CODEX ALIMENTARIUS COMMISSION

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Food and Agriculture Organization of the United Nations

World Health Organization

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**CX 4/35.2** **CL 2013/25-CF**
**August 2013**

**TO:** Codex Contact Points
Interested International Organizations

**FROM:** Secretariat, Codex Alimentarius Commission
Joint FAO/WHO Food Standards Programme
Viale delle Terme di Caracalla, 00153 Rome, Italy
E-mail: codex@fao.org

**SUBJECT:** **Request for comments on MLs for fumonisins in maize and maize products and associated sampling plans**[1]

**DEADLINE**: **15 November 2013**

**COMMENTS**:

**To:**

Secretariat,
Codex Alimentarius Commission,
Joint FAO/WHO Food Standards Programme,
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## BACKGROUND

1. The 4th Session of the Committee on Contaminants in Food (April 2010) agreed to retain the proposed draft maximum levels for fumonisins in maize and maize products and sampling plans at Step 4 until further advice was provided by JECFA.[2]

2. The 6th Session of the Committee (March 2012) agreed to suspend development of the proposed draft MLs for fumonisins for and sampling plans for one year until consideration of a discussion paper to identify the gaps in the *Code of Practice for Prevention and Reduction of Mycotoxin Contamination in Cereals* (CAC/RCP 51-2003)[3] and the need for a separate code of practice for fumonisins in maize and whether there were any other measures to control fumonisins in maize.[4]

3. The 7th Session of the Committee (April 2013) noted that the work on the possible revision of the *Code of Practice for Prevention and Reduction of Mycotoxin Contamination in Cereals* would not impact on the MLs for fumonisins and sampling plans and agreed that the MLs and sampling plans should be further discussed at the next session of the Committee.

4. It was agreed that the proposed draft MLs for fumonisins in maize and maize products and associated sampling plans previously discussed at the 6th session of the Committee would be circulated for comments and a revised proposal for proposed draft MLs for fumonisins in maize and maize products and associated sampling plans would be prepared by Brazil for comments and consideration by the 8th session of the Committee.[5]

### Request for comments

5. Comments are hereby requested at Step 3 on the proposed drat MLs for fumonisins in maize and maize products and associated sampling plans as presented to the 6th Session of the Committee and reproduced in the Annex for convenience. Background information providing the rationale for the MLs and sampling plans can be found in working document CX/CF 12/6/18 available from the Codex website at the following address: ftp://ftp.fao.org/codex/meetings/cccf/cccf6/cf06_18e.pdf.

6. Governments and international organizations wishing to provide comments should do so in writing as directed above. In submitted comments, Codex members and observers are invited to take due consideration of the background information provided in CX/CF 12/6/18 and the discussion that took place at the 6th sessions of the Committee.

## ANNEX

The following maximum levels (ML) of fumonisins (FB1 + FB2) are presented for consideration by the Committee:

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1 Reports, agendas and working documents of Codex meetings are available on the Codex website: http://www.codexalimentarius.org/ by clicking on "Meetings and Reports".
2 ALINORM 10/33/41, paras. 86-95.
3 Codex standards and related texts are available on the Codex website: http://www.codexalimentarius.org/ by clicking on "Standards".
4 REP12/CF, paras 83-96.
5 REP13/CF, paras. 127-133.

| Commodity | Maximum level for fumonisins (FB1+FB2), µg/kg |
|---|---|
| Corn/maize grain, unprocessed | 5000 |
| Corn/maize flour/meal | 2000 |

The following sampling plans for fumonisins (FB1 + FB2) are presented for consideration by the Committee. The operating characteristic curves describing the performance of this and other sampling plan are shown here below.

**Corn/maize grain, unprocessed sampling plan**

| | |
|---|---|
| Maximum level | 5000 µg/kg FB1 + FB2 |
| Increments | 50 x 100 g |
| Aggregate sample size | 5 kg |
| Sample preparation | dry grind with a suitable mill (Romer Mill) |
| Laboratory sample size | 1 kg |
| Number of laboratory samples | 1 |
| Test portion | 25 g test portion |
| Method | HPLC |
| Decision rule | If the fumonisin-sample test result for the laboratory samples is equal or less than 5000 µg/kg, then accept the lot. Otherwise, reject the lot. |

**Corn/maize flour/meal**

| | |
|---|---|
| Maximum level | 2000 µg/kg FB1 + FB2 |
| Increments | 10 x 100 g |
| Aggregate sample size | 1 kg |
| Sample preparation | none |
| Laboratory sample size | 25 g test portion |
| Number of laboratory samples | 1 |
| Test portion | same as laboratory sample |
| Method | HPLC |
| Decision rule | If the fumonisin-sample test result is equal or less than 2000 µg/kg, then accept the lot. Otherwise, reject the lot. |

## SAMPLING PLANS FOR FUMONISINS IN CORN/MAIZE GRAIN AND CORN/MAIZE FLOUR/MEAL

### 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 a fumonisin test procedure and an accept/reject level. A fumonisin test procedure consists of three steps: sample selection, sample preparation and analysis or fumonisin quantification. The accept/reject level is a tolerance usually equal to the Codex maximum level (ML).

**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 shelled maize 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 fumonisin for chemical analysis.

**Operating Characteristic (OC) Curve** – a plot of the probability of a accepting a lot versus lot concentration for a specific sampling plan design. The OC curve provides an estimate of the chances of rejecting a good lot (exporter's risk) and the chances of accepting a bad lot accepted (importer's risk) by a specific fumonisin sampling plan design. A good lot is defined as having a fumonisin concentration below the ML; a bad lot is defined as having a fumonisin concentration above the ML.

### SAMPLING PLAN DESIGN CONSIDERATIONS

1. Sampling statistics are based upon the variability and fumonisin distribution among laboratory samples of shelled maize (Whitaker et al, 1998; Whitaker et al, 2007). The laboratory sample size is expressed in number of maize kernels for statistical purposes. The shelled maize kernel count was assumed to be 3000 kernels per kg. The kernel count per kg can be used to convert laboratory sample size from number of kernels to mass and vice versa.
2. Variability estimates associated with sampling, sample preparation, and analysis and the negative binomial distribution (Whitaker et al, 2007) are used to calculate operating characteristic (OC) curves that describe the performance of the proposed fumonisin-sampling plan. The analytical variance reflects the analytical variability within a single laboratory, which is lower than the analytical variability among laboratories.

#### Material to be sampled

3. Each lot, which is to be examined for fumonisin, must be sampled separately. Lots larger than 50 tonnes should be subdivided into sublots to be sampled separately. If a lot is greater than 50 tonnes, the number of sublots is equal to the lot weight in tonnes divided by 50 tonnes. It is recommended that a lot or a sublot should not exceed 50 tonnes. The minimum lot weight should be 500 kg.
4. Taking into account that the weight of the lot is not always an exact multiple of 50 tonnes sublots, the weight of the sublot may exceed the mentioned weight by a maximum of 25%.
5. 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.
6. In most cases any truck or container will have to be unloaded to allow representative sampling to be carried out.

**Incremental Sample**

7. Procedures used to take incremental samples from a lot of shelled maize are extremely important. Every individual kernel 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 kernel in the lot from being chosen.

8. Since there is no way to know if the contaminated maize 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.

9. The number of incremental samples to be taken from a lot (sublot) depends on the weight of the lot and the size of the aggregate sample. The suggested minimum weight of the incremental sample should be approximately 100 grams for lots of 50 metric tonnes (50,000 kg).

**Static Lots**

10. A static lot can be defined as a large mass of shelled maize 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 maize is 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.

11. 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.

12. 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:

    SF = (LT x IS)/(AS x IP).

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

**Dynamic Lots**

14. Representative aggregate samples can be more easily produced when selecting incremental samples from a moving stream of shelled maize 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).

15. 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 maize flow past the sampling point.

16. 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.

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

    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).

18. 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 as a function of S, V, D, and MR.

    SF = (S x V) / (D x MR).

**Packaging and Transportation of Samples**

19. 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.

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.

**SAMPLE PREPARATION**

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

22. As the distribution of fumonisin 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.

23. 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 fumonisin cross-contamination.

**Test portion**

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

25. 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**

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 specific method. A list of possible criteria and performance levels are shown in Table 1 (EC Regulation No 401/2006). Utilizing this approach, laboratories would be free to use the analytical method most appropriate for their facilities.

**Table 1.** Performance criteria for Fumonisin B1 and B2.

| | Precision | | |
|---|---|---|---|
| **Level (µg/kg)** | **RSDr (%)** | **RSDR (%)** | **Recovery (%)** |
| ≤500 | ≤30 | ≤60 | 60 to 120 |
| > 500 | ≤20 | ≤30 | 70 to 110 |

**PERFORMANCE OF SEVERAL FUMONISIN SAMPLING PLANS FOR SHELLED MAIZE**

27. The performance of each sampling plan design is described by an operating characteristic (OC) curve. Each OC curve was determined by using variability relationships for sampling, sample preparation, and analysis (Equations 1, 2, 3, and 4) and the negative binomial distribution (Whitaker et al., 1998 and Whitaker et al. 2007). The equations describing the sampling variance ($S^2_s$) for any sample size ns in number of kernels, the sample preparation variance ($S^2_{sp}$) for the Romer mill and any size test portion nss in g, and the analytical variance ($S^2_a$) for LC using any number of aliquots na are shown in Equations 1, 2, and 3, respectively, as a function of fumonisin concentration C in mg/kg.

| | | |
|---|---|---|
| Sampling | $S^2_s = (3{,}300/ns)\ 0.033\ C^{1.75}$ | (1) |
| Sample Prep | $S^2_{sp} = (25/nss)\ 0.011\ C^{1.59}$ | (2) |
| Analytical | $S^2_a = (1/na)\ 0.014\ C^{1.44}$ | (3) |
| Total variance | $S^2_t = S^2_s + S^2_{sp} + S^2_a$ | (4) |

Effect of Increasing the Size of a Single Laboratory Sample Tested Per Lot

28. OC describing the performance of the fumonisin sampling plan for shelled maize using laboratory sample sizes of 1, 2, and 5 kg and maximum levels (ML) of 10, 5, 2, and 1 mg/kg are shown in Figures 1, 2, 3, and 4, respectively. As sample size increases, the chances of rejecting lots (chances of rejecting a lot = 1.0 – chances of accepting a lot) with concentrations below the ML decreases (reduces false positives) and the chances of accepting lots with concentrations above the ML decreases (reduces false negatives).

29. An OC curve showing the effect of a 10 kg samples with a 5 mg/kg ML is shown in Figure 5.

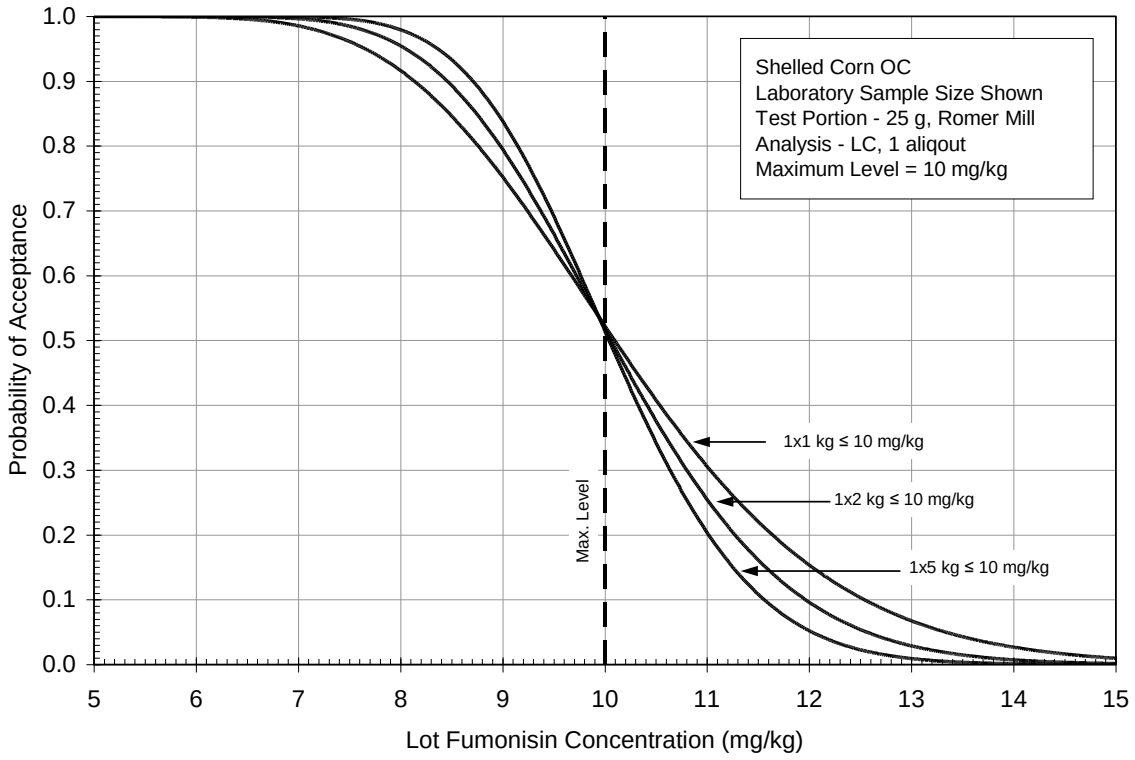


**Figure 1.** Operating characteristic curves showing the performance of sampling plan designs that use 1, 2, and 5 kg samples to detect fumonisin in lots of shelled maize for a maximum level of 10 mg/kg.

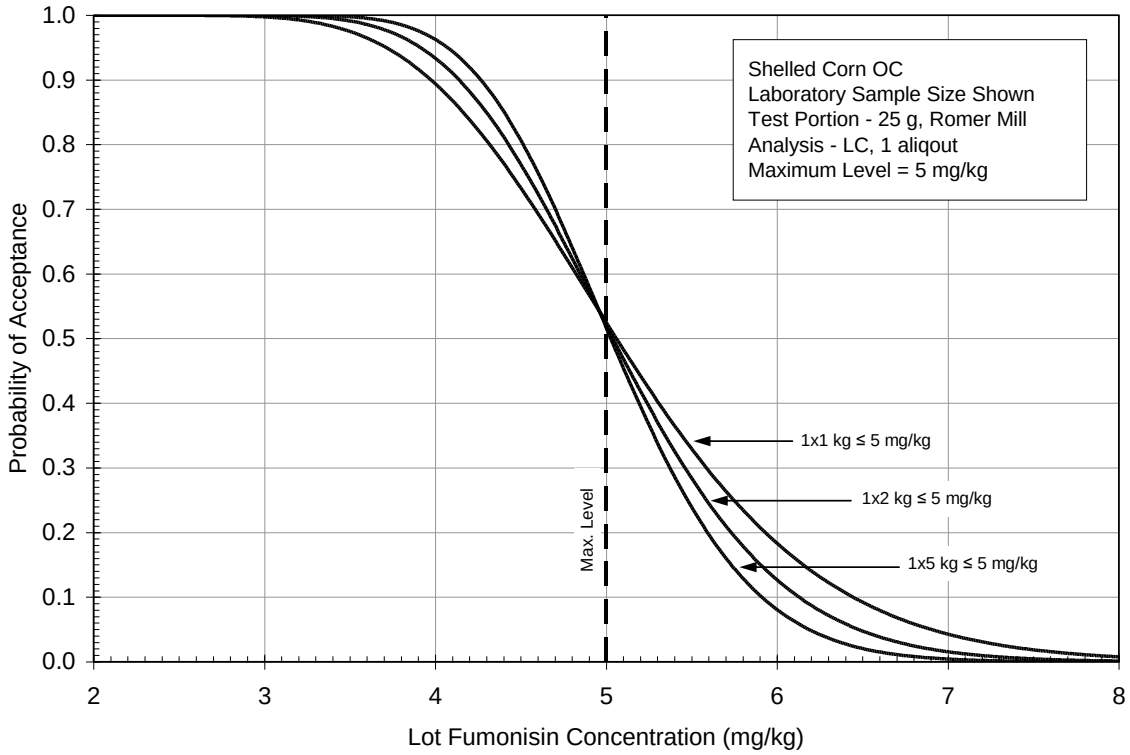


**Figure 2.** Operating characteristic curves showing the performance of sampling plan designs that use 1, 2, and 5 kg samples to detect fumonisin in lots of shelled maize for a maximum level of 5 mg/kg.

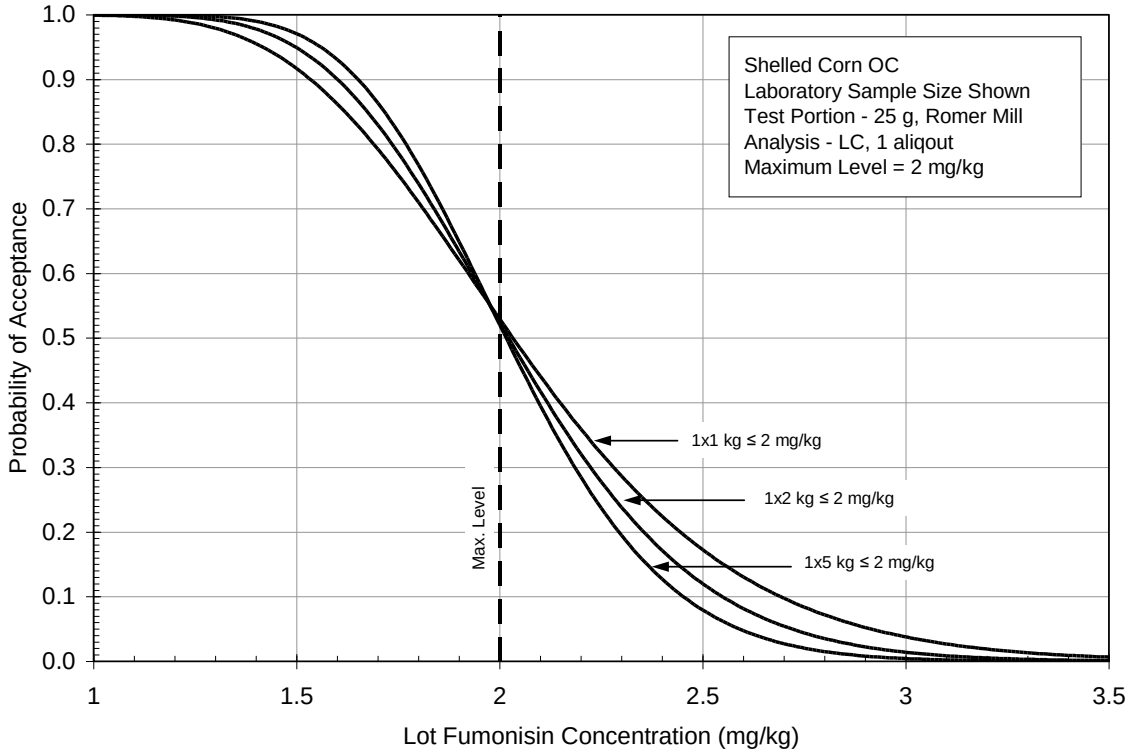


**Figure 3.** Operating characteristic curves showing the performance of sampling plan designs that use 1, 2, and 5 kg samples to detect fumonisin in lots of shelled maize for a maximum level of 2 mg/kg.

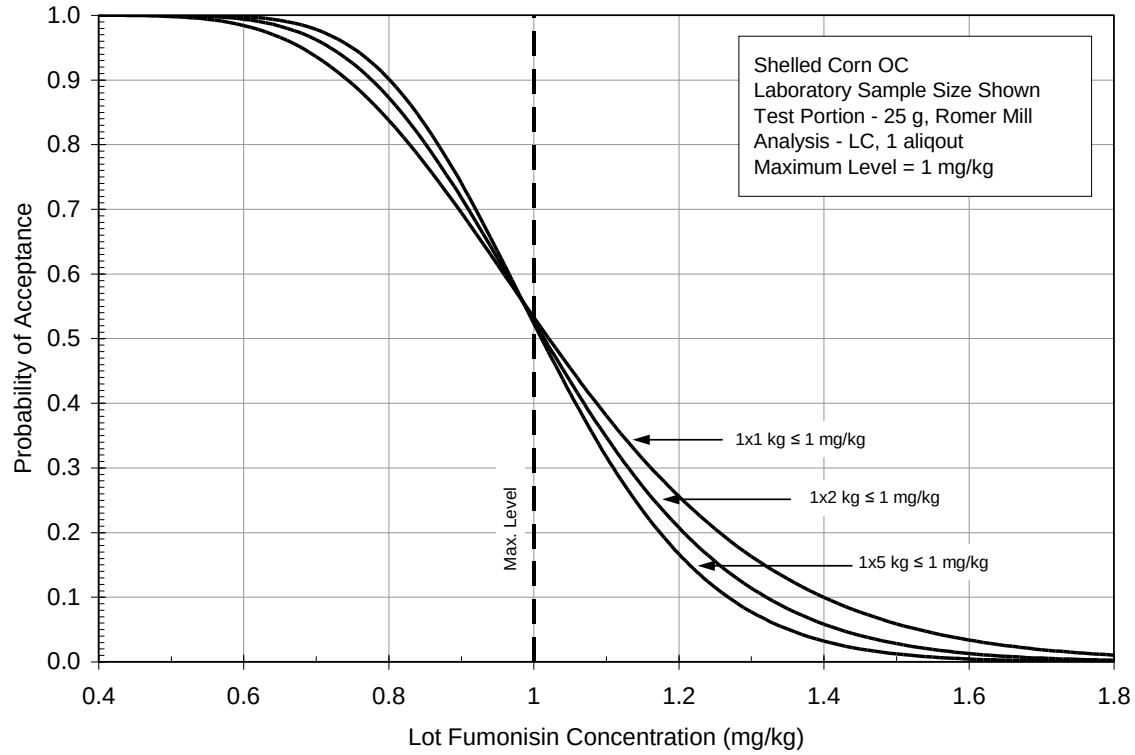


**Figure 4.** Operating characteristic curves showing the performance of sampling plan designs that use 1, 2, and 5 kg samples to detect fumonisin in lots of shelled maize for a maximum level of 1 mg/kg.

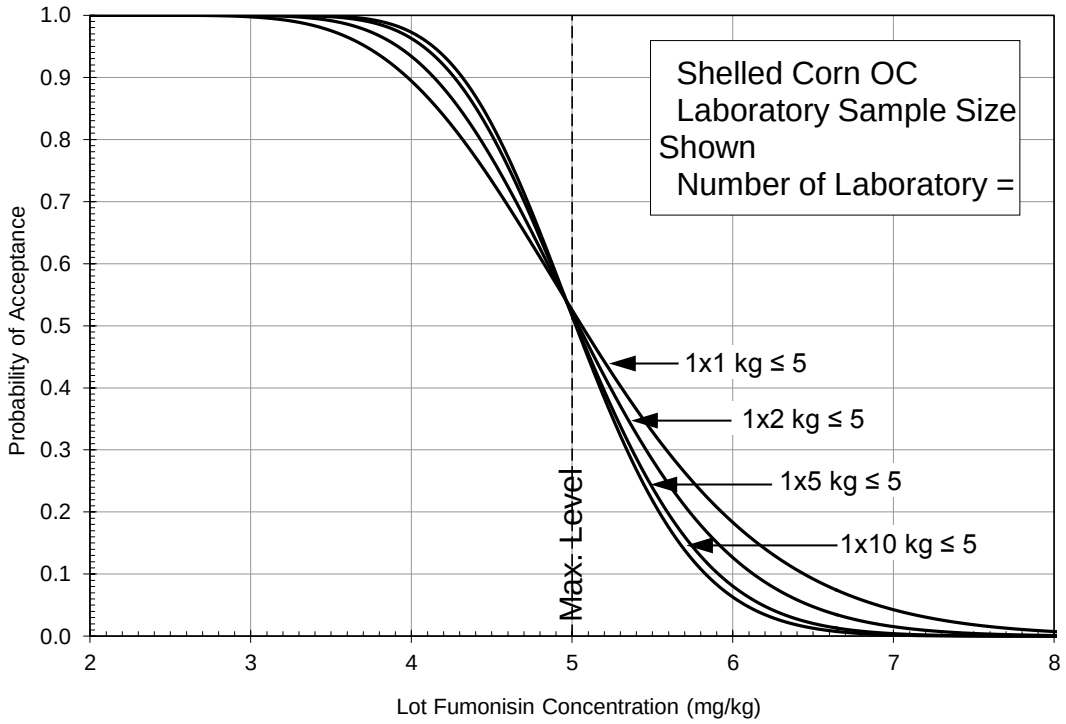


**Figure 5.** OC curves showing the effect of using a 1, 2, 5, and 10 kg sample with a 5 mg/kg maximum level on the chances of accepting (rejecting) lots at various lot concentrations.

30. Each sampling plan in Figures 1, 2, 3, 4, and 5 show the effect of increasing the size of a single laboratory sample on the chances of accepting and rejecting lots over a wide range in lot fumonisin concentrations. For each maximum level, as sample size increases the chances of rejecting lots (chances of rejecting a lot = 1.0 – chances of accepting a lot) with concentrations below the ML decreases (reduces false positives) and the chances of accepting lots with concentrations above the ML decreases (reduces false negatives). Increasing sample size has the desirable effect of reducing both false positives and false negatives at the same time.

Effect of Increasing Number of Laboratory Samples Tested Per Lot

31. Operating Characteristic curve describing the performance of the fumonisin sampling plan for shelled maize where the number 1.0 kg laboratory samples increases from 1 to 2 samples and maximum levels vary from 1, 2, 5, and 10 mg/kg are shown in Figures 6, 7, 8, and 9, respectively. Both laboratory samples must test less than the ML for the lot to be accepted. The operating characteristic curve reflects uncertainty associated with using 1 or 2 laboratory samples of size 1.0 kg, sample comminution in a Romer Mill, 25 g test portion, and quantification of fumonisin in the test portion by HPLC.

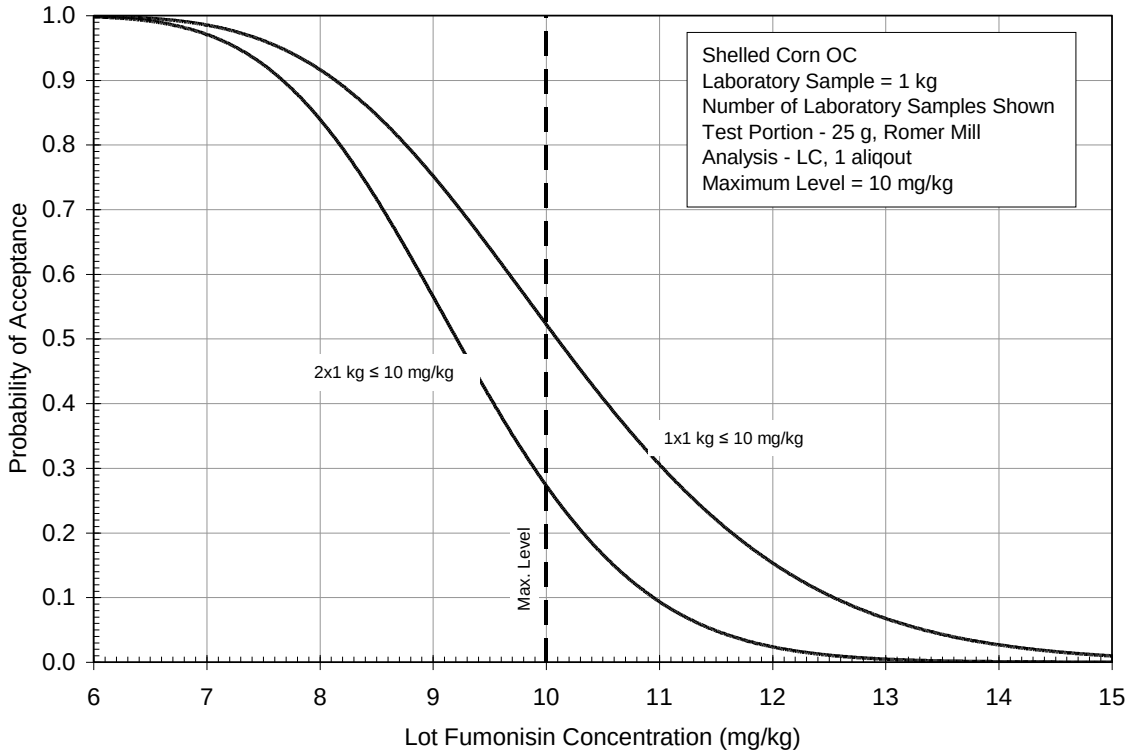


**Figure 6.** Operating characteristic curves showing the performance of sampling plan designs that use 1 or 2 samples of size 1.0 kg each to detect fumonisin in lots of shelled maize for a maximum level of 10 mg/kg.

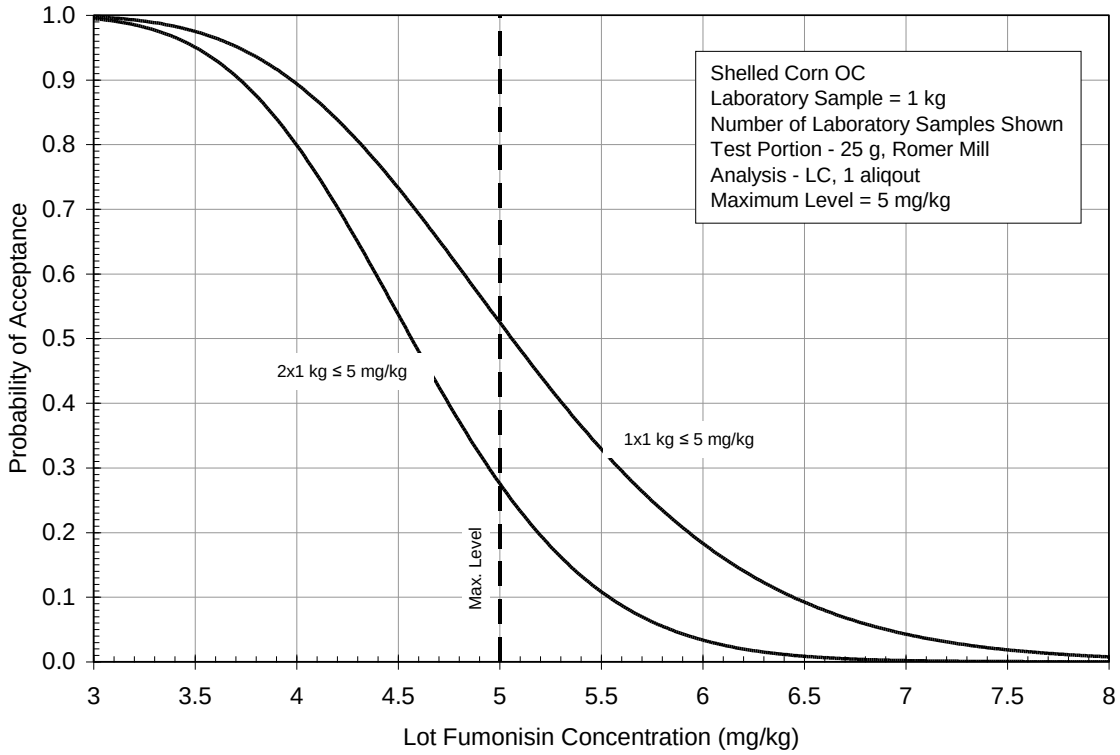


**Figure 7.** Operating characteristic curves showing the performance of sampling plan designs that use 1 or 2 samples of size 1.0 kg each to detect fumonisin in lots of shelled maize for a maximum level of 5 mg/kg.

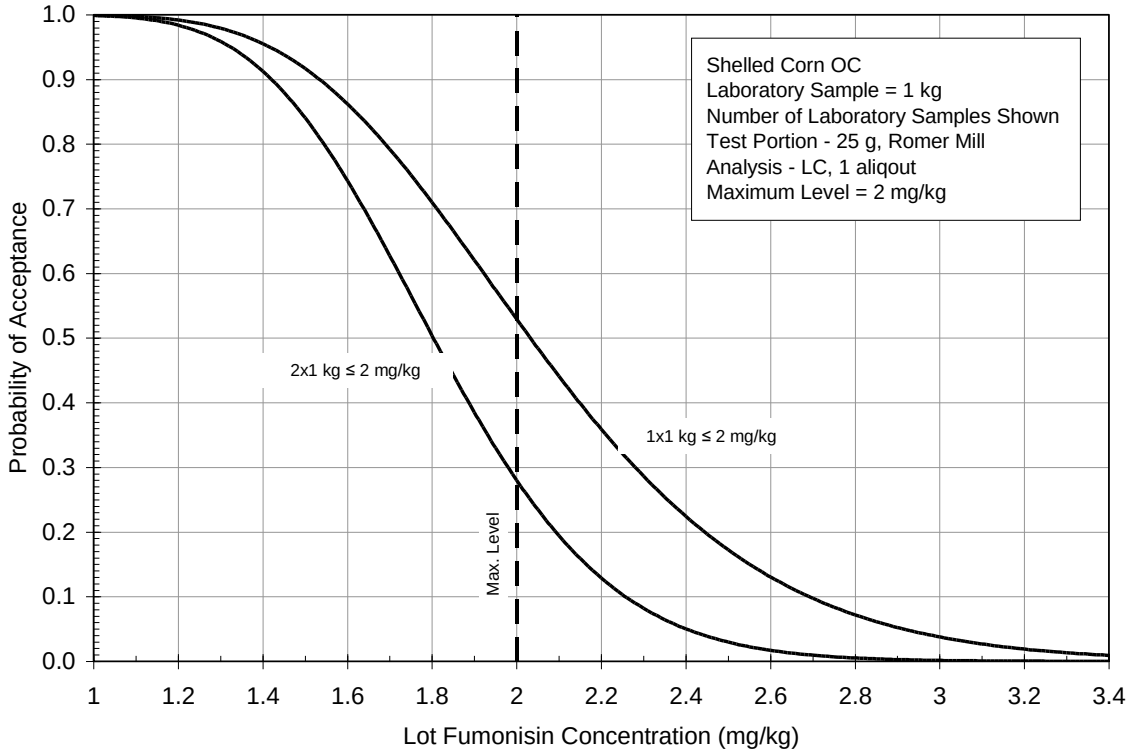


**Figure 8.** Operating characteristic curves showing the performance of sampling plan designs that use 1 or 2 samples of size 1.0 kg each to detect fumonisin in lots of shelled maize for a maximum level of 2 mg/kg.

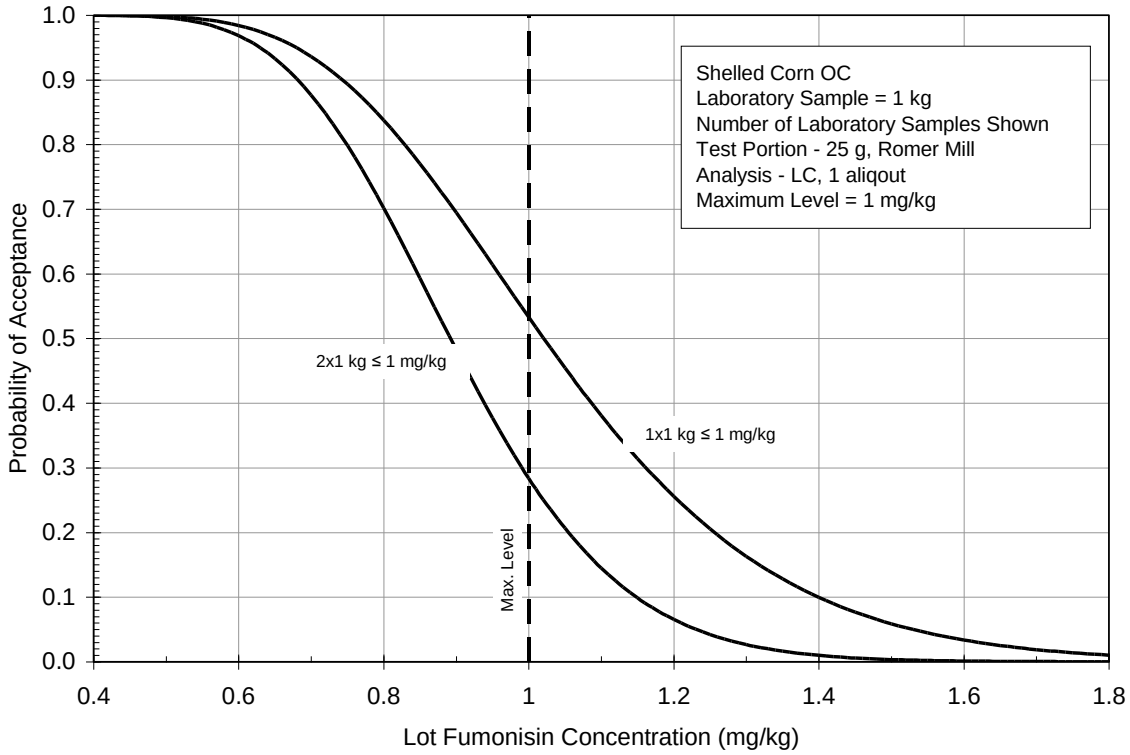


**Figure 9.** Operating characteristic curves showing the performance of sampling plan designs that use 1 or 2 samples of size 1.0 kg each to detect fumonisin in lots of shelled maize for a maximum level of 1 mg/kg.

32. For each maximum level, as the number of laboratory samples increase from 1 to 2 samples (each sample = 1.0 kg), the chances of rejecting lots with concentrations below the ML increases (increases false positives) and the chances of accepting lots with concentrations above the ML decreases (reduces false negatives). Increasing the number of samples tested per lot is an effective method of reducing the chances of a false negative, but has a high cost to the exporter in that it increases the chances of false positives.

33. The OC curves show in Figures 1 to 9 provide an indication of the interaction between maximum level, laboratory sample size, and number of laboratory samples can be used to minimize the chances of accepting lots with fumonisin concentrations above a certain level. For example, if a sampling plan was to be designed that did not accept more than 10% of the lots at 6 mg/g or higher, then either 1 x 5 kg <= 5 mg/kg (Figure 2) or 2 x 1 kg <= 5 mg/kg (Figure 7) would satisfy that criterion.

**PERFORMANCE OF SEVERAL FUMONISIN SAMPLING PLANS FOR MAIZE FLOUR/MEAL**

34. Since no sampling data is available for fumonisin and maize flour or maize meal, the variability associated with selecting a test portion from a sample comminuted with the Romer mill (Equation 2) is used to estimate the sampling variance for maize flour/meal. The total variability of the fumonisin test procedure for corn/maize flour (or any comminuted material) is the sum of the sampling variance and analytical variance. Since the material is comminuted, there is usually no sample preparation variance. Although no laboratory data is available, it is likely that the sampling variability for flour (Vsf) would be much lower than the sampling variability for grain comminuted with the Romer mill (Vsr) because the particle size of the processed flour is assumed to be much smaller than maize comminuted with the Romer mill (Vsf << Vsr). It is assumed that the analytical variability would be about the same for both processed flour and grain comminuted with the Romer mill. The sampling variance would be a larger component of the total variance when sampling grain comminuted with the Romer mill. The performance of the sampling plan is affected by the particle size. Using Equation 5 below for sampling variance would predict the need for a larger sample than would be predicted if a smaller sampling variance was used that more accurately reflected the particle size of maize flour. For a given sample size, smaller the particle size (more particles per unit mass), lower the sampling variability, lower the good lots rejected (exporter's risk), and lower the bad lots accepted (importer's risk) (Whitaker BT, *personal communication, 2012*).

35. Operating characteristic curves developed for sampling comminuted shelled maize (maize flour/meal) with specific sample sizes and analytical methods were calculated using variances described in Equations 2 and 3 which were measure by Whitaker et al, 1998. The sampling and analytical variances are:

Sampling Variance = $(25/ns)\ 0.011\ C^{1.59}$ (5)

Analytical variance = $(1/na)\ 0.014\ C^{1.44}$ (6)

Where ns is the size of the comminuted sample in grams and na is the number of aliquots quantified by LC.

Sampling variance (Equation 5) reflects a particle size distribution consistent with comminuting shelled maize with the Romer mill and is the sample preparation variance for shelled maize (Equation 2).

Analytical variance (Equation 6) reflects quantification of fumonisin in one aliquot by LC methods.

36. The effects of sample size and number of samples on the OC curves are shown in Figures 10 and 11, respectively, for an ML of 2 mg/kg. The effects of increasing sample size and the number of samples on the OC curve is similar to that described above for shelled maize.

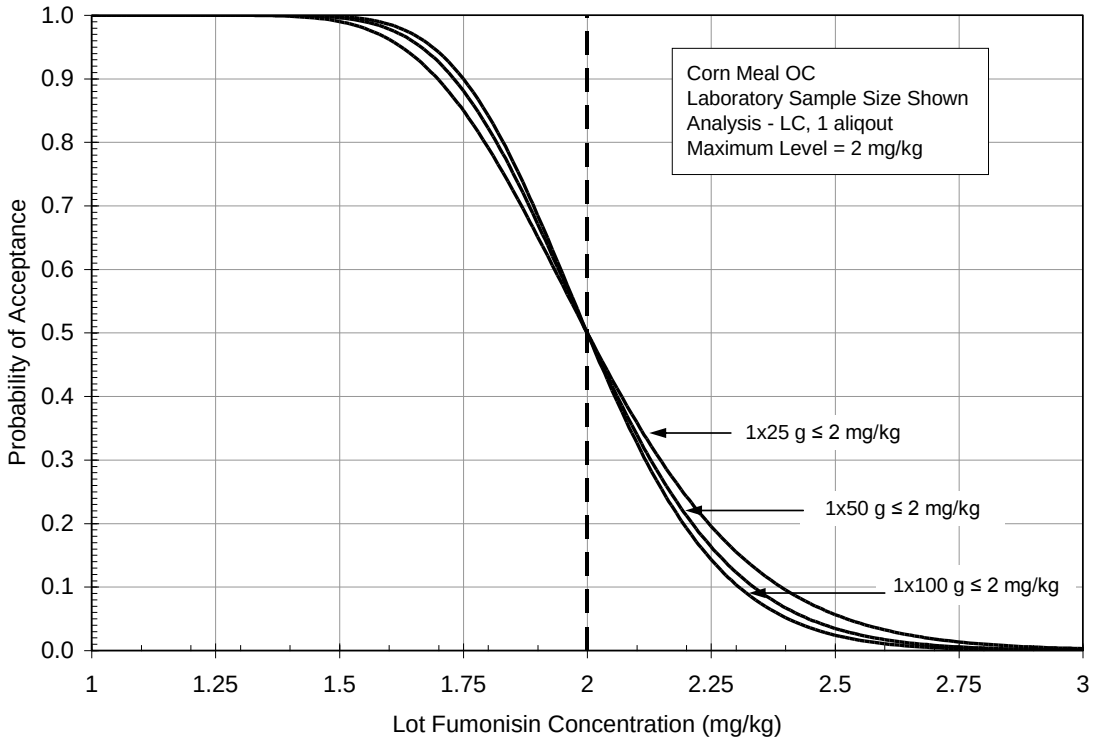


**Figure 10.** Operating characteristic curves showing the performance of sampling plan designs that use 25, 50, and 100 g samples to detect fumonisin in lots of maize flour/meal for a maximum level of 2 mg/kg.

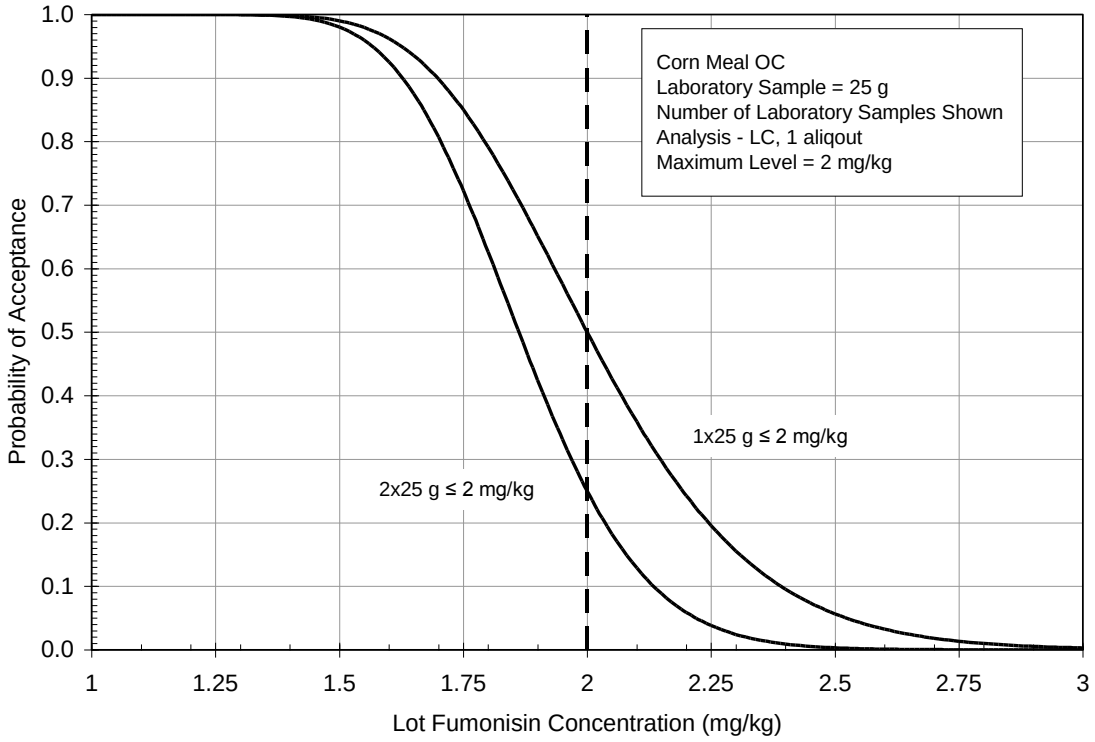


**Figure 11.** Operating characteristic curves showing the performance of sampling plan designs that use 1 or 2 samples of size 25 g each to detect fumonisin in lots of maize flour/meal for a maximum level of 2 mg/kg.