codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

JOINT OFFICE: Viale delle Terme di Caracalla 00100 ROME Tel: 39 06 57051 www.codexalimentarius.net Email: codex@fao.org Facsimile: 39 06 5705 4593

Agenda Item 8 **CX/CF 10/4/8**
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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON CONTAMINANTS IN FOODS

4th Session
Izmir, Turkey, 26 – 30 April 2010

PROPOSED DRAFT MAXIMUM LEVELS FOR FUMONISINS IN MAIZE AND MAIZE-PRODUCTS AND ASSOCIATED SAMPLING PLANS (N10-2009)

Codex Members and Observers wishing to submit comments at Step 3 on the above matter, 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 **12 April 2010**. Comments should be directed:

to:	with a copy to:
Ms Tanja Åkesson Codex Contact Point Ministry of Agriculture, Nature and Food Quality P.O. Box 20401 2500 EK The Hague The Netherlands Tel.: +31 70 378.4045 Fax: +31 70 378.6141 E-mail: t.z.j.akesson@minlnv.nl - ***preferably*** -	Secretary, Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Viale delle Terme di Caracalla, 00153 Rome, Italy Fax: +39 (06) 5705 4593 E-mail: codex@fao.org - ***preferably*** -

BACKGROUND

1. The 3rd Session of the Codex Committee on Contaminants in Foods agreed to initiate work on establishing maximum levels and associated sampling plans for fumonisins (FB1 and FB2) in maize and some maize-based products[1] which was approved by the 32nd Session of the Codex Alimentarius Commission[2]. The Committee further agreed to request JECFA to review the available toxicology and occurrence data in order to carry out a re-evaluation of fumonisins in maize and maize products and that, based on the outcome of this JECFA re-evaluation, the maximum level might be revised[1]. Further details on the discussion of this matter can be found in the report of the last session of the Committee[3].

2. In developing the proposed draft maximum levels and associated sampling plans, the Brazilian Delegation received incidence data from Argentina, Australia, Belgium, China, Finland, Japan, Nigeria, Norway, the United Kingdom and the United States of America. Operating characteristic (OC) curves which supported the discussion on sampling plans were submitted by the USA.

REQUEST FOR COMMENTS

3. Proposed draft maximum levels for fumonins in maize and maize products and associated sampling plans are presented for comments at Step 3 in Annexes I and II. Background information supporting the proposed draft maximum levels and accompanying sampling plans are contained in the Annex III.

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1 ALINORM 09/32/41, para. 101, App.VII.
2 ALINORM 09/32/REP, App. VI.
3 ALINORM 09/32/41, paras. 96-101.

ANNEX I

PROPOSED DRAFT MAXIMUM LEVELS FOR FUMONISINS (FB1 + FB2) IN MAIZE AND MAIZE PRODUCTS

The following maximum levels of fumonisins (FB1 + FB2) in maize and maize-based products are presented for comments and consideration by the Committee.

Commodity	Maximum level for fumonisins (FB1+FB2), mg/kg
Corn/maize grain, unprocessed	5
Corn/maize flour/meal	2
Popcorn grain	2
Maize-based baby food	0.5
Maize-based breakfast cereals, snacks and chips	1

Considering:

(a) Data provided by national governments allowed a conclusion that, in most countries, the incidence of fumonisins in corn/maize grain and corn flour is high, with a mean FB1+FB2 level of about 1 and 0.5 mg/kg, respectively. The total intake of fumonisins from the consumption of corn/maize grain and related products, including corn flour, at these contamination levels reached the PMTDI for diets H and I, which include countries in Africa and Latin America. While in Africa the maize is subject to very little processing and is mainly home-grown, in some countries in Latin America, there is a high consumption of nixtamalized maize products.

(b) The mean level of fumonisins in breakfast cereals varied widely among countries, reaching 0.51 mg/kg FB1+FB2 in USA. It is important to point out that bound fumonisins found in extruded maize products, such as breakfast cereal, are not detected by the usual extraction procedure and the exposure to fumonisins 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. 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.

(c) Assessment of the compliance and efficiency of the Code of practice for the prevention and reduction of mycotoxin contamination in cereals, on the fumonisin levels on maize is necessary. However, data from the literature or provided by the national governments did not allow to performed this assessment.

(d) In certain communities where the staple foods are maize and maize-based products, the co-occurrence of fumonisins, a strong cancer promoter, and aflatoxins, which are proven human carcinogens, is a cause for concern. Possible synergistic or combined effects of these mycotoxins on human health should be investigated.

(e) The establishment of a maximum level (ML) of fumonisin (FB1+FB2) in corn/maize grain was considered based on the incidence data provided by the countries. A ML of 2 mg/kg would imply in the exclusion of 27 % of the lots from the market; a ML of 5 and 10 mg/kg would decrease significantly this percentage (to 1.4 and 0.3 %, respectively), with the mean intake representing 30-40 % of the PMTDI. A limit of 5 mg/kg would mean that 30% of the 95th percentile consumers will exceed the PMTDI.

(f) Taking into consideration the risk from exposure and the impact on international trade, a ML of 5 mg/kg for FB1+FB2 in corn/maize grain, unprocessed, is recommended.

ANNEX I

(g) The establishment of a ML for fumonisin (FB1+FB2) in corn/maize flour/meal was considered based on the incidence data provided by the USA and European countries. The highest dataset was provided by the USA, indicating that about 8% of the lots would be excluded from the market if a ML of 2 mg/kg be established for this commodity.

(h) Data on popcorn was submitted by Japan, China, European countries and USA. A total of 252 samples were analyzed, from which 75 % of them with positive results for FB1; highest level was 1.6 mg/kg FB1+FB2. A ML of 2 mg/kg for FB1+FB2 in popcorn grain is recommended.

(i) Data in only 86 samples of baby food analyzed on European countries were submitted. Most of the samples had no FB1 and the highest level of FB1+FB2 was 0.32 mg/kg. A ML of 0.5 mg/kg for FB1+FB2 in maize-based baby food is recommended.

(j) Data on breakfast cereals, chips and snacks were provided by European countries, Japan, Australia and USA. A total of 431 samples were analyzed with almost 60 % of them with detected FB1. Maximum values of FB1+FB2 were 2.3 mg/kg in Japan and 1.67 mg/kg in USA; maximum levels in other countries were below 1 mg/kg.

(k) It is important to emphasize that levels on breakfast cereals, chips and snacks concern only the free fumonisins and additional data on bound residues in these commodities are necessary. Furthermore, these products are not submitted to any processing before consumption and they consumption by children is important. A ML of 1 mg/kg for FB1+FB2 in maize-based breakfast cereals, chips and snacks is recommended.

(l) In addition to bound residue data, consumption data on breakfast cereals, chips, snacks and tortillas are necessary to complete the dietary risk assessment of fumonisins.

ANNEX II

PROPOSED DRAFT SAMPLING PLAN FOR FUMONISINS

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 the sampling plan are shown in Annex III.

Maize grain

Maximum level	5 mg/kg FB1 + FB2
Increments	50x100g
Aggregate sample size	5 kg
Sample preparation	dry grind with a suitable mill (Romer Mill)
Laboratory sample size	1 kg
Number of laboratory samples	2
Test portion	25 g test portion
Method	LC
Decision rule	If the fumonisin-sample test result for both laboratory samples is equal or less than 5 mg/kg, then accept the lot. Otherwise, reject the lot.

Maize flour/meal

Maximum level	2 mg/kg FB1 + FB2
Increments	10 x 100 g
Aggregate sample size	1 kg
Sample preparation	none
Laboratory sample size	50 g
Number of laboratory samples	1
Test portion	same as laboratory sample
Method	LC
Decision rule	If the fumonisin-sample test result for both laboratory samples is equal or less than 5 mg/kg, then accept the lot. Otherwise, reject the lot.

Popcorn

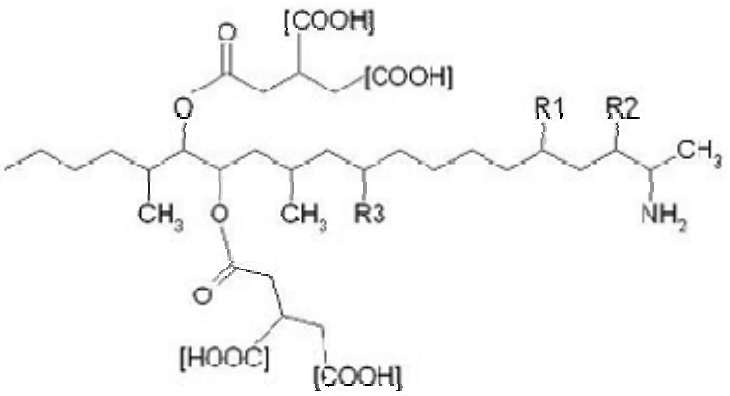
Maximum level	2 mg/kg FB1 + FB2
Increments	50x100g
Aggregate sample size	5 kg
Sample preparation	dry grind with a suitable mill (Romer Mill)
Laboratory sample size	1 kg
Number of laboratory samples	2
Test portion	25 g test portion
Method	LC
Decision rule	If the fumonisin-sample test result for both laboratory samples is equal or less than 2 mg/kg, then accept the lot. Otherwise, reject the lot.

ANNEX III

This Annex contains background information in support of the proposed draft maximum levels and associated sampling plans as presented in Annexes II and III.

INTRODUCTION

1. Fumonisins are mycotoxins 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 (Plattner et al., 1996, 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 and most toxic naturally occurring analogues (Sydenham et al., 1992a,b Thiel et al., 1992). 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)



Fumonisin B1: R1= OH; R2= OH; R3= OH
Fumonisin B2: R1= H; R2= OH; R3= OH
Fumonisin B3: R1= OH; R2= OH; R3= H

Figure 1. Chemical structure of FB1, FB2 and FB3

2. Fumonisins are produced mainly by *Fusarium verticillioides* (Sacc.) Nirenberg(synonym *F. moniliforme* Sheldon) (teleomorph, *Gibberella moniliformis*), *Fusarium proliferatum* (Matsushima) and *Fusarium nygamai*, as well as *Alternaria alternata f. sp. lycopersici* (Marasas et al., 2001; Rheeder et al., 2002).

3. FB2 was detected in cultures of *Aspergilus 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 Spaks, 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. Ross et al. (1992) estimated the ratio of FB1/FB2 in naturally contaminated maize as approximately 3:1. Some authors estimated that FB1 accounts for approximately 70% of the total fumonisins found in nature (Nelson et al., 1993 Marasas, 2001; Wang et al. 2008a,b). FB3 can be found at low incidence, with the ratio between FB3 and FB1 varying from 0.34 to 0.87 (Bacon et al., 1992; Sydenham et al., 1991; Chulze et al., 1999).

ANNEX III

7. Data on the occurrence of FB1, FB2 and FB3 on 169 maize samples submitted by the Chinese Government to this Committee have shown that, in average, FB1 accounted for 73.8 % (± 5.6%) and FB2 for 16.1 % (± 5.0%) of the total fumonisins found in the samples; the mean ratio FB1:FB2:FB3 was 10:2.3:1.4; about 99% of the samples were positive for FB1 and 85% for FB3. In its last evaluation on fumonisins, the JECFA (2001) estimated the ratio FB1:FB2:FB3 as 10:3:1.

8. 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

9. In 2001, the JECFA at its 56th meeting, evaluated extensive technical, biochemical and toxicological data on fumonisins as well as data on human dietary exposure to fumonisins. Laboratory animal and *in vitro* studies have shown disruption of lipid metabolism as the initial site of action of fumonisin. 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).

10. In animal species studies revised by the JECFA, the liver and kidney were found to be a target for FB1; in mice, the liver was more sensitive than the kidney. The non-observed-effect-level (NOEL) for renal cancer in Fischer 344N rats was 0.67 mg/kg bw/day, and the NOEL for renal toxicity was 0.2 mg/kg bw/day. The NOEL for liver cancer in male BD IX rats was 0.8 mg/kg bw/day, and the NOEL in feed-restricted female B6C3F_1 mice was 1.9 mg/kg bw/day.

11. A Provisional Maximum Tolerable Daily Intake (PMTDI) of 2 µg/kg bw/day was allocated by the JECFA to FB1, FB2 and FB3 alone or in combination, on the basis of a NOEL of 0.2 mg/kg bw/day and a safety factor of 100.

12. Human epidemiological studies revised 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. Geographical differences in demography, ethnic groups, genetic susceptibility, culture, economy and nutritional status all affect the rates of disease; however, some common risk factors are emerging, such as having maize as the main dietary staple and, to some extent, a low socioeconomic status. Furthermore, high incidences of oesophageal cancer have been associated with limited diets consisting mainly of wheat or maize and low contents of certain minerals and vitamins.

13. The JECFA evaluation included reports on 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 (FAO/WHO, 2001).

14. In a more recent study conducted in China, the status of FB1 contamination in food samples in areas of high and low incidences of oesophageal and liver cancer was investigated (Sun et al., 2007). Higher fumonisin levels were found in the high cancer incidence areas, suggesting a possible contributing role of FB1 in human esophageal- and hepato-carcinogenesis.

15. In a review of the toxicological profile of fumonisins in laboratory animals and epidemiological studies in humans, Marasas et al (2004) indicated that fumonisins area potential risk factor for NTD, craniofacial anomalies, and other birth defects arising from neural crest. This was later supported by a study conducted by Missmer et al. (2006) with Mexican-American women, finding an association between increasing maternal serum sphinganine:shingosine ratio (Sa:So) with increase risk of NTD in the offspring.

ANNEX III

16. Theumer et al (2002) demonstrated that subchronic FB1 intake could affect the small intestine and alter the interleukin profile and some main functions of macrophages in antitumor activity in rats. Posterior *in vitro* studies showed that the co-exposure to fumonisins and aflatoxin B1 (AFB1) produced a higher liver toxicity, with respect to their individual administration, inducing apoptosis and mitotic hepatocytes. Furthermore, the mixture of fumonisins and AFB1 induced different immunobiological effects in comparison to the individual action of the same toxins (Theumer et al. 2003). Although FB1 is poorly absorbed and metabolized in the intestine, some studies have shown that it induces intestinal disturbances (abdominal pain or diarrhea) (Bouhet and Oswald, 2007).

17. The International Agency for Research on Cancer (IARC) has classified FB1 as possibly carcinogenic to humans (Group 2B) (IARC, 2002).

SAMPLING PLANS

18. A study conducted by Whitaker et al (1998) describing the sampling variance associated with the testing of shelled maize for fumonisin was evaluated by the JECFA in 2001. In this study, 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. A nested design used to determine the variation was: selection of 10 batches with a wide range of fumonisin concentrations; from each batch, 10 comminuted test samples were taken randomly, and two 25-g portions were taken from each by riffle division. FB1, FB2 and FB3 were determined by AOAC official method 995.15. Sampling, sample preparation, and analytical variances were found to be functions of fumonisin concentration and are described in Annex I by the Equations 1, 2, and 3, respectively.

19. 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 (Romer mill and 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.

20. The sampling plan for fumonisin analysis in various commodities included in the report by JECFA is shown on Table 1. This plan assumes that a minimum of 30 batches of food should be sampled from each country or region; the coefficient of variation of the sampling plan should be no more than 30% and the coefficient of variation of the complete analytical method should be no more than 10%.

Table 1 - Proposed sampling plans for fumonisins analysis (FAO/WHO, 2001)

Commodity	Increments (n x y grams)	Aggregate size (kg)	Notes
Whole (shelled) maize	50 x 100	5.0	Whitaker et al. (1998): Sampling variation for fumonisins in maize similar to that reported for aflatoxins
Corn-on-the-cob	50 cobs	7.5	Assuming that core of cob contributes about 30% of total weight of cob and that a cob yields about 100 g of kernels
Maize flour, maize meal, maize grits, processed maize foods (e.g. cornflakes, tortilla chips, popcorn, muffin mix, starch)	10 x 100	1.0	Assumed that sampling variance for these commodities was similar to that associated with aflatoxin in comminuted feeds; suggested sampling plan associated with sampling precision of 12.5% for aflatoxin in comminuted feeds

21. The performances of several fumonisin sampling plan designs for shelled maize are shown in Annex I for 1, 2, and 5 kg sample sizes and maximum levels of 1, 2, 5, and 10 mg/kg. Operating characteristic (OC) curve that describe the performance of each sampling plan design were determined by using Equations 1, 2, and 3 and the negative binomial distribution (Whitaker et al., 1998 and Whitaker et al, 2007) to compute the operating characteristic curves.

ANNEX III

METHODS OF ANALYSIS

22. Fumonisins are polar molecules, soluble in water and in polar solvents and thus ideally suited for determination by reversed-phase HPLC. As they lack a significant UV chromophopore, low levels of fumonisins can be detected after derivatization of sample extracts followed by fluorescent detection**.** In general, fumonisin can be extracted from maize or maize based products using methanol-water or acetonitrile-water. Clean up using C18 cartridges, SAX or immunoaffinity columns are normally used (Sydenham et al., 1996; Solfrizzo et al., 2001, Caldas and Silva, 2007). The Limit of Quantification (LOQ) of the HPLC/fluorescent methods are in the range of 0.02 to 0.5 µg/kg. 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/ total fumonisins (Sydenham et al., 1996c).

23. ELISA methods have received a lot of attention lately because they can be used for rapid screening purposes under field conditions or in the laboratory (Castells et al., 2008). Maragos et al. (2001) detected FB1 using Fluorescence Polarization detection, with a LOD of 0.5 µg/kg. Wang et al. (2008**b)** extracted FB1, FB2, FB3 and FB4 with ACN:water and analysed them by HPLC coupled with an evaporative laser scattering detector.

24. 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) when compared to 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.

25. Fumonisins 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 FB_1). 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.

OCCURRENCE IN FOOD

26. The worldwide occurrence of fumonisins in food has been well documented and reviewed in the literature and by the JECFA (FAO/WHO, 2001). Although fumonisins are found mainly in maize and maize-based products, the sporadic natural occurrence of fumonisins in other food commodities, such as sorghum, barley, rice and coffee has been reported (Noonim et al., 2010; Ghali et al., 2009; Park et al., 2009). Because of their water solubility, fumonisins are unlikely to bioaccumulation 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).

27. Lower contamination levels of fumonisins detected in heat treated food, such as pre-cooked corn flour, snack and corn 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 corn 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 corn-based foods, such as tortilla chips and maize chips analyzed.

28. Tables 2 shows data on the occurrence of fumonisins on corn/maize in some countries from work published in the literature. The studies cover corn/maize that has been harvested in the last 10 years (crop year starting from 1999). Samples were collected in the field, in stored rooms, at the marked and/or in the household. FB3 was only analyzed in some studies. In most studies, FB1 was detected in all samples analyzed; the mean concentration varied widely among the studies, ranging from 0.5 mg/kg (samples from the market in China or from the field in Croatia) to 15 mg/kg in Italy (samples from the field).

ANNEX III

Table 2: Incidence of fumonisin in corn/maize reported in the literature

Country	Origin	Fumonisin	Analyzed /positive	Crop year	LOD/LOQ mg/kg	Mín., mg/kg	Max., mg/kg	Mean, mg/kg	SD (mg/kg)	Ref.
Brazil (SC)	Field	FB1	76/76	2000	NR	NR	NR	1.89	1.9	Westhuizen et al., 2003
		FB2	76/76		NR	NR	NR	0.7	0.76	
		FB3	76/76		NR	NR	NR	0.28	0.28	
		total	76/76		NR	0.02	18.74	2.87	0.287	
Brazil (Paraná)	Field, reception, pre-drying	FB1	490/490	2003/ 2004	0.027/-	0.03	12.68	1.53	NR	Silva et al., 2008
		FB2	490/344		0.035/-	<0.035	7.89	0.775	NR	
		Total	490/490		-	0.03	18.78	1.92	NR	
Brazil (MT, RS, BA, SP)	freshly harvested	FB1	200/196	2005	-/0.015	<0.015	9.67	1.75	58% of 149 sum>2	Rocha et al., 2009
		FB2	200/149			<0.015	3.16	0.48		
China	household	FB1	259/230	2001-2002	0.016/-	<0.016	25.5	1.59	NR	Sun et al., 2007
China	market	FB1	65/28	2005	-/0.03	0.21	1.8	0.48	NR	Wang et al., 2008a
China (Linxian)	market, granary, household	FB1	104/42	2005-2006	-/3 ng/µL	< 0.025	3.2	0.735	NR	Wang et al., 2008b
China	field	FB1	282/281	2005	0.002/-	< 0.02	71.12	6.66/ 1.57[d]	NR	Gong et al., 2009
Croátia	field	FB1	49/49	2002	0.01/-	0.142	1.378	0.459	0.311	Domijan et al., 2005
		FB2	49/3		0.01/-	0.068	3.08	68.4, 0.109, 3.08		
Guatemala	Field, storage, market	FB1	1421/572	2000-2004	0.3/-	NR	NR	0.935	NR	Torres et al., 2007
	local markets[c]	FB1+FB2+ FB3	236/209	2005	NR	NR	43.08	3.55	0.39	
Italy	Field trials	FB1	40/40	2004	-/0.004	0.37	64.15	15.05	2.08*	Cavaliere et al., 2007
		FB2	40/40		-/0.002	0.19	37.09	6.74	1.02*	
		FB3	40/40		NR	0.04	16.69	2.76	0.36*	
Iran	feed and human consumption	FB1	52/52	2000	0.01/-	<0.01	11.02	2.04	NR	Ghiasian et al., 2006
		FB2	52/18		0.01/-	<0.01	3.364	0.558	NR	
		FB3	52/16		0.01/-	<0.01	0.9	0.153	NR	
Iran	Dried maize	FB1	49/48	2000	0.014/-	<0.014	12.9	6.14	3.08	Yazdanpanah et al., 2006
Spain	From Argentina	Total (B1+B2+ B3)	92/92	2002 - 2004	0.025/-	0.337	10.61	2.60	1.81	Castells et al.,2008
West Africa	0 to 6 months of storage	FB1+FB2+ FB3	144/ NR	1999-2003	0.25/-	NR	NR	1.90[a]	NR	Fandohan et al., 2005

a. mean of 36 sets of data; NR= not reported; * lower SD (0.05); PHC=primary; hepatocellular carcinoma; c. Total/ FB1 = 1.32; d. mean/median

29. Information on the levels of fumonisins in corn/maize, corn/maize products and other commodities was submitted to the Committee by twelve countries around the word. A summary of the information received is shown on Table 3.

ANNEX III

Table 3. Data on the levels of fumonisins submitted by national governments

Country	Commodities/samples analysed	Fumonisins	Sampling period
Argentina	Corn/maize	FB1 and FB2	2005-2007
Australia	Corn/maize products and other commodities	FB1 and FB2	2003
Brazil	Corn/maize	FB1 and FB2	1999 - 2006
Belgium	Corn/maize, its products and other commodities	FB1, FB2 and FB3	2008 -2009
China	Corn/maize and other products	FB1, FB2 and FB3	2008-2009
Finland	Corn/maize products	FB1 and FB2	2006-2009
Japan[a]	Corn/maize, its products and other commodities	FB1, FB2 and FB3	2004-2009
Nigeria	Corn/maize	FB1	2003
Norway	Corn/maize and other products	FB1 and FB2	1999-2001
South Africa	Corn/maize	Total fumonisins (FB1 + FB2 + FB3)	2003 - 2008
United Kingdom	Corn/maize and its products	FB1, FB2 and FB3	2003 - 2009
United States[b]	Corn/maize and its products	FB1 and FB2	2004 - 2008

[a.] Data from the Ministry of Health, Labor and Welfare and Ministry of Agriculture, Forestry and Fisheries; [b] summary data

30. The distributions of FB1 + FB2 levels in corn/maize in Argentina, Belgium, Brazil, China, Japan, Nigeria, South Africa and United Kingdom are shown in Figure 2. Whenever only data on FB1 or total fumonisin (FB1 + FB2 + FB3) were submitted, FB1 and/or FB2 were estimated based on the FB1:FB2:FB3 ratio of 10:3:1 (FAO/WHO, 2001). With the exception of Brazil, the distributions were positively skewed, with the asymmetric tail extending towards high values. Using the Chi-Square test, these populations did not fit ($p<0.01$) the normal, lognormal and gamma distribution. They also did not fit the normal distribution using the Shapiro-Wilk test. Clearly, the distribution of the Brazilian data is distinct from those found for the other country. Data distribution from Nigeria and South Africa are similar, probably due to similar climate and agricultural practices.

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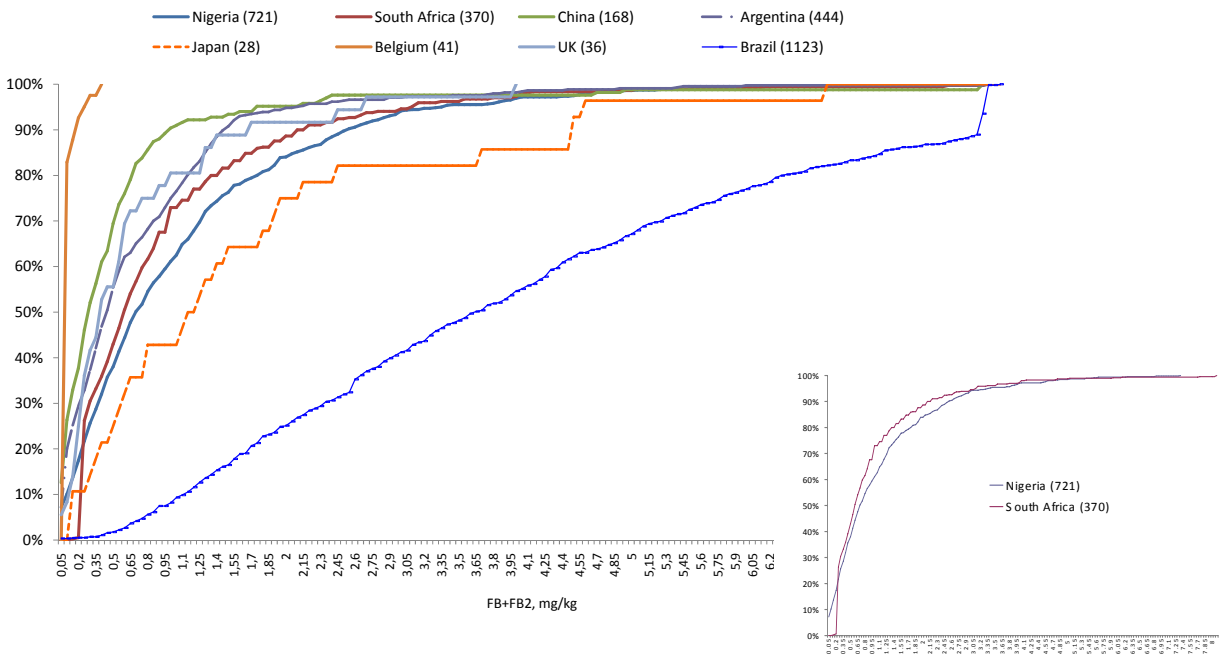

Figure 2. Cumulative distribution of FB1 + FB2 in corn/maize of data submitted by the national governments. In parenthesis, the number of samples analyzed. In detail, the cumulative distributions for Nigeria and South Africa

31. Table 4 shows a descriptive summary of the raw data received. From 36 (UK) to 1123 samples (Brazil) were analyzed, with the number of positive samples (≥ LOD/LOQ for FB1) ranging from 0 to 100% (Table 4). The mean was lower or around 1 mg/kg in all cases, except in Brazil, where a mean of 4.4 mg/kg was found. Higher maximum value was also reported in Brazil (45.4 mg/kg). The summary data submitted by the United States are shown on Table 5.

Table 4. Descriptive summary of the data on fumonisin level (F1+B2) from raw data submitted by the national governments

Country	Analyzed	%Positive	Mean[a]	Sd	median	25th P	75th P	Min.	Max.
Argentina	444	90	0.73	1.1	0.44	0.15	1.0	0.01	15.8
Belgium	41	98.9	0.10	0.064	0.075	0.075	0.086	0.075	0.395
Brazil	1123	99.7	4.4	3.4	3.64	2.0	5.76	0.045	45.4
China	168	31.7	0.62	1.7	0.28	0.087	0.560	0.002	19.1
Japan	28	100	1.67	1.67	1.18	0.52	2.0	0.11	6.7
Nigeria[b]	721	97.2	1.1	1.13	0.70	0.290	1.42	0.01	7.34
Norway	12	0	0.04	-	0.02	-	-	0.02	0.02
South Africa	370	75.0	0.96	1.15	0.59	0.230	1.17	0.144	11.7
UK	36	100	0.69	0.84	0.37	0.203	0.79	0.019	3.97
Total	**2994**	**76.9**	**1.1**						

Sd=Standard deviation. A. The mean was estimated considering non detected samples at the limit of detection or, when provided, the limit of quantification; b. values < 0.01 mg/kg were considered as non-detected

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Table 5. Summary data on FB1 + FB2 (mg/kg) on corn/maize submitted by the United States[a]

Year	Analyzed	% positive	Mean	Sd	Min.	Max.	% > 2
2004	64	46.9	0.91	0.79	0.11	4.38	23.3
2005	39	59.0	0.31	0.31	0.1	2.44	4.4
2006	41	63.4	0.82	0.91	0.11	4.68	19.2
2007	19	78.9	1.1	0.94	0.13	5.53	46.7
2008	24	87.5	0.48	0.33	0.11	1.48	0
Total	**187**	**67.1[b]**	**0.72[b]**	**NR**	**0.1**	**4.68**	**18.7[b]**

[a] Samples were collected by Food and Drug Administration (FDA) field personnel during routine investigations of firms that stored and/or distributed domestic foods in accordance with FDA Compliance Program for Mycotoxins in Domestic and Imported Foods using AOAC Int. analytical procedures; LOQ =0.1 mg/kg; [b] Estimated mean from the above values

32. Table 6 shows the percentage of the samples analyzes in each country with FB1+FB2 in corn/maize above certain levels. In average, about 33 % of the samples analyzed were above 1 m/kg, 18 % above 2 mg/kg and 5% above 5 mg/kg. Over 90 % of the Brazilian samples were above 1 mg/kg.

Table 6. Percentage of corn/maize samples with FB1+ FB2 levels above certain levels, in mg/kg, according to the raw data submitted by national governments

Country	Samples analyzed	% > 1	% > 2	% > 4	% > 5	% > 10
Argentina	444	25.0	5.2	1.8	0.9	0.2
China	168	9.5	4.8	2.4	1.2	0.6
Belgium	41	0	0	0	0	0
Brazil	1123	91.8	74.8	45.2	32.8	6.4
Japan	28	57.1	25.9	14.3	3.6	0
Nigeria	721	38.8	16.0	3.1	1.4	0
South Africa	370	27.0	11.4	2.7	1.4	0.3
UK	36	18.4	8.3	0	0	0
USA	187	-	18.7	-	- _	-
Total	**3118**	**33.4 [a]**	**18.3[a]**	**8.7[a]**	**5.2 [a]**	**0.9[a]**

a. Estimated mean from the above values

33. A summary of the data on corn/maize products submitted by national governments are shown on Table 7. The variety of products that were analyzed is very high, and only the most common products are listed. In general, mean levels for FB1+FB2 were below 0.10 mg/kg. The highest mean levels are found in corn/maize meal, flour or polenta, products with different size grit obtained after grain degermination and milling. In USA, 9.6 % of the cornmeal samples had levels above 2 mg/kg and 6.4 % of the corn flour samples had levels above 2 mg/kg. The levels found in immature corn (sweet corn or canned corn) were low, with the exception of data from USA (mean of 0.67 mg/kg). Breakfast cereals, tortilla and popcorn had mean levels from 0.20 to 0.60 mg/kg in most cases.

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Table 7. Levels of fumonisins on corn/maize products submitted by national governments

Country	Commodity	Analyzed	% positive[a]	Result	Min.[b]	Max.	Mean[c]	Sd
Belgium	Baby food	40	0	B1+B2	<0.075	<0.075	-	-
Finland	Baby food	1	0	B1+B2	<0.03	<0.03	-	-
UK	Baby food	46	26	B1+B2	<0.04	0.32	0.06	0.05
Australia	Cornflake/breakfast cereals	12	0	B1+B2	<0.10	<0.10	-	-
Belgium	Cornflake/breakfast cereals	51	39.2	B1+B2	<0.075	6.7	0.238	0.93
Finland	Cornflake/breakfast cereals	9	66.7	B1+B2	<0.03	0.64	0.07	0.08
Japan	Cornflake/breakfast cereals	101	40.6	B1	<0.01	0.103	0.015	0.012
				B2	<0.01	0.019	0.010	0.001
UK	Cornflake/breakfast cereals	64	87.5	B1+B2	<0.02	0.73	0.13	0.13
USA	Cornflake/breakfast cereals	21	57.1	B1+B2	<0.2	2.3	0.51	-
Belgium	Corn chips	28	53.6	B1+B2	<0.075	0.30	0,17	0,29
Finland	Corn chips	9	66.7	B1+B2	<0.03	0.20	0.07	0.07
Japan	Corn snack	90	85.6	B1	<0.002	1.67	0.068	0.18
				B2	<0.002	0.60	0.019	0.064
UK	Corn snack	46	76.1	B1+B2	<0.02	0.30	0.09	0.06
Finland	Corn bread/cake	5	40	B1+B2	<0.03	0.52	0.127	22
UK	Corn bread/pasta	11	63.6	B1+B2	<0.02	1.05	0.23	0.35
Finland	Corn, canned	5	0	B1+B2	<0.03	<0.03	-	-
USA	Corn, canned	15	80	B1+B2	<0.2	2.57	0.67	-
Australia	Sweet corn	4	0	B1+B2	<0.1	<0.1		
Japan	Sweet corn	177	8.5	B1	<0.01/ <0.002	0.036	0.008	0.004
				B2	<0.01/ <0.002	0.015	0.008	0.004
UK	Sweet/baby corn	32	90.6	B1+B2	<0.02	0.25	0.08	0.05
Finland	Corn grits	2	100	B1+B2	0.03	0.138	0.084	-

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Country	Commodity	Analyzed	% positive[a]	Result	Min.[b]	Max.	Mean[c]	Sd
Japan	Corn grits	53	100	B1	<0.002	1.38	0.15	0.25
				B2	<0.002	0.59	0.042	0.09
USA	Corn grits	45	51.1	B1+B2	<0.2	1.3	0.345	-
USA	Corn flour[d]	130	61.5	B1+B2	<0.2	3.6	0.52	-
Finland	Corn/maize flour	7	85.7	B1+B2	<0.03	1.68	0.51	0.72
UK	Corn/maize flour	69	87	B1+B2	<0.04	2.7	0.49	0.57
UK	Corn/maize meal and polenta	22	100	B1+B2	0.04	7.8	1.2	1.8
USA	Cornmeal[c]	268	62.7	B1+B2	<0.2	4.07	0.53	-
Belgium	Polenta	20	90	B1+B2	<0.075	1.85	0.59	0.58
Japan	cornstarch	35	28.6	B1	<0.002	0.063	0.004	0.010
				B2	<0.002	0.017	0.003	0.003
Belgium	Popcorn	60	20	B1+B2	<0.075	0.26	0.087	0.034
China	Popcorn	3	100	B1+B2	0.044	0.246	0.105	0.12
Finland	Popcorn	2	0	B1+B2	<0.03	-	-	-
Japan	Popcorn	69	76.8	B1	<0.002	0.354	0.049	0.070
				B2	<0.002	0.094	0.012	0.016
UK	Popcorn	9	44.4	B1+B2	<0.2	0.58	0.13	0.19
USA	Popcorn	109	16.5	B1+B2	<0.02	1.63	0.59	-
Finland	Taco	1	100	B1+B2	0.30	-	-	-
Belgium	Tortilla	30	36.7	B1+B2	<0.075	0.84	0.20	0.27
UK	Tortila/tortilla chips/tako	56	92.8	B1+B2	<0.02	1.4	0.27	0.24
Japan	Corn soup, liquid	63	0	B1	<0.01	-	-	-
				B2	<0.01	-		
Japan	Corn soup, powder	46	15.2	B1	<0.01	0.013	0.010	0.0004
				B2	<0.01	-	-	-
UK	Corn oil	19	26.3	B1+B2	<0.01	0.08	0.05	0.02

[a] for FB1; [b] levels < correspond to the LOQ of FB1 and/or FB2; [c] assuming samples < LOQ are at LOQ level; [c] 9.6 % of the samples with levels above 2 mg/kg; [d] 6.4 % of the samples with levels above 2 mg/kg

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AGRICULTURAL, TECHNOLOGICAL AND COMMERCIAL ASPECTS

Agricultural Approaches

34. The Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals, including annex on Fumonisins (CAC/RCP 51-2003) recommends that the time of harvest for maize should be carefully planned, as maize grown and harvested during warm months may have fumonisin levels significantly higher than maize grown and harvested during cooler months of the year.

35. While maize is very sensitive to water loss and goes into drought stress at about 0.98 water activity (a_w), *Fusarium* species can grow well below that value and down to about 0.90 a_w. Hence, the fungus can happily grow under drought stress conditions and fumonisin control in crops where fungus infection occurred pre-harvest is extremely difficult (Pitt JI, personal communication, 2009). Drought stress can be minimized by irrigation (Cavaliere *et al.,* 2007). Similarly, fertilization can be used to minimize nutrition stress and optimal planting and weed control methods can be used to minimize population stress (Hassegawa et al., 2008).

36. The results from investigations on agronomic practices indicate that: (a) fungal infection rates are higher in crops planted in fields previously planted with maize, particularly when residues from the maize crops were left in the field, (b) the incidence of *Fusarium* kernel rot is higher in warm climates under drought conditions, and (c) freshly harvested maize should be dried to a suitable moisture level immediately and stored (Bacon and Nelson, 1994; Munkvold and Desjardins, 1997; Warfield and Gilchrist, 1999; Miller, 1994; Fandohan et al, 2005).

37. Fungal infection and mycotoxin production in organically and conventionally grown produce is still an extremely controversial issue (Magkos et al., 2006) and there is no scientifically evidence that the differences observed between conventional and organic foodstuffs would lead to any objectively measurable effect on consumer health (Ariño et al., 2007).

Stability of fumonisin during processing

38. 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

39. 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). Afolabi et al. (2006) proposed the visible sorting of maize grain as a technique to reduce fumonisin levels by subsistence farmers.

40. 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 1987). Fumonisins are not expected to be destroyed during this process and are found in all fractions, with higher concentration in bran and germ (Katta et al. 1997, Brera et al, 2004). Rensik (2006) showed that germ and bran had fumonisins levels 29 fold higher than corn meal and corn grits, 13 fold higher than corn flour and 3 fold higher than whole maize.

41. The effects of heating on the stability of fumonisins depend on the process, the temperature and heating time. Significant reduction of fumonisin levels occurs during processes at temperatures > 150 °C, such as those used for dry or moist maize meal production (Scott & Lawrence, 1995), frying maize chips (Jackson et al., 1997), baking, roasting and alkaline cooking (Castelo et al. 1998, Jackson et al. 1997, Katta et al. 1999). De Girolamo et al., (2001) showed that flaking, cooking and toasting processes reduced FB1 levels at 48 to 70% and that the addition of glucose increased the reduction percentage at almost 90 % during cooking and toasting processes.

42. Extrusion processing is used extensively in the production of breakfast cereal, snack and textured foods. Bullerman et al. (2007; 2008) found the greatest reduction of fumonisins occurring at extrusion temperatures of 160 ^{o}C or higher and in the presence of glucose (up to 87%). About 60% of the FB1 species detected in corn (spiked and fermented) extruded with glucose was *N*-(deoxy-D-fructos-1-yl) FB1, in addition to hydrolyzed FB1 (HFB1) and N-carboxymethyl FB1 (Castelo et al. 2001, Bullerman et al., 2008). Seefelder et al (2003) have shown that FB1 and HFB1 are able to bind to polysaccharides and proteins via their two tricarballylic acid side chains.

43. Voss et al (2008) evaluated the toxicity of maize grits spiked with FB1 extruded with 10% glucose fed to rats. With one exception, the fumonisin B1-spiked and fermented extrusion products caused moderately severe kidney lesions and reduced kidney weights, effects typically found in fumonisin-exposed rats. Lesions in rats fed contaminated grits after extrusion with glucose were significantly less severe and not accompanied by kidney weight changes. The authors concluded that extrusion with glucose supplementation is potentially useful for safely reducing the toxicity of fumonisins in maize-based products. Lu et al (2002) had reached the same conclusion and shown that glucose bind to fumonisins via the amino group.

44. 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.

45. 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). 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.

46. Palencia et al (2003) found that tortillas prepared using the traditional nixtamalization method of Mayan communities contained FB1, FB2 and FB3 and their hydrolyzed counterparts. There were equimolar amounts of FB1 and HFB1 in the tortillas, but the total fumonisins were reduced by 50%. They also found a reduced sphinganine elevation in cells treated with extracts of tortillas compared with cells treated with extracts of contaminated maize.

47. Ethanol fermentation of fumonisin contaminated maize results in very little degradation of the toxins; most of the toxins remain in the distiller's grains, thin stillage and distiller's soluble fraction (Bennett and Richard, 1996; Bothast et al., 1992). Fumonisins have also been found in beer, indicating that the toxins persist under the conditions (temperature, pH) prevailing during the brewing process (Scott & Lawrence, 1995; Scott et al., 1997; Hlywka & Bullerman, 1999).

48. Visconti et al. (1996) found that gamma-irradiation (15 kGY) effectively sterilized the maize flour, but caused only about a 20% reduction in its fumonisin content. Ferreira-Castro et al (2007) found possible decreased fumonisins levels by irradiating maize with 5 or 10 kGy; however, at 2kGy, the survived fungi (36%) were able to produce more fumonisins than the fungi in the control samples. Aziz et al. (2007) found that the viable counts of Fusarium in seeds decreased by increasing the radiation dose levels; 7 kGy was sufficient for complete destruction of FB1 in wheat and maize.

HUMAN EXPOSURE AND RISK ASSESSMENT

49. Exposure to fumonisins is thought to occur 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.

50. The JECFA (FAO/WHO, 2001) conducted an international intake estimate for FB1 using incidence data found in 349 samples of maize imported, mainly, from Europe, South America and the USA, and consumed in the Netherlands. This distribution was taken as representative of the maize available in trade throughout the world. The levels of FB1 in the samples analyzed were shown to be distributed log-normally, with a mean of 1.36 mg/kg. The distribution was combined with food consumption data from the GEMS/Food regional diets for Maize all (GC-645), which includes maize, maize flour, sweet maize (on-the-cob and kernels) and popcorn.

51. Three scenarios were examined. In the first scenario, the per-capita consumption of maize was combined with the distribution of concentrations of fumonisins to yield a distribution of FB1 intake. In the second scenario, a hypothetical distribution of maize consumption was estimated by assuming that it is log-normally distributed in each diet, with a standard deviation equal to 66% of the mean consumption. The third scenario was intended to mimic the worst case, in which the only grain that a person consumes is maize.

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52. The mean FB1 intake in scenarios 1 and 2 ranged from 0.2 µg/bw/day in the European diet to 2.4 µg/kg bw/day in the African diet, for a body weight of 60 kg. The intake at the 97.5th percentile in scenario 2 ranges from 1.4 µg/kg bw/day in Europe to 16.3 µg/kg bw/day in Africa. Assuming a ratio of FB1:FB2:FB3 of 10:3:1, the total fumonisin intake (FB1 + FB2 + FB3) was estimated by adding 40% to the estimated FB1 intake. The total intake represents 14 % of PMTDI established by the JECFA (2 µg/kg bw/day) in Europe, 70 % PMTDI in Latin America and 160% PMTDI in Africa.

53. The predicted intake of fumonisins in the third scenario ranged from 5.2 µg/kg bw/day in Europe to 10 µg/kg bw/day in Far Eastern. The 95th percentile intake ranged from 23 to 47 µg/kg bw/day person in these diets. The JECFA emphasized that the number of individuals covered by this scenario is extremely small on a global basis and consists primarily of rural subsistence farmers, who are not representative of national or GEMS/Food regional populations.

54. The dietary intake of FB1 + FB2 through the consumption of maize grain (GC 0645: excluding flour, oil and beer; as no significant levels of fumonisins are expected to remain in oil and beer) according to the 13 GEMS/FOOD Consumption Cluster Diets (WHO, 2006) was calculated for this discussion paper using the mean levels of 1 mg/kg, 2 mg/kg and 5 mg/kg. The intake represented up to 1 % of PMTDI at all levels in seven of the thirteen diets. The highest intakes occurred with the diets C (Africa and Middle East), D (Europe and Middle East), K (Latin America) and M (Europe and Latin America). The intake exceeded 100 % PMTDI only at the highest FB1+FB2 level, in the diet C.

Table 8. Daily intake estimates (in µg/person) for fumonisins in Maize (GC 645: excl. flour, oil and beer) at levels of 1, 2 and 5 mg/kg in the 13 Cluster Diets*

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**
1 mg/kg													
Intake, µg/person	0	1.4	51.4	11.9	0.2	0.2	0.6	0	0.1	0	7.7	0	19.4
% PMTDI	0	1	40	10	0	0	1	0	0	0	6	0	20
2 mg/kg;													
Intake, µg/person	0	2.9	102	23.8	0.5	0.3	1.3	0.1	0.1	0.1	15.3	0	38.7
% PMTDI	0	2	90	20	0	0	1	0	0	0	10	0	30
5 mg/kg													
Intake, µg/person	0.1	7.2	257	59.4	1.2	0.8	3.2	0.2	0.3	0.2	38.3	0	96.8
% PMTDI	0	6	210	50	1	1	3	0	0	0	30	0	80

*a body weight of 60 kg was used for all clusters, with exception of G and L (55 kg).

55. Table 9 shows the intake calculation for FB1+FB2 through the consumption of corn/maize flour at the concentration levels of 0.5 mg/kg (mean found in most of the dataset on Table 7 for corn flour/meal and polenta), 1 and 2 mg/kg. The intake did not exceed the PMTDI for any diet at the 0.5 mg/kg level; it did exceed over two times the PMTDI at the two higher levels for diets H (South and Central America) and I (South and East Africa).

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Table 9. Intake estimates (in µg/person) for fumonisins in Maize flour (CF 1255) at FB1+FB2 levels of 0.5, 1 and 2 mg/kg in the 13 Cluster Diets*

	A	B	C	D	E	F	G	H	I	J	K	L	M
0.5 mg/kg													
Intake, µg/person	34.5	7.7	25.7	8.3	7.4	1	144	124.4	103.4	23.9	23.1	5.3	10.8
% PMTDI	30	6	20	7	6	1	10	100	90	20	20	5	9
1 mg/kg;													
Intake, µg/person	68.9	15.4	51.3	16.6	14.7	2	28.8	248.8	206.7	47.8	46.2	10.5	21.5
% PMTDI	60	10	40	10	10	2	30	210	170	40	40	10	20
2mg/kg;													
Intake, µg/person	137.8	30.8	102.6	33.2	29.4	4.0	57.6	497.6	413.4	95.6	92.4	21.0	43.0
% PMTDI	110	30	90	30	20	3	50	410	340	80	80	20	40

* a body weight of 60 kg was used for all clusters, with exception of G and L (55 kg).

56. When considering chronic exposure, it is reasonable to use the mean levels of FB1+FB2 in maize grain (1 mg/kg; Tables 4 and 5) and maize flour (0.5 mg/kg; Table 7) found in the data provided by national governments to estimate the total intake from the consumption of these two commodities. In this estimation, the intake ranged from 1 % of PMTDI in the F cluster diet to 100 % of PMTDI in the H cluster diet. The inclusion of maize germ (including oil), maize beer and popcorn (commodities for which there are available consumption data in the GEMS/FOOD cluster diet dataset) at the same contamination level as maize flour, did not lead to any exceedance of the PMTDI. In this case, cluster diet I also have the intake reaching the PMTDI. For the cluster diets H and I, the consumption of maize flour represented over 90 % of the total fumonisin intake.

57. A chronic dietary exposure assessment of fumonisins (FB1+FB2) from the consumption of maize-based products was conducted in Brazil using a national household budget survey to estimate consumption data (Caldas and Silva, 2007). Mean level of FB1+FB2 in corn meal/corn flour was 3.2 mg/kg. The intake represented 24.1% of the PMTDI for the total population and 355% PMTDI for consumers-only (high consumers), indicating a need for setting safe regulatory levels for fumonisins in maize and maize products in Brazil.

58. In the EU SCOOP Task estimation of the dietary intake of FB1 + FB2 in Europe, the average daily intake represented 0.8 to 13.2% PMTDI for the whole population and 22.3% PMTDI for infants (EC, 2006). In a total diet study performed in France, the 95th percentile of exposure represented 3.2 % of the PMTDI for adults and 8.7 % PMTDI for children (3 to 14 years). For adults, alcoholic beverages contributed with over 50% of the intake; for children, breakfast cereals contributed with over 90 % of the intake (Leblanc et al., 2005). In the Netherlands, it was conservatively estimated that 97% of individuals with gluten intolerance had a daily intake of FB1 of at least 1 µg, and 37% had an intake of at least 100 µg, while the proportions of the general population exposed to these concentrations were 49% and 1%, respectively (de Nijs et al., 1998b). In Denmark, an estimate for an 'eater' shows that the intake of fumonisins will not exceed 0.4 µg/kg bw/day (Petersen and Thorup, 2001).

59. A study conducted in the USA concluded that no human risk of renal toxicity would be expected at the maize contamination levels and consumption patterns for the consumers-only population in the United States. They also suggested that reducing maize consumption would have a greater impact in lowering human risk to kidney damage than lowering the level of fumonisins permitted in maize by a similar factor (Humpreys et al., 2001).

60. In Guatemala, Torres et al (2007) reported that 50% of the maize samples from the markets in 2005 contained fumonisin levels that would result in exposures exceeding the PMTDI. The women intake in three different areas of the country was 3.5 to 15.6 μg/kg bw/day.

61. Yazdanpanah et al. (2006) estimated the exposure of individuals of two provinces of Iran to FB1 and FB2 through the consumption of maize from 1998-2000. The mean intake ranged from 0.009 to 0.34 μg/kg bw/day, with a maximum at 0.71 μg/kg bw/day.

62. In South Africa, fumonisin exposure (FB1+FB2) in Bizana, an area of relatively low oesophageal cancer incidence, was 3.43 +/- 0.15 μg/kg bw/day, significantly lower ($p < 0.05$) than that in Centane, an area of high oesophageal cancer incidence. In both regions, the intake exceeded the PMTDI for fumonisins (Shephard et al., 2007).

63. In Korea, the mean and 95th percentile estimated daily intake of fumonisins (FB1 + FB2) was 0.03 μg/kg bw/day (1.5% PMTDI) and 0.08 μg/kg bw/day (4.0% PMTDI), respectively (Chung, et al, 2008).

64. In Mexico, urinary FB1 was compared with dietary intake after tortilla consumption (Gong et al., 2008). The geometric mean was 35.0, 63.1, and 147.4 pg/mL for the low, medium, and high consumer groups, respectively. Urinary FB1 was correlated with maize intake, however, this biomarker was only considered feasible for high level exposure populations.

65. In China, eight healthy adult volunteers consumed for 1 month a normal diet containing their homegrown maize potentially containing FB1 ranging from 0.08 to 41.1 mg/kg; the estimated daily FB1 intakes ranged from 0.4 to 740 μg/kg bw/day. Analysis of urinary Sa:So ratio before and after consumption suggested that sphingolipid metabolism of humans could be affected by FB1 intake, and may be useful for evaluating high FB1 exposure; males were more sensitive to FB1 disruption of sphingolipid metabolism than females (Qiu and Liu, 2001).

66. Sphinganine and sphingosine were measured in urines of residents in Argentina and Brazil with high maize consumption and compared with urine samples collected in areas with very low or no maize consumption. Mean Sa:So ratio was 1.27 in urine of subjects with high maize consumption ($n = 123$) and 0.36 in controls ($n = 66$) and the difference was statistically significant ($p<0.001$). A similar fumonisin intake was recorded for the Argentinean and Brazilian populations, but the mean Sa:So ratio in Brazil (1.57) was significantly higher ($p<0.05$) than that of Argentina (0.69), suggesting no association with fumonisin exposure (Solfrizzo et al., 2004).

RISK MANAGEMENT CONSIDERATIONS AND PUBLIC HEALTH CONCERNS

67. Current technology cannot prevent fumonisin contamination of maize crops before harvest. The incidence and levels of fumonisins in maize 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.

68. 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).

69. The Korea Food and Drug Administration had notified the World Trade Organisation (WTO)(SPS(G/SPS/N/KOR/283, 6 June 2008) to set maximum limits for fumonisins (FB1+FB2) as 4 mg/kg for maize and 2 mg/kg for maize grits and flour (excepted germ).

70. Table 10 shows the impact of various enforcement limits for maize grain on the % of PMTDI for fumonisins, using the cluster diet C (Europe and Middle East; the population with the highest intake, Table 8) based on the fumonisin distribution in maize grain in South Africa and Nigeria (N=1091). The two African countries showed similar distribution profiles (Figure 2 and Table 4) and are important corn/maize exporters. The mean intake of fumonisins represented an increase of 30 % in the % of PTMDI when the limit was increased from 2 to 5 mg/kg; this increase, however, represented a considerable reduction on the percentage of samples excluded from the market. The increase in % PTMDI was not important when the limit increased from 5 to 10 mg/kg.

ANNEX III

Table 10. Potential % of PMTDI for fumonisins from the consumption of maize grain (GC 645: excl. flour, oil and beer) in the cluster Diet C when various limits are imposed and enforced

Limit (mg/kg)	% of PMTDI						
	Mean	Minimum	Maximum	50th	90th	95th	% excluded
1	20	0	40	20	40	40	33.4
2	30	0	90	20	60	70	18.3
5	40	0	210	30	100	130	5.2
10	40	0	330	30	100	140	0.9
None	40	0	500	30	100	140	0

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ANNEX III

SAMPLING PLANS FOR FUMONISINS IN MAIZE AND MAIZE PRODUCTS

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, shown in Equations 1 thru 4, 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, shown in Equation 1 through 4, 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 plans.

3. The analytical variance (Equation 3) reflects the analytical variability within a single laboratory, which is lower than the analytical variability among laboratories.

4. The issue of correcting the analytical test result for recovery is not addressed in this Annex. However, EC Regulation No 401/2006 (Table 1) can be used as a possible standard for several performance criteria for analytical methods including suggestions for the range of acceptable recovery rates.

5. Maximum levels need to be defined before a final decision can be made about a sampling plan design.

FUMONISIN TEST PROCEDURE AND MAXIMUM LEVELS

6. A fumonisin-sampling plan is defined by a fumonisin test procedure and a maximum level. Since a fumonisin maximum level has not been defined by members of CCCF, sampling plans for a range of MLs are evaluated in this Annex.

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7. The maximum levels for fumonisin in shelled maize evaluated in this Annex are 1, 2, 5, and 10 mg/kg.

8. Choice of the number and size of the laboratory sample is a compromise between minimizing risks (false positives and false negatives) and costs related to sampling and restricting trade. A range of laboratory sample sizes (1, 2, and 5 kg) when using either 1 or 2 laboratory samples to accept or reject a lot are evaluated.

9. Sample preparation – dry grind with a suitable mill and a 25 g test portion.

10. Analytical method – performance based (EC Regulation No 401/2006).

11. Decision rule – If the fumonisin-sample test result is less than the stated ML, then accept the lot. Otherwise, reject the lot.

12. To assist member countries implement a sampling plan, sample selection methods, sample preparation methods, and analytical methods required to quantify fumonisin in laboratory samples taken from bulk lots of shelled maize are described in the following sections.

SAMPLE SELECTION

Material to be sampled

13. 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.

14. 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%.

15. 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.

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

Incremental Sample Selection

17. 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.

18. 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.

Number of Incremental Samples for Lots of varying weight

19. 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. Once the number and size of the laboratory sample(s) has been defined by CCCF, specific recommendations can be made for varying lot sizes.

Weight of the Incremental Sample

20. The suggested minimum weight of the incremental sample should be approximately 100 grams for lots of 50 metric tonnes (50,000 kg).

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Static Lots

21. 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.

22. Taking incremental samples from a static lot usually requires the use of probing devices to select product from the lot. The probing devices should be specifically designed for the commodity and type of container. The probe should (1) be long enough to reach all products, (2) not restrict any item in the lot from being selected, and (3) not alter the items in the lot. As mentioned above, the aggregate sample should be a composite from many small incremental samples of product taken from many different locations throughout the lot.

23. For lots traded in individual packages, the sampling frequency (SF), or number of packages that incremental samples are taken from, is a function of the lot weight (LT), incremental sample weight (IS), aggregate sample weight (AS) and the individual packing weight (IP), as follows:

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

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

Dynamic Lots

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

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

27. Cross-cut samplers should be installed in the following manner: (1) the plane of the opening of the diverter cup should be perpendicular to the direction of the flow; (2) the diverter cup should pass through the entire cross sectional area of the stream; and (3) the opening of the diverter cup should be wide enough to accept all items of interest in the lot. As a general rule, the width of the diverter cup opening should be about two to three times the largest dimensions of items in the lot.

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

    S=(D x LT) / (T x V),

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

29. If the mass flow rate of the moving stream, MR (kg/sec), is known, then the sampling frequency (SF), or number of cuts made by the automatic sampler cup can be computed as a function of S, V, D, and MR.

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

Packaging and Transportation of Samples

30. 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.

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Sealing and Labeling of Samples

31. Each laboratory sample taken for official use shall be sealed at the place of sampling and identified. A record must be kept of each sampling, permitting each lot to be identified unambiguously and giving the date and place of sampling together with any additional information likely to be of assistance to the analyst.

SAMPLE PREPARATION

Precautions

32. 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.

Homogenization - Grinding

33. 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.

34. 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

35. The suggested weight of the test portion taken from the comminuted laboratory sample should be approximately 25 grams.

36. Procedures for selecting the 25 g test portion from the comminuted laboratory sample should be a random process. If mixing occurred during or after the comminution process, the 25 g test portion can be selected from any location throughout the comminuted laboratory sample. Otherwise, the 25 g test portion should be the accumulation of several small portions selected throughout the laboratory sample.

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

ANALYTICAL METHODS

Background

38. A criteria-based approach, whereby a set of performance criteria is established with which the analytical method used should comply, is appropriate. The criteria-based approach has the advantage that, by avoiding setting down specific details of the method used, developments in methodology can be exploited without having to reconsider or modify the specific method. The performance criteria established for methods should include all the parameters that need to be addressed by each laboratory such as the detection limit, repeatability coefficient of variation (within lab), reproducibility coefficient of variation (among lab), and the percent recovery necessary for various statutory limits. Analytical methods that are accepted by chemists internationally (such as AOAC) may be used. These methods are regularly monitored and improved depending upon technology.

Performance Criteria for Methods of Analysis

39. Using EC Regulation No 401/2006, a list of possible criteria and performance levels are shown in Table 1. 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.

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

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FUMONISIN TEST PROCEDURE AND MAXIMUM LEVELS

40. A fumonisin-sampling plan is defined by a fumonisin test procedure and a maximum level. Since a fumonisin maximum level has not been defined by members of CCCF, a range of MLs are evaluated in this Annex.

41. Maximum levels for fumonisin in shelled maize evaluated in this Annex are 1, 2, 5, and 10 mg/kg.

42. 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. A range of laboratory sample sizes (1, 2, and 5 kg) when using either 1 or 2 laboratory samples to accept or rejects a lot are evaluated

43. Sample preparation – dry grind with a suitable mill and a 25 g test portion

44. Analytical method – performance based (see Table 1)

45. Decision rule – If the fumonisin test result is less than the stated ML, then accept the lot. Otherwise, reject the lot.

PERFORMANCE OF SEVERAL FUMONISIN SAMPLING PLANS FOR SHELLED MAIZE

46. The performances of several fumonisin sampling plan designs for shelled maize using 1, 2, and 5 kg laboratory sample sizes and maximum levels of 1, 2, 5, and 10 mg/kg are shown in Figures 1 through 8 (see Table 6 Discussion Paper on Fumonisins, 3rd Session CCCF, Agenda Item 9(a), CX/CF/09/3/09, February 2009 for a discussion of limits). 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 $S^2_{sp} = (25/nss)\ 0.033\ C^{1.59}$ (2)

Analytical $S^2_a = (1/na)\ 0.033\ C^{1.44}$ (3)

Total variance - $S^2_t = S^2_s + S^2_{sp} + S^2_a$ (4)

Effect of Increasing Laboratory Sample Size

47. Operating Characteristic curve describing the performance of the fumonisin sampling plan for shelled maize using laboratory sample sizes of 1, 2, and 5 kg and maximum levels of 1, 2, 5, and 10 mg/kg are shown in Figures 1, 2, 3, and 4, respectively. The operating characteristic curve reflects uncertainty associated with using 1, 2, and 5 kg laboratory sample sizes, sample comminution in a Romer Mill, 25 g test portion, and quantification of fumonisin in the test portion by LC.

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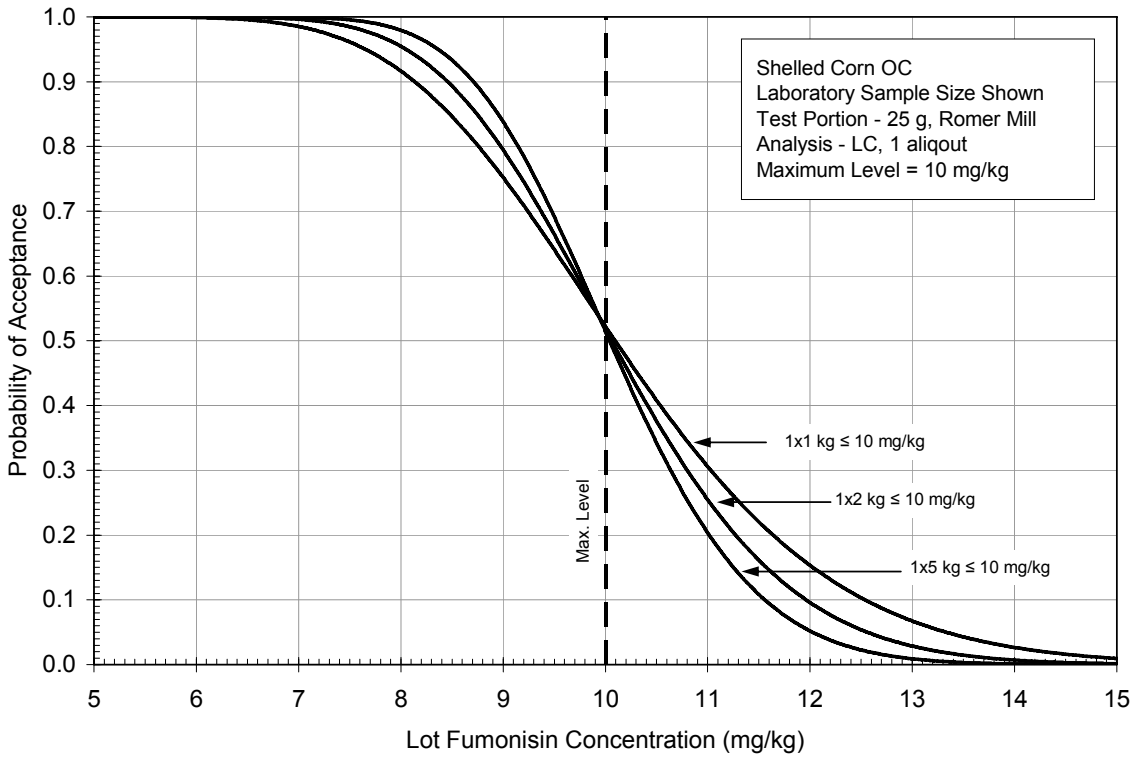

Figure 1. Operating characteristic curves showing the performance of sampling plan designs that use 1, 2, and 5 kg samples to detect fumonisin in lots of shelled maize for a maximum level of 10 mg/kg.

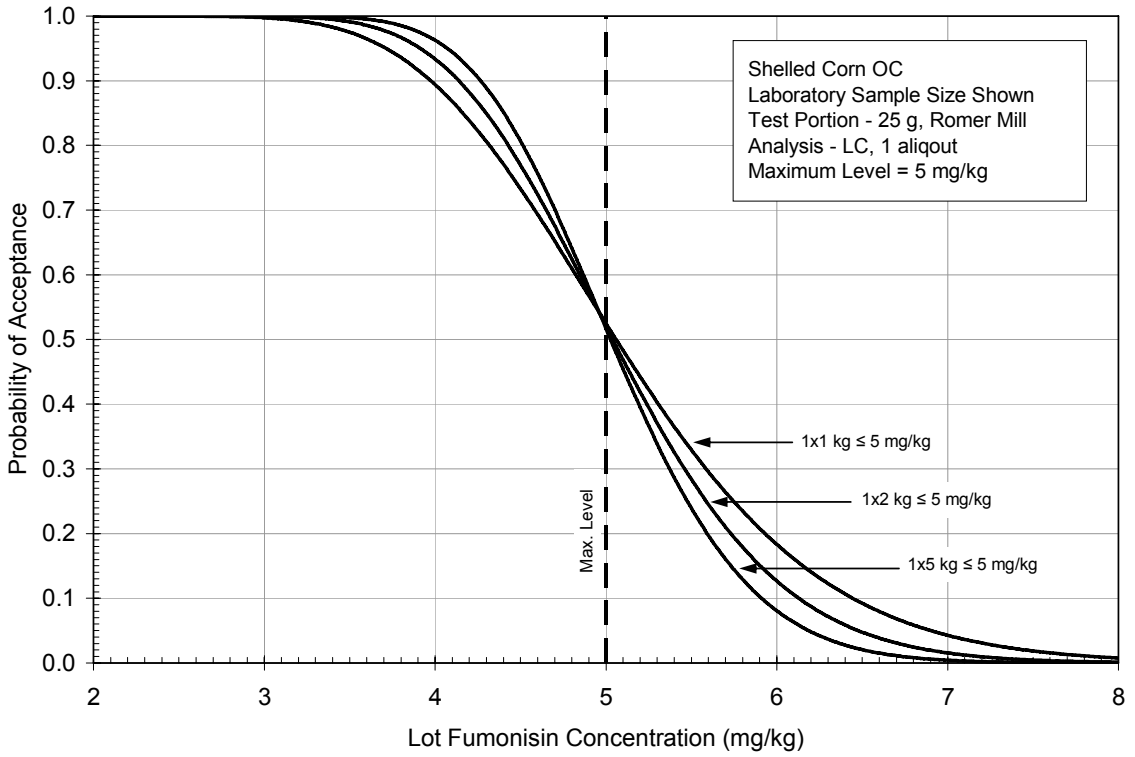

Figure 2. Operating characteristic curves showing the performance of sampling plan designs that use 1, 2, and 5 kg samples to detect fumonisin in lots of shelled maize for a maximum level of 5 mg/kg.

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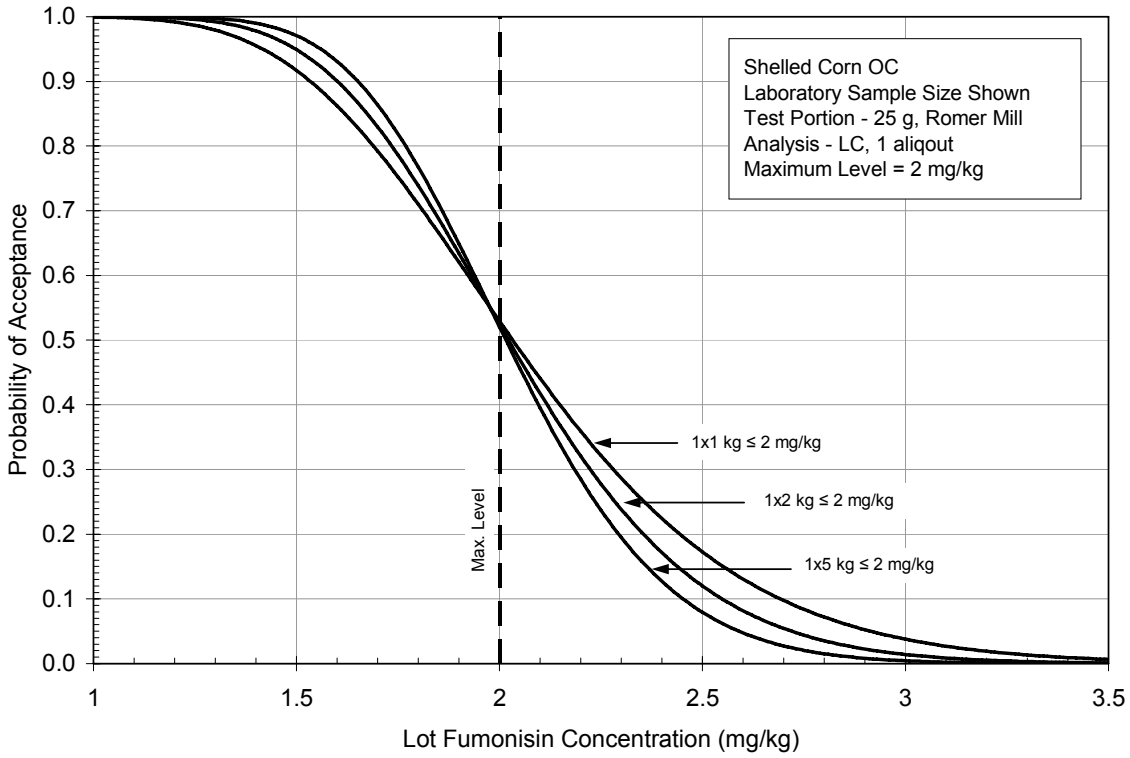

Figure 3. Operating characteristic curves showing the performance of sampling plan designs that use 1, 2, and 5 kg samples to detect fumonisin in lots of shelled maize for a maximum level of 2 mg/kg.

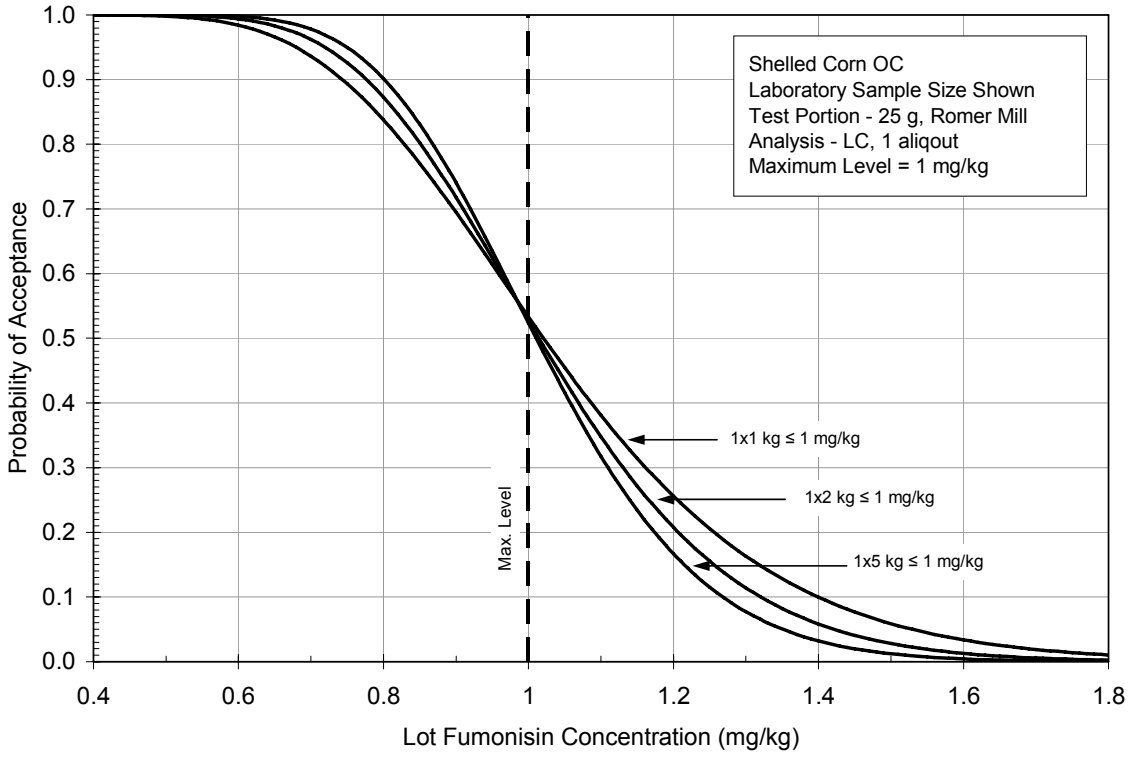

Figure 4. Operating characteristic curves showing the performance of sampling plan designs that use 1, 2, and 5 kg samples to detect fumonisin in lots of shelled maize for a maximum level of 1 mg/kg.

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48. Each sampling plan in Figures 1, 2, 3, and 4 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

49. 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 5, 6, 7, and 8, respectively. The operating characteristic curve reflects uncertainty associated with using 1 or 2 laboratory sample 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.

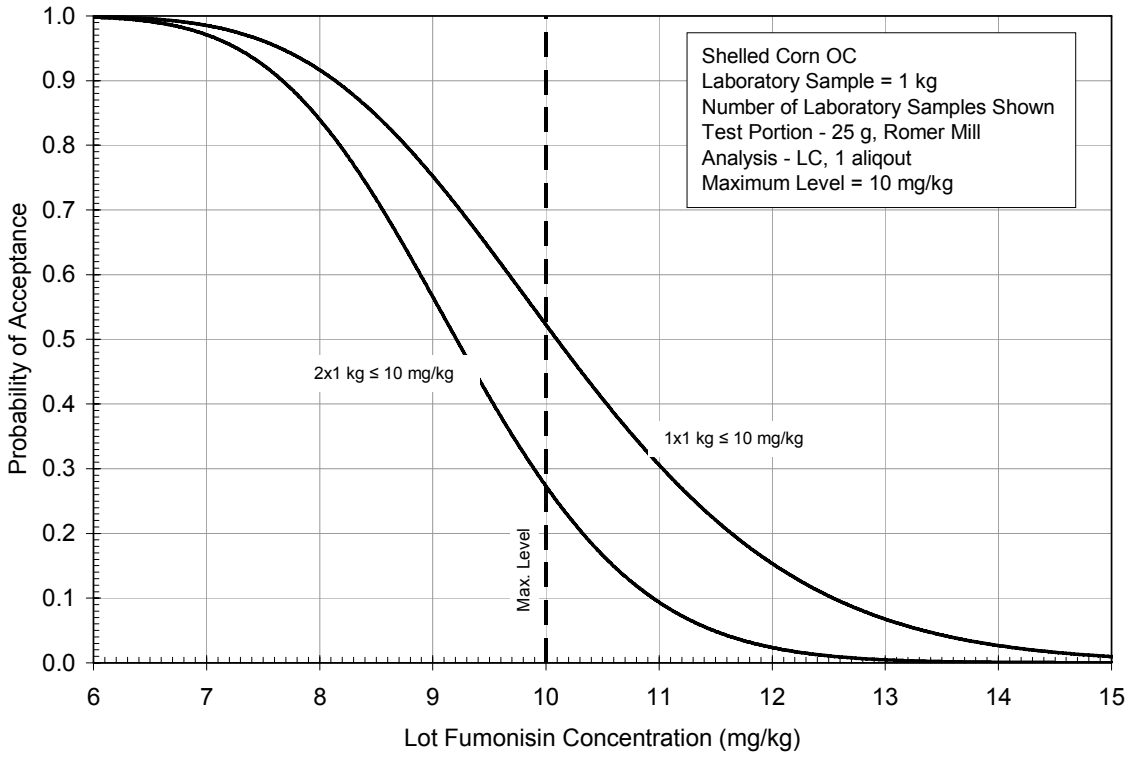


Figure 5. Operating characteristic curves showing the performance of sampling plan designs that use 1 or 2 samples of size 1.0 kg each to detect fumonisin in lots of shelled maize for a maximum level of 10 mg/kg.

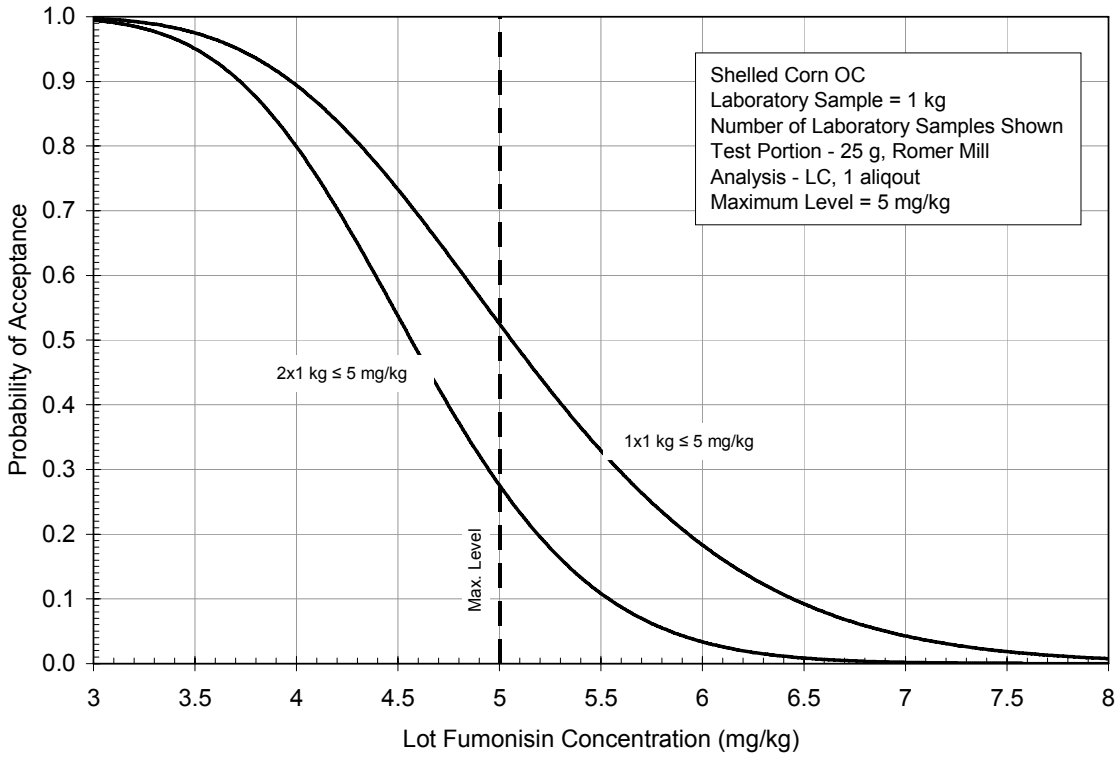


Figure 6. Operating characteristic curves showing the performance of sampling plan designs that use 1 or 2 samples of size 1.0 kg each to detect fumonisin in lots of shelled maize for a maximum level of 5 mg/kg.

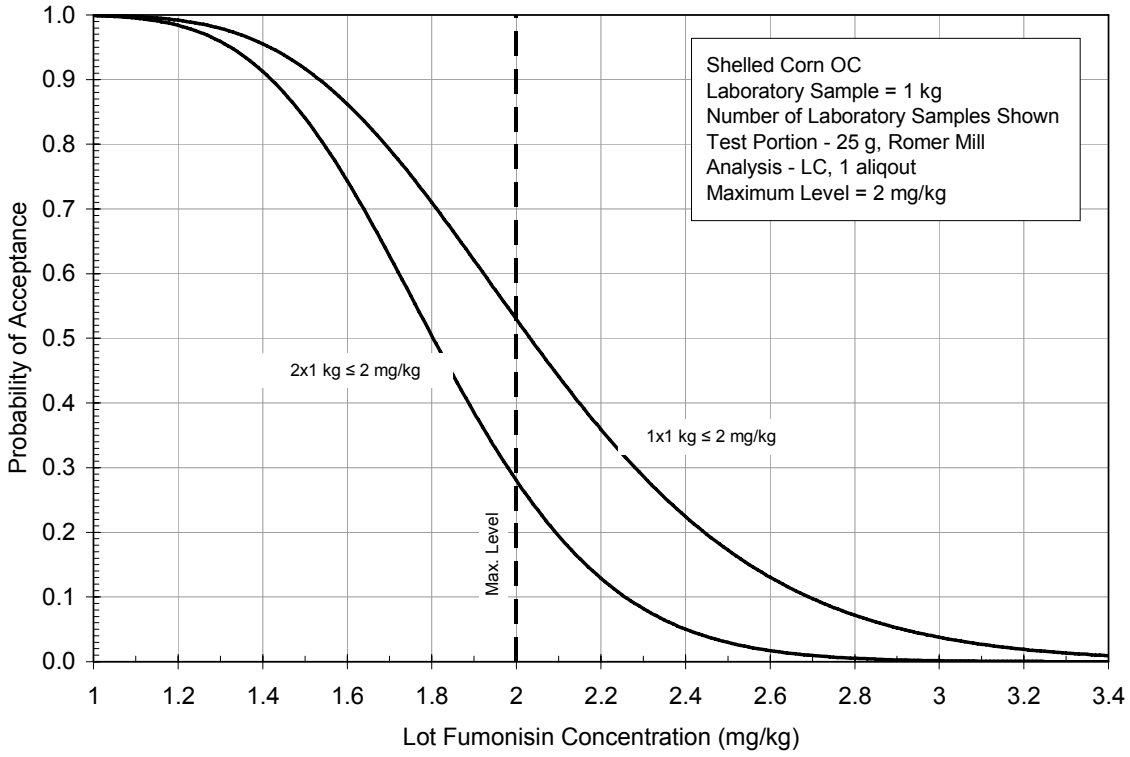


Figure 7. Operating characteristic curves showing the performance of sampling plan designs that use 1 or 2 samples of size 1.0 kg each to detect fumonisin in lots of shelled maize for a maximum level of 2 mg/kg.

ANNEX III

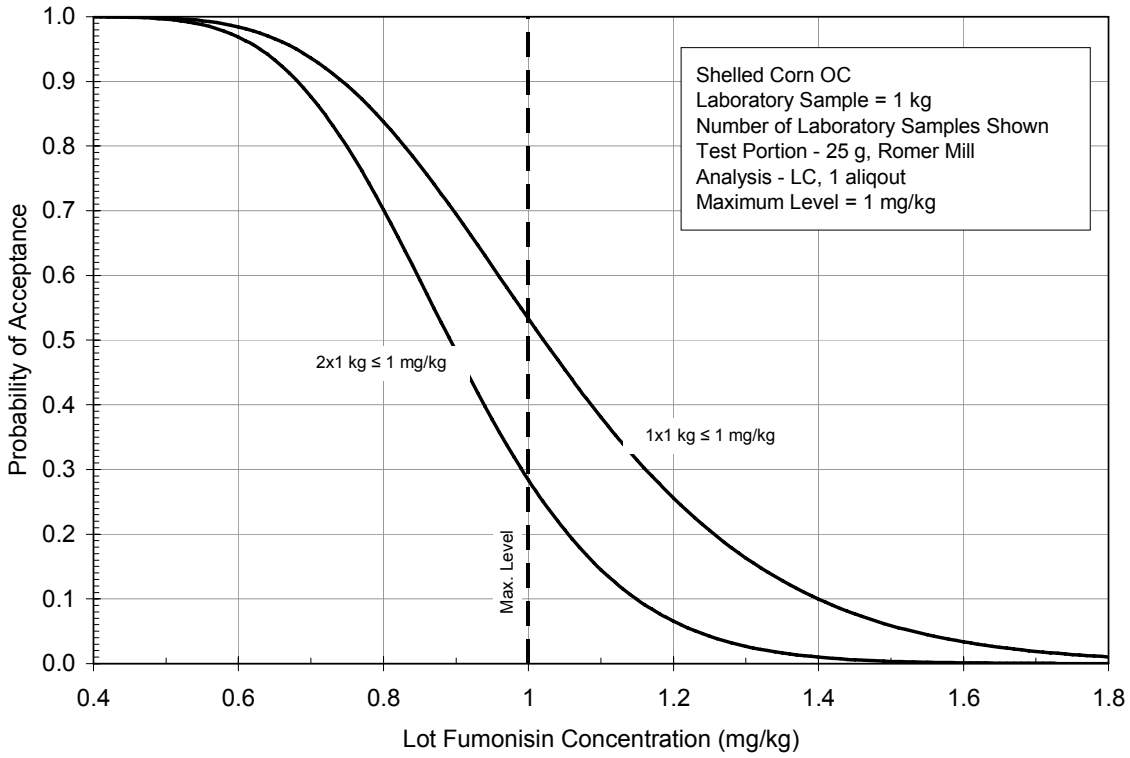


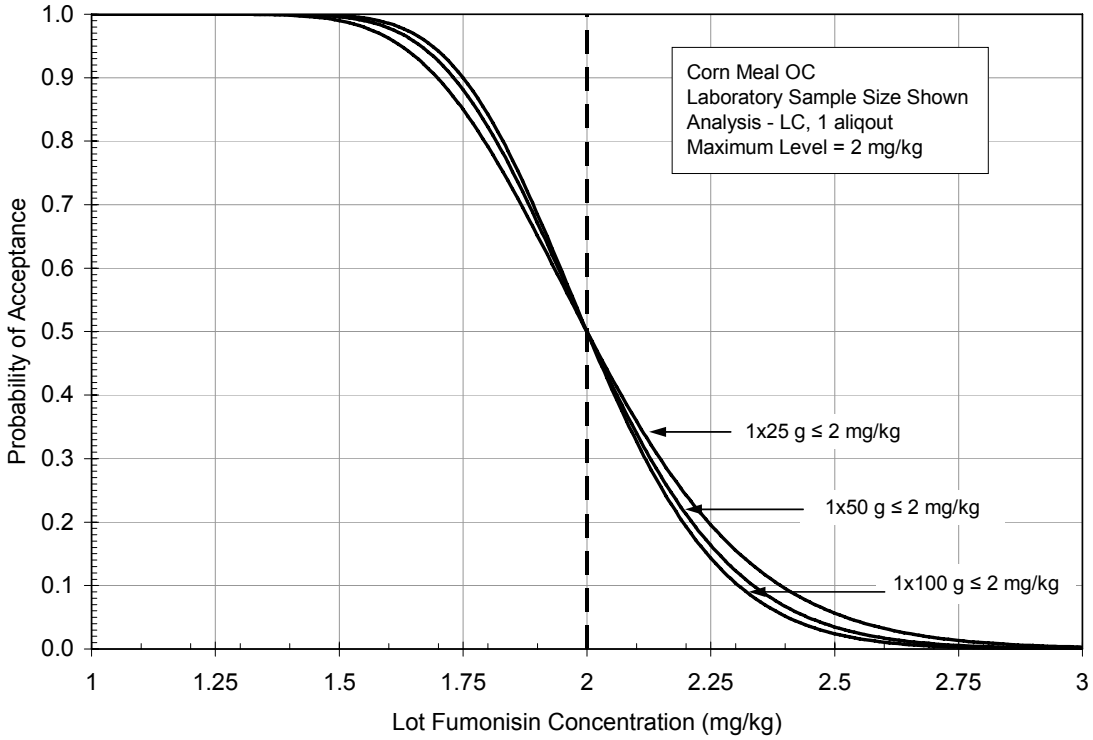
Figure 8. Operating characteristic curves showing the performance of sampling plan designs that use 1 or 2 samples of size 1.0 kg each to detect fumonisin in lots of shelled maize for a maximum level of 1 mg/kg.

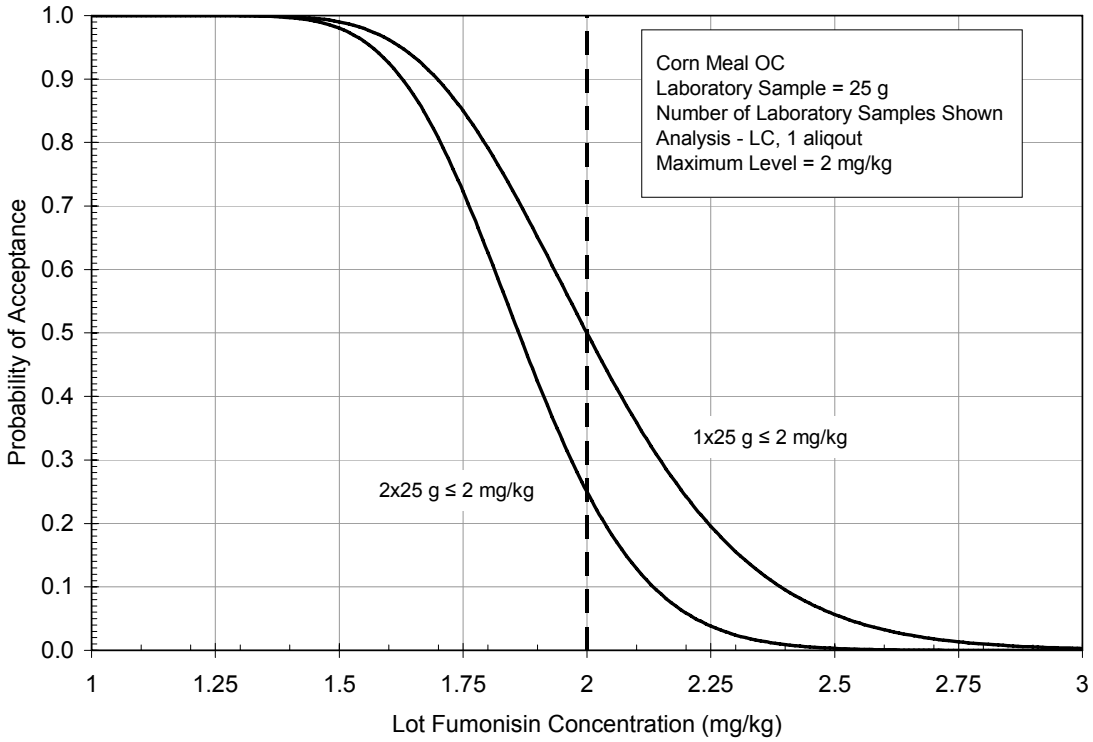
50. Figures 5, 6, 7, and 8 show the effect of increasing the number of laboratory samples that must all test below the ML on the chances of accepting or rejecting lots over a wide range in lots fumonisin concentrations. For each maximum level, as the number of samples increase from 1 to 2 samples (each 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.

51. As the OC curves show in Figures 1 to 8, 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 CCCF did not want more than 10% of the lots at 6 mg/g or higher to be accepted by a sampling plan, then either 1x5kg<=5mg/kg (Figure 2) or 2x1kg<=5mg/kg (Figure 6) would satisfy that criterion.

ANNEX III

Sampling Comminuted Shelled Maize (Maize Flour) for Fumonisin





Operating characteristic curves developed for sampling comminuted shelled maize (maize flour) with specific sample sizes and analytical methods were calculated using variances measure by Whitaker et al, 1998. The sampling and analytical variances are:

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Sampling Variance = (25/ns) 0.011 $C^{1.59}$ (1)

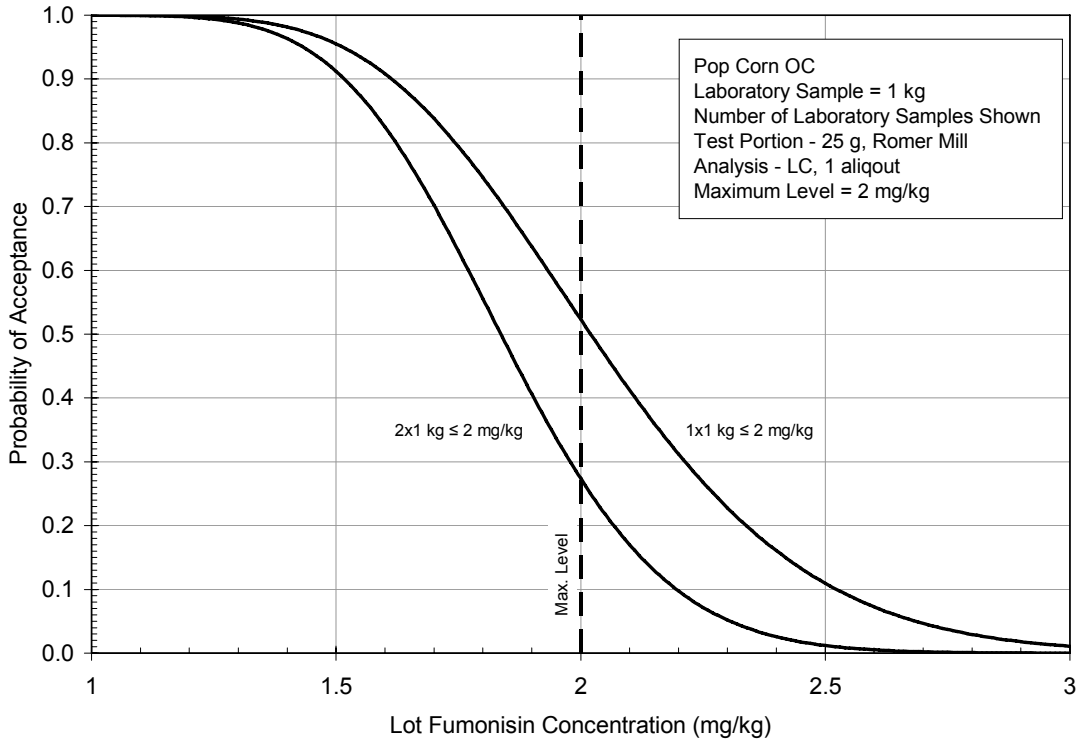
Analytical variance = (1/na) 0.014 $C^{1.44}$ (2)

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

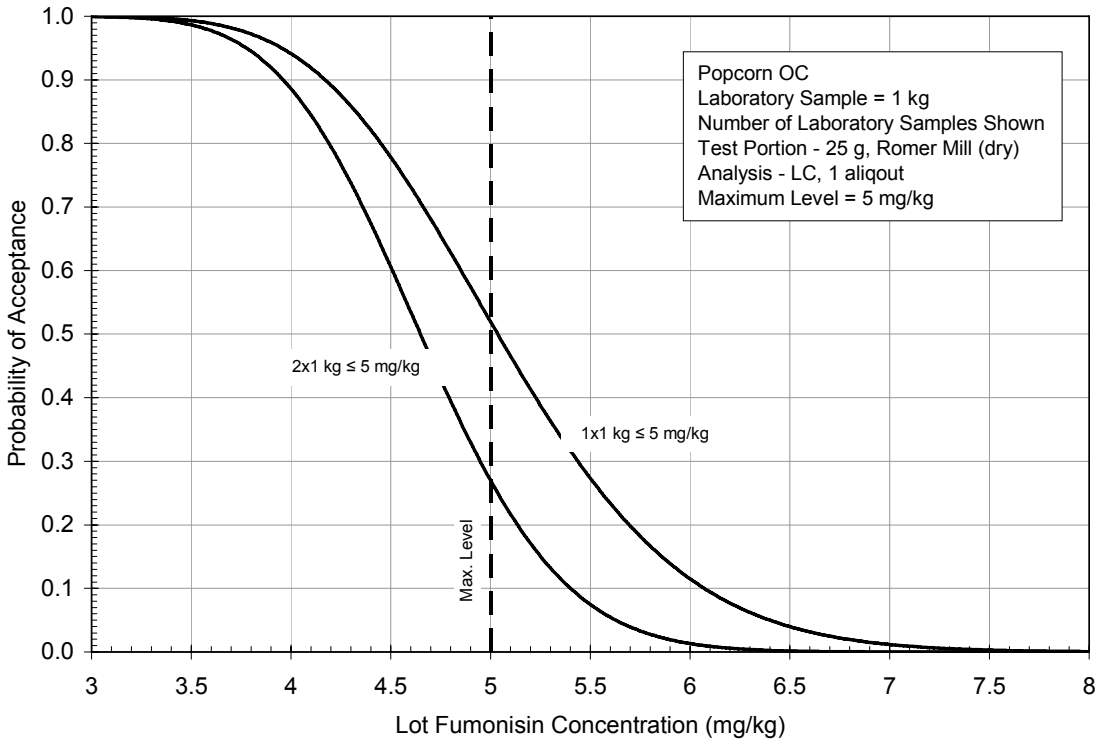
Sampling variance (Equation 1) reflects a particle size distribution consistent with comminuting shelled maize with the Romer mill.

Analytical variance (Equation 2) reflects quantification of fumonisin in one aliquot by LC methods.

Sampling Popcorn for Fumonisin



ANNEX III



Operating characteristic curves developed for sampling popcorn with specific sample sizes, sample preparation, and analytical methods were calculated using variances measure by Whitaker et al, 1998. The sampling, sample preparation, and analytical variances are:

Sampling Variance = (3300/ns) 0.033 $C^{1.75}$ (1)

Sample Prep Variance = (25/nss) 0.011 $C^{1.59}$ (2)

Analytical variance = (1/na) 0.014 $C^{1.44}$ (3)

Sampling variance (Equation 1) reflects a kernel count of 73 popcorn kernels per 10 g. This count per gram was an average of counts per gram that varied from 59 to 94 kernels per 10 g (Iowa State University, http://www.ag.iastate.edu/centers/cad/popcorn.html). The number of kernels, ns, in 1 kg laboratory sample is 8030.

Sample preparation variance reflects comminution of the laboratory sample with a Romer mill and a 25 g test portion removed from the laboratory sample for extraction by LC.

Analytical variance (Equation 2) reflects quantification of fumonisin in one aliquot by LC methods.