Solving for T in Equation 2,

T = (5.0 cm x 20,000 kg)/(20 kg x 20 cm/sec) = 250 sec.

31. If the lot is moving at 500 kg per minute, the entire lot will pass through the sampler in 40 minutes (2400 sec) and only 9.6 cuts (9 incremental samples) will be made by the cup through the lot (Equation 3). This may be considered too infrequent, in that too much product (2,083.3 kg) passes through the sampler between the time the cup cuts through the stream.

Packaging and Transportation of Samples

32. Each laboratory sample shall be placed in a clean, inert container offering adequate protection from contamination, sunlight, 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. Samples should be stored in a cool dark place.

Sealing and Labelling of Samples

33. 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.

SAMPLE PREPARATION

Precautions

34. Sunlight should be excluded as much as possible during sample preparation, since aflatoxin gradually breaks down under the influence of ultra-violet light. Also, environmental temperature and relative humidity should be controlled and not favor mold growth and aflatoxin formation.

Homogenization - Grinding

35. As the distribution of aflatoxin is extremely non-homogeneous, laboratory samples should be homogenized by grinding the entire laboratory sample received by the laboratory. Homogenization is a procedure that reduces particle size and disperses the contaminated particles evenly throughout the comminuted laboratory sample.

36. The laboratory sample should be finely ground and mixed thoroughly using a process that approaches as complete homogenization as possible. Complete homogenization implies that particle size is extremely small and the variability associated with sample preparation (Annex I) approaches zero. After grinding, the grinder should be cleaned to prevent aflatoxin cross-contamination.

37. The use of vertical cutter mixer type grinders that mix and comminute the laboratory sample into a paste represent a compromise in terms of cost and fineness of grind or particle size reduction[4]. A better homogenization (finer grind), such as a liquid slurry, can be obtained by more sophisticated equipment and should provide the lowest sample preparation variance[5].

Test portion

38. The suggested weight of the test portion taken from the comminuted laboratory sample should be approximately 50 grams. If the laboratory sample is prepared using a liquid slurry, the slurry should contain 50 g of nut mass.

39. Procedures for selecting the 50 g test portion from the comminuted laboratory sample should be a random process. If mixing occurred during or after the comminution process, the 50 g test portion can be selected from any location throughout the comminuted laboratory sample. Otherwise, the 50 g test portion should be the accumulation of several small portions selected throughout the laboratory sample.

40. It is suggested that three test portions be selected from each comminuted laboratory sample. The three test portions will be used for enforcement, appeal, and confirmation if needed.

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4 Ozay, G., Seyhan, F., Yilmaz, A., Whitaker, T., Slate, A., and Giesbrecht, F. 2006. Sampling hazelnuts for aflatoxin: Uncertainty associated with sampling, sample preparation, and analysis. J. Association Official Analytical Chemists, Int. 89:1004-1011.
5 Spanjer, M., Scholten, J., Kastrup, S., Jorissen, U., Schatzki, T., Toyofuku, N. 2006. Sample comminution for mycotoxin analysis: Dry milling or slurry mixing?, Food Additives and Contaminants, 23:73-83.

ANALYTICAL METHODS

Background

41. 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 specific 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 (within lab), reproducibility coefficient of variation (among lab), and the percent recovery necessary for various statutory limits. 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

42. A list of criteria and performance levels are shown in Table 2. Utilizing this approach, laboratories would be free to use the analytical method most appropriate for their facilities.

Table 2: Specific Requirements with which Methods of Analysis Should Comply

Criterion	Concentration Range (ng/g)	Recommended Value	Maximum Permitted Value
Blanks	All	Negligible	n/a
Recovery	1 to 15	70 to 110%	n/a
	>15	80 to 110%	n/a
Precision or Relative Standard Deviation RSD_R (Reproducibility)	1 to 120	Equation 4 by Thompson	2 x value derived from Equation 4
	>120	Equation 5 by Horwitz	2 x value derived from Equation 5
Precision or Relative Standard Deviation RSD_r (Repeatability)	1 to 120	Calculated as 0.66 times Precision RSD_R	n/a
	>120	Calculated as 0.66 times Precision RSD_r	n/a

n/a = not applicable

43. The detection limits of the methods used are not stated. Only the precision values are given at the concentrations of interest. The precision values are calculated from equations 4 and 5 developed by Thompson[2] and Horwitz and Albert[6], respectively.

Equation 4: $RSD_R = 22.0$ (for $C \leq 120$ ng/g or $c \leq 120 \times 10^{-9}$)

Equation 5: $RSD_R = 2^{(1-0.5 \log c)}$ (for $C > 120$ ng/g or $c > 120 \times 10^{-9}$)

where:
- RSD_R = the relative standard deviation calculated from results generated under reproducibility conditions
- RSD_r = the relative standard deviation calculated from results generated under repeatability conditions = $0.66 RSD_R$
- c = the aflatoxin concentration ratio (i.e. 1 = 100g/100g, 0.001 = 1,000 mg/kg)
- C = aflatoxin concentration or mass of aflatoxin to mass of treenuts (i.e. ng/g)

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6 Horwitz, W. and Albert, R. 2006. The Horwitz ratio (HorRat): A useful index of method performance with respect to precision. J. Association of Official Analytical Chemists, Int., 89:1095-1109.

Annex I

Uncertainty, as measured by the variance, associated with sampling, sample preparation, and analytical steps of the aflatoxin test procedure used to estimate aflatoxin in almonds, hazelnuts, and pistachios.

Sampling data for almonds, hazelnuts, and pistachios were supplied by the United States, Turkey, and Iran, respectively.

Variance estimates and the negative binomial distribution[1] were used to compute operating characteristic curves for each treenut in Annex II. Sampling, sample preparation, and analytical variances associated with testing almonds, hazelnuts, and pistachios are shown in Table 1 below.

Because of the computational complexities associated with use of the negative binomial distribution to compute operational characteristic (OC) curves for various sampling plan designs, the effect of various laboratory sample sizes, various numbers of laboratory samples, and various maximum levels on the performance (OC curves) of sampling plan designs is provided at the website address http://www5.bae.ncsu.edu/usda/www/ResearchActDocs/treenutwg.html.

Table 1. Variances[a] associated with the aflatoxin test procedure for each treenut.

Test Procedure	Almonds	Hazelnuts	Pistachios
Sampling[b,c]	$S^2_s = (7,730/\text{ns})5.759C^{1.561}$	$S^2_s = (10,000/\text{ns})4.291C^{1.609}$	$S^2_s = 8,000/\text{ns})7.913C^{1.475}$
Sample Prep[d]	$S^2_{sp} = (100/\text{nss})0.170C^{1.646}$	$S^2_{sp} = (50/\text{nss})0.021C^{1.545}$	$S^2_{sp} = (25/\text{nss})2.334C^{1.522}$
Analytical[e]	$S^2_a = (1/\text{na})0.0484C^{2.0}$	$S^2_a = (1/\text{na})0.0484C^{2.0}$	$S^2_a = (1/\text{na})0.0484C^{2.0}$
Total variance	$S^2_s + S^2_{sp} + S^2_a$	$S^2_s + S^2_{sp} + S^2_a$	$S^2_s + S^2_{sp} + S^2_a$

a/ Variance = S^2 (s, sp, and a denote sampling, sample preparation, and analytical steps, respectively, of aflatoxin test procedure)

b/ ns = laboratory sample size in number of shelled nuts, nss =test portion size in grams, na = number of aliquots quantified by HPLC, and C = aflatoxin concentration in ng/g total aflatoxin.
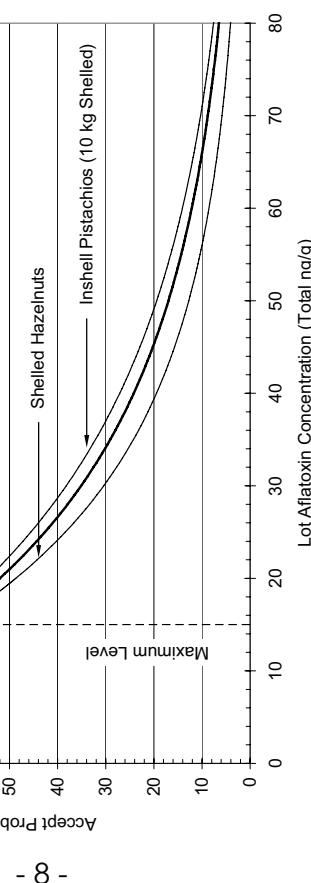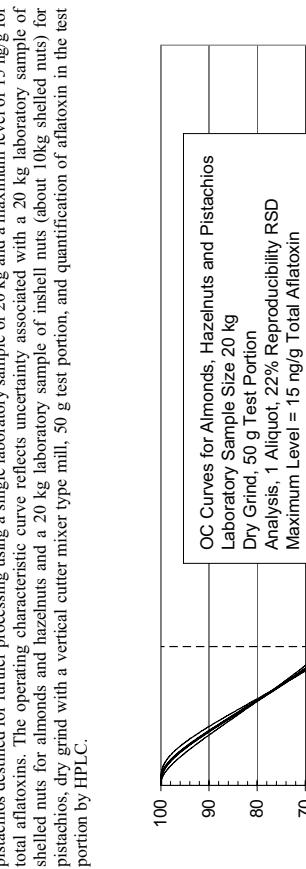
c/ Shelled nut count/kg for almonds, hazelnuts, and pistachios is 773, 1000, and 1600, respectively.

d/ Sample preparation for almonds, hazelnuts, and pistachios reflect Hobart, Robot Coupe, and Marjaan Khatman type mills, respectively. Laboratory samples were dry ground into a paste for each treenut.

e/ Analytical variances reflect FAPAS recommendation for upper limit of analytical reproducibility uncertainty. A relative standard deviation of 22% is considered by Thompson[2] (based upon FAPAS data) as an appropriate measure of the best agreement that can be obtained between laboratories. An analytical uncertainty of 22% is larger than the within laboratory uncertainty measured in the sampling studies for the three treenuts.

44. Equations 4 and 5 are generalized precision equations, which have been found to be independent of analyte and matrix but solely dependent on concentration for most routine methods of analysis.

45. Results should be reported on the edible portion of the sample.

Operating Characteristic curve describing the performance of the aflatoxin sampling plan for ready-to-eat almonds, hazelnuts, and pistachios using two laboratory samples of 10 kg each and a maximum level of 10 ng/g for total aflatoxins, dry grind with a vertical cutter mixer type mill, 50 g test portion, and quantification of aflatoxin in the test portion by HPLC.

OC Curves for Almonds, Hazelnuts and Pistachios
Laboratory Sample Size 2x10 kg
Dry Grind, 50 g Test Portion
Analysis, 1 Aliquot, 22% Reproducibility RSD
Maximum Level = 10 ng/g Total Aflatoxin

Shelled Almonds
Shelled Hazelnuts
Inshell Pistachios (2x5 kg Shelled)
Maximum Level

Accept Probability (%)
Lot Aflatoxin Concentration (Total ng/g)

Operating Characteristic Curves describing the performance of draft aflatoxin sampling plans for almonds, hazelnuts, and pistachios.

Treenuts Destined for Further Processing

Operating Characteristic curve describing the performance of the aflatoxin sampling plan for almonds, hazelnuts, and pistachios destined for further processing using a single laboratory sample of 20 kg and a maximum level of 15 ng/g for total aflatoxins. The operating characteristic curve reflects uncertainty associated with a 20 kg laboratory sample of shelled nuts for almonds and hazelnuts and a 20 kg laboratory sample of inshell nuts (about 10kg shelled nuts) for pistachios, dry grind with a vertical cutter mixer type mill, 50 g test portion, and quantification of aflatoxin in the test portion by HPLC.

OC Curves for Almonds, Hazelnuts and Pistachios
Laboratory Sample Size 20 kg
Dry Grind, 50 g Test Portion
Analysis, 1 Aliquot, 22% Reproducibility RSD
Maximum Level = 15 ng/g Total Aflatoxin

Shelled Almonds
Shelled Hazelnuts
Inshell Pistachios (10 kg Shelled)
Maximum Level

Accept Probability (%)
Lot Aflatoxin Concentration (Total ng/g)