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CODEX ALIMENTARIUS COMMISSION

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World Health Organization

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Agenda Item 7 **CX/CF 12/6/10**
January 2012

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON CONTAMINANTS IN FOODS

Sixth Session
Maastricht, The Netherlands, 26 – 30 March 2012

PROPOSED DRAFT MAXIMUM LEVELS FOR TOTAL AFLATOXINS IN DRIED FIGS INCLUDING SAMPLING PLANS

(AT STEP 3)

Codex Members and Observers wishing to submit comments at Step 3 on the proposed draft maximum level for Total Aflatoxins in Dried Figs and associated sampling plans, including possible implications for their economic interests, should do so in conformity with the *Uniform Procedure for the Elaboration of Codex Standards and Related Texts* (Codex Alimentarius Commission Procedural Manual) before **29 February 2012**. Comments should be directed:

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BACKGROUND

1. The Delegation of Turkey, as Chair of the electronic Working Group on Dried Figs, introduced the document highlighting the main issues associated with the establishment of the proposed maximum level (ML) of 10 µg/kg as laid down in working document CX/CF 11/43/7 at the 5th session of the Codex Committee on Contaminants in Food (CCCF), which was held from March 21 to 25, 2011 in The Hague. In particular, the Delegation informed the Committee that the proposed ML ensures protection of consumers' health while ensuring fair trade practices as consumption of dried figs as such or as ingredients was lower than other products traded worldwide such as tree nuts for which the same level had been established.

2. The Committee noted that there was wide support for the proposed ML of 10 µg/kg; however some delegations stated that it was not possible to agree with the proposed ML without having full clarity about the sampling plan. Other delegations also emphasized the importance of the sampling plans in view of the heterogeneous distribution of aflatoxins in dried figs. This would in turn allow proper enforcement of the ML. It was noted that the proposed ML represented a good balance between cost benefits of dried fig production and protection of human health.

3. The Delegation of Turkey highlighted that the sampling plan referred to in CX/CF 11/5/7 was already in use for many years in European countries. As regards the need for accompanying sampling plans, the Delegation explained that sampling plans were closely linked to the ML therefore there should first be agreement with the proposed ML before pursuing a development of sampling plans although reference was made in the working document to sampling plans. The Delegation agreed that sampling plans would be described and justified together with the proposed level for consideration by the 6th session of the Committee.

4. The Committee agreed to return the Proposed Draft Maximum Level for Dried Figs to Step 2/3 so that the sampling plans according to the proposed ML of 10 µg/kg can be developed for consideration by the 6th session of the Committee[1].

[1] REP11/CF, paras 44 – 50.

5. This document has been prepared by Turkey with contributions from Algeria, Argentina, Brazil, China, Costa Rica, Croatia, Dominican Republic, Egypt, the European Union, Ghana, India, Iran, Japan, Kenya, Mali, Mexico, Morocco, Spain, the Syrian Arab Republic, Thailand, Tunisia, the United Kingdom, the United States of America, Zambia, WHO, FAO, and the INC.

6. The proposed draft Maximum Level for Total Aflatoxins in Dried Figs is presented in Annex I. The supportive information for the proposed draft ML that led the 5th Session of the Committee to conclude on the development of sampling plans associated to a proposed draft ML of 10 µg/kg of total aflatoxins in dried figs is not reproduced in this paper but it is available for consultation in CX/CF 11/5/7[2]. The proposed draft associated sampling plans is presented in Annex II. A detailed report on the rational for the proposed sampling plans is given in Annex III. The List of Participants is in Annex IV.

REQUEST FOR COMMENTS

7. Codex Members and Observers are kindly invited to send their comments on the proposed draft Maximum Level for Total Aflatoxins (Annex I) and the Proposed draft Associated Sampling Plans (Annex II) for consideration by the 6th Session of the Codex Committee on Contaminants in Foods.

[2] This working document is available for downloading at: ftp://ftp.fao.org/codex/meetings/cccf/cccf5/cf05_07e.pdf

ANNEX I

PROPOSED DRAFT MAXIMUM LEVEL FOR TOTAL AFLATOXINS IN DRY FIGS

(At Step 3)

Ready-to-eat dried figs	**Maximum Level for Total Aflatoxins** **10 µg/kg**

ANNEX II

PROPOSED DRAFT SAMPLING PLAN FOR AFLATOXIN CONTAMINATION IN DRIED FIGS

(At Step 3)

The development of the proposed draft sampling plan for aflatoxin contamination in dried figs closely follows the format used by CCCF to develop sampling plan for aflatoxin contamination in treenuts (almonds, pistachios, hazelnuts, and Brazil nuts). The evaluation of the performance of the proposed aflatoxin sampling plan for dried figs is based upon results from a sampling study conducted together with Aydın Provincial Directorate under the leadership of TUBITAK Marmara Research center in Turkey. A detailed copy of the report describing the results of the sampling study is provided in Annex IV.

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 level. An aflatoxin test procedure consists of three steps: sample selection of sample(s) of a given size, sample preparation and aflatoxin quantification. The accept/reject level 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 dried figs 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 dried figs – dried figs, which are not intended to undergo an additional processing/treatment that have proven to reduce levels of aflatoxin.

Operating Characteristic (OC) Curve – a plot of the probability of accepting a lot versus lot concentration when using a specific sampling plan design. The OC curve also 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 commercially classify dried figs mostly as "ready-to-eat" (RTE). As a result, maximum levels and sampling plans are proposed for only ready-to-eat dried figs.

2. The performance of the proposed draft sampling plan was computed using the variability and aflatoxin distribution among laboratory samples of dried figs taken from contaminated lots (Annex IV). Because the dried fig count per kg is different for different varieties of dried figs, the laboratory sample size is expressed in number of dried figs for statistical purposes. However, the dried fig count per kg for each variety of dried figs can be used to convert laboratory sample size from number of dried figs to mass and vice versa.

3. Uncertainty estimates (variances) associated with sampling, sample preparation, and analysis and the negative binomial distribution[3] are used to calculate operating characteristic (OC) curves that describe the performance of the proposed aflatoxin-sampling plans for dried figs.

4. The analytical variance measured in the sampling study reflects within laboratory variance and was replaced with an estimate of 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[4]. 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 dried figs. The within laboratory analytical uncertainty for dried figs can be found in study results described in Annex III.

5. 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.

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[3] 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.

[4] 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.

AFLATOXIN TEST PROCEDURE AND MAXIMUM LEVELS

6. 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.

7. The maximum level for "ready-to-eat" dried figs is 10 ng/g total aflatoxins.

8. 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 plan use two 10 kg aggregate samples of dried figs.

9. The RTE sampling plan has been designed for enforcement and controls concerning total aflatoxins in bulk consignments (lots) of dried figs traded in the export market.

   Maximum level – 10 ng/g total aflatoxins

   Number of laboratory samples – 2

   Laboratory sample size – 10 kg

   Sample preparation – water-slurry grind and a test portion that represents 55 g mass of dried figs

   Analytical method – performance based (see Table 2)

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

   The operating characteristic curve describing the performance of the sampling plan for the ready-to-eat dried figs is shown in section 47 at the end of this Annex.

10. To assist member countries implement the above Codex sampling plan, sample selection methods, sample preparation methods, and analytical methods required to quantify aflatoxin in laboratory samples taken from bulk dried fig lots are described in the following sections.

SAMPLE SELECTION

Material to be sampled

11. Each lot, which is to be examined for aflatoxin, must be sampled separately. Lots larger than 15 tonnes should be subdivided into sublots to be sampled separately. If a lot is greater than 15 tonnes, the number of sublots is equal to the lot weight in tonnes divided by 15 tonnes. It is recommended that a lot or a sublot should not exceed 15 tonnes. For a 20 kg aggregate sample, the minimum lot weight should be 500 kg.

12. Taking into account that the weight of the lot is not always an exact multiple of 15 tonnes, the weight of the sublot may exceed the mentioned weight by a maximum of 25%.

13. 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.

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

Incremental Sample Selection

15. Procedures used to take incremental samples from a dried fig lot are extremely important. Every individual fig 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.

16. Since there is no way to know if the contaminated figs 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.

17. Since the number and size of the laboratory sample(s) do not vary for lot (sublot) sizes above 0.5 tonnes (Table 1), the number and size of the incremental samples must vary with lot (sublot) size to maintain a constant aggregate sample size of 20 kg. For small lots (lot sizes below 0.5 tonnes), the size of the aggregate sample is reduce so that the aggregate sample size doesn't exceed a significant portion (4 to 6%) of the lot or sublot size.

Number and Size of Incremental Samples for Lots of varying weight

18. The number and size 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 and size of incremental samples to be taken from lots or sublots of various sizes. The number of incremental samples varies from 30 to 100 for lot sizes from 0.5 to 15 tonnes, respectively. The number and size of the laboratory samples for lot weights below 0.5 tonnes is shown in Table 1.

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)	Laboratory Sample Size (KG)	Number of Laboratory Samples
15.0 ≥ T > 10.0	100	200	20	10	2
10.0 ≥ T > 5.0	80	250	20	10	2
5.0 ≥ T > 2.0	60	334	20	10	2
2.0 ≥ T > 1.0	40	500	20	10	2
1.0 ≥ T > 0.5	30	667	20	10	2
0.5 ≥ T > 0.2	20	500	10	10	1
0.2 ≥ T > 0.1	15	400	6	6	1
0.1 ≥ T	10	300	3	3	1

a/ Minimum aggregate sample size = laboratory sample size of 20 kg for lts above 0.5 tonnes

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, 200 g = 20000/100

19. The suggested minimum weight of the incremental sample for lots (sublots) above 0.5 tonnes varies from 200 grams for a 15 tonne lot to 667 grams for a 0.5 tonne lot. The suggested minimum weight of the incremental sample for lots (sublots) below 0.5 tonnes is shown in Table 1.

Static Lots

20. A static lot can be defined as a large mass of dried figs 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 dried figs 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.

21. 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.

22. 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).

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

Dynamic Lots

24. Representative aggregate samples can be more easily produced when selecting incremental samples from a moving stream of dried figs 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).

25. 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.

26. 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.

27. 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).

28. 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).

29. 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.

30. 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

31. 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

32. 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

33. 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

34. As the distribution of aflatoxin is extremely non-homogeneous, the 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.

35. 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 approaches zero. After grinding, the grinder should be cleaned to prevent aflatoxin cross-contamination.

36. 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[5]. 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[6].

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[5] 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.

[6] 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.

Test portion

37. 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 fig mass.

38. 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.

39. 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

40. 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 analytical method. The performance criteria established for analytical 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

41. 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

42. The detection limits of the methods used are not stated. Only the precision values are given at the concentrations of interest. The precision values (expressed as a%) are calculated from equations 4 and 5 developed by Thompson[3] and Horwitz and Albert[7], respectively.

Equation 4: $RSD_R = 22.0$

Equation 5: $RSD_R = 45.25C^{-0.15}$

where:

- RSD_R = the relative standard deviation calculated from results generated

[7] 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.

- under reproducibility conditions
- RSD_r = the relative standard deviation calculated from results generated under repeatability conditions = $0.66RSD_R$
- C = aflatoxin concentration or mass of aflatoxin to mass of dried figs (i.e. ng/g)

43. 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.

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

UNCERTAINTY, AS MEASURED BY THE VARIANCE, ASSOCIATED WITH THE SAMPLING, SAMPLE PREPARATION, AND ANALYTICAL STEPS OF THE AFLATOXIN TEST PROCEDURE USED TO DETECT AFLATOXIN IN DRIED FIGS

45. From the sampling study described in Annex IV, the sampling, sample preparation, and analytical variances associated with the aflatoxin test procedure for dried figs are shown in Table 3.

Table 3. Variances[a] associated with the aflatoxin test procedure for each dried figs

Test Procedure	Variances for Dried Figs
Sampling[b,c]	$S^2_s = (590/ns)2.219C^{1.433}$
Sample Prep[d]	$S^2_{sp} = (55/nss)0.01170C^{1.465}$
Analytical[e]	$S^2_a = (1/na)0.0484C^{2.0}$
Total	$S^2_t = S^2_s + S^2_{sp} + S^2_a$

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

b/ ns = laboratory sample size in number of dried figs, nss =test portion size in grams of fig mass, na = number of aliquots quantified by HPLC, and C = aflatoxin concentration in ng/g total aflatoxins.

c/ Count/kg for dried figs averaged 59/kg.

d/ Sample preparation variance reflects a water-slurry method and a test portion that reflects 55 g fig mass.

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 dried figs.

OPERATING CHARACTERISTIC CURVE DESCRIBING THE PERFORMANCE OF THE DRAFT AFLATOXIN SAMPLING PLAN FOR READY-TO-EAT DRIED FIGS

46. The operating characteristic curve describing the performance of draft aflatoxin sampling plans for ready-to-eat dried figs is shown in Figure 1.

47. Operating characteristic (OC) curves for various sampling plan designs that reflect 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 in Annex IV.

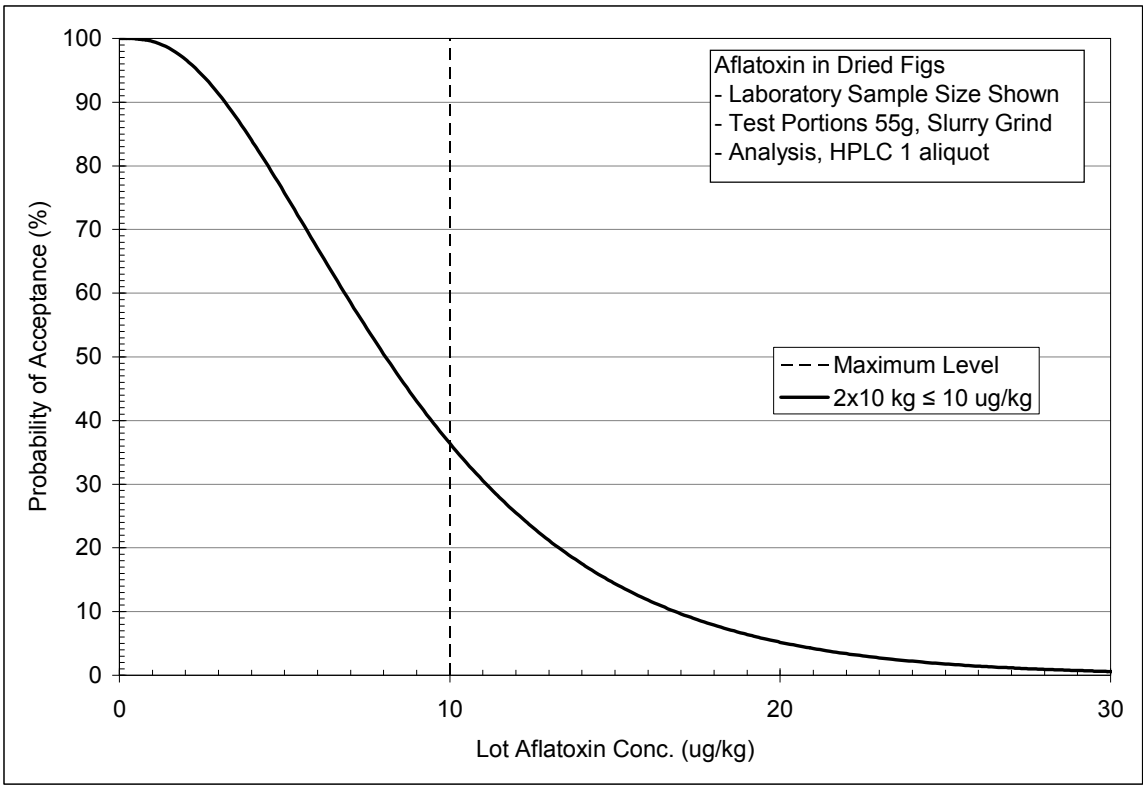

Figure 1. Operating characteristic (OC) curve describing the performance of the aflatoxin sampling plan for ready-to-eat dried figs using two laboratory samples of 10 kg each and a maximum level of 10 ug/kg total aflatoxins, water-slurry comminution method, test portion that reflects 55 g fig mass, and quantification of aflatoxin in a the test portion by HPLC.

ANNEX III

BACKGROUND INFORMATION IN SUPPORT OF THE ASSOCIATED SAMPLING PLANS

General Overview

The development of a method to evaluate the performance of an aflatoxin sampling plan is an essential part of designing sampling plans and establishing a maximum level for dried figs. The aflatoxin concentration of a bulk lot is estimated by measuring the aflatoxin in samples taken from the lot. Because of the variability associated with the aflatoxin test procedure (sampling, sample preparation, and analysis) the true aflatoxin concentration of a bulk lot cannot be determined with 100% certainty. As a result, some good lots (lot concentration below the maximum level) will be rejected by a sampling plan (seller's risk) and some bad lots (lot concentration greater than the maximum level) will be accepted by a sampling plan (buyer's risk). Evaluating the performance of an aflatoxin-sampling plan requires knowledge of the variability and distribution among replicated sample test results taken from a contaminated lot.

The objectives of the sampling study were to develop a method to predict the performance of aflatoxin sampling plan designs, which requires knowledge of the variability and distribution among sample test results for dried figs, and predict the performance of several aflatoxin sampling plan designs.

All samples were collected, prepared, and analyzed for aflatoxin under the leadership of Dr Hayrettin Ozer with the TUBITAK Marmara Research Center in Turkey. Statistical analysis of the aflatoxin test results were conducted with the assistance of Dr. Thomas Whitaker and Mr. Andrew Slate (USDA/ARS retired). The experimental procedure and resulting statistical analysis are very similar to that used by CCCF to develop aflatoxin maximum levels and sampling plans for treenuts (almonds, pistachios, hazelnuts, and Brazil nuts).

The study objectives and results were reported in three phases:

Phase 1 - The variability associated with an aflatoxin test procedure that used 10 kg samples, water slurry comminution method, test portions with 55 g of fig mass, and a HPLC analytical method was determined for each of 20 lots. The total variance of the aflatoxin test procedure was partitioned into sampling, sample preparation, and analytical variance components. These three variance equations are shown in ANNEX II. From these variance results, one can determine how much each step of the aflatoxin test procedure contributes to the total variability. This knowledge will help determine how to best reduce the total variability of the aflatoxin test procedure and obtain more precise estimates of the true aflatoxin concentration in a bulk lot of dried figs.

Phase 2 – For each lot, the observed aflatoxin distribution among 16 sample test results was compared to the Negative Binomial, Compound Gamma, Lognormal, and Normal distributions. Using goodness-of-fit test, the Negative Binomial distribution was chosen to simulate the observed aflatoxin distribution among sample test results. The Negative Binomial distribution was used to predict the distribution among aflatoxin test results for test procedures other than that used in the experimental design.

Phase 3 – Using the variance and distribution information (Phase 1 and 2), a computer model was developed to predict the probability of accepting or rejecting dried fig lots using a specific sampling plan design. A sampling plan is defined by an accept/reject level and an aflatoxin test procedure. The aflatoxin test procedure is defined by number and size samples, sample preparation method (type mill and test portion size), analytical procedure, and number of aliquots quantified. The accept probabilities are used to compute an operating characteristic (OC) curve specific to a sampling plan design. The OC curve is used to predict the good lots rejected (seller's or exporter's risk) and the bad lots accepted (buyer's or importer's risk).

Because of the uncertainty associated with the aflatoxin test procedure (variances developed in Phase 1), two types of mistakes can be made when using an aflatoxin-sampling plan to classify lots as good (lot concentration below a maximum level) or bad (lot concentration above a maximum level). Sometimes good lots will test "bad" by the sampling plan and be rejected. Good lots rejected are often called false positives or seller's risk. Sometimes bad lots will test "good" by the sampling plan and be accepted. Bad lots accepted are often called false negatives or buyer's risk. Definition of seller and buyer depends where in the market system the lot is being tested. If lots are tested in the export market, the buyer and seller are importers and exporters, respectively. The buyer's and seller's risks associated with an aflatoxin-sampling plan can be predicted with the help of an operating characteristic (OC) curve.

A generalized OC curve is shown in **Figure 2.1**. An OC curve indicates the chances of accepting a lot at a given concentration by a specific sampling plan design. The chances of rejecting lots can also be determined by subtracting the percent lots accepted from 100%. Lots with aflatoxin levels below the maximum level that are rejected by a sampling plan represent the seller's risk of good lots rejected by a sampling plan design. Lots with aflatoxin levels above the maximum level that are accepted by a sampling plan represent the buyer's risk of bad lots accepted by a sampling plan design. The seller's risk (good lots rejected) is represented by the area above the OC curve and below the maximum level. The buyer's risk (bad lots accepted) is represented by the area below the OC curve and to the left of the maximum level. A good sampling plan should try to minimize the buyer's and seller's risks for available resources.

An example of an OC curve for the aflatoxin test procedure used in the experimental study (10 kg sample, a test portion that represents 55 g fig mass (where the sample is comminuted using a water-slurry method), 1 aliquot (where aflatoxin is quantified using HPLC methods) and a 10 ug/kg accept/reject level) is shown graphically in **Figure 2.2**. If a 10 kg sample is taken from a lot with unknown aflatoxin concentration, the sample is comminuted using a water-slurry method, the aflatoxin is extracted from a test portion that represents 55 g fig mass, and the aflatoxin value in one aliquot quantified using HPLC is less than or equal to 10 ug/kg (accept/reject level), the lot is accepted, otherwise the lot is rejected. The percent lots accepted by this sampling plan design with different lot aflatoxin concentrations are described by the OC curve in **Figure 2.2**. For example, about 87% of lots with a true concentration of 5 ug/kg will be accepted (13% rejected) by the sampling plan described above. Other points along the OC curve can be seen in **Figure 2.1**. For example, about 60, 23, and 8% of lots with concentrations at 10, 20, and 30 ug/kg will be accepted by the above sampling plan design, respectively. Most all lots with concentrations above 40 ug/kg are rejected by the sampling plan.

Reducing the variability associated with the aflatoxin test procedure will reduce both the buyer's risk and the seller's risk associated with a sampling plan design. The variability of the aflatoxin test procedure can be reduces by increasing sample size (or number of samples of a given size), grinding the sample into smaller particles, increasing test portion size, increasing the number of aliquots quantified for aflatoxin, and using a more precise analytical method (ie., HPLC versus TLC). **Figure 2.3** shows three OC curves reflecting 5, 10, and 20 kg laboratory sample sizes. All three sampling plans use an accept/reject level of 10 ug/kg. As sample size increases, the OC curves get steeper around the maximum level of 10 ug/kg. As a result, the areas representing the seller's risk and buyers' risk get smaller indicating that both risks get smaller as sample size increases. Increasing sample size is often the first approach taken to reduce uncertainty and risks because, as shown in the Phase 1 study, the sampling step of the aflatoxin test procedure for dried figs accounts for most of the total variability (99% at 10 ug/kg).

Changing the accept/reject level of a sampling plan design relative to the maximum level can also be used to reduce either the seller's risk or the buyer's risk, but not both risks at the same time. **Figure 2.4** shows three OC curves for accept/reject limits of 5, 10, and 15 ug/kg. It is assumed that the maximum level is constant at 10 ug/kg. When the accept/reject level is equal to the maximum level (10 ug/kg), both the buyer and the seller share in the risks associated with the sampling plan. If the accept/reject level is reduced to a value below the regulatory level (5 versus 10 ug/kg), the OC curve representing 5 ug/kg shifts to the left. When compared to the OC curve for a 10 ug/kg accept/reject level, the area representing the seller's risk increases and the area representing the buyer's risk decreases. Reducing the accept/reject level to a value below the maximum level reduces the buyer's risk, but increases the seller's risk. Often importers will contract for product where they specify that export lots must meet an accept/reject level below their maximum level because it reduces the importer's risk, but it forces the exporter to take the largest share of the risk.

If the accept/reject level is increased to a value above the maximum level (15 versus 10 ug/kg), the OC curve representing 15 ug/kg shifts to the right. When compared to the OC curve for a 10 ug/kg accept/reject level, the area representing the seller's risk decreases and the area representing the buyer's risk increases. So increasing the accept/reject level above the maximum level increases the buyer's risk, but reduces the seller's risk. Using an accept/reject level greater than the maximum level is not used often, but is used sometimes early in the market system when a processor can clean up or reduce contamination by processing the product and removing contamination. The processor knows that using processing methods such as color sorting will reduce contamination and increase the chances that the lot may pass inspection later in the market system.

Multiple samples can also be used to reduce the buyer's risk. **Figure 2.5** shows three OC curves representing the use of 1, 2, or 3 ten kg samples (1x10 kg, 2x10 kg, and 3x10 kg) where all samples must test less than or equal to the accept/reject level of 10 ug/kg total aflatoxin (same as the maximum level) in order for the lot to be accepted. The OC curves shift to the left, as more samples are required to all test less than the accept/reject level. If any one of the multiple samples tests greater than the accept/reject level, the lot is rejected (even if the average of the multiple sample values is less than or equal to the accept/reject level). The effect of requiring multiple samples to all test less than or equal to the accept/reject level is similar to using an accept/reject level that is less than the maximum level with a single sample (**Figure 2.4**). This sampling plan design is often used late in the market system for ready-to-eat products to reduce the chances of accepting product with concentrations above the maximum level. However, the seller's risk of rejecting good lots increases as the number of samples tested increases.

The above examples demonstrate how OC curves can be used to evaluate the performance of sampling plan designs and how to change the design elements to reduce risks associated with a sampling plan. The seller in discussion with the buyer of dried figs must establish the risk levels that each will tolerate. Once there is agreement, the dried fig industry can evaluate various sampling plan designs in an effort to find a design that meets the stated objectives of both the buyer and seller.

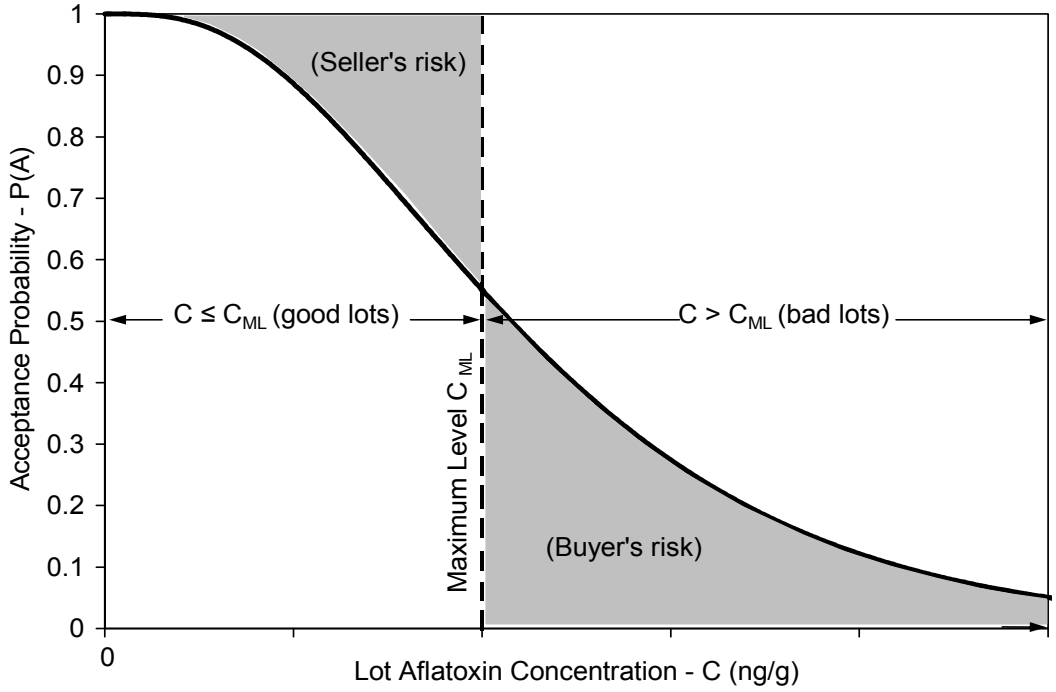

Figure 2.1. Typical shape of an operating characteristic curve used to evaluate the buyer's risk (false negative or bad lots accepted) and seller's risk (false positive or good lots rejected) associated with a sampling plan.

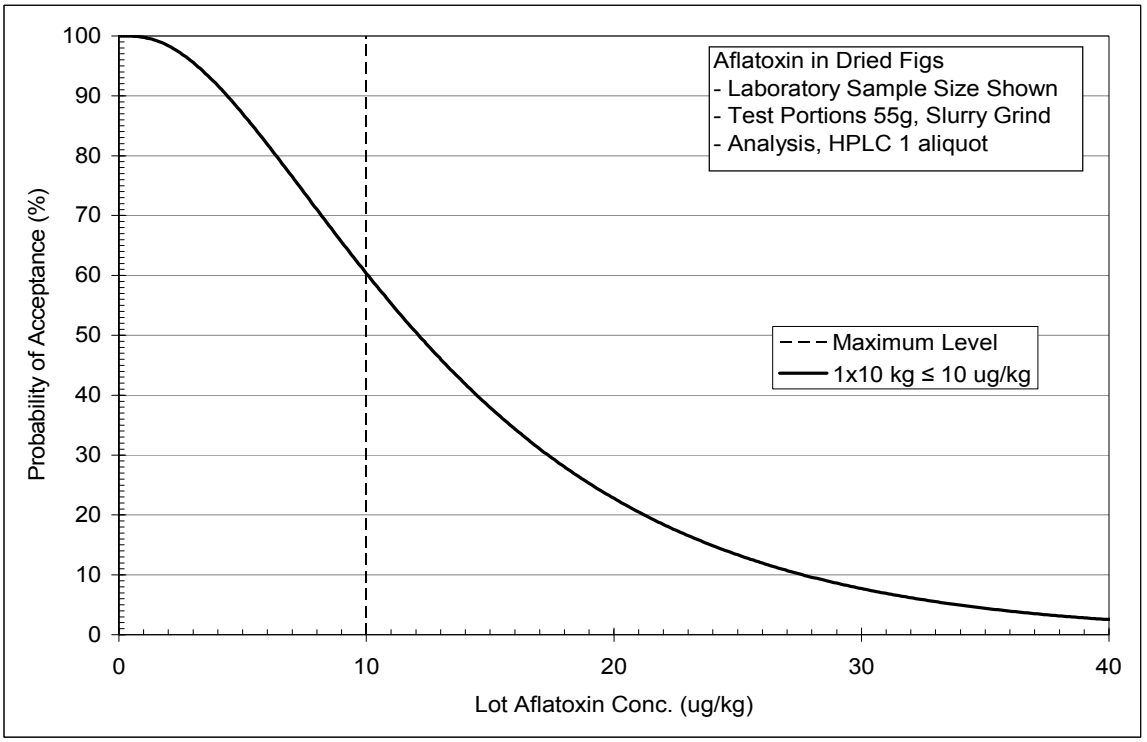

Figure 2.2. Operating characteristic curve showing the performance of an aflatoxin sampling plan that uses a single 10 kg sample, water-slurry comminution, 1 test portion with 55 g fig mass, quantify aflatoxin in 1 aliquot using HPLC methods, and an accept/reject level of 10 ug/kg total aflatoxin.

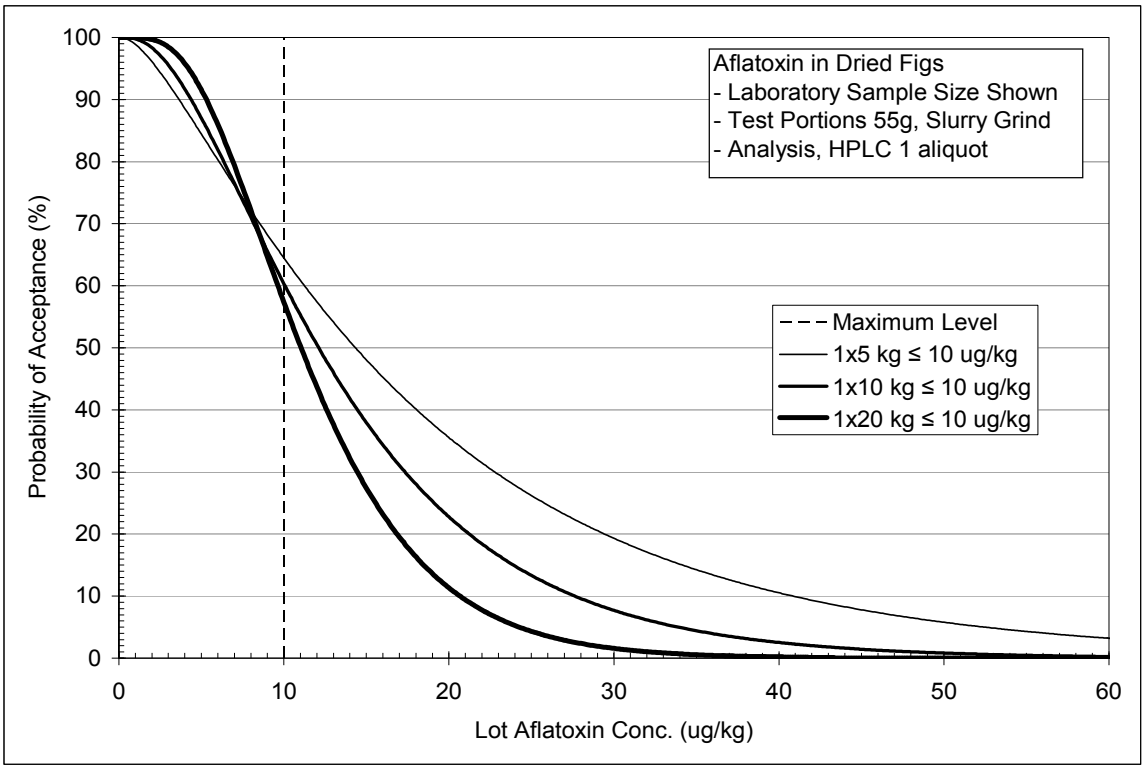

Figure 2.3. Three operating characteristic (OC) curves describing performance of three aflatoxin sampling plans for dried figs that use either a 5, 10 and 20 kg sample, water-slurry comminution, 1 test portion with 55 g fig mass, 1 aliquot, HPLC, and an accept/reject level = 10 ug/kg total aflatoxins.

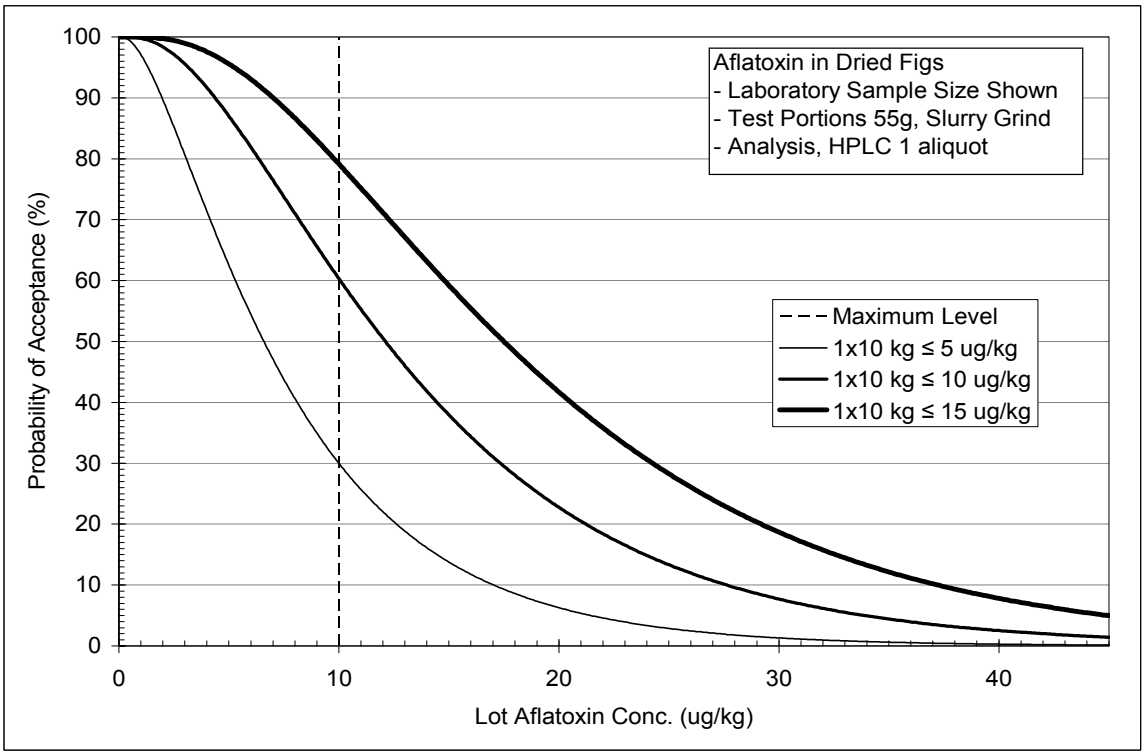

Figure 2.4. Three operating characteristic (OC) curves that describe the performance of three aflatoxin sampling plans for dried figs using a 10 kg sample, water-slurry comminution, 1 test portion with 55 g fig mass, 1 aliquot HPLC, and three accept/reject limits of 5, 10 and 15 ug/kg total aflatoxins.

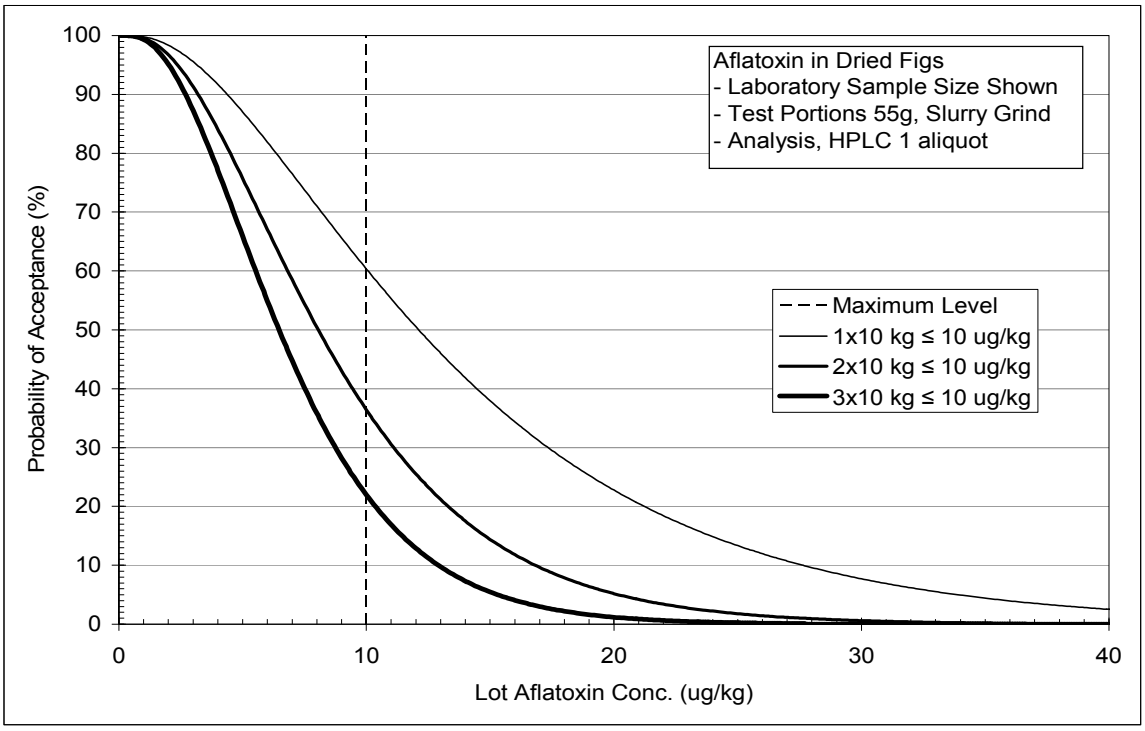

Figure 2.5. Three operating characteristic (OC) curves describing the performance of three aflatoxin sampling plans for dried figs using 1x10 kg, 2x10 kg and 3x10 kg multiple samples, water-slurry comminution, 1 test portion with 55 g fig mass, 1 aliquot, HPLC, and an accept/reject level of 10 ug/kg. All samples must test ≤ 10 ug/kg total aflatoxin for a lot to be accepted.

ANNEX IV

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