CODEX ALIMENTARIUS COMMISSION

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World Health Organization

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SAMPLING PLANS FOR TOTAL AFLATOXINS IN CERTAIN CEREALS AND CEREAL-BASED PRODUCTS INCLUDING FOODS FOR INFANTS AND YOUNG CHILDREN (At Step 4)

(Prepared by the Electronic Working Group chaired by Brazil and co-chaired by India)

Codex members and observers wishing to submit comments at Step 3 on sampling plans should do so as instructed in CL 2023/20-CF available on the Codex webpage[1]

BACKGROUND

1. The 15th Session of the Codex Committee on Contaminants in Foods (CCCF15, 2022) agreed[2] on maximum levels (MLs) for total aflatoxins in maize grain, destined for further processing; flour meal, semolina and flakes derived from maize; husked rice; polished rice; sorghum grain, destined for further processing and cereal-based food for infants and young children. The Committee also decided to re-establish an Electronic Working Group (EWG), chaired by Brazil and co-chaired by India, to further develop the sampling plan taking into account the possibility to harmonize the sampling plans for maize grain; flour, meal, semolina and flakes with the sampling plan for deoxynivalenol (DON) and fumonisins; and the sampling plan for cereal-based foods for infants and young children with the sampling plan for DON.

WORK PROCESS

2. The present document was initially elaborated considering the information received in response to circular letter CL 2022/46-CF on the ratios of aflatoxin AFB1, AFB2, AFG1 and AFG2 in maize grain, maize products, husked rice, polished rice, sorghum grain and cereal-based foods for infant and young children (see Appendix III). In the first draft, countries were asked to comment on the performance criteria methods prepared considering a 50:50 ratio of AFB1:AFB2+AFG1+AFG2. Different scenarios (1, 5, 10 and 30 Kg to laboratory sample weight and 25 and 50 g to test portion size to maize grain) were also tested, using the online FAO Mycotoxin Sampling Tool).
3. After the document had been circulated, comments received from countries suggested that different ratios of AFB1: AFB2+AFG1+AFG2, such as 80:20 and 90:10, should also be tested.
4. For the final round of comments, EWG members were invited to consider if it was feasible to assume the sampling plans to DON and Fumonisins in maize flour and maize meal could apply to AF contamination on maize flour, meal, semolina and flakes derived from maize, sorghum, husked and polished rice. This approach was suggested since data on the variation in sampling, sampling preparation and analysis did not become available in the first round.
5. The final document was elaborated considering the comments received in reply to CL 2022/46-CF and from EWG members as well as complementary information obtained through the FAO Mycotoxin Sampling Tool, such as sampling variance, sample preparation variance, analysis variance, total variance at Codex ML/'Regulatory Limit', probability of mischaracterizing a lot as "compliant" with the ML and Total error at Codex ML/'Regulatory Limit'.
6. Final recommendations were prepared in order to contemplate the different views presented during the EWG discussions.

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1 Codex webpage/Circular Letters: http://www.fao.org/fao-who-codexalimentarius/resources/circular-letters/en/.
Codex webpage/CCCF/Circular Letters: http://www.fao.org/fao-who-codexalimentarius/committees/committee/related-circular-letters/en/?committee=CCCF

2 REP22/CF15, para. 154

SUMMARY OF THE DISCUSSION IN THE EWG

7. The EWG observed that among the food commodities in discussion there is information on sampling variation, preparation and analysis only for maize grain in the FAO mycotoxin sampling tool. CL 2022/46-CF requested comments on sampling plans for total aflatoxins for cereals and cereal-based foods, including foods for infants and young children, asking for data on the typical ratio of the four aflatoxins in naturally contaminated samples of the cereals for which MLs were established and on the variation in sampling, sampling preparation and analysis for husked rice, polished rice and sorghum. Brazil, Canada, Chile, European Union (EU), Iran, Japan, Republic of Korea, Saudi Arabia and the United States of America (USA) provided information in response to CL 2022/46-CF. Their comments are included in Appendix III for information.

KEY POINTS OF DISCUSSION

Lack of information concerning AFB1: AFB2+AFG1+AFG2 ratio.

8. One country questioned whether it would not be possible to use data from GEMS/Food to obtain the ratio of aflatoxins (AFB1: AFB2+AFG1+AFG2) present in each type of sample considered in this document. For aflatoxins, data submitted to GEMS/Food included information on individual aflatoxins (AFB1, AFB2, AFG1, AFG2), the sum of AFB1 plus AFB2 and total aflatoxins, which could generate up to 6 entries per sample. However, there was not a pattern of data submission, most countries submitted only the total aflatoxins (sum of AFB1, AFB2, AFG1 and AFG2) or individual values for aflatoxin B1. On the other hand, when individual data was submitted, it was only possible to gather the information of a sample if the "serial number" was provided, what did not always happen. At CCCF14, an approach to estimate aflatoxins ratio in samples submitted to the GEMS/Food was presented, using only the data in which it was possible to have the individual values of occurrence of aflatoxins. However, the values presented were not well accepted by the Committee at that time as they did not cover all the cereals and cereal products under discussion and that the proportion may vary among commodities[3].

9. Therefore, for this version, information was requested directly from countries with the intention of obtaining more detailed information on samples submitted to GEMS/Food. All countries reported the frequency of AFB1 being higher than 50% of total AF, but not all of them submitted data to support different scenarios of 80:20 and 90:10 ratio. If the scenario 50:50 was adopted for the same ML, a LOQ of ≤ 3.0 µg/kg for the method should be achieved for AFB1. The methodological performance criteria will be then very restrictive if AFB2+AFG1+AFG2 was lower than 50%. The benefit of adopting these criteria to less toxic congeners should be evaluated.

Need for guidance on the sum of components

10. Some members identified the need for guidance when the concentration of one aflatoxin is lower than LOQ and how to sum up components. This issue is being addressed in the work to establish "Guidance on data analysis for the development of maximum levels and improved data collection" (under discussion within CCCF).

Assuming the sampling plans for DON and fumonisins on maize flour and maize meal could apply to AF contamination in maize flour, meal, semolina and flakes derived from maize, sorghum, husked and polished rice.

11. Maize flour, meal, semolina and flakes derived from maize and cereal-base foods for infants and young children: There was general agreement that since comminution of grain will occur during processing of flour meal, semolina, flakes derived from maize and cereal based food for infants and young children, this will reduce the heterogeneity of the materials with respect to AFs. This fact supports the alignment of sampling plans for DON and fumonisins in maize flour and maize meal and cereal-based foods for infant and young children with the sampling plans for aflatoxins in the same commodities.

12. Sorghum, husked and polished rice: A country pointed out that the FAO Mycotoxin Sampling Tool does not have data for mycotoxins in sorghum, husked rice, or polished rice, so the Tool cannot be used to simulate variance or OC curves for different sampling scenarios for these grains. Additionally, considering: i) the impact of sample weight and test portion size on the total variance in the scenarios modelled for each aflatoxin, DON and fumonisins in maize grain, and; ii) that sorghum, husked and polished rice are not comminuted during processing (comminution reduces heterogeneity), the alignment of the sampling plan for DON and fumonisins in maize flour and meal was not supported by one country and, therefore, a more conservative approach was proposed to align the sampling plan with the one being proposed for aflatoxins in maize grain.

[3] CX/CF 21/14/10-Part II, paras. 2 and 7

Aligning the sampling plan for AFT in maize grain with those of DON and fumonisins

13. Some countries did not agree to align the sampling plan for AFT in maize grain with those of DON and fumonisins as there is a greater heterogenicity as shown by the FAO mycotoxin tool. A country reported that, as per their experience, using a higher laboratory sample weight and a higher portion size would result in a lower probability of misclassifying a lot of maize.

14. Although there was general support of assuming 5 kg as laboratory sample weight and 25g as test portion size and not assume the sampling plans to DON and fumonisins (1 kg and 25g) for maize grain, only one country did not support this option. In their view, the practicality of increasing the laboratory sample from 5 to 10 kg should be discussed by CCCF members. More information is detailed in Appendix II, paragraphs 19 and 20.

CONCLUSIONS

15. The proposals that have attempted to address the concerns expressed by various delegations regarding methods of analysis and sampling plans are detailed in Appendix II, including a detailed technical analysis of such proposals.

16. The FAO Mycotoxin Sampling Tool does not have data for mycotoxins in sorghum, husked rice, or polished rice, so the Tool cannot be used to simulate variance or OC curves for different sampling scenarios for these grains.

17. Given that sorghum, husked rice and polished rice are not comminuted during processing (comminution reduces heterogeneity), it was considered not appropriate to align the sampling plan for aflatoxins in these cereals with those for DON and fumonisins in maize flour and maize meal. As maize grain is larger than sorghum and rice and that it is expected that increasing the cereal grain would generate more heterogeneity, it is expected that the proposed sampling plan for aflatoxin in maize grain may also be applicable to sorghum and rice. Therefore, it is proposed to align the sampling plans for aflatoxins in sorghum, husked rice and polished rice with the proposed sampling plan for aflatoxins in maize grain.

18. Comminution of grain will occur during processing of flour meal, semolina, flakes derived from maize and cereal-based food for infants and young children, this will therefore reduce the heterogeneity of the materials with respect to AFs. This fact supports the alignment of sampling plans for aflatoxins in maize flour and maize meal and cereal-based foods for infant and young children with the sampling plans for DON and fumonisins in maize, flour and maize meal and for DON in cereal-based food for infant and young children.

RECOMMENDATIONS

19. Based on the previous discussion and weighing the applicability and cost of analysis, the EWG recommends CCCF is to consider:

    (i) the adoption of 5 kg as laboratory sample size and 25 g as test portion size for maize grain, destined for further processing.

    (ii) the alignment of the sampling plans for sorghum, husked rice and polished rice with the proposed sampling plan for aflatoxins in maize grain.

    (iii) the alignment of the sampling plans for flour meal, semolina and flakes derived from maize and cereal-based foods for infant and young children with DON and fumonisins sampling plans.

    (iv) proposed sampling plans for the selected food categories as shown in Appendix I based on the conclusions provided in paragraphs 15-18 and the data/information provided in Appendix II, including their readiness for final adoption by the Codex Alimentarius Commission (CAC46, 2023).

APPENDIX I

SAMPLING PLANS FOR TOTAL AFLATOXINS IN CERTAIN CEREALS AND CEREAL-BASED PRODUCTS INCLUDING FOODS FOR INFANTS AND YOUNG CHILDREN (For comments)

Sampling plans for aflatoxin (AFB1+AFB2+AFG1+AFG2) in maize grain, destined for further processing.

Maximum level	15 µg/kg AFB1+AFB2+AFG1+AFG2
Increments	Increments of 100g, depending on the lot mass (≥0.5 tons)
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm – 20 mesh)
Laboratory sample size	5 kg
Number of laboratory samples	1
Test portion	25 g
Method	Selected according to the established performance criteria
Decision rule	If the sum of test results of AFB1, AFB2, AFG1 and AFG2 for the laboratory sample is equal to or less than 15 µg/kg, accept the lot. Otherwise, reject the lot.

Sampling plans and performance criteria for aflatoxin (AFB1+AFB2+AFG1+AFG2) in flour meal, semolina and flakes derived from maize

Maximum level	10 µg/kg AFB1+AFB2+AFG1+AFG2
Increments	10 x 100g
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm – 20 mesh), if necessary for coarse samples
Laboratory sample size	1 kg
Number of laboratory samples	1
Test portion	25g test portion
Method	Selected according to the established performance criteria
Decision rule	If the sum of test results of AFB1, AFB2, AFG1 and AFG2 for the laboratory sample is equal to or less than 15 µg/kg, accept the lot. Otherwise, reject the lot

Sampling plans and performance criteria for aflatoxin (AFB1+AFB2+AFG1+AFG2) in husked rice

Maximum level	20 µg/kg AFB1+AFB2+AFG1+AFG2
Increments	Increments of 100g, depending on the lot mass (≥0.5 tons)
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm – 20 mesh)
Laboratory sample size	5 kg
Number of laboratory samples	1
Test portion	25g
Method	Selected according to the established performance criteria
Decision rule	If the sum of test results of AFB1, AFB2, AFG1 and AFG2 for the laboratory sample is equal to or less than 15 µg/kg, accept the lot. Otherwise, reject the lot

Sampling plans and performance criteria for aflatoxin (AFB1+AFB2+AFG1+AFG2) in polished rice

Maximum level	5 µg/Kg AFB1+AFB2+AFG1+AFG2
Increments	Increments of 100g, depending on the lot mass (≥0.5 tons)
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm – 20 mesh)
Laboratory sample size	5 kg
Number of laboratory samples	1
Test portion	25g
Method	Selected according to the established performance criteria
Decision rule	If the sum of test results of AFB1, AFB2, AFG1 and AFG2 for the laboratory sample is equal to or less than 15 µg/kg, accept the lot. Otherwise, reject the lot

Sampling plans and performance criteria for aflatoxin (AFB1+AFB2+AFG1+AFG2) in sorghum

Maximum level	10 µg/kg AFB1+AFB2+AFG1+AFG2
Increments	Increments of 100g, depending on the lot size (≥0.5 tons)
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm – 20 mesh)
Laboratory sample size	5 kg
Number of laboratory samples	1
Test portion	25g
Method	Selected according to the established performance criteria
Decision rule	If the sum of test results of AFB1, AFB2, AFG1 and AFG2 for the laboratory sample is equal to or less than 15 µg/kg, accept the lot. Otherwise, reject the lot

Sampling plans and performance criteria for aflatoxin (AFB1+AFB2+AFG1+AFG2) in cereal-based food for infants and young children

Maximum level	5 µg/kg AFB1+AFB2+AFG1+AFG2
Increments	10 x 100g
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm – 20 mesh), if necessary for coarse samples
Laboratory sample size	1 kg
Number of laboratory samples	1
Test portion	25g
Method	Selected according to the established performance criteria
Decision rule	If the sum of test results of AFB1, AFB2, AFG1 and AFG2 for the laboratory sample is equal to or less than 15 µg/kg, accept the lot. Otherwise, reject the lot

Sampling plans and performance criteria for aflatoxin (AFB1+AFB2+AFG1+AFG2) in cereal-based food for infants and young children destined for food aid programs

Maximum level	10 µg/kg AFB1+AFB2+AFG1+AFG2
Increments	10 x 100g
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm – 20 mesh), if necessary for coarse samples
Laboratory sample size	1 kg
Number of laboratory samples	1
Test portion	25g
Method	Selected according to the established performance criteria
Decision rule	If the sum of test results of AFB1, AFB2, AFG1 and AFG2 for the laboratory sample is equal to or less than 15 µg/kg, accept the lot. Otherwise, reject the lot

Definitions:

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	It is defined by an aflatoxin test procedure and an accept/reject level. An aflatoxin test procedure consists of three steps: sample selection, sample preparation and analysis or aflatoxin 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 cereal grains and cereal-based products 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 in such a way to ensure that the laboratory sample is still representative of the sublot sampled.
Test portion	A portion of the comminuted laboratory sample. The entire laboratory sample should be comminuted in a mill. A portion of the comminuted laboratory sample is randomly removed for the extraction of the aflatoxin for chemical analysis.

SAMPLING PLAN DESIGN CONSIDERATIONS

MATERIAL TO BE SAMPLED

1. Each lot of cereal grains and cereal-based products, which is to be examined for AFs, must be sampled separately. Lots larger than 50 tons should be subdivided into sublots to be sampled separately. If a lot is greater than 50 tons, the lot should be subdivided into sublots according to Table 1.

Table 1. Subdivision of cereal grains sublots according to lot size – Maize grain, sorghum, polished rice and husked rice

Lot size (t)	Maximum size or minimum number of sublots	Number of incremental samples	Minimum sample size (kg)
≥ 1500	500 tons	100	5
> 300 and < 1500	3 sublots	100	5
≥ 100 and < 300	100 tons	100	5
≥ 50 and < 100	2 sublots	100	5
< 50	-	3-100*	5

*see Table 3

Table 2. Subdivision of cereal grains sublots according to lot size - flour meal, semolina, and flakes derived from maize and cereal based food for infants and young children and cereal based food for infants and young children destined for food aid programs

Lot size (t)	Maximum size or minimum number of sublots	Number of incremental samples	Minimum sample size (kg)
≥ 1500	500 tons	100	1
> 300 and < 1500	3 sublots	100	1
≥ 100 and < 300	100 tons	100	1
≥ 50 and < 100	2 sublots	100	1
< 50	-	3-100*	1

*see Table 4

2. Considering that the size of the lot is not always an exact multiple of the size of sublots, the size of the sublot may exceed the mentioned size by a maximum of 20%.

INCREMENTAL SAMPLE

3. The suggested minimum size of the incremental sample of cereal grains and cereal-based products should be 100 g for lots ≥ 0.5 tons.

4. For lots less than 50 tons of cereal grains and cereal-based products, the sampling plan must be used with 3 to 100 incremental samples, depending on the lot size. For very small lots (< 0.5 tons) 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 3. Number of incremental samples of cereal grains to be taken depending on the size of the lot- Maize grain, sorghum, polished rice and husked rice

Lot size (t)	Number of incremental samples	Minimum laboratory sample size (kg)
≤ 0.05	3	5
> 0.05 - ≤ 0.5	5	5
> 0.5 - ≤ 1	10	5
> 1 - ≤ 3	20	5
> 3 - ≤ 10	40	5
> 10 - ≤ 20	60	5
> 20 - < 50	100	5

Table 4. Number of incremental samples of cereal grains to be taken depending on the size of the lot - flour meal, semolina, flakes derived from maize and cereal based food for infants and young children and cereal based food for infants and young children destined for food aid programs

Lot size (t)	Number of incremental samples	Minimum laboratory sample size (kg)
≤ 0.05	3	1
> 0.05 - ≤ 0.5	5	1
> 0.5 - ≤ 1	10	1
> 1 - ≤ 3	20	1
> 3 - ≤ 10	40	1
> 10 - ≤ 20	60	1
> 20 - < 50	100	1

STATIC LOTS

5. A static lot can be defined as a large mass cereal grains and cereal-based products 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 cereal grains and cereal-based products 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.

6. 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.

7. 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 size (LT), incremental sample size (IS), aggregate sample size (AS) and the individual packing size (IP), as follows:

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

8. The sampling frequency (SF) is the number of packages sampled. All sizes 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 cereal grains and cereal-based products 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 cereal 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.

SEALING AND LABELLING OF SAMPLES

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 PRECAUTIONS

16. Sunlight should be excluded as much as possible during sample preparation, since aflatoxin gradually breaks down under the influence of ultra-violet light. Also, environmental temperature and relative humidity should be controlled and not favor mould growth and aflatoxin formation.

HOMOGENIZATION - GRINDING

17. As the distribution of aflatoxin is extremely non-homogeneous, laboratory samples should be homogenized by grinding the entire laboratory sample received by the laboratory. Homogenization is a procedure that reduces particle size and disperses the contaminated particles evenly throughout the comminuted laboratory sample.

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 (Annex I) approaches zero. After grinding, the grinder should be cleaned to prevent aflatoxin cross-contamination.

19. The use of vertical cutter mixer type grinders that mix and comminute the laboratory sample into a paste represent a compromise in terms of cost and fineness of grind or particle size reduction. A better homogenization (finer grind), such as a liquid slurry, can be obtained by more sophisticated equipment and should provide the lowest sample preparation variance.

TEST PORTION

20. The suggested mass of the test portion taken from the comminuted laboratory sample should be approximately 25 g. If the laboratory sample is prepared using a liquid slurry, the slurry should contain 25 g of sample mass.

21. 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.

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 is shown in Table 5. Utilizing this approach, laboratories would be free to use the analytical method most appropriate for their facilities.

Table 5. Method criteria for total aflatoxins in cereals, considering AFB1: AFB2+AFG1+AFG2 of 50:50.

Commodity	Analyte	ML (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Precision (%)	Minimal applicable range (µg/kg)	Recovery (%)
Maize grain	AF B1+B2+G1+G2	15	≤ 3	≤ 6	≤44	8.4 - 21.6	60-115
	AFB1	-	≤1.5	≤ 3.0	≤44	4.2 - 10.8	60-115
	AFB2	-	≤ 0.5	≤ 1	≤44	1.4 - 3.6	40-120
	AFG1	-	≤ 0.5	≤ 1	≤44	1.4 - 3.6	40-120
	AFG2	-	≤ 0.5	≤ 1	≤44	1.4 - 3.6	40-120
Maize flour, meal, semolina and flakes derived from maize; Sorghum grain; cereal-based foods for infants and young children for food aid programs	AF B1+B2+G1+G2	≤10	≤2	≤4	≤44	5.6 - 14.4	60-115
	AFB1	-	≤1.0	≤2.0	≤44	2.8 - 7.2	60-115
	AFB2	-	≤0.33	≤0.67	≤44	0.9 - 2.4	40-120
	AFG1	-	≤0.33	≤0.67	≤44	0.9 - 2.4	40-120
	AFG2	-	≤0.33	≤0.67	≤44	0.9 - 2.4	40-120
Husked Rice	AF B1+B2+G1+G2	20	≤4	≤8	≤44	11.2 - 28.8	60-115
	AFB1	-	≤2.0	≤4.0	≤44	5.6 - 14.4	60-115
	AFB2	-	≤0.67	≤1.33	≤44	1.9 - 4.8	60-115
	AFG1	-	≤0.67	≤1.33	≤44	1.9 - 4.8	60-115
	AFG2	-	≤0.67	≤1.33	≤44	1.9 - 4.8	60-115
Polished Rice; Cereal-based food for infants and young children	AF B1+B2+G1+G2	5	≤1	≤2	≤44	2.8 - 7.2	40-120
	AFB1	-	≤0.5	≤1	≤44	1.4 - 3.6	40-120
	AFB2	-	≤0.17	≤0.33	≤44	0.5 - 1.2	40-120
	AFG1	-	≤0.17	≤0.33	≤44	0.5 - 1.2	40-120
	AFG2	-	≤0.17	≤0.33	≤44	0.5 - 1.2	40-120

APPENDIX II
SUMMARY OF THE DISCUSSION IN THE EWG AND
ANALYSIS OF THE DATA/INFORMATION PROVIDED IN REPLY TO CL 2022/46-CF
(For information)

1. Due to high differences in analytical limits of detection (LODs) and quantification between methods available to determine total aflatoxins (AFT) content, determining the ratios of AF B1, B2, G1 and G2 in cereals and cereal-derived products is challenging, particularly when comparing results from different studies and when attempting to combine data for similar foods.

2. It was considered the upper bound maximum level (UB) concentrations, assuming that all values of the different congeners reported at concentrations below the limit of quantification are equal to the limit of quantification (LOQ).

3. In general, there are a great diversity of reported ratio of AFB1:AFB2:AFG1:AFG2 from different countries and food products. However, all countries informed a ratio of 50% or higher of AFB1:AFB1+AFB2+AFG1+AFG2. Table 1 provides information on response to CL 2022/46-CF on AFs ratios taking into account three different scenarios: 50%, 80% and 90% of AFB1:AFB1+AFB2+AFG1+AFG2.

4. Brazil reported data from 353 samples in which AFB1 was quantified (LOQ = 0.06 µg/kg), being: polished rice (72 samples), husked rice (4), maize grain (276) and maize flour (1). The percentage of B1 ranged from 18.4% to 99.8%, with mean of 88.5% and standard deviation of 13.3%. The percentage of samples in which the AFB1 concentration was higher than 50% of AFB1:AFB1+AFB2+AFG1+AFG2 were 95.8%, 100%, 96.4% and 100%, respectively, for the food commodities listed above. The percentage of samples in which the AFB1 concentration was greater than 80% of AFB1:AFB1+AFB2+AFG1+AFG2, values were 88.9%, 100%, 83.7% and 100%, respectively. The LOQ value was utilized for any congener without a quantifiable amount for summing AFB1+AFB2+AFG1+AFG2 value.

5. Canada reported that in Canadian surveys of aflatoxins in maize grain, corn flour, corn meal, husked (brown) rice, polished (white) rice, sorghum grain and grain-based infant cereals, AFB1 was the most often detected, while AFB2 and AFG1 were only occasionally detected, but when they were, they usually co-occurred with AFB1. While there were many samples in which only AFB1 was detected, those samples containing several aflatoxins typically had total aflatoxin concentrations comprising over 80% AFB1 with the remainder being AFB2 or a combination of AFB2 and AFG1. AFG2 was not detected.

6. Chile noted that among hundreds of samples analysed, most of their results were below the LOQ and so it was not possible to build a trend in relation to the ratio of AFs on their surveillance data.

7. The EU noted that there were only limited data available in which all four aflatoxins have been quantified separately and where at least one of the four aflatoxins has been quantified in relevant cereals and cereal products (280 samples). The data were presented in four groups: maize grain (19 samples), flour, meal, semolina and flakes derived from maize (46 samples), husked rice and polished rice (214 samples) and sorghum (1 sample). No samples of cereal-based foods for infants and young children were available in which all four aflatoxins were quantified separately. The percentage of samples in which AFB1 concentration was higher than 50% of AFB1:AFB1+AFB2+AFG1+AFG2 were 63.1%, 100%, 96.7% and 100% for those groups, respectively.

8. Japan reported that the data sent were obtained from those imported food which violated the ML for total AFs in Japan (33 samples of maize and 7 samples of maize meal/flour). For these samples total AFs were calculated in two methods: one assuming <LOQ=0; and the other assuming <LOQ=LOQ. When assuming <LOQ=LOQ for calculation, the total AFs can be >10 µg/kg. The percentage of samples in which AFB1 concentration was higher than 50% of AFB1:AFB1+AFB2+AFG1+AFG2 were 90.9% and 100%. Considering that AFB1 >80% of total AFs, percentage of samples will be 60.6 and 71.4%, respectively.

9. Republic of Korea informed that, for polished and brown rice, only aflatoxin AFB1 and AFB2 were detected, being 100% of AFB1 or AFB1 (>90%) and AFB2 (<10%) present. For maize, the ratio was AFB1:AFG1 (50%:50%). For sorghum the ratios were calculated with the mean of the samples; it appears that AFG1 was dominant regardless of storage conditions. However, it is difficult to find a typical ratio of aflatoxins in sorghum.

10. Saudi Arabia reported that the percentage of AFB1 in maize grain was 50%, in semolina and flakes derived from maize was 60%, and in husked and polished rice 65.4%. The AFB2 percentages were 50%, 40% and 35%, respectively. The total percentages of G1 and G2 were zero. This information was submitted by Saudi Arabia delegation however raw data was not submitted, therefore completed information on table 1 is not described.

11. The United States of America (USA)informed on two datasets. For a set of 10 naturally contaminated samples of human food products (maize grain and milled maize products) with total aflatoxins ≥ 10 ppb, the ratio of AFB1:AFB2:AFG1:AFG2 was 15.5:1:0.349:0.158. The percentage of B1 ranged from 84.7% to 95% with a mean of 90.7% and standard deviation of 2.95%. For a set of 155 naturally contaminated maize grain samples, the AFB1:AFB2:AFG1:AFG2 ratio was 16:1:0.013:0.044. The percentage of B1 ranged from 84.1% to 100% with a mean of 93.8% and standard deviation of 2.84%. AFB1 contributions of 90% or greater were reported for 93% and 70% of maize grain and milled maize products, respectively.

12. The received data shown that AFB1 is the most frequently present in contaminated samples and according to JECFA report[1], most of the available toxicological data is related to AFB1, the relative potency assigned to these congeners is AFB1 > AFG1 >> (AFB2, AFG2). Data submitted by countries are summarized in Table 1.

Table 1: Percentage of samples with AFB1 concentrations higher that 50%, 80% and 90% of AFB1:AFB1+AFB2+AFG1+AFG2 (AFB1 concentration > % total AFs)

Country Commodity (n)	AFB1 concentration > 50% total AFs % Samples (n)	AFB1 concentration > 80% total AFs % Samples (n)	AFB1 concentration > 90% total AFs % Samples (n)
Japan			
Maize grain (33)	90.9 (30)	60.6 (20)	3.0 (1)
Maize meal/flour (7)	100 (7)	71.4 (5)	14.3 (1)
Brazil			
Polished rice (72)	95.8 (69)	88.9 (64)	56.9 (41)
Husked rice (4)	100 (4)	100 (4)	75.0 (3)
Maize grain (276)	96.4 (266)	83.7 (231)	68.5 (189)
Maize products (1)	100 (1)	100 (1)	100 (1)
USA			
Maize grain and maize products (10)	100 (10)	100 (10)	*(*)
Maize grain (155)	100 (155)	100 (155)	*(*)
Saudi Arabia			
Maize grain (*)	100 (*)	*(*)	*(*)
Maize products (*)	100 (*)	*(*)	*(*)
Rice, polished / husked (*)	100 (*)	*(*)	*(*)
Republic of Korea			
Polished rice (*)	100 (*)	*(*)	*(*)
Husked rice (*)	100 (*)	*(*)	*(*)
Maize (*)	100 (*)	*(*)	*(*)
EU			
Maize grain (19)	63 (12)	*(*)	*(*)
Maize flour/meal (45)	100 (45)	*(*)	*(*)
Rice, polish /husked (213)	97 (206)	*(*)	*(*)
Sorghum (1)	100 (1)	*(*)	*(*)

(*) not informed

[1] WHO Food Additives Series, No. 74; FAO JECFA Monographs 19 bis Safety evaluation of certain contaminants in food: prepared by the eighty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). ISBN 978-92-4-166074-7

13. Data informed by several delegations showed that AFB1 is the most prevalent aflatoxin in cereal and cereal-based products. Different scenarios of AFB1 ratio occurrence were presented in order to find the best way to propose the performance criteria of the methods, considering that the maximum limits were established for total aflatoxins. For example, if the scenario of 90:10 ratio was selected for a ML of 15 µg/kg, the performance criteria of the method should achieve a LOQ of ≤ 5.4 µg/kg for AFB1. On the other hand, if the scenario 50:50 was adopted for the same ML, a LOQ of ≤ 3.0 µg/kg the method should be achieved for AFB1. Considering this information and also bearing in mind the highest toxicity of AFB1, it seems reasonable to adopt lower proportions of AFB1 ratio to the sum of total AFs. Additionally, a lower proportion rate, such as 50:50, would make the methods of analysis feasible, since they would not push the LOQs of AFB2, AFG1 and AFG2 to a level that will not be achievable for most countries.

14. Below, is shown three different scenarios[2]: 50:50, 80:20 and 90:10 ratio of AFB1: AFB2+AFG1+AFG2. Taking into account the discussion on the paragraph 16, the CCCF is invited to consider Scenario 1.

    a) Maize grain:

    Scenario 1: 50:50 of AFB1:AFB1+AFB2+AFG1+AFG2 ratio

Analyte	ML (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Precision (%)	Minimal applicable range (µg/kg)	Recovery (%)
AF B1+B2+G1+G2	15	≤ 3	≤ 6	≤44	8.4 - 21.6	60-115
AFB1	-	≤1.5	≤ 3.0	≤44	4.2 - 10.8	60-115
AFB2	-	≤ 0.5	≤ 1	≤44	1.4 - 3.6	40-120
AFG1	-	≤ 0.5	≤ 1	≤44	1.4 - 3.6	40-120
AFG2	-	≤ 0.5	≤ 1	≤44	1.4 - 3.6	40-120

Scenario 2: 80:20 of AFB1:AFB1+AFB2+AFG1+AFG2 ratio

Analyte	ML (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Precision (%)	Minimal applicable range(µg/kg)	Recovery (%)
AF B1+B2+G1+G2	15	≤ 3	≤ 6	≤44	8.4 - 21.6	60-115
AFB1	-	≤2.4	≤ 4.8	≤44	4.2 – 10.8	60-115
AFB2	-	≤ 0.2	≤ 0.4	≤44	1.4 - 3.6	40-120
AFG1	-	≤ 0.2	≤ 0.4	≤44	1.4 - 3.6	40-120
AFG2	-	≤ 0.2	≤ 0.4	≤44	1.4 - 3.6	40-120

Scenario 3: 90:10 of AFB1:AFB1+AFB2+AFG1+AFG2 ratio

Analyte	ML (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Precision (%)	Minimal applicable range (µg/kg)	Recovery (%)
AF B1+B2+G1+G2	15	≤ 3	≤ 6	≤44	8.4 - 21.6	60-115
AFB1	-	≤2.7	≤ 5.4	≤44	4.2 – 10.8	60-115
AFB2	-	≤ 0.1	≤ 0.2	≤44	1.4 - 3.6	40-120
AFG1	-	≤ 0.1	≤ 0.2	≤44	1.4 - 3.6	40-120
AFG2	-	≤ 0.1	≤ 0.2	≤44	1.4 - 3.6	40-120

    b) Maize flour, meal, semolina and flakes derived from maize; Sorghum grain; cereal-based foods for infants and young children for food aid programs

[2] The MLs are already approved by CCCF, taking into account the Guidelines for Establishing Numeric Values for Method Performance Criteria described in the Procedural Manual of Codex Alimentarius.

Scenario 1: 50:50 of AFB1:AFB1+AFB2+AFG1+AFG2 ratio

Analyte	ML (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Precision (%)	Minimal applicable range (µg/kg)	Recovery (%)
AF B1+B2+G1+G2	≤10	≤2	≤4	≤44	5.6 - 14.4	60-115
AFB1	-	≤1.0	≤2.0	≤44	2.8 - 7.2	60-115
AFB2	-	≤0.33	≤0.67	≤44	0.9 - 2.4	40-120
AFG1	-	≤0.33	≤0.67	≤44	0.9 - 2.4	40-120
AFG2	-	≤0.33	≤0.67	≤44	0.9 - 2.4	40-120

Scenario 2: 80:20 of AFB1:AFB1+AFB2+AFG1+AFG2 ratio

Analyte	ML (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Precision (%)	Minimal applicable range (µg/kg)	Recovery (%)
AF B1+B2+G1+G2	10	≤2	≤4	≤44	5.6 - 14.4	60-115
AFB1	-	≤1.6	≤3.2	≤44	2.8 - 7.2	40-120
AFB2	-	≤0.13	≤0.27	≤44	0.9 - 2.4	40-120
AFG1	-	≤0.13	≤0.27	≤44	0.9 - 2.4	40-120
AFG2	-	≤0.13	≤0.27	≤44	0.9 - 2.4	40-120

Scenario 3: 90:10 of AFB1:AFB1+AFB2+AFG1+AFG2 ratio

Analyte	ML (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Precision (%)	Minimal applicable range (µg/kg)	Recovery (%)
AF B1+B2+G1+G2	10	≤2	≤4	≤44	5.6 - 14.4	60-115
AFB1	-	≤1.8	≤3.6	≤44	2.8 - 7.2	40-120
AFB2	-	≤0.07	≤0.13	≤44	0.9 - 2.4	40-120
AFG1	-	≤0.07	≤0.13	≤44	0.9 - 2.4	40-120
AFG2	-	≤0.07	≤0.13	≤44	0.9 - 2.4	40-120

c) Husked Rice

Scenario 1: 50:50 of AFB1:AFB1+AFB2+AFG1+AFG2 ratio

Analyte	ML (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Precision (%)	Minimal applicable range (µg/kg)	Recovery (%)
AF B1+B2+G1+G2	20	≤4	≤8	≤44	11.2 - 28.8	60-115
AFB1	-	≤2.0	≤4.0	≤44	5.6 - 14.4	60-115
AFB2	-	≤0.67	≤1.33	≤44	1.9 - 4.8	60-115
AFG1	-	≤0.67	≤1.33	≤44	1.9 - 4.8	60-115
AFG2	-	≤0.67	≤1.33	≤44	1.9 - 4.8	60-115

Scenario 2: 80:20 of AFB1:AFB1+AFB2+AFG1+AFG2 ratio

Analyte	ML (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Precision (%)	Minimal applicable range (µg/kg)	Recovery (%)
AF B1+B2+G1+G2	20	≤4	≤8	≤44	11.2 - 28.8	60-115
AFB1	-	≤3.2	≤6.4	≤44	5.6 - 14.4	60-115
AFB2	-	≤0.27	≤0.53	≤44	1.9 - 4.8	60-115
AFG1	-	≤0.27	≤0.53	≤44	1.9 - 4.8	60-115
AFG2	-	≤0.27	≤0.53	≤44	1.9 - 4.8	60-115

Scenario 3: 90:10 of AFB1:AFB1+AFB2+AFG1+AFG2 ratio

Analyte	ML (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Precision (%)	Minimal applicable range (µg/kg)	Recovery (%)
AF B1+B2+G1+G2	20	≤4	≤8	≤44	11.2 - 28.8	60-115
AFB1	-	≤3.6	≤7.2	≤44	5.6 - 14.4	60-115
AFB2	-	≤0.13	≤0.27	≤44	1.9 - 4.8	60-115
AFG1	-	≤0.13	≤0.27	≤44	1.9 - 4.8	60-115
AFG2	-	≤0.13	≤0.27	≤44	1.9 - 4.8	60-115

d) Polished Rice and cereal-based food for infants and young children

Scenario 1: 50:50 of AFB1:AFB1+AFB2+AFG1+AFG2 ratio

Analyte	ML (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Precision (%)	Minimal applicable range (µg/kg)	Recovery (%)
AF B1+B2+G1+G2	5	≤1	≤2	≤44	2.8 - 7.2	40-120
AFB1	-	≤0.5	≤1	≤44	1.4 - 3.6	40-120
AFB2	-	≤0.17	≤0.33	≤44	0.5 - 1.2	40-120
AFG1	-	≤0.17	≤0.33	≤44	0.5 - 1.2	40-120
AFG2	-	≤0.17	≤0.33	≤44	0.5 - 1.2	40-120

Scenario 2: 80:20 of AFB1:AFB1+AFB2+AFG1+AFG2 ratio

Analyte	ML (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Precision (%)	Minimal applicable range (µg/kg)	Recovery (%)
AF B1+B2+G1+G2	5	≤1	≤2	≤44	2.8 - 7.2	40-120
AFB1	-	≤0.8	≤1.6	≤44	1.4 - 3.6	40-120
AFB2	-	≤0.07	≤0.13	≤44	0.5 - 1.2	40-120
AFG1	-	≤0.07	≤0.13	≤44	0.5 - 1.2	40-120
AFG2	-	≤0.07	≤0.13	≤44	0.5 - 1.2	40-120

Scenario 3: 90:10 of AFB1:AFB1+AFB2+AFG1+AFG2 ratio

Analyte	ML (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Precision (%)	Minimal applicable range (µg/kg)	Recovery (%)
AF B1+B2+G1+G2	5	≤1	≤2	≤44	2.8 - 7.2	40-120
AFB1	-	≤0.9	≤1.8	≤44	1.4 - 3.6	40-120
AFB2	-	≤0.03	≤0.07	≤44	0.5 - 1.2	40-120
AFG1	-	≤0.03	≤0.07	≤44	0.5 - 1.2	40-120
AFG2	-	≤0.03	≤0.07	≤ 44	0.5 - 1.2	40-120

SAMPLING PLAN

15. It was not considered appropriate to directly align sampling plans for aflatoxins in cereals and cereal-based products with those for DON and fumonisins. Aflatoxins distribution on cereal and cereal products may result in a higher degree of heterogeneity and therefore measurement error relative to these other mycotoxins. Based on the modelled scenarios from the FAO Mycotoxin Sampling Tool[3], the total variance in relation to the applicable Codex ML is notably greater for aflatoxins compared to DON and fumonisins. Table 2 shows a comparison among different scenarios of sampling plans for DON, fumonisins and aflatoxins in maize grain on the respectively maximum level established, using the FAO Mycotoxin Sampling Tool.

[3] http://tools.fstools.org/mycotoxins/ - version 1.1.

Table 2: FAO Mycotoxin Sampling Tool input parameters and output data generated using different laboratory sample sizes for DON, fumonisins, and aflatoxins in maize/'corn, shelled'.

Sampling plan parameters[1]	**DON**					**Fumonisins**					**Aflatoxins**				
Codex ML/'Regulatory Limit'	2 mg/kg					4 mg/kg					15 µg/kg				
Laboratory sample size (kg)	1	5	5	10	10	1	5	5	10	10	1	5	5	10	10
Test portion size (g)	25	25	50	25	50	25	25	50	25	50	25	25	50	25	50
FAO Mycotoxin Sampling Tool output[2]															
Sampling variance	0.77	0.15	0.15	0.08	0.08	0.42	0.08	0.08	0.04	0.04	182	36	36	18	18
Sample preparation variance	0.09	0.09	0.04	0.09	0.04	0.10	0.10	0.05	0.10	0.05	78	78	39	78	39
Analysis variance	0.02	0.02	0.02	0.02	0.02	0.21	0.21	0.21	0.21	0.21	6.6	6.6	6.6	6.6	6.6
Total variance at Codex ML/'Regulatory Limit'	0.88	0.26	0.21	0.19	0.14	0.73	0.39	0.34	0.35	0.30	267	121	82	103	64
Probability of mischaracterizing a lot as "compliant" with the ML[3]	59	55	55	54	54	54	53	53	53	53	64	60	58	59	57
Other Parameters															
Total error[4] at Codex ML /'Regulatory Limit'	0.94	0.51	0.46	0.44	0.37	0.85	0.62	0.58	0.59	0.55	16	11	9.1	10	8.0

[1]Common parameters input into the FAO Mycotoxin Sampling Tool include: kernel count of 3000 per kg; 'among lab' analytical variance type; number of laboratory samples of 1, number of aliquots of 1.

[2]Sampling variance, Sample preparation variance, Analysis variance and Total variance (the sum of variance due to sampling, sample preparation and analysis) are generated by the FAO Mycotoxin Sampling Tool and can be viewed under the 'Table Results' tab. The Total variance refers to the variance in relation to the 'Regulatory Limit' input by the user in the Tool.

[3]Probability of mischaracterizing a lot as "compliant" with an ML is to "incorrectly" accepting a lot/consignment/cargo of maize with a true concentration that is greater than the Codex ML/'Regulatory Limit' input by the user in the Tool; this may represent a potential health risk in the case of health-based MLs. The probability of "incorrectly" rejecting a consignment/lot/cargo with a true concentration that is less than the 'Regulatory Limit' input by the user in the Tool can be calculated as (100-Probability of "incorrectly" accepting a lot; this may represent an economic risk.

[4]Total error is the square root of the Total variance at the Codex ML/'Regulatory Limit'.

16. It is possible to observe that total variance of DON and fumonisins is much lower than aflatoxins. It can partially be explained by its ML being much higher than the aflatoxins ML (1000x higher). Additionally, it also important to point out that aflatoxins are not homogeneously distributed in lots, what makes the sampling process an important source of variance.

17. Observing the data in Table 2, the different variances among aflatoxins, fumonisins and DON is considerable. This same difference is not observed when evaluating the possibility of lot rejection. The possibility of lots misclassification in different scenarios varies from 57-60% for aflatoxins, 53-54% for fumonisins and 54-59% for DON.

18. Using the online FAO Mycotoxin Sampling Tool, different scenarios were simulated with laboratory sample sizes of 1, 5, 10 and 30 kg and test portion sizes of 25 and 50 g to test maize grain sampling plan, as shown in Figure 1.

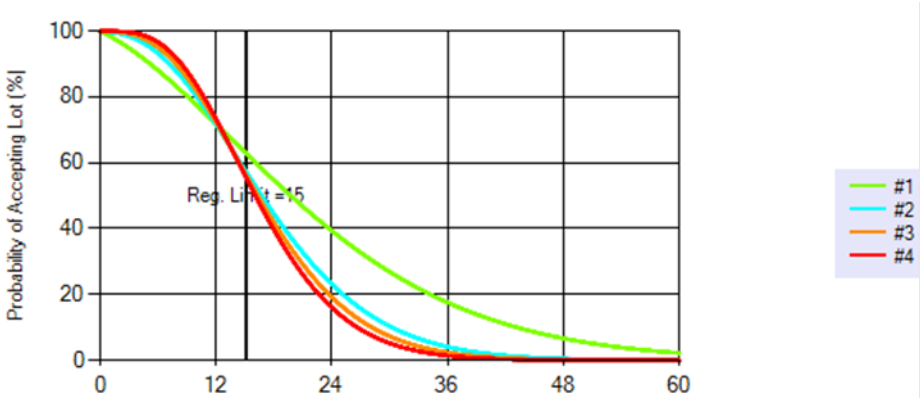

Figure 1: Probability of accepting a lot of corn, shelled (%) for different laboratory sample mass, considering test portion size of 50 g and accept/reject limit of 15 µg/kg. * Laboratory sample size: #1 = 1kg, #2 = 5 kg, #3 = 10 kg and #4 = 30kg

19. It was observed that using 1 kg as laboratory sample had a very distinct behaviour compared to the other sample sizes. It was also observed that 30 kg as laboratory sample size had a very similar behaviour compared to 10 kg. For these reasons, those options were not considered adequate. Another simulation was done, taking into account different test portion sizes of 25 g and 50g, as shown on Figure 2.

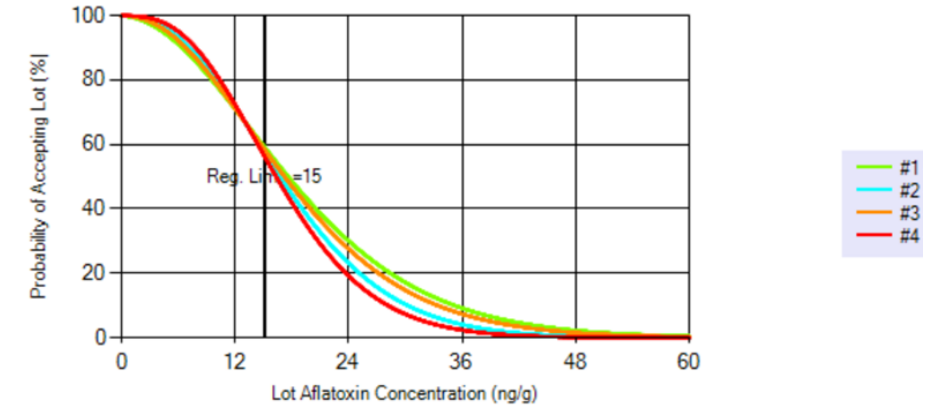

Figure 2: Probability of accepting a lot of corn, shelled (%) considering 5 and 10 kg as laboratory sample mass and either 25g or 50g as test portion. Accept/reject limit of 15 µg/kg. * Laboratory sample size: #1 = 5 kg, #2 = 5 kg, #3 = 10 kg and #4 = 10 kg; Test portion: #1 = 25 g, #2 = 50 g, #3 = 25 g and #4 = 50 g

20. Figure 2 shows that lower levels of contamination up to the LM established by the CCCF and above 48 µg/kg, the sampling plans performed similarly. Otherwise, lots with contamination levels between 15 and 48 µg/kg, the OC #4 (10 kg as laboratory sample size and 50g as test portion size) is the one that has a lower probability of misclassifying the lot. Considering the applicability and cost of analysis, it seems reasonable to accept the OC #1 (5 kg as laboratory sample size and 25g as test portion size).

APPENDIX III
Comments received in reply to CL 2022/46-CF
(For information)
Original Language Only

GENERAL AND SPECIFIC COMMENTS

MEMBER/OBSERVER - COMMENT
Canada Canada considered surveys of aflatoxins in corn (maize) grain, corn flour, corn meal, husked (brown) rice, polished (white) rice, sorghum grain and grain-based infant cereals conducted by a variety of Canadian government departments and agencies in an effort to provide information on the ratios of aflatoxin (AF) B1, B2, G1 and G2 in these commodities. Both within (i.e. for the four AFs) and between these Canadian surveys, varying analytical limits of detection (LODs) and quantification (LOQs) are reported. For example, the LODs (or LOQs in cases where no LODs were available) within some surveys differ by 10-fold (e.g. 0.01 to 1.0 ng/g) and between surveys by up to 1000-fold (i.e. 0.002 to 4.0 ng/g). These differences make determining the ratios of AF B1, B2, G1 and G2 in cereals and cereal-derived products challenging, particularly when comparisons are made between studies and when attempting to combine data for similar foods from several studies. In general, in the various Canadian surveys for the food commodities noted above, AFB1 was the most often detected, while AFB2 and AFG1 were only occasionally detected but when they were, they usually co-occurred with AFB1. However, the positive detection rate of AFB1 depended on the LOD of the specific survey, ranging from 0.2% in surveys with higher LODs to 58% in surveys with the lowest LODs. While there were many samples in which AFB1 only was detected, those samples containing several aflatoxins typically had total aflatoxin concentrations comprised of over 80% AFB1 with the remainder being AFB2 or a combination of AFB2 and AFG1. AFG2 was not detected in the Canadian data described above, but this may be a result of the higher relative LOD reported for AFG2 compared to the other three AFs in some of these surveys. Canada has submitted data from Canadian surveys conducted within the past 10 years to the Global Environmental Monitoring System (GEMS/Food) database, as per the instructions in past Codex Calls for Data. The LODs (and LOQs when LODs were not available) of these Canadian surveys range from 0.06 ng/g to 4.0 ng/g depending on the survey, year and analyte. Canada notes that surveys with higher LODs (or LOQs) may be of limited use in establishing quantitative ratios between the four aflatoxins. It may therefore be helpful for the EWG to decide upon a maximum LOD (or LOQ, as required) above which data would not be included in the determination of aflatoxin ratios in the cereal grains under consideration. If additional guidance is provided by the electronic working group (EWG) in this regard, Canada could, upon request, support the EWG Chair in determining the AF ratios based on Canadian monitoring data. In addition to the Canadian data in the GEMS/Food database, Canada has two somewhat older surveys of cereal-based foods which may provide useful information. While these surveys fall outside the period of the past 10 years typically requested in the Codex Calls for Data, they employed an LOD of 0.002 ng/g and thus may be of greater use in establishing aflatoxin ratios. The first survey measured AF B1, B2, G1 and G2 in cereal-based foods for infants and young children (Tam et al. 2006), while the second includes measurements of aflatoxins B1 and B2 in a variety of types of rice, including both husked and polished rice (Health Canada, unpublished data). If these data are of interest to the EWG Chair, Canada could provide the raw data from these surveys or a summary of aflatoxin ratios in the relevant foods to the EWG Chair, upon request. Canadian Government References (raw data available upon request to the Canadian delegation to CCCF) Tam J, Mankotia M, Mably M, Pantazopoulos P, Neil RJ, Calway P and Scott PM. 2006. Survey of breakfast and infant cereals for aflatoxins B1, B2, G1 and G2. Food Additives and Contaminants 23(7): 693-699.

MEMBER/OBSERVER - COMMENT
Other references that may provide information in aflatoxin distribution in cereal grains Bullerman, LB and Bianchini, A. 2007. Stability of mycotoxins during food processing. International J. Food Microbiology. 119: 140-146. Castells M, Ramos A, Sanchis V and Marin S. 2007. Distribution of total aflatoxins in milled fractions of hulled rice. J. Agric. Food Chem. 55: 2760-2764. Schaarschmidt, S and Fauhl-Hassek, C. 2019. Mycotoxins during the Processes of Nixtamalization and Tortilla Production. Toxins. 11. The pre-CCCF15 virtual working group (VWG) recommended to CCCF15 (CF15/CRD9 and REP22/15CF, para. 153) to "consider harmonizing the sampling plans for maize grain and flour, meal, semolina and flakes derived from maize with the sampling plan for DON and fumonisins and the sampling plan for cereal-based food for infants and young children with the sampling plan for DON in cereal-based foods for infants and young children, when appropriate." Canada does not consider it appropriate to directly align sampling plans for aflatoxins in cereals and cereal-derived products with those for DON and fumonisins. DON and fumonisins are predominantly formed in the field during plant development, whereas aflatoxin is produced during plant development and storage. Canada supports the points noted in REP22/15CF, para. 152, that sampling plans for total aflatoxins should be adjusted, as needed, in order to take into consideration the unique quality of aflatoxins to be produced both in the field and during storage, which results in a higher degree of heterogeneity and therefore measurement error relative to the other mycotoxins. Using the online FAO Mycotoxin Sampling Tool (http://tools.fstools.org/mycotoxins/ - version 1.1.) ('the Tool'), Canada has demonstrated the impacts on the variance and likelihood of misclassifying consignments of maize in relation to the Codex ML for each DON, fumonisins and aflatoxins when different input values are used for the masses of the laboratory sample (1 to 10 kg) and test portion (25 to 50 g) relative to the values of these parameters in the existing sampling plan for DON in maize and other cereal grains (≥ 1 kg and 25 g, respectively) (GSCTFF, 2019; page 34). This type of analysis is possible as sampling and other experimentally-derived variance data for fumonisins, DON and aflatoxins in shelled maize are incorporated into the Tool. The information generated by the Tool indicates that it is not appropriate to harmonize the sampling plans for maize grain and maize-derived products with the sampling plan for DON and fumonisins. The output from the FAO Mycotoxin Sampling Tool includes tables and figures that Canada has emailed to the Codex Secretariat. This information shows that for all three sets of modelled scenarios, the total variance and total error in relation to the applicable Codex ML is notably greater for aflatoxins compared to DON and fumonisins. The total error at the respective MLs ranged from 53-109% for aflatoxins, 19-41% for DON, and 14-21% for fumonisins. Furthermore, the variance estimates produced by the Tool also indicate the relative contributions of sampling, sample preparation, and the analytical test methods on the total variance of the mycotoxin test procedure. The results demonstrate that the most efficient way to minimize variance and the likelihood of misclassifying compliance with the ML for aflatoxins in maize is to increase the size of the laboratory sample relative to those recommended in the existing sampling plans for DON and fumonisins. Additional improvements can be made by increasing the test portion size. This approach: i) focuses on the parameters, that is sampling and sample preparation, that contribute most significantly to the total variance (Whitaker and Dickens, 1979; Whitaker et al., 2010; ii) is less resource intensive than increasing the number of laboratory samples; and iii) has been used for other mycotoxin-food commodity combinations (Broggi et al., 2007; Kumphanda et al., 2019; Pitt et al., 2018; Tittlemier et al., 2013; United States Department of Agriculture, 2022). It should be noted that increasing the degree of comminution of the laboratory sample also reduces variance, but the degree of comminution is not incorporated into the Tool as a user-defined parameter.

MEMBER/OBSERVER - COMMENT
Canada recommends that CCCF determine the acceptable degree of total error and target likelihood of misclassifying a consignment relative to an existing Codex ML and consider targeting the same values in each Codex sampling plan for mycotoxins in cereal grains and cereal-derived foods. If CCCF agreed that XX% total error is acceptable, then the recommended laboratory sample size would be expected to vary between sampling plans for different mycotoxin-cereal grain (or cereal grain-based foods) combinations. References Cited: Broggi, L.E., Pacin, A.M., Gasparovic, A., Sacchi, C., Rothermel, A., Gallay, A. and Resnik, S., 2007. Natural occurrence of aflatoxins, deoxynivalenol, fumonisins and zearalenone in maize from Entre Rios Province, Argentina. Mycotoxin Research 23: 59-64. Kumphanda, J., Matumba, L., Whitaker, T.B., Kasapila, W. and Sandahl, J., 2019. Maize meal slurry mixing: an economical recipe for precise aflatoxin quantitation. World Mycotoxin Journal 12: 203-212. Pitt, J.I., Boesch, C., Whitaker, T.B. and Clarke, R., 2018. A systematic approach to monitoring high preharvest aflatoxin levels in maize and peanuts in Africa and Asia. World Mycotoxin Journal 11: 485-491. Tittlemier, S.A., Gaba, D. and Chan, J.M., 2013. Monitoring of Fusarium Trichothecenes in Canadian Cereal Grain Shipments from 2010 to 2012. Journal of Agricultural and Food Chemistry 61: 7412-7418. United States Department of Agriculture, 2022. Mycotoxin Test Kit Evaluation. Available at: https://www.ams.usda.gov/services/fgis/standardization/tke Accessed June 27, 2022. Whitaker, T.B. and Dickens, J.W., 1979. Variability associated with testing corn for aflatoxin. Journal of the American Oil Chemists' Society 56: 789-794. Whitaker, T.B., Slate, A.B., Doko, M.B., Maestroni, B.M. and Cannavan, A., 2010. Sampling Procedures to Detect Mycotoxins in Agricultural Commodities. Springer.
Chile Chile agradece la oportunidad de presentar observaciones sobre los planes de muestreo para el total de aflatoxinas en cereales y productos a base de cereales, incluidos alimentos para lactantes y niños pequeños. Al respecto, Chile quisiera comentar lo siguiente: - Chile ha realizado varios cientos de análisis para determinar la presencia de aflatoxinas totales en alimentos como los considerados en esta carta circular a lo largo de una decada, no obstante lo anterior, la mayor parte de nuestros resultados arrojan resultados inferiores al límite de cuantificación del método usado, por lo que no nos es posible construir una tendencia en relación al ratio entre las aflatoxinas, a partir de nuestra vigilancia. - Dicho lo anterior, quisiéramos compartir lo indicado por el artículo de Sulyok et.al. “Uncommon occurrence ratios of aflatoxin B1, B2, G1 and G2 in maize and groundnuts from Malawi”, publicado en Mycotoxin Research en septiembre del 2014, donde se indica que, se han reportado a modo general diferentes ratios de ocurrencia de las cuatro aflatoxinas, y que todos coinciden en que la concentración de AFB1 por lo general, supera la mitad de la suma de las aflatoxinas y que AFB2 y AFG2 se encuentran en las concentraciones más bajas. - El artículo citado también indica que, la proporción de las concentraciones de aflatoxinas B y G estarían muy influenciadas por los ciclos de temperatura, lo que podría implicar que los ratio de concentración de AFB y AFG pudieran ser dependientes de la región, no obstante no dispone de datos de ocurrencia en este aspecto.

MEMBER/OBSERVER - COMMENT

European Union

European Union Competence - European Union Vote

The European Union (EU) wishes to provide following information and comments in reply to the CL 2022/46-CF

A) Information /data on the typical ratio of the four aflatoxins in naturally contaminated samples of maize grain; flour, meal, semolina and flakes derived from maize, husked rice, polished rice and sorghum grain and cereal-based foods for infants and young children.

The EU has only limited data available in which all four aflatoxins have been quantified separately and where at least one of the four aflatoxins has been quantified in relevant cereals and cereal products (280 samples). The data are presented in four groups: maize grain (19 samples), flour, meal, semolina and flakes derived from maize (46 samples), husked rice and polished rice (214 samples) and sorghum (1sample). No samples of cereal based for infants and young children are available in which all four aflatoxins were quantified separately.

Table 1: maize grain (19 samples)

	No of samples > LOQ	Range of ratio sum/AFsingle	Comments (details on the range of ratio)
AFB1	19	1.0 – 4.0	5 with ratio 1.0, 3 with ratio 1.1, 1 with ratio 1.2, 3 with ratio 1.7, 2 with ratio 2.8, 2 with ratio 3.0, and 1 with ratio 4.0
AFB2	12	2.5 - 21	3 with ratio 2.5, 1 with ratio 4.0, 2 with ratio 4.5, 2 with ratio 4.6, 1 with ratio 6.3, 1 with ratio 8.1, 1 with ration 18.0 and 1 with ratio 21.0
AFG1	5	4.0 - 4.6	1 with ratio 4.0, 2 with ratio 4.5, 2 with ratio 4.6
AFG2	5	4.0 - 4.6	1 with ratio 4.0, 2 with ratio 4.5, 2 with ratio 4.6

Table 2: flour, meal, semolina and flakes derived from maize (46 samples)

	No of samples > LOQ	Range of ratio sum/AFsingle	Comments (details on the range of ratio)
AFB1	45	1.0 – 2.0	37 with ratio 1.0, 5 with ratio 1.1, 1 with ratio 1.2, 1 with ratio 1.3 and 1 with ratio 2.0
AFB2	6	8.4 – 19.0	1 with ratio 8.4, 1 with ratio 13.7, 1 with ratio 14.0, 1 with ratio 14.6, 1 with ratio 15.2 and 1 with ratio 19.0
AFG1	6	1.0 – 35.1	1 with ratio 1.0, 1 with ratio 2.3, 1 with ratio 9.8, 1 with ratio 13.7, 1 with ratio 19.7 and 1 with ratio 35.1
AFG2	0	--	

MEMBER/OBSERVER - COMMENT

Table 3: husked rice and polished rice (214 samples)

	No of samples > LOQ	Range of ratio sum/AFsingle	Comments (details on the range of ratio)
AFB1	213	1.0 – 4.0	120 with ratio 1.0, 59 with ratio 1.1, 2 with ratio 1.2, 3 with ratio 1.3, 2 with ratio 1.4, 3 with ratio 1.5, 2 with ratio 1.6, 1 with ratio 1.7, 14 with ratio 2.0, 1 with ratio 2.2, 1 with ratio 2.5, 1 with ratio 3.8 and 4 with ratio 4.0
AFB2	85	2.0 – 17.7	14 with ratio 2.0, 5 with a ratio between 2 - 3, 4 with a ratio 4.0, 14 with a ratio between 4.5 - 10, 40 with a ratio between 10 - 15 and 8 with a ration between 15 and 17.7
AFG1	9	1.4 – 43.7	1 with ratio 1.4, 1 with ratio 1.7, 4 with ratio 4.0, 1 with ratio 5.5, 1 with ratio 8.3 and 1 with ratio 43.7
AFG2	11	1.0 -5.5	1 with ratio 1.0, 1 with ratio 2.7, 1 with ratio 3.3, 1 with ratio 3.7, 5 with ratio 4.0, 1 with ratio 4.3 and 1 with ratio 5.5

Table 4: sorghum (1 sample)

	No of samples > LOQ	Range of ratio sum/AFsingle	Comments (details on the range of ratio)
AFB1	1	1.6	
AFB2	1	11.0	
AFG1	1	3.7	
AFG2	0	--	

B) Information on the variation in sampling, sampling preparation and analysis for husked rice, polished rice and sorghum grain.

In a technical report of the European Normalisation Committee, a comparison of the level of homogenisation between dry milling of a sample versus the slurry method was performed. (CEN/TR 15298:2006 - Foodstuffs - Sample comminution for mycotoxins analysis - Comparison between dry milling and slurry mixing). The matrices however used for this comparison were not husked rice, polished rice and sorghum grain and also not other cereals or cereal based products (but peanuts and tree nuts). It was concluded that slurries contain smaller particles than dry milled samples and thus generate the lowest possible coefficients of variation (CV) values which in turn leads to better sample homogenisation.

As regards the performance criteria to be applied in case the maximum level applies to a sum of different components, the EU is of the opinion that in this case the same performance criteria apply to both the sum and the individual components of the sum.

MEMBER/OBSERVER - COMMENT
C) Comments on sampling plans for AFT for maize grain and flour, meal, semolina, and flakes derived from maize, as well as for cereal-based foods for infants and young children. The EU is of the opinion that the sampling plan and decision rule as already established in CXS 193-1995 for the control of Codex MLs for deoxynivalenol (for cereal grains (wheat, maize and barley) destined for further processing; for flour, meal, semolina and flakes derived from wheat, maize or barley; for cereal-based foods for infants and young children) and for fumonisins (B1 + B2) (maize grain, maize flour and maize meal). are also applicable for the control of aflatoxins in maize grain and flour, meal, semolina and flakes derived from maize as well for the control of aflatoxins in cereal-based foods for infants and young children. The sampling provisions for the control of deoxynivalenol in cereal grains (wheat, maize and barley) are also applicable for the control of aflatoxins in husked rice, polished rice and sorghum grain. From an enforcement point of view it is important that the sampling procedures for the control of mycotoxins in cereals and cereal based products are aligned so that the same representative sample of the lot can be used for the control of compliance with maximum levels for several mycotoxins at the same time as there are multi-mycotoxin methods of analysis available that enable to analyse reliably several mycotoxins at sufficient level of sensitivity and compliant with the analytical performance criteria established for the individual mycotoxins. In that context, it is appropriate to consider this sampling procedure also for the control of the Codex MLs for ochratoxin A in wheat barley and rye.
Japan We submmit following information concerring imported foods. ○Explanation about the submitted Data https://docs.google.com/document/d/1fjbSq0Qa39zmlIUBpK3rLn1L7-ZY6t5T/edit?usp=sharing&ouid=116692258879162744514&rtpof=true&sd=true ○Data on aflatoxins ratio in maiz https://docs.google.com/spreadsheets/d/167CmwZEgm5YSWhH7vThAzbzVeoOnD_zZ/edit?usp=sharing&ouid=116692258879162744514&rtpof=true&sd=true
Republic of Korea We submit following information for the request a : information/data on the typical ratio of the four aflatoxins in naturally contaminated samples of maize grain; flour, meal, semolina and flakes derived from maize, husked rice, polished rice, sorghum grain and cereal-based foods for infants and young children. – Polished and brown rice: only aflatoxin B1 & B2 were detected. Generally, either 100% of AFB1 or AFB1 (>90%) and AFB2 (<10%). – Maize: the trend was AFB1:AFG1=50%:50%. – Sorghum: the ratios calculated with the mean of the samples appear that AFG1 was dominant regardless of storage conditions. However, there were many individual samples which ratios did not match with each mean under the condition (refer to the reference). It would be difficult to find a typical ratio of aflatoxins in sorghum.

MEMBER/OBSERVER - COMMENT
Saudi Arabia Knowledge of the aflatoxins ratio and distribution in food may affect the choice of suitable aflatoxin quantitation methods and appropriate regulations in food. Although there has been some discrepancy in the reported prevalence rates of the four aflatoxins, all sources agree that B1 concentration typically surpasses 50% of the total aflatoxins and that B2 and G2 occur at the lowest concentrations. The results of many experiments suggest that temperature cycling and population ratios of different fungal strains on various matrices have a significant impact on the ratio of aflatoxins concentrations that are produced. These findings show that the concentration ratios of aflatoxins B1,B2,G1, and G2 could be depending on geographic location; nevertheless, there are very little occurrence data on this particular . In the table (1) data on the typical ratio of the four aflatoxins in naturally contaminated samples of maize grain, semolina and flakes derived from maize and rice ,husked rice, polished rice. Saudi Arabia would like to add information related to products such as maize grain, semolina and flakes derived from maize, and Rice ,husked rice, polished rice that have total aflatoxin100%. However, B1 (%) of maize grain is 50%, and semolina and flakes derived from maize is 60%, and 65.4% of Rice ,husked rice, polished rice. B2 (%) of maize grain is 50% and 40% of semolina and flakes derived from maize, and 34.5% of Rice ,husked rice, polished rice. The total percentages of G1 and G2 are zero.
United States of America The United States appreciates the opportunity to provide comments in response to CL 2022/46-CF, which requests comments on a) information/data on the typical ratio of the four aflatoxins in naturally contaminated samples of maize grain; flour, meal, semolina and flakes derived from maize, husked rice, polished rice, sorghum grain and cereal-based foods for infants and young children, and b) information on the variation in sampling, sampling preparation and analysis for husked rice, polished rice and sorghum grain. Information on the observed aflatoxin isomer ratios (B1:B2:G1:G2) in maize and maize products is provided below based on samples analyzed by the US Food and Drug Administration and USDA Federal Grain Inspection Service (FGIS): • Maize and milled maize product samples: 15.5:1:0.349:0.158 o This ratio is based on analysis by FDA of 10 naturally contaminated maize and milled maize product samples (with total aflatoxins ≥ 10 ppb) that were analyzed using an HPLC/Fluorescence method based on AOAC 991.31 with limits of quantitation (LOQ) of 0.48-6.2 ppb B1, 0.16-1.05 ppb B2, 0.2-0.72 ppb G1, and 0.06-0.32 ppb G2. o The percentage of B1 ranged from 84.7% to 95% with a mean of 90.7% and standard deviation of 2.95%. o The percentage of B2 ranged from 3.7% to 8.5% with a mean of 6.4% and a standard deviation of 1.88%. o The LOQ value was utilized for any isomer without a quantifiable amount. • Maize samples: 16:1:0.013:0.044 o This ratio is based on analysis by FGIS of 155 naturally contaminated maize samples that were analyzed using an HPLC/Fluorescence method based on AOAC 994.08 with limits of detection (LOD) of 0.15 ppb B1, 0.045 ppb B2, 0.30 ppb G1, and 0.060 ppb G2. o The percentage of B1 ranged from 84.1% to 100% with a mean of 93.8% and standard deviation of 2.84%. o The percentage of B2 ranged from 0% to 15.9% with a mean of 5.9% and a standard deviation of 2.73%. o The LOD value was utilized for any isomer without quantifiable amount. We do not currently have data on aflatoxin isomer ratios for husked rice, polished rice, sorghum grain and cereal-based foods for infants and young children.

APPENDIX IV

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