codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 2 **CX/MAS 09/30/2**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING
Thirtieth Session
Balatonalmádi, Hungary, 9 - 13 March 2009

MATTERS REFERRED TO THE COMMITTEE BY THE CODEX ALIMENTARIUS COMMISSION AND OTHER CODEX COMMITTEES

A. GENERAL DECISIONS OF THE COMMISSION

Amendments to the Procedural Manual

The Commission considered the proposed amendments to the "*Relations between Commodity Committees and General Committees*" and endorsed the recommendation of the 61st Session of the Executive Committee to adopt the proposed amendments with the following changes:

- Include a reference to "*CODEX STAN 193-1995*" to the first paragraph of the Section on Contaminants;
- Replace the term "revisions" with "amendments" in the same section for consistent use of those terms that were defined in the Procedural Manual; and
- Delete the proposed inclusion of a reference to "contaminants" in the section on methods of analysis of pesticide residues in food so that this section cover the relations between the Committee on Methods of Analysis and Sampling, on one hand, and the Committees on Pesticide Residues and on Residues of Veterinary Drugs in Foods on the other (ALINORM 08/31/REP para 17-18 and Appendix IV).

B. DECISIONS OF THE COMMISSION RELATED TO THE WORK OF THE COMMITTEE

Proposed Amendment to the *Working Instructions for the Implementation of the Criteria Approach*

The Commission adopted the Proposed Amendment and agreed that the written comments presented in ALINORM 08/31/4A should be referred to the Committee on Methods for Analysis and Sampling for consideration in view of their technical nature (ALINORM 08/31/REP, para. 19 and Appendix V). This question will be considered under **Agenda Item 6**.

Guidelines on Criteria for Methods for the Detection and Identification of Foods Derived from Biotechnology

Some delegations expressed their concerns on this proposal for the following reasons: currently no Codex provisions on foods derived from biotechnology required methods of analysis; the scope of the work proposed was not clear enough; and it might duplicate the work of other organisations in the same area. These delegations therefore proposed to develop the criteria as an FAO/WHO document rather than Codex Guidelines.

Many delegations expressed the view that foods derived from biotechnology were a high priority for Codex and of great importance to many countries at the national level, and that the detection and identification of genetically modified material was essential in order to ensure food safety and to address consumer concerns. These delegations therefore supported new work and recalled that this question had been discussed extensively in the Committee on Methods of Analysis and Sampling for several sessions and that progress should not be delayed. Several delegations stressed the need for technical guidance on methodology applying

to GM foods, and especially for developing countries, and the need to facilitate harmonisation at the international level to prevent barriers to trade.

The Delegation of the United States of America proposed to return the project document to the Committee in order to broaden the scope of the work proposed as it should not be limited to genetically modified material but was also relevant to allergens and contaminants. However, taking into account the significant efforts made by the Committee to develop this proposal, the Delegation could support new work with the following amendment to paragraph 2 of the project document so that it would read: "Recognizing the difficulties with the practical application of new technology in this area, the Committee proposed to develop recommendations with respect to criteria for methods of analysis and for quality control measures that should be introduced in laboratories offering GM analyses". The Secretariat clarified that the project document was not a document for adoption by the Commission and could not be modified as it had been prepared by the Committee to provide supporting information for the proposal for new work.

The Commission approved new work on the Guidelines on the Criteria and recommended that the Committee consider the concerns and recommendations regarding the scope expressed at the current session (ALINORM 08/31/REP, para. 94 to 97). The Proposed Draft Guidelines will be considered under **Agenda Item 7**.

Standard for Food Grade Salt (CODEX STAN 150-1984)

The Commission noted that in the above standard, Part I, 4.7, reference was made to "Instructions on Codex Sampling Procedures" (CX/MAS 1-1987). The Commission noted the explanation given by the Secretariat that CX/MAS 1-1987 had been prepared by the Committee on Methods of Analysis and Sampling but had not been adopted by the Commission in 1987. It was used in practice as a recommendation from the Committee to other committees. The reference was included in CODEX STAN 150-1987 when the sampling plan for salt was endorsed by the Committee in 1988. The Commission agreed to invite the Committees on Food Additives and on Methods of Analysis and Sampling to decide how to refer to definitions in the section or whether the section was needed (ALINORM 08/31/REP, para 88).

The Committee is invited to consider how to proceed with the section on sampling of food grade salt.

Committee on Natural Mineral Waters: Amendments to the Standard on Natural Mineral Waters

The Commission noted the proposal of Kenya to initiate new work on the completion of the Section on methods of analysis in the Codex Standard on Natural Mineral Waters (CODEX STAN 108-1981) in view of the fact that, in the standard, there was no indication of specific methods of analysis and sampling procedures available for a number of chemical substances mentioned in Sections 3.2.17 (Surface active agents), 3.2.18 (Pesticides and PCBs), 3.2.19 (Mineral oil) and 3.2.20 (Polynuclear aromatic hydrocarbons), and the proposal to revise the Section on Hygiene to make it easier to use and consistent with the Recommended International Code of Hygienic Practice for Collecting, Processing and Marketing of Natural Mineral Waters (CAC/RCP 33-1985). The Chair of the Committee on Natural Mineral Waters also noted that the proposal from Kenya had been presented orally at the last session of the Committee on Natural Mineral Waters, however it had not been examined by the Committee as it fell outside the mandate given to the Committee by the 30th Session of the Commission. The Commission further noted that the project document had been considered at the last session of the Executive Committee and, after some discussion, agreed to refer the issue on the methods of analysis raised in Project Document 22 to the Committees on Contaminants in Foods, on Pesticide Residues and on Methods of Analysis and Sampling for review in their respective areas of competence as a matter of priority, especially whether further work was warranted and desirable.

The Commission also requested the Committees concerned, as mentioned above, to inform the Executive Committee and the Commission about their findings in order to allow the Commission to take an informed decision on this matter at its next session (ALINORM 08/31/REP, para. 106-108). The Committee is invited to consider the methods of analysis for natural mineral waters under the relevant agenda items.

C. MATTERS ARISING FROM THE EXECUTIVE COMMITTEE

Committee on Contaminants in Foods (CCCF)

The Committee discussed the need to refer the Proposed Draft Aflatoxin Sampling Plan for Almonds, Hazelnuts and Pistachios (ALINORM 08/31/41, Appendix IX), currently at Step 5/8, to the Committee on Methods of Analysis and Sampling in order to ensure consistency with general sampling texts, the criteria approach for methods of analysis and existing methods for the determination of aflatoxins. Some Members pointed out that the Proposed Draft Sampling Plan was directly related to the Draft Maximum Levels for Total Aflatoxins in Almonds, Hazelnuts and Pistachios forwarded to the Commission for adoption and that

the maximum levels could not be adopted without the sampling plans. The Committee recognised the importance of the adoption of these maximum levels in order to protect consumers' health and therefore recommended that the Commission adopt the Proposed Draft Sampling Plan as proposed by the CCCF and forward it to CCMAS for further consideration (ALINORM 08/31/3A, para 49).

The Committee is invited to consider the Sampling Plan, as adopted by the Commission and attached in the **Annex.**

D. MATTERS ARISING FROM THE LAST SESSION OF THE CCMAS

Standard for Sugars: Method for Determination of Colour in Plantation and Mill White Sugar

The Committee considered the recommendations of the Committee on Sugars for the methods for determination of colour in plantation and mill white sugar as requested by the Commission.

The Delegation of Brazil proposed that Method GS2/3-9 should be included as an alternative method for determination of colour since the principle of the method was similar to Method GS9/1/2/3-8, was equivalent and was widely used. The Delegation of the EC was of the opinion that the recommendations of the CCS should be supported. The Delegation of the United Kingdom informed the Committee that the International Commission for Uniform Methods of Sugar Analysis (ICUMSA) under the chairmanship of a representative of British Sugar, UK , would be discussing this matter at its next meeting in October 2008 and proposed that the Committee should request an information paper from ICUMSA on its decisions regarding the methods for sugar before further consideration of the methods. In addition, the Delegation of the United Kingdom proposed that the Committee should request that ICUMSA should reconsider the numbering of its methods since the current numbering system was confusing to those not familiar with the analysis of sugar.

In view of the discussion, the Committee agreed to postpone consideration and endorsement of the methods for determination of colour in sugar to its next session pending inputs from ICUMSA.

This question will be considered under **Agenda Item 5**.

ANNEX

AFLATOXIN 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[1] are used to calculate operating characteristic (OC) curves that describe the performance of the proposed aflatoxin-sampling plans (Annex II).

[1] 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[2]. 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.

[2] 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 selection methods, 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 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 = 20000/10

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 x LT) / (T x 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 x V) / (D x 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[3]. 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[4].

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.

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.

[3] 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.

[4] 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.

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[5], respectively.

    Equation 4: $RSD_R = 22.0$ (for $C \leq 120$ ng/g or $c \leq 120x10^{-9}$)

    Equation 5: $RSD_R = 2^{(1-0.5\log c)}$ (for $C > 120$ ng/g or $c > 120x10^{-9}$)

    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 = $0.66RSD_R$
    - 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)

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.

[5] 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]	$S^2_s = (7{,}730/ns)5.759C^{1.561}$	$S^2_s = (10{,}000/ns)4.291C^{1.609}$	$S^2_s = 8{,}000/ns)7.913C^{1.475}$
Sample Prep[d]	$S^2_{sp} = (100/nss)0.170C^{1.646}$	$S^2_{sp} = (50/nss)0.021C^{1.545}$	$S^2_{sp} = (25/nss)2.334C^{1.522}$
Analytical[e]	$S^2_a = (1/na)0.0484C^{2.0}$	$S^2_a = (1/na)0.0484C^{2.0}$	$S^2_a = (1/na)0.0484C^{2.0}$
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.

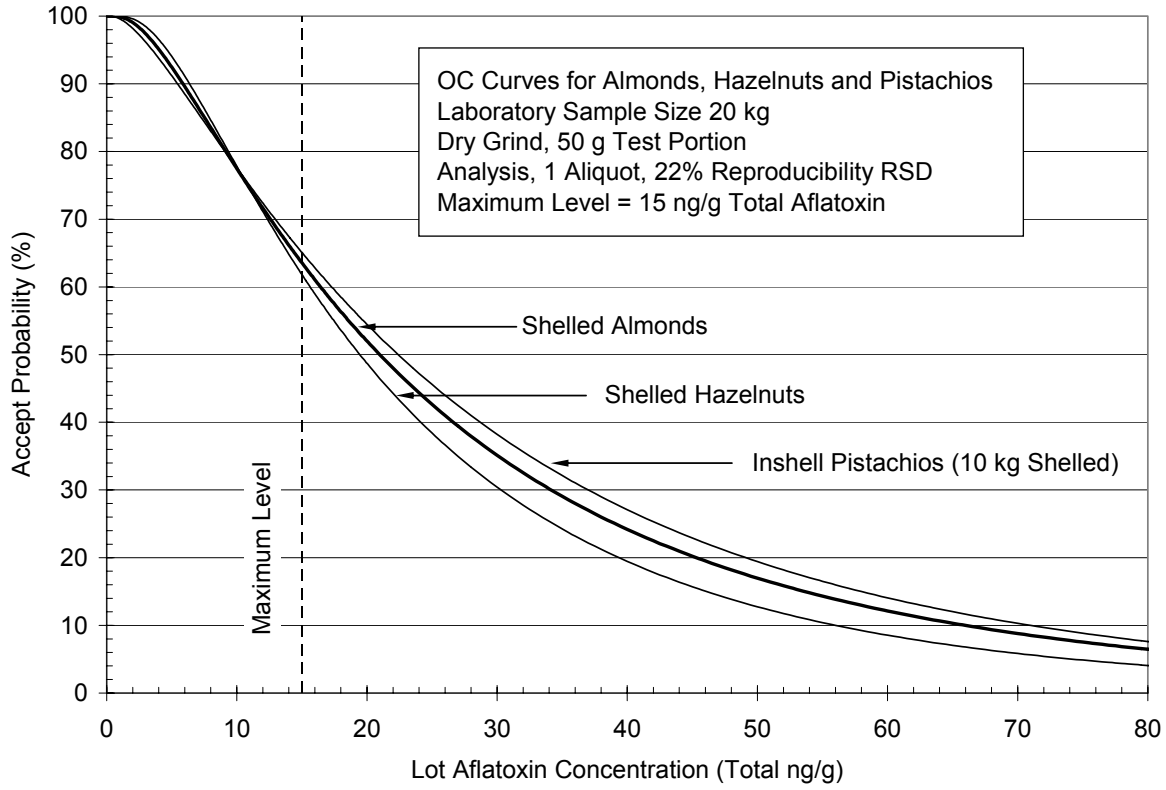
e/ Analytical variances reflect FAPAS recommendation for upper limit of analytical reproducibility uncertainty. A relative standard deviation of 22% is considered by Thompson[2] (based upon FAPAS data) as an appropriate measure of the best agreement that can be obtained between laboratories. An analytical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

Annex II

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.



Ready-to-Eats Treenuts

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.