



**Food and Agriculture
Organization of
the United Nations**



**World Health
Organization**

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Agenda Item 9

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**JOINT FAO/WHO FOOD STANDARDS PROGRAMME
CODEX COMMITTEE ON CONTAMINANTS IN FOODS**

Eighth Session

The Hague, The Netherlands, 31 March – 4 April 2014

**PROPOSED DRAFT MAXIMUM LEVELS FOR FUMONISINS IN MAIZE AND MAIZE PRODUCTS
AND ASSOCIATED SAMPLING PLANS**

(AT STEP 4)

(Prepared by Brazil)

Codex Members and Observers wishing to submit comments at Step 3 on the proposed draft *maximum levels for fumonisins in maize and maize products and associated sampling plans* (see Appendix I), 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 **10 March 2014**. Comments should be directed:

to:

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Note: Supportive information presented in Appendix II is not subject to comments.

BACKGROUND

1. The 3rd session of the Committee on Contaminants in Foods (March 2009) agreed¹ to initiate work on the establishment of maximum levels for fumonisins in maize and maize products and associated sampling plans. This work was approved² by the 32nd session of the Codex Alimentarius Commission.
2. The 4th session of the CCCF (March 2010) considered the maximum levels and associated sampling plans and noted that fumonisins in maize and maize products were proposed for evaluation by JECFA. The Committee therefore agreed to suspend the work until the finalization of the JECFA evaluation.³
3. The 6th session of the CCCF (March 2012) considered maximum levels for fumonisins in maize and maize products based on outcome of the 74th JECFA Meeting including comments provided at the 4th session of the CCCF. However, in view of the variety of views on the maximum levels, the Committee agreed to suspend work on the maximum levels for one year until the outputs of a discussion paper to identify gaps on management measures to control fumonisin contamination in the *Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals* (CAC/RCP 51-2003) and the need for a separate code of practice for fumonisins in maize.⁴

¹ ALINORM 09/32/31, para. 101 and Appendix VII.
² ALINORM 09/32/REP, Appendix VI.
³ ALINORM 10/33/41, paras. 93-95.
⁴ REP11/CF, paras. 90-95.

4. The 7th session of the CCCF (April 2013) noted that work on the possible revision of the *Code of Practice for Prevention and Reduction of Mycotoxin Contamination in Cereals* (CAC/RCP 51-2003) would not impact on the MLs for fumonisins and sampling plans and agreed that the maximum levels and sampling plans should further be discussed at the next session of the Committee. The Committee agreed that the maximum levels for fumonisins in maize and maize products and associated sampling plans previously discussed at the 6th session would be circulated for comments through a circular letter and a revised proposal would be prepared by Brazil for consideration by the 8th session.⁵

INTRODUCTION

5. The delegation of Brazil revised the document presented at the 6th session of the CCCF in light of comments submitted at that session and in reply to CL 2013/25-CF (comments received from Argentina, Canada, Colombia, Japan and Kenya). In addition, the following was considered: the concept that the product to which the maximum levels apply should be clearly defined; the fact that fumonisins are not carried over from feed to animal products; and the need for harmonizing the sampling plans for fumonisins and DON in maize.
6. The conclusions and recommendations of the revision process including the proposed draft maximum levels and associated sampling plans for fumonisins in maize and maize products are presented in Appendix I. Background information in support of the maximum levels and sampling plans are provided in Appendix II.

REQUEST FOR COMMENTS

7. Codex Members and Observers are invited to send their comments on the proposed maximum levels for fumonisins in maize and maize products including the associated sampling plans as indicated in the box on the first page. While considering the maximum levels and sampling plans, due consideration should be given to the conclusions accompanying these proposals.

⁵ REP13/CF, para. 133.

APPENDIX I

**PROPOSED DRAFT MAXIMUM LEVELS FOR FUMONISINS IN MAIZE AND MAIZE PRODUCTS
AND ASSOCIATED SAMPLING PLANS**

CONCLUSIONS

- (a) Data provided by national governments to the 74th JECFA showed that, in most countries, the incidence of fumonisins in maize grain is high (70%), with a global mean total fumonisin upper bound level of 1681 µg/kg and a maximum of 5921 µg/kg.
- (b) The implementation of a ML of 5000 µg/kg in maize grain would lead to rejections in 6 of the 13 GEMS/Food Clusters (up to 88% of rejected samples in Cluster A). A ML of 2000 µg/kg in maize flour/meal leads to 57 and 53% of rejected samples in Clusters G and K, respectively.
- (c) Setting a ML of 5000 µg/kg in maize grain had an important impact on the dietary exposure, mostly in Clusters A, D and G. No significant additional impact was found in most clusters when the ML is set at 2500 µg/kg.
- (d) The 74th JECFA concluded that implementation of a ML of 5000 µg/kg in maize grain and of 2000 µg/kg in maize flour/meal, could significantly reduce exposure to total fumonisins (by more than 20%) in six Clusters (A, B, D, F, G, K). The final intake would exceed the PMTDI by 35% in Cluster A, by 15% in Clusters C and K by over 3 times in Cluster H.
- (e) Maize grain is a commodity that will be submitted to further processing before be ready for consumption, including milling, nixtamalization and thermal treatment, which will decrease the levels of fumonisins present. Hence, the exposure from the consumption of maize grain, unprocessed, conducted by the JECFA overestimates the real exposure to fumonisin through the consumption of ready-to-eat maize products.
- (f) Fumonisins are detected in popcorn and maize products, such as breakfast cereals and maize-based baby food. However, the data available is still limited to allow a proposal of MLs for these commodities.
- (g) It is important to point out that bound fumonisins found in extruded maize products (such as breakfast cereals) and some fermented maize products (such as kenkey and banku) are not detected by the usual extraction procedure and the exposure to fumonisins from the consumption of these food cannot be fully assessed. Studies available have shown that the levels found in the bound form are higher than what is found in the free form.
- (h) Efforts should be made to broaden the survey of bound fumonisins and generate consumption data at international level in extruded maize products that would allow a full risk assessment for fumonisins.
- (i) Data were available to allow a proposal for a sampling plan for fumonisin in maize grain. The sampling plan design should be harmonized with that proposed for deoxynivalenol in maize.
- (j) Although no sampling studies were available for maize flour/meal, data from Romer mill comminuted grain allowed the construction of operating curves and the proposal of a sampling plan for fumonisin in maize flour/meal.

RECOMMENDATIONS

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

Commodity	Maximum level for fumonisins (FB1+FB2), µg/kg
Maize grain, unprocessed*	5000
Maize flour/meal	2000

* Intend for human consumption only

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

Maize grain, unprocessed sampling plan

Maximum level	5000 µg/kg FB1 + FB2
Increments	increments of 100 g, depending on the lot weight (≥ 50 tonnes) (harmonized with the DON sampling plan for maize)
Aggregate sample size	5 kg (lot ≥ 50 tonnes)
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm - 20 mesh)
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, accept the lot. Otherwise, reject the lot.

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, accept the lot. Otherwise, reject the lot.

SAMPLING PLANS FOR FUMONISINS IN MAIZE GRAIN AND 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

Material to be sampled

- Each lot of maize, 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 lot should be subdivided into sublots according to Table 1.

Table 1. Subdivision of maize sublots according to lot weight

Lot weight (ton)	Weight or number of lots	Number of incremental sample	Aggregate sample weight
≥ 1500	500	100	5
> 300 and < 1500	3 sublots	100	5
≥ 50 and ≤ 300	100 tonnes	100	5
< 50	-	3-100*	1-5

* see table 2

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

Incremental Sample

- The suggested minimum weight of the incremental sample should be approximately 100 grams for lots of 50 metric tonnes (50,000 kg) or higher
- For lots less than 50 tonnes, the sampling plan must be used with 10 to 100 incremental samples, depending on the lot weight, resulting in an aggregate sample of 1 to 5 kg. For very small lots (≤ 0.5 tonnes) a lower number of incremental samples may be taken, but the aggregate sample uniting all incremental samples shall be also in that case at least 1 kg. Table 2 may be used to determine the number of incremental samples to be taken.

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

Lot weight (ton)	Number of incremental sample
≥ 0.05	3
> 0.05 - ≤ 0.5	5
> 0.5 - ≤ 1	10
> 1 - ≤ 3	20
> 3 - ≤ 10	40
> 10 - ≤ 20	60
> 20 - ≤ 50	100

Static Lots

- 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 subplot may not be accessible.
- 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.
- 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:

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

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

Dynamic Lots

9. 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).
10. 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.
11. 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.
12. The size of the aggregate sample (S) in kg, taken from a lot by a cross cut sampler is:

$$S = (D \times LT) / (T \times V),$$
 where D is the width of the diverter cup opening (cm), LT is the lot size (kg), T is interval or time between cup movement through the stream (seconds), and V is cup velocity (cm/sec).
13. 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.

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

Packaging and Transportation of Samples

14. 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.
15. 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

16. 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.
17. 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.
18. 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

19. The suggested weight of the test portion taken from the comminuted laboratory sample should be approximately 25 g.
20. 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.
21. 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

22. 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 3 (EC Regulation No 401/2006). Utilizing this approach, laboratories would be free to use the analytical method most appropriate for their facilities.

Table 3. Performance criteria for Fumonisin B1 and B2.

Level ($\mu\text{g}/\text{kg}$)	Precision		Recovery (%)
	RSDr (%)	RSDR (%)	
≤ 500	≤ 30	≤ 60	60 to 120
> 500	≤ 20	≤ 30	70 to 110

ANNEX

PERFORMANCE OF SEVERAL FUMONISIN SAMPLING PLANS FOR SHELLED MAIZE

1. 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 n_s in number of kernels, the sample preparation variance (S^2_{sp}) for the Romer mill and any size test portion n_{ss} in g, and the analytical variance (S^2_a) for LC using any number of aliquots n_a are shown in Equations 1, 2, and 3, respectively, as a function of fumonisin concentration C in mg/kg.

Sampling $S^2_s = (3,300/n_s) 0.033 C^{1.75}$ (1)

Sample Prep $S^2_{sp} = (25/n_{ss}) 0.011C^{1.59}$ (2)

Analytical $S^2_a = (1/n_a) 0.014 C^{1.44}$ (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

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

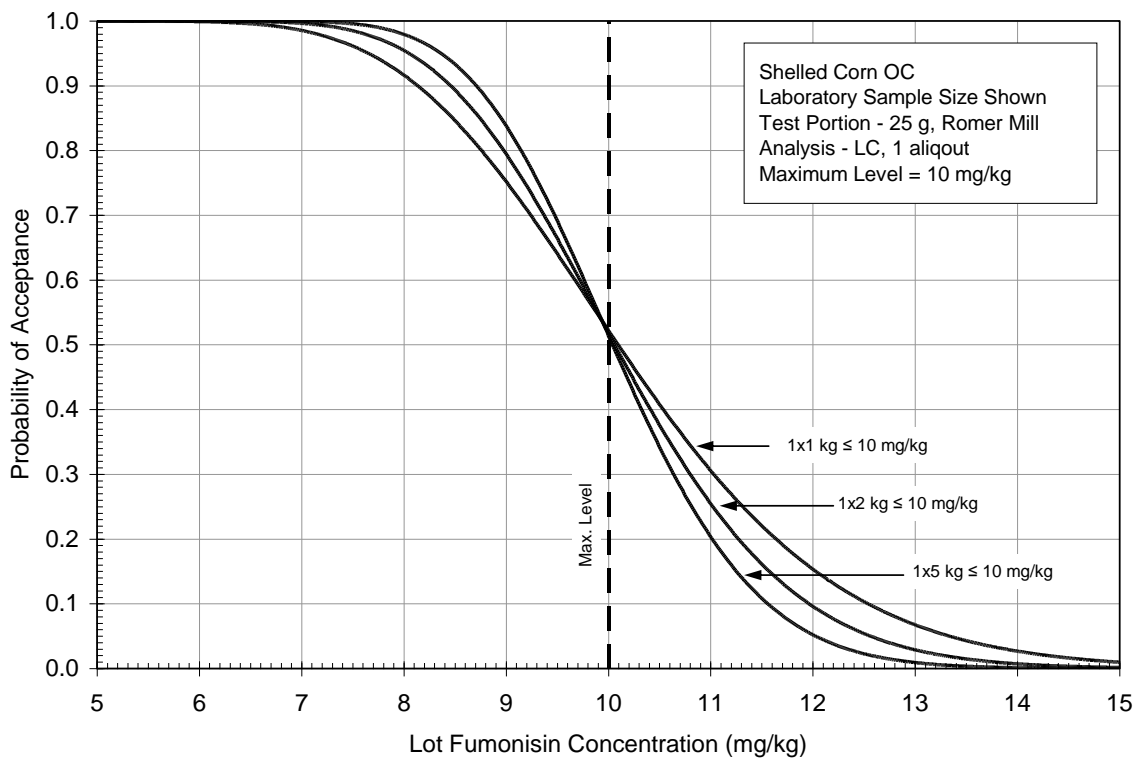


Figure 1. Operating characteristic curves showing the performance of sampling plan designs that use 1, 2, and 5 kg samples to detect fumonisin in lots of shelled maize for a maximum level of 10 mg/kg.

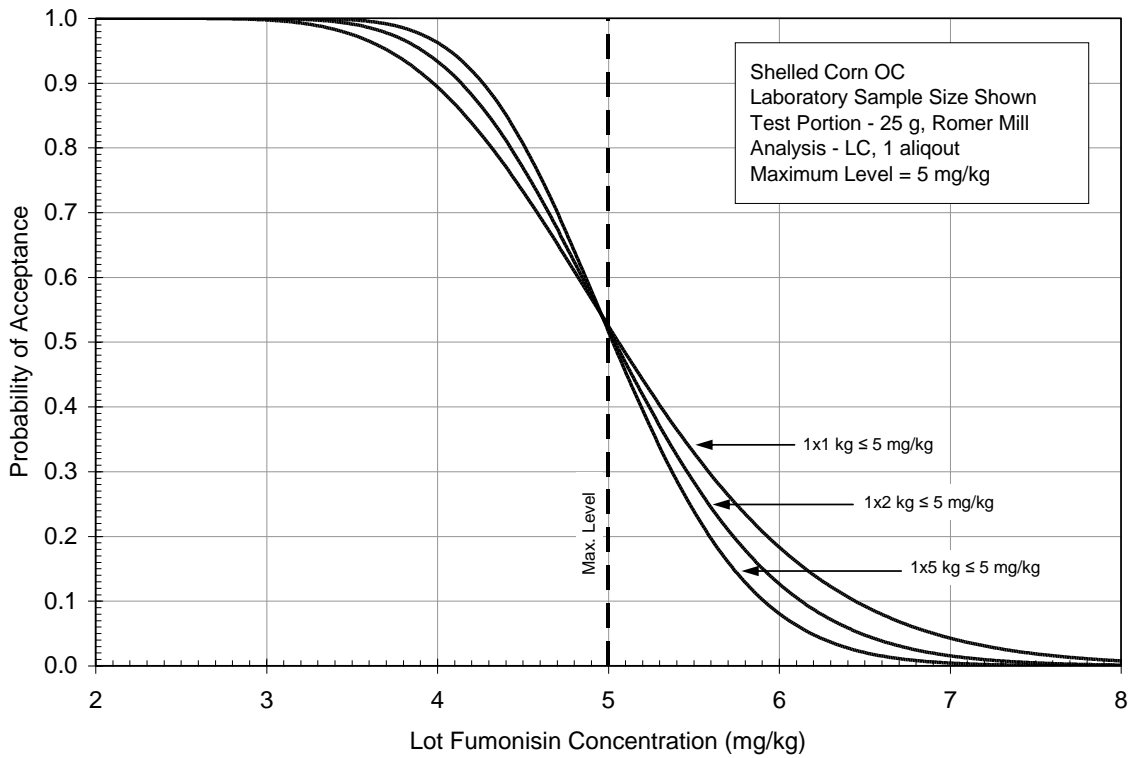


Figure 2. Operating characteristic curves showing the performance of sampling plan designs that use 1, 2, and 5 kg samples to detect fumonisin in lots of shelled maize for a maximum level of 5 mg/kg.

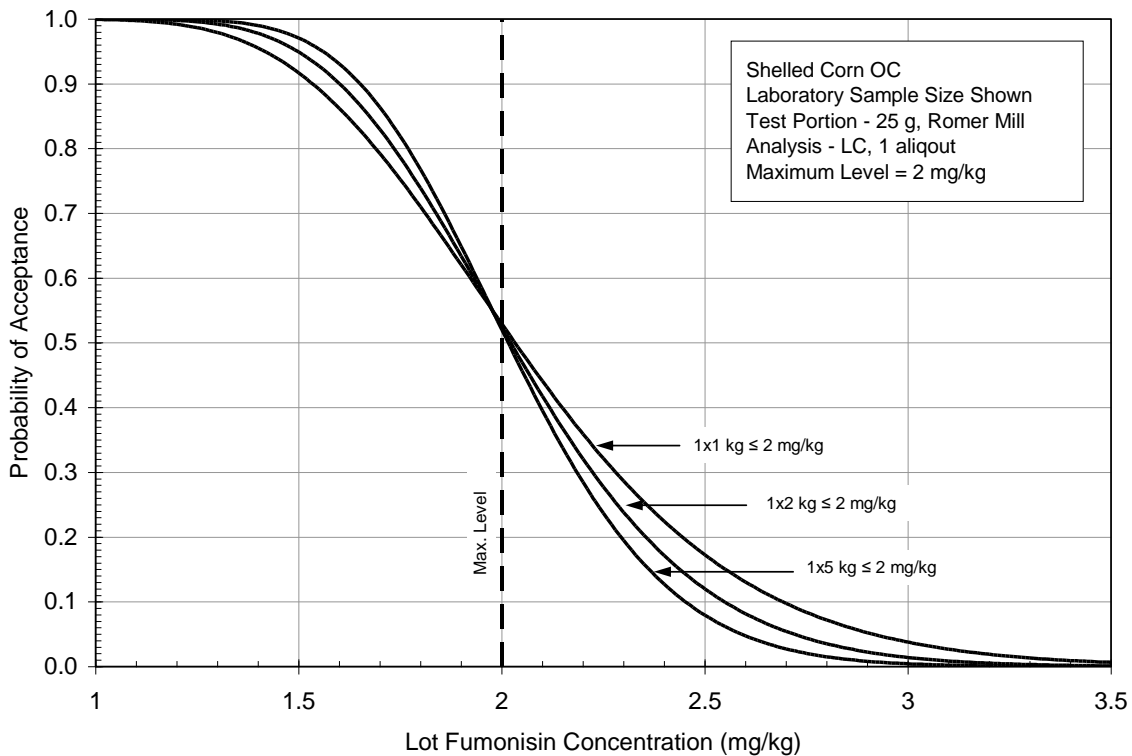


Figure 3. Operating characteristic curves showing the performance of sampling plan designs that use 1, 2, and 5 kg samples to detect fumonisin in lots of shelled maize for a maximum level of 2 mg/kg.

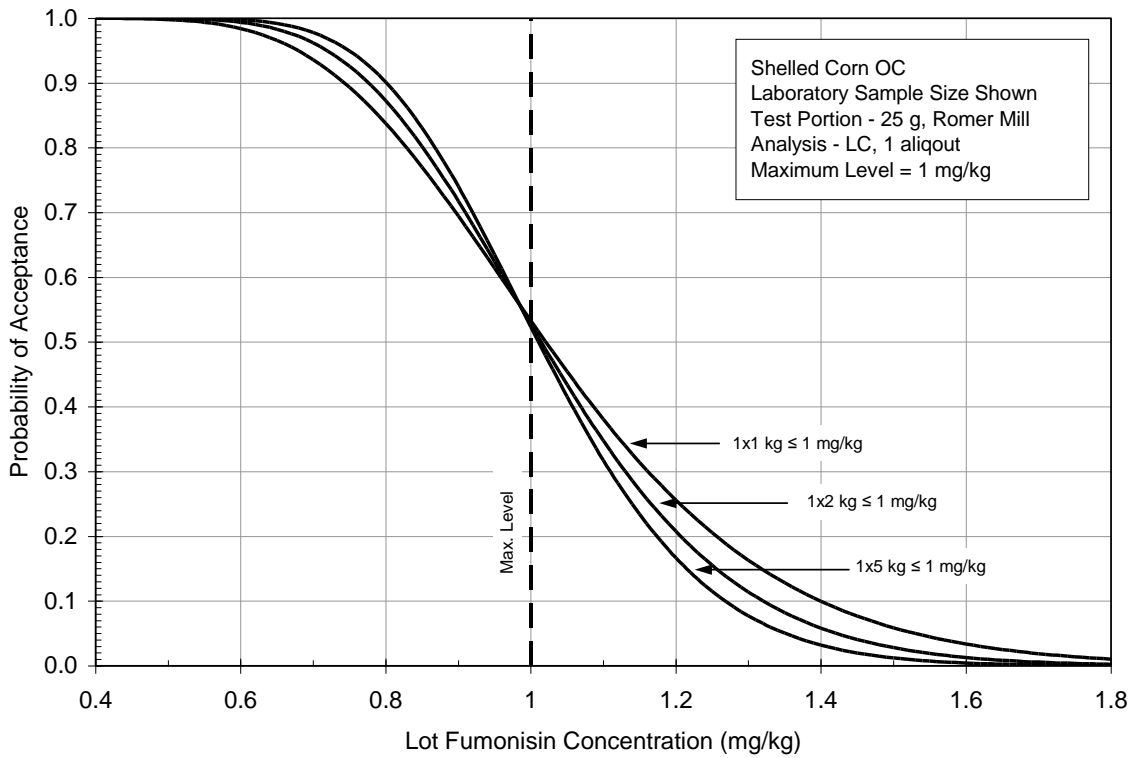


Figure 4. Operating characteristic curves showing the performance of sampling plan designs that use 1, 2, and 5 kg samples to detect fumonisin in lots of shelled maize for a maximum level of 1 mg/kg.

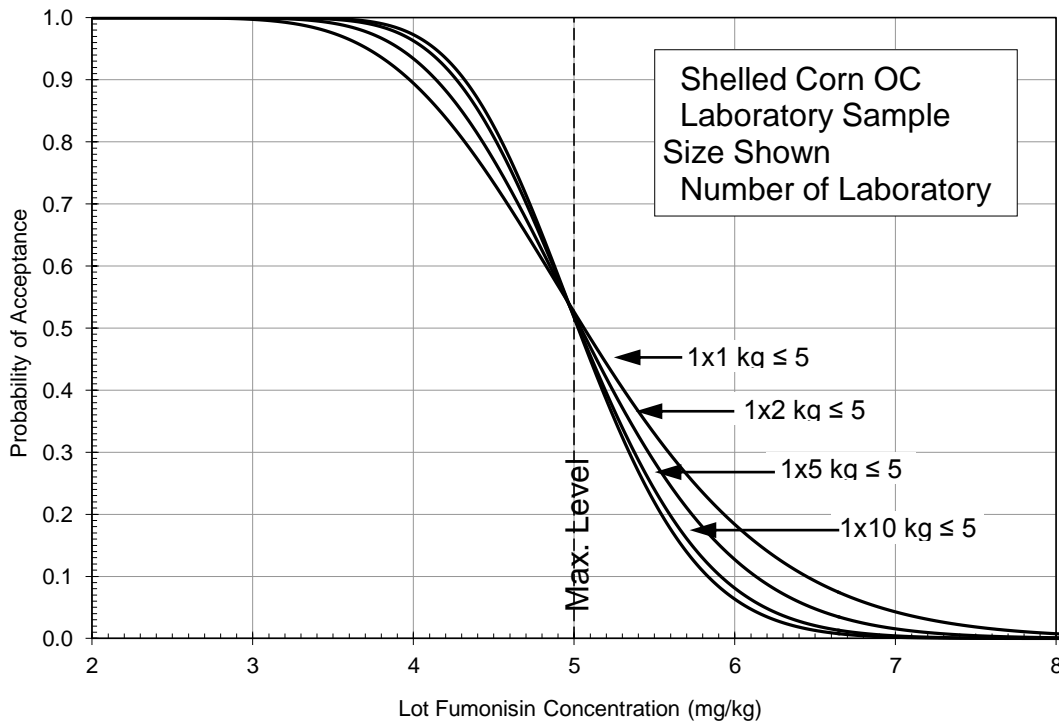


Figure 5. OC curves showing the effect of using a 1, 2, 5, and 10 kg sample with a 5 mg/kg maximum level on the chances of accepting (rejecting) lots at various lot concentrations.

- 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

- 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 appropriated Mill, 25 g test portion, and quantification of fumonisin in the test portion by HPLC.

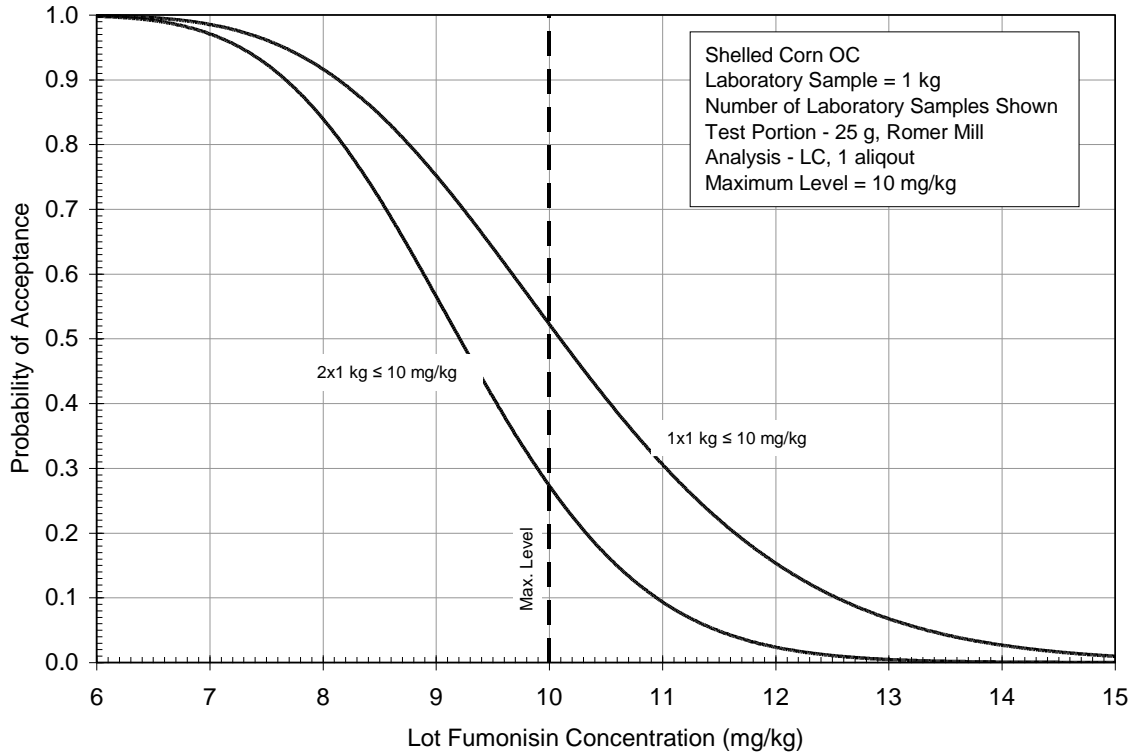


Figure 6. Operating characteristic curves showing the performance of sampling plan designs that use 1 or 2 laboratory samples of size 1.0 kg each to detect fumonisin in lots of shelled maize for a maximum level of 10 mg/kg.

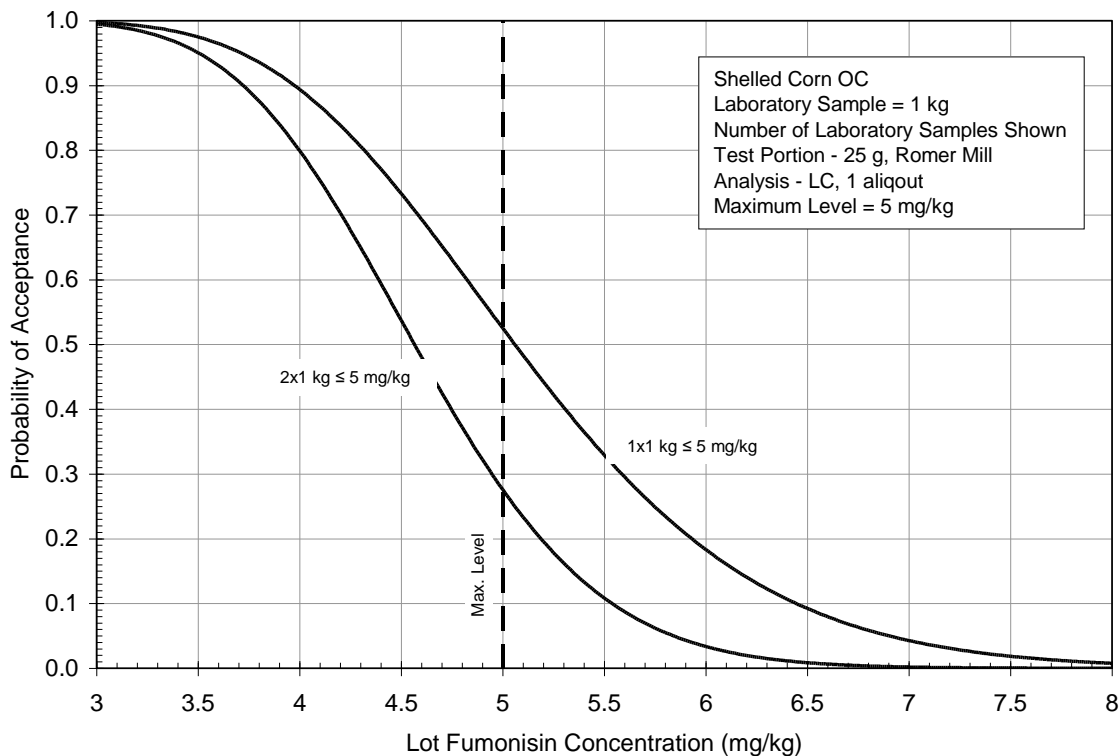


Figure 7. Operating characteristic curves showing the performance of sampling plan designs that use 1 or 2 laboratory samples of size 1.0 kg each to detect fumonisin in lots of shelled maize for a maximum level of 5 mg/kg.

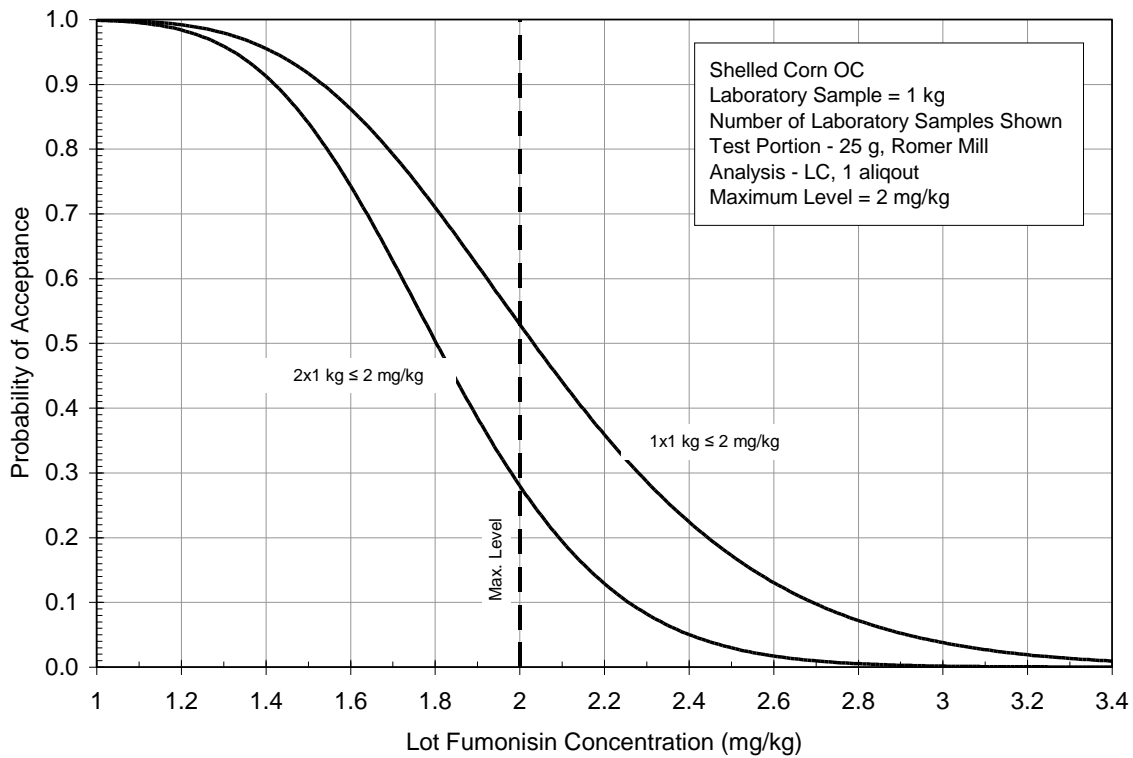


Figure 8. Operating characteristic curves showing the performance of sampling plan designs that use 1 or 2 laboratory samples of size 1.0 kg each to detect fumonisin in lots of shelled maize for a maximum level of 2 mg/kg.

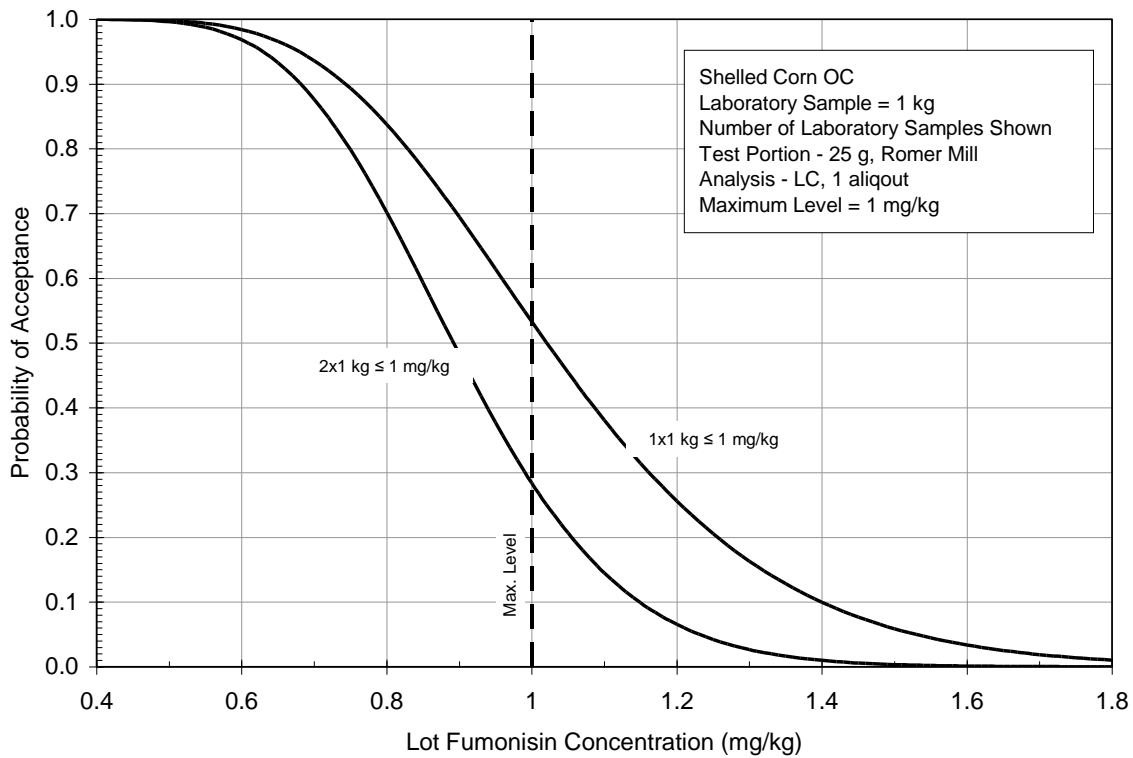


Figure 9. Operating characteristic curves showing the performance of sampling plan designs that use 1 or 2 laboratory samples of size 1.0 kg each to detect fumonisin in lots of shelled maize for a maximum level of 1 mg/kg.

6. 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 laboratory 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.
7. 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 1x5kg<=5mg/kg (Figure 2) or 2x1kg<=5mg/kg (Figure 7) would satisfy that criterion.

PERFORMANCE OF SEVERAL FUMONISIN SAMPLING PLANS FOR MAIZE FLOUR/MEAL

8. 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 appropriated mill (Equation 2) is used to estimate the sampling variance for maize flour/meal. The total variability of the fumonisin test procedure for 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 (V_{sf}) would be much lower than the sampling variability for grain comminuted with the Romer mill (V_{sr}) because the particle size of the processed flour is assumed to be much smaller than maize comminuted with the appropriated mill ($V_{sf} \ll V_{sr}$). It is assumed that the analytical variability would be about the same for both processed flour and grain comminuted with the appropriated 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).
9. 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:

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

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

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.

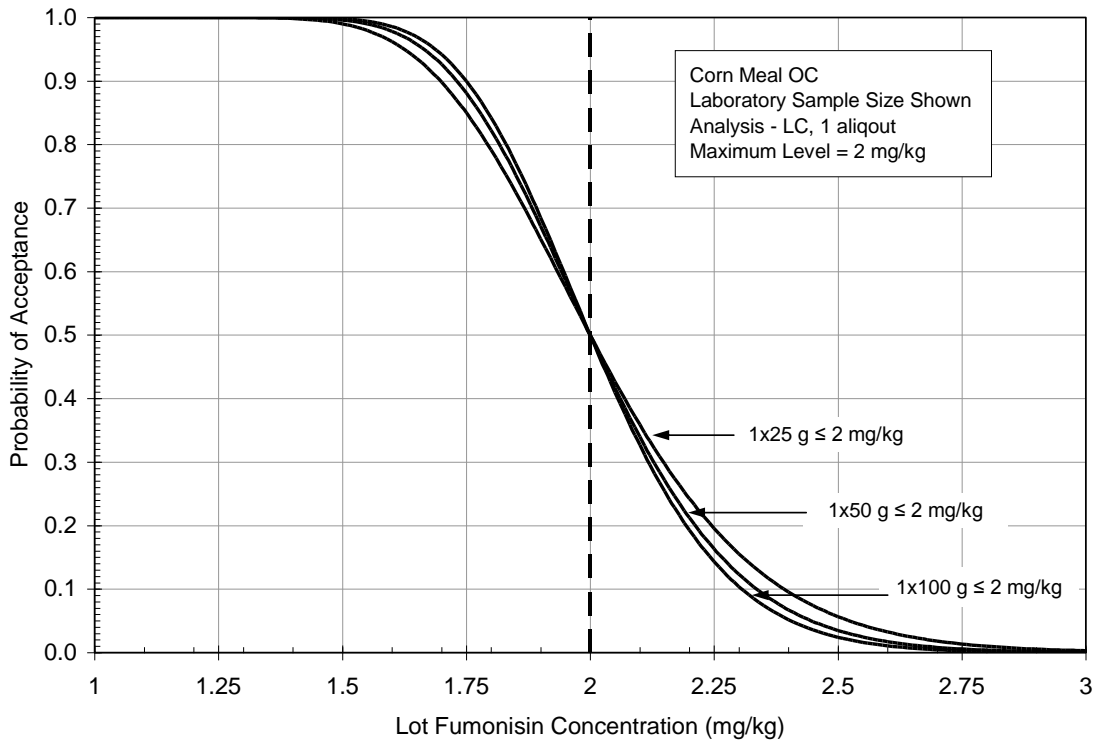


Figure 10. Operating characteristic curves showing the performance of sampling plan designs that use 25, 50, and 100 g samples to detect fumonisin in lots of maize flour/meal for a maximum level of 2 mg/kg.

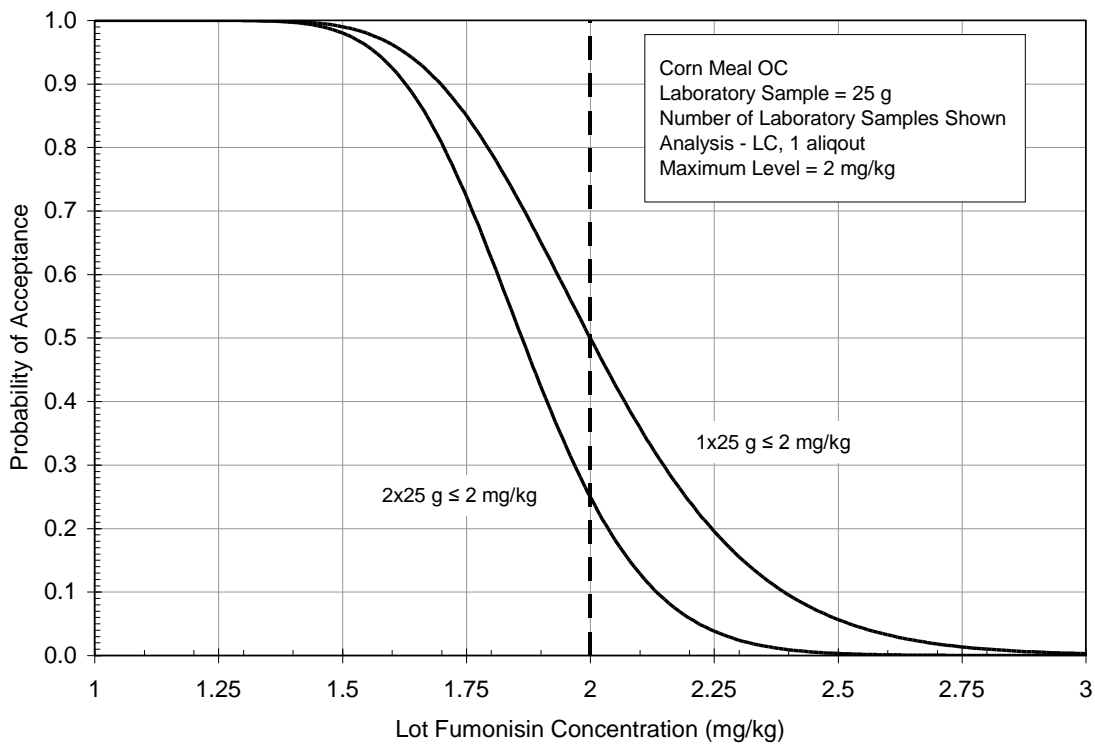


Figure 11. Operating characteristic curves showing the performance of sampling plan designs that use 1 or 2 samples of size 25 g each to detect fumonisin in lots of maize flour/meal for a maximum level of 2 mg/kg.

APPENDIX II

BACKGROUND INFORMATION ON THE ELABORATION OF THE PROPOSED MAXIMUM LEVELS AND SAMPLING PLANS

INTRODUCTION

1. Fumonisin is a mycotoxin structurally related to a group of diesters of propane-1, 2, 3-tricarboxylic acid and various 2-amino-12, 16-dimethylpolyhydroxyeicosanes in which the C14 and C15 hydroxyl groups are esterified with the terminal carboxyl group of tricarboxylic acid (Figure 1). At least 18 fumonisin analogues have been identified and these have been classified into series A, B, C and P based on their chemical structure (Sewram et al., 2005, Torres et al., 2007, Kumar et al., 2008). The B series, consisting mainly of fumonisin B1 (FB1), and fumonisin B2 (FB2), are believed to be the most abundant naturally occurring analogues (WHO, 2011). Recently, 28 isomers of FB1 were detected in a solid rice culture infected with *Fusarium verticillioides*, by RP-HPLC/ESI-TOFMS and RP-HPLC/ESI-ITMS (Bartok et al., 2010)

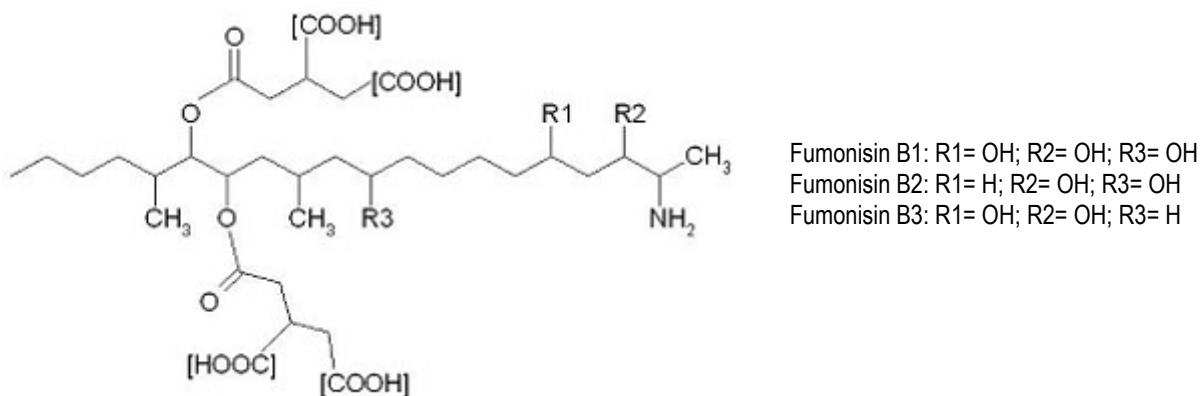


Figure 1. Chemical structure of FB1, FB2 and FB3

2. Fumonisin is produced mainly by *Fusarium verticillioides* (Sacc.) Nirenberg (synonym *F. moniliforme* Sheldon) (teleomorph, *Gibberella moniliformis*), *Fusarium proliferatum* (Matsushima) and *Fusarium nygamai* (Marasas et al., 2001; Rheeder et al., 2002).
3. FB2 was detected in cultures of *Aspergillus niger* for the first time by Frisvad et al. (2007). Later, it was shown that *A. niger* strains were able to produce FB2 and FB4 on grapes and raisins (Morgensen et al., 2010) as well as FB2 on coffee (Noonim et al., 2010). A new FB6 has been isolated, together with FB2, from stationary cultures of the fungus *A. niger* NRRL 326 by Månsson et al. (2010).
4. Biological interactions between the maize plant, and the fungus are complex and may have diametrically opposing results (Yates and Sparks, 2008). *F. verticillioides* grows within the maize plant as an endophyte (Bacon and Hinton, 1996) - an interaction of benefit to the plant growth in other members of the Gramineae (Clay, 1990; Yates et al., 2005). However, under plant stress growth conditions, the symptomless endophytic relationship may convert to a disease and/or Mycotoxin producing interaction (Bacon and Nelson, 1994; Abbas et al., 2006).
5. The mechanisms that trigger the conversion of the fungi-plant relationship from that of a symptomless one to a cause of disease for the plant and a mycotoxin-producing interaction have not yet been identified (Yates and Sparks, 2008). Nevertheless, it is possible that water stress and insect predation, factors that have been related to the onset of the deleterious aspects of this fungal plant interaction (Dowd, 2003), might be involved in the process.
6. The extent of maize contamination with fumonisins varies with geographical location, agricultural practices, and the maize genotype (Jackson and Jablonski, 2004). The levels of fumonisins are also influenced by environmental factors such as temperature, humidity, drought stress and the extent of rainfall during the pre-harvest and harvest periods; storage of the harvested maize kernels under improper moisture conditions can result in additional accumulation of fumonisins (Bacon and Nelson, 1994). Higher levels of fumonisins are usually found in maize kernels produced in the warmer regions of the world (Shelby et al., 1994; Miller, 1999).

BIOLOGICAL ASPECTS

7. Fumonisin was evaluated by the JECFA at its 56th (2001) and 74th meetings (2011). Laboratory animal and *in vitro* studies evaluated by both meetings have shown disruption of lipid metabolism as the initial site of action of fumonisins. The proposed lipid-based mechanism involves inhibition of ceramide synthase, a key enzyme in the biosynthesis of sphingolipids, and changes in the polyunsaturated fatty acid and phospholipid pools. Both lead ultimately to lipid-mediated alterations in signaling and metabolic pathways crucial to the cell growth, death and differentiation (FAO/WHO, 2001; FAO/WHO, 2011).

8. In experimental animal studies, the extent of disruption of sphingolipid metabolism correlates well with toxicity and has been used successfully to demonstrate structure–activity relationships, tissue specificity, strain/sex susceptibility and the efficacy of intervention strategies in poultry, fish, rats, mice, pigs and horses. However, the level of accumulation of bioactive lipid metabolites or depletion of lipids that constitutes a quantitative measure of adverse physiological effects is not known and the JECFA concluded that disruption of lipid metabolism cannot be used as a toxicological end-point for the final evaluation (FAO/WHO, 2011).
9. In animal species studies revised by both JECFA meetings, the liver and kidney were found to be a target for FB1. The 56th JECFA allocated a Provisional Maximum Tolerable Daily Intake (PMTDI) of 2 µg/kg bw/day to FB1, FB2 and FB3 alone or in combination, on the basis of a NOEL of 0.2 mg/kg bw/day (renal toxicity on mice) and an uncertainty factor of 100.
10. The 74th JECFA identified four animal studies as suitable for providing data for BMD (bench mark dose) estimation. Modeling was carried out using the USPA's BMD software and the models resulting in acceptable fits ($P > 0.05$) were selected to derive BMD₁₀ and BMDL₁₀ values for a BMR (bench mark response) of 10% extra risk. The lowest BMDL₁₀ was 165 µg/kg bw per day for pure FB1 (megalocytic hepatocytes in male mice). Using a uncertainty factor of 100, the meeting derived a PMTDI of 2 µg/kg bw/day, retaining the previously established group PMTDI.
11. Human epidemiological studies revised by the two fumonisins evaluations conducted by the JECFA have indicated an association between the occurrence of *Fusarium verticillioides* on maize and the incidence of oesophageal cancer in various regions of the world. However, a dose–response relationship has not yet been established, nor has a toxicological mechanism been elucidated. Furthermore, geographical differences in demography, ethnic groups, genetic susceptibility, culture, economy and nutritional status all affect the rates of oesophageal cancer.
12. A study investigating the link between fumonisin exposure and childhood stunting in the United Republic of Tanzania evaluated by the 74th JECFA indicated that infants whose estimated fumonisin exposure exceeded the PMTDI of 2 µg/kg bw were significantly shorter and weighed less than those whose exposure was below this PMTDI. These results are in agreement with animal studies in which fumonisins exposure was associated with reduced weight gain and feed conversion efficiency (FAO/WHO, 2011).
13. The JECFA evaluated studies that describe higher rates of neural tube defects (NTD) in areas of South Africa, China and USA at times when the maize based foods consumed contained relatively high levels of fumonisins. As folate deficiency has been implicated in the development of NTD, the blockage of folate uptake by fumonisins may have been a factor in this regard. A new study of NTD incidence among Mexican Americans on the Texas/Mexico border, combined with toxicological and earlier epidemiological studies, confirms that fumonisin exposure in pregnant women may be a contributing factor to increased NTD risk in their babies (FAO/WHO, 2011).
14. The International Agency for Research on Cancer (IARC) has classified FB1 as possibly carcinogenic to humans (Group 2B) (IARC, 2002).

METHODS OF ANALYSIS

15. ELISA methods can be used for rapid screening purposes under field conditions or in the laboratory (Castells et al., 2008). These methods, however, generally overestimate the concentration of fumonisins present in samples as a result of antibodies' cross reactivity to compounds that are structurally related to fumonisins (Bird et al., 2002; Jackson and Jablonski, 2004).
16. In general, fumonisin can be extracted from maize or maize based products using methanol-water or acetonitrile-water and the extract cleaned up using C18 cartridges, SAX or immunoaffinity columns (Sydenham et al., 1996; Solfrizzo et al., 2001, Caldas and Silva, 2007). Fumonisin is suited for determination by reversed-phase HPLC, but as they lack a significant UV chromophore, derivatization is necessary before detection (Ndube et al., 2011). HPLC/fluorescence is the official AOAC-IUPAC method [995.15] for maize kernels at concentrations of 0.5 - 8 µg/g FB1 or 0.8 -12.8 µg/g total fumonisins (Sydenham et al., 1996). Wang et al. (2008) analyzed FB1, FB2, FB3 and FB4 with by HPLC coupled with an evaporative laser scattering detector without any prior derivatization of the samples (LOQ of 0.3 mg/kg).
17. LC/MS or LC/MS-MS methods have been extensively used in the last years as they provide quantitative analysis, as well as fumonisins identity confirmation. Silva *et al.* (2009) has shown that LC/MS-MS provides higher sensitivity (12 µg/kg for FB1 and FB2) than LC/MS (40 µg/kg for both fumonisins), and fluorescence detection (20 µg/kg for FB1 and 15 µg/kg for FB2), and also showed to be more precise.
18. An interlaboratory validation study for fumonisins B₁ and B₂ in maize was reported by Senyuva et al. (2010), involving 12 laboratories in 11 countries. The sample was extracted with acetonitrile-methanol-water, cleaned-up with immunoaffinity column and fumonisins analysed by LC/MS. Relative standard deviation values for within laboratory repeatability (RSD_r) ranged from 1.9 to 12.6% and for between laboratory reproducibility (RSD_R) from 18.2 to 25.5%. HorRat values for r and R were all less than 2 indicating that the method is suitable as a regulatory method for the enforcement of limits for fumonisins in maize.

19. Fumonisin bound to starch and proteins found in food subject to heat during processing, such as cereal breakfast and tortillas, cannot be detected by conventional analysis. In a method described by Kim et al(2003), the protein-bound FB1 was extracted with 1% sodium dodecylsulfate (SDS), the bound fumonisin hydrolyzed with 2 N KOH, the extract cleaned-up on an OASIS polymeric SPE and fumonisins determined by HPLC as HFB1 (hydrolyzed FB₁). This method was further improved by Park et al (2004) by complexing the SDS with methylene blue, and eliminating its interference in the HPLC analysis. Dall'Asta et al. (2008) reported a LC/MS/MS method for the simultaneous quantification of free and masked fumonisins in maize and maize based products.
20. The 74th JECFA identified that the current problems relating to analytical methods include the lack of suitable reference materials for method validation and the unavailability of standards, particularly for the hydrolysed fumonisins (HFB₁, HFB₂, HFB₃) and FB₆.

SAMPLING PLANS

21. A sampling plan is defined by a sampling step (number and size of the laboratory sample taken from lot), sample preparation step (comminution and test portion size), an analytical step, and an accept/reject limit (usually equal to the ML). The OC curve reflects the variability (sum of sampling, sample prep, and analytical variability) and distribution among sample test result for that particular mycotoxin and commodity and accept/reject limits for various MLs. 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 (Whitaker et al., 2007).
22. In a study conducted by Whitaker et al (1998) on fumonisin in shelled maize, a bulk sample of about 45 kg was taken from each of 24 batches of shelled maize which had been harvested from 24 fields in North Carolina, USA. Each bulk sample was riffle-divided into 32 test samples of 1.1 kg each, and these were comminuted in a Romer mill. At a contamination level of 2 mg/kg in a given batch, the coefficient of variation (CV) associated with sampling (1.1 kg sample shelled maize) was 17%, the CV associated with sample preparation (25 g test portion) was 9.1%, and the CV for analysis (HPLC and 1 aliquot quantified) was 9.7%. These values were independent of the fumonisin type. The coefficient of variation associated with the total test procedure was 45%, which was of the same order of magnitude as that for measuring aflatoxin in shelled maize by a similar test procedure.
23. In a sampling study conducted with food-grade shelled maize marketed in Nigeria in 2002, an average of 17 test samples, 100 g each, were taken from each of the 86 maize lots (Whitaker et al., 2007). Each test sample was finely ground by using in a Romer mill and a 25 g analytical subsample was taken for analysis of FB1 by HPLC. The variance, standard deviation and coefficient of variation among 100 g samples used to detect fumonisin in a shipment of maize with a true concentration of 2 µg/g were 1.91, 1.38 and 69% respectively. The uncertainty associated with the fumonisin test procedure was similar in magnitude to the uncertainty previously found by the authors in USA (Whitaker et al., 1998).
24. The number of incremental samples taken from a lot is directly proportional to lot size. As the lot size increases, the number of incremental samples selected from the lot increases. If the aggregate sample is bigger than the designated laboratory sample size, the laboratory sample is randomly removed from the aggregate sample. In general, lot size for maize and cereals vary from small trailers to trucks to railcars to large ships, and fumonisin sampling rates can be similar to that suggested for deoxynivalenol (DON) (REP13/CF⁶, Appendix III; CX/MAS 14/35/3) (Whitaker BT, personal communication, 2013).
25. Currently, there are no available studies that evaluate sampling plans for fumonisins in maize flour/meal. In the CX/CF 10/4/87, various operating curves (OC) and a sampling plan for maize flour/meal was proposed to the 4th CCCF. They were prepared by Dr. Thomas Whitaker using laboratory data from his study conducted in the USA with maize grain (Whitaker et al., 1998). Normal distribution was used to calculate the OC curves (Thomas Whitaker, *personal communication*).
26. The total variability of the fumonisin test procedure for maize flour (or any comminuted material) is the sum of the sampling variance and analytical variance. Since the material is comminuted, there is no sample preparation variance. Although no laboratory data is available, it is likely that the sampling variability for flour (V_{sf}) would be much lower than the sampling variability for grain comminuted with the Romer mill (V_{sr}) because the particle size of the processed flour is much smaller than that comminuted with the Romer mill (V_{sf}<<V_{sr}). 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. When using data from maize grain comminuted in a Romer mill, a bigger sample will be predicted than required by processed flour for the same risk levels, i.e., less sample of processed flour would be required for the same risk levels (Whitaker BT, *personal communication*, 2012).

⁶ Reports of Codex meetings including those of the Committee on Contaminants in Foods are available on the Codex website at: <http://www.codexalimentarius.org/meetings-reports/en/>

⁷ Working documents for sessions of the Committee on Contaminants in Foods are available on the Codex website at: <http://www.codexalimentarius.org/meetings-reports/en/> or by accessing the direct ftp-link: <ftp://ftp.fao.org/codex/meetings/cccf/>

AGRICULTURAL ASPECTS

27. The Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals (CAC/RCP 51-2003) was established in an attempt to control and manage mycotoxins contamination worldwide. The Code of Practice is currently under revision by the CCCF (CX/CF 14/8/14⁸), and among others, recommends the use of statistical models to predict the maize harvest time to minimize fumonisin level. Additionally, the Code indicates that processing may reduce fumonisin levels in the final product.

STABILITY OF FUMONISIN DURING PROCESSING

28. The fate of fumonisin during processing is affected by many factors, including the temperature, moisture of the product, the toxin concentration in the raw product and the presence of other ingredients in the processed food. Processing operations include sorting, milling (dry and wet), heat, extrusion and nixtamalization
29. Sorting and cleaning may lower fumonisin concentration by removal of contaminated material, but do not destroy the mycotoxins. Broken maize kernels contain near 10 times higher levels of fumonisins than intact ones. Strategies to separate healthy from contaminated kernels include removing the contaminated maize in the buoyant fraction after treatment with saturated sodium chloride solution (Shetty&Bhat, 1999) and sequentially passing stored maize kernels through cleaning equipment followed by a gravity table (Malone et al., 1998).
30. Afolabi et al. (2006) collected maize samples from different farms in Nigeria that had been sorted by farmers as good or poor quality. Twelve of 13 poor quality samples contained fumonisins (1.4 to 110 µg/g), as did the five good quality samples (0.2 to 3.7 µg/g), suggesting that visual sorting is an efficient procedure to reduce the exposure to fumonisins.
31. Van der Westhuizen et al. (2011) evaluated the effect of visual sorting and washing maize kernels, procedures normally used by South African rural populations, to reduce fumonisins levels. In the laboratory, the optimized sorting reduced fumonisin levels by 71% and an additional 13% reduction was achieved with 10 min of washing at room temperature.
32. Levels of fumonisins in the milled products vary greatly with the milling conditions, being distributed in milling streams approximately according to their occurrence in the maize seed structure (Scudamore & Patel, 2009). Wet-milling is used to obtain maize starch, germ and fibers. Dry-milling gives rise to the bran (obtained from the removal of pericarp) and the germ, followed by the fractions obtained by decreasing particle size - grits, corn meal and flour (Alexander et al., 1987). The lowest levels are found in the grits and flours, which are mostly derived from the endosperm, and are further closely related to particle size. Higher concentrations are found in bran and germ (Katta et al. 1997, Brera et al, 2004), which may contain fumonisins levels 29 fold higher than corn meal and corn grits, 13 fold higher than corn flour and 3 fold higher than whole maize (Resnik, 2006).
33. Baking, frying and extrusion cooking of corn at high temperatures (> 190°C) also reduces fumonisin concentrations in foods, with the amount of reduction achieved depending on cooking time, temperature, recipe, and other factors. However, the chemical fate of fumonisins in baked, fried, and extruded foods is not well understood and it is not known if the reduced concentrations result from thermal decomposition of fumonisins or from their binding to proteins, sugars or other compounds in food matrices (Humpf and Voss, 2004).
34. Dall'Asta et al. (2009) showed that bound fumonisins occurred at higher levels than the free forms in all 21 samples of maize-based products analyzed. Median concentration was 1.43 mg/kg for snacks, 0.15 mg/kg for pasta and bread and 0.09 mg/kg for cornflakes. The authors concluded that the occurrence of bound or masked mycotoxins should be considered during risk assessment studies.
35. Nixtamalization is a process for making masa for tortillas and other maize products involving boiling and soaking maize in a solution of calcium hydroxide. The process can reduce fumonisin concentration from 50 to 80%, with 35 to 60% of fumonisin being detected in its hydrolyzed form (Burns et al., 2008; Dombrink-Kurtzman et al., 2000; Palencia et al., 2003). Modified nixtamalization procedure, incorporating various combinations of hydrogen peroxide and sodium bicarbonate in addition to calcium hydroxide, has been reported to give a 100% reduction of FB1, however, the masa product exhibited about 60% of the toxicity of the untreated maize using a brine shrimp assay procedure (Park et al., 1996). Burns et al. (2008) suggested that mycotoxin-in-corn matrix interactions during nixtamalization reduce the bioavailability and toxicity of FB1 in rats.

OCCURRENCE IN FOOD

36. The occurrence of fumonisins in maize and other crops around the world vary considerably depending on many factors including environmental conditions, extent of insect damage, hybrid of maize planted and agronomic practices employed.

⁸ Working documents for the 8th session of the Committee on Contaminants in Foods are available on the Codex website at: <http://www.codexalimentarius.org/meetings-reports/en/> or by accessing the direct ftp-link: <ftp://ftp.fao.org/codex/meetings/cccf/cccf8/>

37. This occurrence has been well documented and reviewed in the literature and by the JECFA (FAO/WHO, 2001; FAO/WHO, 2011). Because of their water solubility, fumonisins are unlikely to bioaccumulate in animal tissues; they have either not been detected or are detected at extremely low levels in milk, eggs and meat (Prelusky et al., 1996; Miller et al., 1996). Low levels of fumonisins have been detected in commercial beer, probably as a result of the use of maize grits as an adjunct in replacement of, or in addition to the traditional use of barley in the brewing process (Scott and Lawrence, 1995; Hlywka and Bullerman, 1999; Maenetje et al., 2007).
38. The 74th JECFA (FAO/WHO, 2011) received information on the natural occurrence of fumonisins from a number of countries (Argentina, Australia, Brazil, Canada, China, Ghana, Japan, Republic of Korea, Singapore, United Republic of Tanzania, Uruguay, USA) and results submitted by member states of the EU (Austria, Belgium, Cyprus, Czech Republic, Estonia, France, Germany, Hungary, Lithuania, Luxembourg, the Netherlands, Slovakia and Spain) through EFSA. Additionally, data published in the open literature from 47 countries were also considered in the evaluation. Most of the occurrence data were for FB1, FB2 or FB3, with recent studies showing a few samples naturally contaminated with 3-*epi*-FB3, FB4 and FB6. All occurrence data were classified according to the food groups used in the GEMS/Food consumption Cluster diets (WHO, 2006).
39. Data on 10 354 samples analyzed for FB1 in food are shown in Table 1 (43% from the Americas, 34% from Asia, 12% from Africa, 10% from Europe and 0.1% from Oceania) (FAO/WHO, 2011). More than 80% of the samples analyzed referred to maize. Mean FB1 levels in maize vary widely between and within the GEMS/Food Clusters, with the minimum lower-bound and maximum upper-bound levels of 84 and 4323 µg/kg, respectively, respectively. Upper-bound level for the global total mean for FB1 in maize was 1260 µg/kg and for all other commodities did not exceed 100 µg/kg

Table 1. Levels of fumonisin B1 in food commodities (FAO/WHO, 2011)

Commodity	No. of individual samples	% sample < LOD or LOQ	Global total* mean (µg/kg)		Mean (µg/kg) across GEMS/Food Clusters (Lower and upper bound estimates)	
			Lower bound	Upper bound	Minimum	Maximum
Barley	175	82	35	44	0	212
Buckwheat	95	100	0	3	0	3
Figs dried	230	25	238	250	238	250
Groundnuts shelled	16	81	97	105	97	105
Maize	8569	30	1237	1260	84	4323
Millet	42	86	0	8	0	8
Oats	17	88	1	10	1	10
Rice	242	96	2	31	0	100
Sorghum	17	76	35	74	0	151
Soya bean (dry)	137	53	33	34	0	84
Sweet maize, kernels	740	66	84	94	0	397
Wheat	74	80	38	48	0	221

* Global total lower and upper bound mean obtained pooling the data across all GEMS/Food Clusters; when calculating the mean values, samples in which the concentration was below the LOQ or LOD were assumed to have a value of zero (lower-bound estimates) or the limit itself (upper-bound estimates).

40. Data on 12 392 samples on total fumonisin are shown in Table 2 (49% from the Americas, 27% from Asia, 13% from Europe, 11% from Africa and 0.1% from Oceania). More than 80% of the samples referred to maize. Mean FB1 levels in maize vary widely between and within the GEMS/Food Clusters, with the minimum lower-bound and maximum upper-bound levels of 174 and 5921 µg/kg, respectively.
41. Lower contamination levels of fumonisins detected in heat treated food, such as pre-cooked maize flour, snack and maize flake samples, found in many studies can be explained by the bound fumonisins formed during processing and which cannot be detected by the usual analytical methods (Seefelder et al., 2003; Lu et al., 2002). Kim et al. (2003) found an average of 2.6 times more FB1 present in bound form in maize flakes compared to conventional analysis. Park et al. (2004) found about 1.3 times more FB1 in the bound form compared with extractable FB1 in the 15 samples of alkali-processed maize-based foods, such as tortilla chips and maize chips analyzed.

Table 2. Levels of total fumonisins in food commodities (74th JECFA; FAO/WHO, 2011)

Commodity	No. of individual samples	Global total* mean (µg/kg)		Mean (µg/kg) across GEMS/Food Clusters (Lower and upper bound estimates)	
		Lower bound	Upper bound	Minimum	Maximum
Barley	123	51	102	0	340
Buckwheat	96	0	10	0	10
Maize	10759	1651	1681	174	5921
Millet	42	0	18	0	18
Oats	26	1	23	0	200
Rice	207	1	59	0	104
Soya bean (dry)	106	6	11	0	30
Sweet corn, kernels	939	131	164	0	549
Wheat	94	0	29	0	200

* Global total lower and upper bound mean obtained pooling the data across all GEMS/Food Clusters; when calculating the mean values, samples in which the concentration was below the LOQ or LOD were assumed to have a value of zero (lower-bound estimates) or the limit itself (upper-bound estimates).

42. Information on the natural occurrence of fumonisins in feed materials was drawn from data received from a number of countries (Brazil, China, Japan, Norway, South Africa and Uruguay), results submitted by member states of the EU through EFSA (Belgium, Estonia, France, Hungary, Lithuania, the Netherlands and Slovakia), one commercial feed supplier and published surveys. Data on 7060 samples originating from Africa (4.5%), America (13.3%), Asia (69.8%), Europe (4.5%) and Australia and New Zealand (7%) were selected for evaluation by the 74th JECFA (Table 3). The levels found in maize feed are within the same range as those found in maize for human consumption (Table 2).

Table 3. Levels of fumonisins (B1+B2+B3) in feed (74th JECFA; FAO/WHO, 2011)

Commodity	No. of individual samples	% sample < LOD or LOQ	Mean concentration*	
			Lower bound	Upper bound
Cereals	384	87	97	313
Corn gluten meal	18	-	3807	3807
Distiller's Dried Grains with Solubles	185	14	1077	1110
Distiller's grains plus solubles	78	23	825	883
Finished feed	2353	30	691	765
Maize	1927	24	1565	1625
Other feed	1391	75	339	524
Rice	20	95	38	275
Silage	248	79	184	383
Soy and soybean products	362	93	52	283
Wheat	88	89	28	250

*Samples in which the concentration was below the LOQ or LOD were assumed to have a value of zero (lower-bound estimates) or the limit itself (upper-bound estimates).

43. Although the JECFA was requested by CCCF to evaluate the co-occurrence of fumonisins with other mycotoxins, this evaluation could not be performed because concurrent levels of fumonisins and other mycotoxins in individual samples were not available (FAO/WHO, 2011).

HUMAN EXPOSURE AND RISK ASSESSMENT

44. Exposure to fumonisins to occurs mainly from the consumption of maize and maize-based products. The amount of intake varies depending on fumonisin levels in maize/maize-products and the amount of maize/maize-products consumed by different individuals or populations.

45. The 74thJECFA reviewed national evaluations performed by the European Union (collectively), the Netherlands, Portugal, China, Mexico-USA, Spain, the Republic of Korea, South Africa, the United Republic of Tanzania, Brazil, Italy, France and Guatemala. Unprocessed maize was often the only source of fumonisins considered, but in some of the studies, other cereals and cereal-based products were also taken into account. Most of these reports included dietary exposure estimates for fumonisin B1 only. Most of the estimates were below 1 µg/kg bw per day for the general population but particularly high exposure levels of fumonisin B1 (up to 7.6 and 33.3 µg/kg bw per day for mean and high consumers, respectively) and total fumonisins (up to 10.6 and 44.8 µg/kg bw per day for mean and high consumers, respectively) were registered in specific regions and population groups (FAO/WHO, 2011).
46. The 74th JECFA conducted international exposure estimates using the consumption Cluster diets from GEMS/Food (FAO/WHO, 2011). Concentration data for both FB1 and total fumonisins from each Cluster have been pooled to derive summary representative concentrations for each Cluster. The commodities considered in the dietary assessment were barley, buckwheat, figs dried, groundnuts shelled, maize, millet, rice, soya bean (dry), sweet corn, kernels and wheat. For each commodity, when concentration data were not available for a Cluster, the global total lower and upper bound means were used to assess exposure (Tables 2 and 3). A standard body weight of 60 kg was used in the assessment.
47. For the upper-bound scenario, the total dietary exposure to FB1 was estimated to range from 0.3 µg/kg bw per day (Cluster L) to 6.2 µg/kg bw per day (Cluster A). Total dietary exposure to total fumonisins was higher than 2.0 µg/kg bw per day (the fumonisins PMTDI) in Clusters A and C (countries in central and northern Africa), G (Asia), and H and K (Central and South America countries). Similar results were found for the lower bound scenario
48. The contribution of maize to the total exposure to total fumonisins ranged from 18% (Cluster E, upper-bound scenario) to 99% (Cluster H, lower-bound scenario). Only under the upper-bound scenario did wheat contribute to the exposure to total fumonisins, with percentages ranging from 1% (Clusters A, H and M) to 44% (Cluster D).

RISK MANAGEMENT CONSIDERATIONS AND PUBLIC HEALTH CONCERNS

49. Guideline levels of fumonisins (FB1+FB2+FB3) in food in the United States include 2 mg/kg for degermed, dry-milled maize products (<2.5% fat content) and 3 mg/kg for popcorn grain (USFDA, 2001). In the European Community, the maximum limit (FB1+FB2) is 4 mg/kg for unprocessed maize, 1 mg/kg for maize intended for direct human consumption, 0.8 mg/kg for maize-based breakfast cereals and maize-based snacks, 0.2 mg/kg for processed maize-based foods and baby foods for infants and young children; ML on milling fractions of maize depends on the particle size – 1.4 mg/kg for > 500 µm and 2 mg/kg for ≤ 500 µm (EC No 1126/2007).
50. The Korea Food and Drug Administration (KFDA) adopted in 2009 a ML for fumonisins (FB1+FB2) of 4 mg/kg for maize and 2 mg/kg for their simple processed (ground, cut) products and their flour. The KFDA notified the World Trade Organization (G/SPS/N/KOR/334, 8 July 2009) to set a ML (FB1+FB2) as 1 mg/kg for processed grain products in which the content of maize, simple processed maize products or maize flour combined constitutes at least 50% of the total product content, biscuits containing at least 50% of maize, cereals and maize product for popcorn use as 4 mg/kg for maize and 2 mg/kg for maize grits and flour (excepted germ) (CRD 18, 4th CCCF).
51. The 74th JECFA evaluated the impact of the establishment of MLs in maize and maize products on the dietary exposure assessment (FAO/WHO, 2011). For this, all samples for which the upper-bound mean concentration of total fumonisins exceeded its ML were excluded from the calculation.
52. The percentages of rejected samples after implementation of the MLs proposed in CX/CF 10/4/8 at the 4th CCCF is presented in Table 4. MLs did not result in rejected samples in the majority of commodities and Clusters. Overall, only 11% of the samples were excluded.
53. The implementation of ML of 5000 µg/kg for maize grain would lead to 88 and 28% of rejected samples in GEMS/Food Clusters A and D, respectively. Implementation of a ML of 2500 µg/kg would not lead to a change in rejection rate in Cluster A and increase the rejection to 40% in Cluster D. Decreasing the recommendation from 5000 to 2500 µg/kg increases the rejection level from 2 to 31% in Cluster H, 12 to 32% in Cluster K and 1 to 33% in Cluster M.
54. Rejections of maize flour/meal occurred in 4 Clusters, being higher than 50% in Clusters G and K. Rejection of popcorn and corn breakfast cereals and snacks only occurred in Cluster K. No rejection occurred in any Cluster at a ML of 500 µg/kg of maize-based baby food.

Table 4. Effect of the implementation of the proposed Codex MLs in the proposed food categories on the rejection of samples per GEMS/Food Cluster (modified from FAO/WHO, 2011)

Commodity	Proposed ML, µg/kg	GEMS/Food Cluster	Number of samples	% of rejected samples after implementation of MLs
Corn/maize grain, unprocessed	5000	Cluster A	280	88%
Corn/maize grain, unprocessed	5000	Cluster D	172	28%
Corn/maize grain, unprocessed	5000	Cluster K	2296	12%
Corn/maize grain, unprocessed	5000	Cluster J	337	2%
Corn/maize grain, unprocessed	5000	Cluster H	805	2%
Corn/maize grain, unprocessed	5000	Cluster M	1047	1%
Corn / maize flour / meal	2000	Cluster G	331	57%
Corn / maize flour / meal	2000	Cluster K	721	53%
Corn / maize flour / meal	2000	Cluster B	554	16%
Corn / maize flour / meal	2000	Cluster M	781	4%
Popcorn grains	2000	Cluster K	70	17%
Corn breakfast cereals/snacks	1000	Cluster K	102	11%

55. The 74th JECFA also evaluated the impact of a range of hypothetical MLs for the categories “Corn/maize grain, unprocessed” and “Corn / maize flour / meal” on the rejection of samples (Tables 5 and 6). No or little effect was noticed on the international exposure estimates resulting from the implementation of MLs higher than what was proposed in CX/CF 10/4/8 for the two categories (5000 and 2000 µg/kg, respectively).

Table 5. Effect of the implementation of a range of hypothetical MLs for “Corn/maize grain, unprocessed” on the rejection of samples per GEMS/Food Cluster (FAO/WHO, 2011)

GEMS/Food Cluster	Number of samples	% of rejected samples after the implementation of MLs (µg/kg)					
		500	1000	2500	5000	7000	10000
Cluster A	280	100%	96%	88%	88%	3%	
Cluster B	300	31%	31%	31%			
Cluster C	20	100%	100%				
Cluster D	172	51%	51%	40%	28%	28%	6%
Cluster E	96	58%	58%				
Cluster F	0						
Cluster G	431	60%	49%	27%			
Cluster H	805	90%	31%	31%	2%		
Cluster I	572	29%	22%				
Cluster J	337	27%	27%	9%	2%		
Cluster K	2296	100%	95%	32%	12%	3%	
Cluster L	339	8%	8%				
Cluster M	1047	88%	33%	33%	1%	1%	1%

Table 6. Effect of the implementation of a range of hypothetical MLs for “Corn/maize flour/meal” on the rejection of samples per GEMS/Food Cluster (FAO/WHO, 2011)

GEMS/Food Cluster	Number of samples	% of rejected samples after the implementation of MLs (µg/kg)					
		250	500	1000	2000	3000	4000
Cluster A	0						
Cluster B	554	63%	42%	16%	16%		
Cluster C	0						
Cluster D	0						
Cluster E	70	53%	53%				
Cluster F	0						
Cluster G	331	85%	85%	85%	57%	57%	43%
Cluster H	3	100%	100%				
Cluster I	101	9%	9%				
Cluster J	0						
Cluster K	721	87%	85%	76%	53%	32%	11%
Cluster L	878	15%	13%	4%			
Cluster M	781	90%	12%	4%	4%	4%	2%

56. The effect of the implementation of various MLs for maize grain and maize flour/meal on the chronic dietary exposure to total fumonisins was evaluated by the 74th JECFA for the GEMS/Food Consumption Cluster diets (Table 7). Reduction in the exposure from all commodities occurred in 9 Clusters (from 6% to 68%). The implementation of MLs of 5000 and 2000 µg/kg for maize grain and maize flour/meal, respectively, decreased considerably the exposure in Cluster A (from 8.4 to 2.7 µg/kg bw/day) and G (from 2.9 to 1.2 µg/kg bw/day). For Cluster H, the highest impact in the exposure occurred when the ML for maize grain was set at 2500 µg/kg, but no ML scenario would bring the exposure in this Cluster to levels below the PMTDI of 2 µg/kg bw/day. In order to bring the exposure for all Clusters except Cluster H to a level below the PMTDI, a ML for maize grain should be set at 1000 µg/kg.
57. The 74th JECFA concluded that adverse effects from fumonisin exposure may occur and that reduction of exposure to fumonisin and other toxins produced by *F. verticillioides* is highly desirable, particularly in areas of the world where maize is a major dietary staple food and where high contamination can occur. As fumonisins do not carry over from feed to animal products in significant amounts, the occurrence of fumonisins in feed was considered not to be a human health concern.
58. The 74th JECFA concluded that implementation of the MLs proposed in CX/CF 10/4/8 (5000 and 2000 µg/kg for maize grain, unprocessed and maize flour/meal, respectively) could significantly reduce exposure (by more than 20%) to total fumonisins in six Clusters (A, B, D, F, G, K). The main contribution to reduction was due to the proposed ML for “maize grain, unprocessed”. No or little effect on the international exposure estimates was noticed as a result of implementing MLs higher than those proposed in CX/CF 10/4/8. It was noted that implementation of the proposed MLs would result in rejection of 1–88% of “maize grain, unprocessed” and 4–57% of “maize flour/meal” across the Clusters.

Table 7. Effect of the implementation of a range of hypothetical MLs and the proposed Codex MLs, on the international estimate of chronic dietary exposure to total fumonisins (FAO/WHO, 2011)

ML ^a for corn/maize grain, unprocessed (µg/kg)	ML ^a for corn/maize flour/meal (µg/kg)	GEMS/Food consumption Cluster regions (µg/kg bw per day)												
		A	B	C	D	E	F	G	H	I	J	K	L	M
No limits	No limits	8.4	1.9	2.3	1.9	1.2	0.4	2.9	7.3	2.0	1.0	3.0	0.4	2.0
10 000	4000	8.4	1.9	2.3	1.6	1.2	0.4	1.3	7.3	2.0	1.0	2.9	0.4	1.8
10 000	2000	8.4	1.4	2.3	1.6	1.2	0.4	1.2	7.3	2.0	1.0	2.8	0.4	1.8
7000	3000	8.3	1.9	2.3	0.8	1.2	0.4	1.2	7.3	2.0	1.0	2.6	0.4	1.8
5000	2000	2.7	1.4	2.3	0.8	1.2	0.3	1.2	6.9	2.0	0.9	2.3	0.4	1.8
2500	1000	2.7	0.8	2.3	0.4	1.2	0.3	0.8	3.1	2.0	0.7	2.0	0.4	0.9
1000	500	1.4	0.5	1.5	0.3	1.1	0.2	0.6	3.0	1.1	0.5	0.8	0.3	0.9
500	250	0.4	0.4	1.5	0.3	1.1	0.2	0.5	2.2	1.0	0.5	0.5	0.3	0.6
5000	4000	2.7	1.9	2.3	0.8	1.2	0.3	1.3	6.9	2.0	0.9	2.4	0.4	1.8
5000	3000	2.7	1.9	2.3	0.8	1.2	0.3	1.2	6.9	2.0	0.9	2.3	0.4	1.8
5000	1000	2.7	1.4	2.3	0.8	1.2	0.3	1.2	6.9	2.0	0.9	2.3	0.4	1.8
5000	500	2.7	1.3	2.3	0.8	1.2	0.3	1.2	6.9	2.0	0.9	2.3	0.3	1.8
5000	250	2.7	1.3	2.3	0.8	1.2	0.3	1.2	6.9	2.0	0.9	2.4	0.3	2.3
7000	2000	8.3	1.4	2.3	0.8	1.2	0.4	1.2	7.3	2.0	1.0	2.6	0.4	1.8
2500	2000	2.7	0.8	2.3	0.4	1.2	0.3	0.9	3.1	2.0	0.7	2.0	0.4	0.9
1000	2000	1.4	0.8	1.5	0.3	1.1	0.3	0.8	3.1	1.1	0.5	1.3	0.4	0.9
500	2000	0.6	0.8	1.5	0.3	1.1	0.2	0.7	2.2	1.0	0.5	1.3	0.4	0.7

^aProposed MLs in CX/CF 10/4/8 at the 4thCCCF: 5000 µg/kg for “corn/maize grain, unprocessed”, 2000 µg/kg for “corn/maize flour/meal”,

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