CODEX ALIMENTARIUS COMMISSION

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World Health Organization

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REPORT OF THE PRE-SESSION WORKING GROUP ON SAMPLING PLANS FOR TOTAL AFLATOXINS IN CERTAIN CEREALS AND CEREAL-BASED PRODUCTS INCLUDING FOODS FOR INFANTS AND YOUNG CHILDREN

(Prepared by Brazil as Chair of the Electronic Working Group)

1. A physical meeting was held prior to CCCF16 chaired by Brazil to discuss agenda item 7 - Sampling plans for total aflatoxins in certain cereals and cereal-based products including foods for infants and young children. The Electronic Working Group (EWG) chair presented the key points of the discussions occurred in the EWG. Aflatoxins has a heterogenous distribution profile and AFB1, AFB2, AFG1 and AFG2 are not equally distributed in different food commodities.

2. Based on comments received in reply to 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, the EWG chair recommended that a ratio of 50:50 be considered for AFB1: AFB2 + AFG1 + AFG2.

3. During the EWG discussions, it was observed that it was not possible to align the sampling plan for aflatoxin in maize with the one for fumonisin and DON, but that a laboratory sample weight of 5 kg could have a good balance between practicability and performance. Also, there was general agreement that comminution of grain will occur during processing and will reduce the heterogeneity of the materials with respect to Afs, which supports the alignment of sampling plans for DON and Fumonisins in case of maize flour, maize meal and cereal-based foods for infant and young children with the sampling plans for aflatoxins in the same commodities.

4. Regarding rice and sorghum, she discussed that rice grain is smaller than maize, having approximately 50,000 kernels in 1kg as opposed to 3,000 kernels/kg for wheat.

5. Considering comments received in reply to CL 2023/20-CF, a few editorial amendments were made on the sampling plans for total aflatoxins in certain cereals and cereal-based products of Appendix I - CX/CF 23/16/7.

6. The decisions rules were adjusted to align with the corresponding Maximum Levels (ML) adopted by CAC45 for maize grain, destined for further processing; flour meal, semolina and flakes derived from maize; husked rice; polished rice; sorghum; cereal-based foods for infants and young children; and cereal-based food for infants and young children destined for food aid programs.

7. Tables 2 and 4 were excluded considering alignment of sampling plans for DON as established in the General Standard for Contaminants and Toxins in Food and Feed (GSCTFF) CXS 193-1995 and considering that the sampling plans being proposed already establish 10 increments of 100g for flour meal, semolina and flakes derived from maize and cereal-based foods for infants and young children. The remaining tables and the references to them were renumbered accordingly.

8. References made to “lot size” and “lot mass” were altered to lot weight, aligning with the sampling plans for DON and fumonisins.

9. Paragraph 18 was edited replacing the expression “approaches zero” to “is minimized”.

10. Paragraph 19 was excluded as the sampling plans already established that the sample should be prepared using a suitable mil.

11. The sampling plans edited is attached in Appendix I.

KEY POINTS OF DISCUSSION:

12. The chair opened the discussions on the following recommendations:

    a) Adopt the ratio 50:50 of AFB1: AFB2+AFG1+AFG2.

13. European Union (EU) expressed that the four single aflatoxins (aflatoxin B1, B2, G1 and G2) are analysed with the same method of analysis and aflatoxin B1 is, among the four aflatoxins, not the most challenging compound for achieving a certain low limit of quantification. EU is concerned about establishing performance criteria for each aflatoxin component separately and emphasized that the CCMAS informative document on the sum of components also indicates that there may be a different approach for components that do not have a fixed ratio. In addition, the Procedural Manual[1] states that "The approaches described for developing method performance criteria are intended for single-analyte provisions. The approaches described may not be suitable for provisions involving sum of components. There are numerous ways in which methods and limits that involve a sum of components can be converted into method performance criteria, but this should be undertaken with care on a case-by-case basis." EU expressed the opinion that the LOD and LOQ requirements for the single aflatoxins should not depend on the assumed ratio of the concentration of the single aflatoxin in relation to the concentration of the sum of aflatoxins.

14. Japan supported the proposed 50:50 ratio, but expressed concern that LOQ for the other toxins (B2, G1 and G2) may not be achievable in such required levels. If these levels could not be validated, the adoption of proposed LOQ for AFB1 could be assumed as the same for the AFB2, AFG1 and AFG2.

15. In order to reflect these concerns, a footnote was added in the LOD and LOQ in Table 3. Such approach should be evaluated by CCMAS.

    b) Align sampling plans for DON and Fumonisins in case of maize flour, maize meal and cereal-based foods for infant and young children with the sampling plans for aflatoxins in the same commodities (1 kg as laboratory sample weight).

16. There was a general agreement on this recommendation. This fact supports the alignment of sampling plans for DON and Fumonisins in case of maize flour, maize meal and cereal-based foods for infant and young children with the sampling plans for aflatoxins in the same commodities.

    c) Adopt 5 kg as laboratory sample weight for maize grain.

17. EU recognized that higher laboratory sample weight could be burdensome for developing countries, but proposed that it could be equal or higher than 5 kg. This opinion was also supported by Japan.

18. El Salvador expressed the view that laboratory sample weight of 5 kg was already burdensome from developing countries and 1kg would be preferable in terms of practicality in the laboratory due to lack of space in some laboratories.

19. There was a general support of 5kg for maize grain with the amendment to consider laboratory sample equal or higher than 5 kg.

    d) Adopt 5 kg as laboratory sample weight for rice (husked and polished) and for sorghum.

20. EU recognized that higher laboratory sample weight could be burdensome for developing countries, but proposed that laboratory sample weight could add equal or larger than 5 kg on laboratory sample weight. This opinion was also supported by Japan.

21. There was a general support of 5kg for rice (husked and polished) and for sorghum with the amendment to consider laboratory sample equal or higher than 5 kg.

RECOMMENDATIONS:

22. The PWG recommends to CCCF to approve the Sampling Plans for Total Aflatoxins in certain cereals and cereal-based products including food for infants and young children as described in Appendix I and send to CCMAS for endorsement.

[1] 28th Edition CODEX ALIMENTARIUS COMMISSION PROCEDURAL MANUAL. Principles for the establishment of Codex methods of analysis, para. 177, note 2.

APPENDIX I

**SAMPLING PLANS FOR TOTAL AFLATOXINS
IN CERTAIN CEREALS AND CEREAL-BASED PRODUCTS
INCLUDING FOODS FOR INFANTS AND YOUNG CHILDREN**

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 weight (≥0.5 tons)
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm – 20 mesh)
Laboratory sample weight	≥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 weight	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 10 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 weight (≥0.5 tons)
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm – 20 mesh)
Laboratory sample weight	≥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 20 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 weight (≥0.5 tons)
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm – 20 mesh)
Laboratory sample weight	≥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 5 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 weight (≥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 weight	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 10 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 weight	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 5 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 weight	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 10 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 weight – Maize grain, sorghum, polished rice and husked rice

Lot weight (t)	Maximum weight or minimum number of sublots	Number of incremental samples	Minimum laboratory sample weight (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 2

2. Considering that the weight of the lot is not always an exact multiple of the weight of sublots, the weight 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 weight. 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 2. Number of incremental samples of cereal grains to be taken depending on the weight of the lot- Maize grain, sorghum, polished rice and husked rice

Lot weight (t)	Number of incremental samples	Minimum laboratory sample weight (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

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 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).

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 x V) / (D x 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 is minimized. After grinding, the grinder should be cleaned to prevent aflatoxin cross-contamination.

TEST PORTION

19. The suggested weight 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.

20. 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

21. 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 3. Utilizing this approach, laboratories would be free to use the analytical method most appropriate for their facilities.

Table 3. 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

*If those values could not be validated, LOD and LOQ for AFB1 could be assumed for AFB2, AFG1 and AFG2.