CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 10 (b) **CX/CF 21/14/10-Add.2**

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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON CONTAMINANTS IN FOODS

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

Comments submitted by Argentina, Canada, Cuba, Chile, Egypt, Iran, Kenya Mexico, Republic of Korea, United States of America (USA), AOCS (American Oil Chemists' Society) and EURACHEM

Background

1. This document compiles comments received in response to CL 2020/60-CF issued in December 2020.

Explanatory notes on the Annex

2. The comments are hereby compiled in the **Annex** and are presented in table format.

ANNEX

COMMENTS ON SAMPLING PLANS AND PERFORMANCE CRITERIA FOR TOTAL AFLATOXINS IN CERTAIN CEREALS AND CEREAL-BASED PRODUCTS INCLUDING FOODS FOR INFANTS AND YOUNG CHILDREN

GENERAL COMMENTS

COMMENTS	MEMBER/OBSERVER
Argentina recomienda establecer un Plan de Muestreo para los productos citados en el documento. Además, es deseable que los criterios estén unificados al informar los resultados analíticos sobre la porción específica del producto analizado y su representatividad. Términos y definiciones: Destinado a un procesamiento posterior: Se recomienda una nota que explique: Destinado a ser sometido a un tratamiento adicional que reduce el nivel de aflatoxinas antes de que se utilicen como ingredientes en productos alimenticios elaborados u ofrecidos de otro modo para el consumo humano. RSDR: Se recomienda hacer referencia a la Norma CXS 193-1995 para la definición de RSDR o agregar la siguiente nota debajo: RSDR es el desvío estándar relativo calculado a partir de los resultados generados bajo condiciones reproducibles [(SR /) x 100]. Otros comentarios: Método: Aunque mencionar el método HPLC permite un amplio rango de variantes, también excluye otras metodologías basadas en diferentes principios que están actualmente en uso o en desarrollo. La recomendación es no especificar un método analítico, ya que la Norma tiene un enfoque basado en criterios.	**Argentina**
General/overall comment: The Procedural Manual states that the Committee submitting a sampling plan should identify the basis on which the standards have been established: whether every item must comply with the provision or only a specified portion must comply. This should be considered when preparing the final sampling plans. If maximum levels (MLs) for aflatoxins in cereal grains and rice continue to be considered by CCCCF for elaboration, a sampling plan should be developed. The MLs should be confirmed for each commodity before finalizing the sampling plan because the performance criteria established for the method for determination of aflatoxins are associated with the ML.	**Canada**
Chile agradece la oportunidad de presentar observaciones y se revisaron las recomendaciones de esta carta circular y sus comentarios se exponen a continuación: - Chile opina que se deben desarrollar planes de muestreo y criterios de rendimiento para el análisis del total de aflatoxinas para cereales en grano (maíz y sorgo destinados a un posterior procesamiento, arroz pulido y descascarillado), en harina, semolina y hojuelas de maíz, y en productos a base de cereales, incluidos alimentos para lactantes y niños pequeños. Chile apoya el plan de muestreo propuesto, no obstante, presenta su preocupación respecto de cómo implementar esta aplicación en países en vías de desarrollo, en virtud de los recursos económicos asociados a su implementación, para el actual plan de muestreo que propone el grupo de trabajo electrónico.	**Chile**

COMMENTS	MEMBER/OBSERVER
- Respecto del plan como tal, Chile quisiera comentar lo siguiente: * Considerar incluir en lo concerniente a homogenización – trituración, el párrafo 24 del Codex Stan referido al plan de muestreo para el contenido de aflatoxinas en maní, respecto a la utilización de una trituradora de martillo con un cedazo de número 14, dado que constituye una solución intermedia en términos de costo y precisión. También se sugiere considerar incluir la opción de lechada en las alternativas para incrementar la homogenización de la muestra. * Considerar revisar la Tabla n°2 con detalle de valor de masa de incrementales para ajustar el valor de muestra de laboratorio a 1kg, cuando la masa incremental sea superior a 100g, o en su lugar cambiar de 3 a 10 mínimos incrementales, entendiendo que cada incremental es de 100 g. * Considerar indicar el tamaño del tamiz necesario para lograr una completa homogenización de la muestra de laboratorio. * Se solicita especificar con qué solución reactiva se debe limpiar el molino con el cual se homogeniza la muestra de laboratorio, de tal forma de prevenir la contaminación cruzada con aflatoxinas.	
Cuba considera apoyar el documento con el plan de muestreo del totalde aflatoxinas en cereales en grano y en los demás productos.	**Cuba**
Egypt would like to thank the CCCF on the work done and would like to add the following comments: 1. Approval on developing the proposed sampling plan for total aflatoxins in (AFT; AFB1 + AFB2 + AFG1 +AFG2) in cereals grains (maize and sorghum destined for further processing; polished and husked rice); in flour, semolina and flakes derived from maize; and in cereal-based products including foods for infants and young children. 2. Approval on the proposed draft sampling plan attached to Appendix (1) with the following note: a) Providing references to the tables contained in the document with the risk level in the field of decision rules. b) Provide the tables with examples of the decision rule.	**Egypt**
1) Considering the issue of decision rules in the ISO/IEC 17025: 2017, and the importance of the uncertainty of each laboratory for decisions about the results based on agreements with customers, it is recommended that this subject to be considered in the Decision rule item in tables instead of mention of MRL. 2) In Table 3, the limits considered for Recovery do not correspond to the limits defined in the Procedural Manual Codex and should be corrected.	**Iran**
Kenya is in agreement that sampling plan for total aflatoxins in cereal grains destined for further processing; flour, in semolina and flakes derived from maize; and cereal based foods including infant foods should be developed. Rationale: Aflatoxins contamination is heterogenous especially when it comes to samples such as maize and ground nuts which are mostly affected; this is due to the nature of growth of molds i.e in clusters therefore a cluster of Aflatoxins producing fungi growth on a particular quantity of grains can increase levels of aflatoxins significantly.Sampling is a very important process when testing aflatoxins because of distribution of the fungi producing the toxins.	**Kenya**

COMMENTS	MEMBER/OBSERVER
Other comments: Comment 1: Kenya notes that for Cereals grains destined for further processing as per draft, a maximum limit of 15 µg/kg is appropriate/agreeable IF operational prerequisite programs and critical control points are going to be applied in order to ensure that the final product is within the set limits noting that this is a raw material, and not used for direct human consumption. Comment 2: For Analytical methods Kenya proposes inclusion of ELISA method as a rapid method for detection and screening of aflatoxins Rationale for comment 2: There is no significant difference in recovery rate between HPLC and ELISA.	
Si se debe desarrollar un plan de muestreo del total de aflatoxinas en AFT (AFB1 + AFB2 + AFG1 +AFG2) en cereales en grano (maíz y sorgo destinados a un posterior procesamiento, arroz pulido y descascarillado), en ~~la~~ harina~~, la,~~ semolina ~~y las~~ y hojuelas derivadas del maíz y en productos a base de cereales, incluidos alimentos para lactantes y niños pequeños; Se repite el concepto "porción de análisis en" en todas las tablas. Se sugiere eliminarlo en la segunda columna de todas las tablas	**Mexico**
We agree to the development of the sampling plan for total aflatoxins in cereals grains (maize and sorghum destined for further processing; polished and husked rice); in flour, semolina and flakes derived from maize; and in cereal-based products including foods for infants and young children. According to the General Standard for Contaminants in Food and Feed (CXS 193-1995), information on appropriate sampling procedures should be supplied when establishing ML, noting that special attention is required for mycotoxins in some commodities as they may not be homogeneously distributed in the product. Additionally, existing sampling plans for aflatoxins in dried figs, peanuts, and treenuts such as almond, hazelnuts, pistachios, shelled brazil nuts indicate a need to develop a new sampling plan for aflatoxins in cereals which matrices are totally different from the above foods.	**Republic of Korea**
The United States appreciates the opportunity to provide comments in response to CL 2020/60/OCS-CF, which requests input on: (a) whether a sampling plan for total aflatoxins in certain cereal and cereal-based products including foods for infants and young children should be developed and (b) the proposed draft sampling plan for total aflatoxins in certain cereal and cereal-based products including foods for infants and young children as presented in Appendix I of CX/CF 20/14/10-Part II. (a) Since maximum levels (MLs) are under consideration for total aflatoxins in certain cereals and cereal-based products including foods for infants and young children, the United States supports simultaneous consideration of a sampling plan for these commodities. (b) The proposed sampling plan described in Appendix I of CX/CF 20/14/10 – Part II appears to be similar to the Codex sampling plan for deoxynivalenol (DON) in cereal-based foods for infants and young children; in flour, meal, semolina and flakes derived from wheat, maize or barley; and in cereal grains (wheat, maize and barley) destined for further processing (General Standard for Contaminants and Toxins in Food and Feed, CXS 193-1995, Amended in 2019, page 34), as well as the European Union (EU) sampling plan for mycotoxins in cereal grains (European Commission Regulation No 401/2006). While a potentially useful resource, this sampling plan will be challenging to implement if importing countries require exporting countries to sample and test for total aflatoxins according to this plan before exporting large lots of cereal grains. The plan proposes 100 increments of 100 g for all lots greater than 50 tons. In addition, for lots between 50 and 500 tons, various sizes or numbers of sublots are provided. Both the number and size of the increments, as well as the sublot provisions, make this sampling plan impractical to implement at US export facilities and possibly other country export facilities as well. An alternative sampling plan to consider would be based on the international standard, ISO 24333:2009 "Cereals and cereal products – Sampling."	**USA**

COMMENTS	MEMBER/OBSERVER
This standard directly applies to the sampling of cereal grains and cereal products for aflatoxins and other mycotoxins. This standard has no separate definitions of lot and sublot, so the size requirements for a portion of grain apply to lots or sublots. In addition, the size of the incremental samples can range from 300 to 1900 grams, rather than being fixed at 100 grams. Also, in ISO 24333 the minimum number of increments is specified as 25 for 1,500 ton lots and 20 for 500 ton lots, rather than 100 for every lot ≥ 50 tons. Finally, in ISO 24333 the minimum number of increments specified for 15 – 300 ton lots ranges from 3 to 18, rather than a minimum of 60 to 100 in the proposed aflatoxin sampling plan. With regards to the analytical method performance criteria provided in Table 3, the United States notes that to establish an ML, there should be analytical methods available that apply to the concentration ranges specified by Codex for establishing numeric criteria. We recommend that CCCF consult with CCMAS on the best approach for the criteria because total aflatoxins are a "sum of components." The proposed "70 percent of total aflatoxins would be AFB1 and the remaining 30 percent would be distributed equally between AFB2, AFG1 and AFG2" may not be appropriate for all cereal grains. The United States recommends the use of numeric criteria and not the endorsement of specific methods.	
Laboratory sample – the smallest quantity of shelled cereal 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. If nothing less than 1 kg is representative of the "lot" then the laboratory can never remove a smaller portion before comminution and have it still be representative. The entire laboratory must be comminuted. The smaller mass can never be representative.	**AOCS - American Oil Chemists' Society**

SPECIFIC COMMENTS

Maize grain destined for further processing

COMMENTS	MEMBER/OBSERVER
The Analytical Methods section below states that "a criteria-based approach, whereby a set of performance criteria is established with which the analytical method used should comply, is appropriate." Table 3 then goes on to outline requirements for method performance regarding sensitivity, accuracy, and precision. As such, it is recommended that mention of a specific method be removed from these tables. If it is retained, then please specify a detection method as well; if this is the case, it may be helpful to indicate that the method listed is a suggestion, perhaps through adding "e.g. HPLC". Due to its large kernel size and heterogeneity of aflatoxin contamination, a larger laboratory sample should be suggested for maize: 10 kg instead of 1 kg for all maize lots, irrespective of size. For the smallest lots, this can be reconsidered, but in general this will decrease the variability of test results due to sampling a heterogeneous material. The FAO's Mycotoxin Sampling Tool (http://tools.fstools.org/mycotoxins/) estimates that an increase of laboratory sample size from 1 kg to 10 kg will decrease variance by about a factor of 4 at the more stringent proposed ML of 15 ng/g. This will decrease the probability of accepting a non-compliant lot by a factor that ranges from about 2-5x depending on the true lot concentration. Both tons and tonnes are written for different commodities. It is suggested that 'tonnes' be used throughout. This would ensure no confusion between metric tonnes and imperial tons. It is suggested that before moving forward with the sampling plan, the maximum level (ML) should be confirmed for each commodity because the performance criteria established for the method for determination of aflatoxins are based on the ML.	**Canada**
'Number of laboratory samples = 1 ' does not allow for the taking of duplicate samples when required. Proposed change: Explain that a duplicate sample can be taken on some batches for either (1) the estimation of uncertainty from sampling (UfS *), or (2) in cases of doubt in analysing the first test sample. * Ramsey M.H., Ellison S. L. R., and Rostron P.(eds.) (2019) Eurachem/EUROLAB/ CITAC/Nordtest/ AMC Guide: Measurement uncertainty arising from sampling: a guide to methods and approach, Second Edition, Eurachem, ISBN 978-0-948926-35-8 http://www.eurachem.org/index.php/publications/guides/musamp 20 µg/kg AFT or 15 µg/kg AFT - Not clear why there are two ML values Proposed change: Add explanation for why there are two ML values and when to use which value	**EURACHEM**

Flour, meal, semolina and flakes derived from maize

COMMENTS	MEMBER/OBSERVER
Directions should be provided for both flour, meal, semolina and flakes derived from maize and for cereal-based foods for infants and young children to grind in a similar fashion as all of the other commodities listed above, since there is a possibility that the foods will not be in a format that passes through a 20 mesh screen (e.g. flakes).	**Canada**

Cereal-based foods for infants and young children

COMMENTS	MEMBER/OBSERVER
It may be helpful to specify what "cereal-based foods" means here, i.e. whether this ML would apply to the packaged food at retail or to the cereals used as the basis for the infant food. This would speak to individual lots versus bulk sampling requirements.	**Canada**

DEFINITIONS

COMMENTS	MEMBER/OBSERVER
It is suggested that the definition of Sampling plan be revised. At present, the description is more focused on the steps involved in acceptance or rejection of products. The definition should instead include what the plan is, i.e., a procedure to choose or draw separate samples from a given lot for AFT testing to allow for determination of that lot's compliance with the appropriate ML. The idea that the samples need to be of consistent size is important, otherwise the statistical assumptions may not hold. **Incremental sample –** the quantity of material taken from a single random place in the lot or sublot. Although the above tables specify increment size (e.g. 100 g), it is not the increment size that needs to be controlled, but rather the number of increments and minimum mass of aggregate sample. The size of the increment can be calculated from that. This should be reflected in the definition. For example, if at least 100 increments and a minimum mass of at least 10 kg are required, then 100 increments of 100 grams each would meet this requirement. However, if only 50 gram increments are possible with available equipment, one could instead take 200 increments of 50 grams each to attain the required 10 kg sample mass. Taking more increments is never a problem; taking fewer is a problem. **Laboratory sample –** the smallest quantity of shelled cereal 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. There is a concept of minimum mass for sampling a bulk material. If the desired laboratory sample is less than the aggregate sample obtained, the sample needs to be comminuted or course ground (to reduce the particle size) in order to mass reduce down to the lab sample. This step reduces the size of the grains prior to the homogenization step. For example, to go from 10 kg aggregate sample to a 1 kg laboratory sample would require a course grind of the 10 kg aggregate sample and then further mass reduced to 1 kg using correct non-biased sampling methods, rotary splitting is probably the best. Going from an aggregate sample to a lab sample is a sampling step.	**Canada**
Lote: cantidad determinada de un producto alimentario entregado en una sola vez y que presenta, a juicio del agente responsable, características comunes, como el ~~origen~~[origen, la variedad, el tipo ~~de~~ de] envase, el envasador, el expedidor o el marcado. Para que el lector tenga una mejor comprensión del texto, al leer algunas características comunes involucradas. **Plan de muestreo**: se define por un procedimiento de análisis del AFT y un nivel de aceptación o rechazo. Un procedimiento de análisis del AFT consta de tres pasos: selección de la muestra, preparación de la muestra y análisis o cuantificación del AFT. El nivel de ~~aceptación o rechazo~~ aceptación es un valor de tolerancia que generalmente es igual o menor al nivel máximo (NM) del Codex.Por otro lado, el nivel de rechazo es un valor de tolerancia que generalmente es mayor al NM del Codex. Se sugiere refrasear: Debido a que la palabra “aceptación” y “rechazo” juntas pueden ser confusas para el lector.	**Mexico**
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. Aggregate sample’ - often called a ‘composite sample’ – Proposed change: Include the synonym 'composite sample'	**EURACHEM**

SAMPLING PLAN DESIGN CONSIDERATIONS

COMMENTS	MEMBER/OBSERVER
Characteristics specific to sampling of aflatoxins, such as operating characteristic curves, inferences to be made to lots or processes, levels of risk to be accepted and pertinent supportive data would be beneficial to include. The Codex Committee on Methods of Analysis and Sampling (CCMAS) is currently working on the development of a Sampling Plan tool which is hoped to aid Horizontal and Commodity committees in the development of OC (operating characteristic) curves and to understand the important components of sampling plan design including the levels of consumer's and producer's risks. However, sampling for mycotoxins is complex owing to the heterogeneity, large lot sizes, etc., and the plan development may benefit from including these factors.	**Canada**

Material to be sampled

Table 1. Subdivision of maize sublots according to lot weight

COMMENTS	MEMBER/OBSERVER
All of the tables indicate a minimum laboratory sample weight, but the more appropriate term is the ‘minimum aggregate sample mass’. Physical subdivision is not feasible for some bulk grain shipments. Lots carried by truck can range up to about 46 tonnes. Some railcars can hold masses in the range of 90 tonnes. Bulk vessel shipments are in the thousands of tonnes range. While EU regulation 401/2006 uses the same sub-lot concept and definitions, the advantage of this concept isn't clear. Is a rationale available for the minimum laboratory sample weight, and for its relationship with lot weight, sub-lots, and the number of incremental samples? Without a clear scientific rationale and advantage, it is recommended that the concept of sub-lot be avoided. The focus should instead be on ensuring that the entire lot is sampled and the laboratory sample is prepared correctly so that bias is avoided. This should then produce a laboratory sample representative of the lot. Consideration could be given to changing 'weight' to 'mass' to be more accurate.	**Canada**

Incremental Sample

COMMENTS	MEMBER/OBSERVER
The suggested minimum weight of the incremental sample should be 100 grams for lots ≥ 0.5 tons. Please provide a scientific rationale for the minimum increment mass of 100 g. EU 401/2006 states that the "weight of the incremental sample shall be about 100 grams" for sampling cereals and cereal products, and that this document also does not provide a rationale for the incremental sample weight. Please add a description to indicate how the sub-lot, lab sample, and other sizes were selected in Tables 1 and 2. Sampling plans also usually specify the type of sampling, e.g. attribute or variable sampling. In this case, it seems to be variable sampling and for the purpose of compliance. For lots less than 50 tons, 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.	**Canada**

COMMENTS	MEMBER/OBSERVER
According to Table 1: Guidelines for establishing numeric values for the criteria, for 4 µg/kg and above it until 100 µg/kg, accepted recovery is 60-115.	**Iran**

Incremental Sample

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

COMMENTS	MEMBER/OBSERVER
Unless the sample size to lot size ratio gets above a certain value, aggregate sample size should not increase (or decrease) with lot size; this is because of the concept of minimum mass in sampling theory. There are 2 main things to overcome when sampling a bulk material: compositional heterogeneity and distributional heterogeneity. The first is related to what the analyte particles are and how much exist in the material. The second relates to how they are distributed in the material, i.e. are they relatively even throughout or are they in clumps or "hotspots." To overcome distributional heterogeneity one takes many increments. To overcome compositional heterogeneity one needs to sample a certain minimum mass. Both of these requirements do not change with an increase in the lot size. They are factors of the material that is being sampled. Aflatoxin sampling represents a very challenging sampling problem and if it isn't done correctly, results will not be representative and huge sampling error (uncertainty) will exist. In Table 2, could the 100 increments of at least 100 grams each be continued down to the lot weight of 1 tonne? At that point one would be sampling 1% of the material and might consider taking a smaller aggregate sample. The increments below this point should not be reduced this much, though, and should never be fewer than 20. Calculations or experiments to measure sampling variability can be done to determine the numbers for minimum mass (the amount for the aggregate sample) and number of increments. For example, the numbers of incremental samples in Table 2 could be set to 20, 40, 50, 100, 100, 100 and 100.	**Canada**

STATIC LOTS

COMMENTS	MEMBER/OBSERVER
A static lot can be defined as a large mass of ~~shelled~~ cereal grain 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 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. 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. The consistent mass of increments is important to avoid a biased aggregate sample. Increments taken must be of a consistent mass to avoid a biased aggregate sample. It is recommended that this be emphasized here. SF = (LT x IS)/ (AS x IP)	**Canada**

COMMENTS	MEMBER/OBSERVER
It is recommended that this formula be verified to ensure that it conforms to current sampling theory (Pierre Gy and Francis Pitard have written extensively on this). Representative aggregate samples can be more easily produced when selecting incremental samples from a moving stream of shelled cereal 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). The sample size should not be reduced unless the minimum mass is respected. If not, the aggregate must be ground before it can be further mass-reduced. Representative aggregate samples can be more easily produced when selecting incremental samples from a moving stream of ~~shelled~~ cereal grain 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).	
Si se conoce la velocidad de circulación de la masa, MR (kg/seg), entonces la frecuencia del muestreo (SF) o el número de cortes que hace el vaso receptor automático se puede contabilizar como una función de S, V, D y MR. como se define en la siguiente ecuación: SF = (S x V) / (D x MR). Para mayor comprensión del lector	**Mexico**
A static lot can be defined as a large mass of shelled cereal 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 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. Selecting a truly random sample from a static lot can be difficult' This emphasises the need for estimating UfS to assess the quality of the sampling process in the light of the analyte heterogeneity in the sampling target. Proposed change: Add text to emphasise the need for estimating UfS to assess the quality of the sampling process in the light of the analyte heterogeneity in the sampling target (ref *) Ramsey M.H., Ellison S. L. R., and Rostron P.(eds.) (2019) Eurachem/EUROLAB/CITAC/Nordtest/AMC Guide: Measurement uncertainty arising from sampling: a guide to methods and approach, Second Edition, Eurachem, ISBN 978-0-948926-35-8 http://www.eurachem.org/index.php/publications/guides/musamp	**EURACHEM**

SAMPLE PREPARATION

COMMENTS	MEMBER/OBSERVER
The laboratory sample should be finely ground and mixed thoroughly using a process that approaches as complete homogenization as possible. Complete homogenization implies that particle size is extremely small, and the variability associated with sample preparation approaches zero. After grinding, the grinder should be cleaned to prevent AFT cross-contamination. Mixing ground sample can be problematic and introduce heterogeneity. Most grinding equipment can't obtain a uniform particle size, so there are always larger particles and fines. When mixing, fines typically fall to the bottom and side of the container while larger particles sit on top. This should ideally be considered when removing a test portion.	**Canada**

TEST PORTION

COMMENTS	MEMBER/OBSERVER
The suggested ~~weight~~ mass of the test portion taken from the comminuted laboratory sample should be approximately 25 g or 50g, depending on the product analysed. When analytical method used requests smaller test portions, this can be applied since the performance of the method still fits the purpose and does not impact the uncertainty of the result. The struck text is incorrect; sampling can have a large impact on the result, often larger than the method and instrument uncertainty. When the analytical method used requests smaller test portions, this can be applied since the performance of the method still fits the purpose and does not impact the uncertainty of the result. Procedures for selecting the test portion from the comminuted laboratory sample should be a random process. ~~If mixing occurred during or after the comminuting process, the~~ The test portion ~~can be selected from any location throughout the comminuted laboratory sample. Otherwise, the test portion~~ should be the accumulation of several small portions selected throughout the laboratory sample. Substantive/technical - Mixing is often problematic, as any movement of the comminuted grain can lead to stratification of particles of different densities and/or shapes. If the stratification is associated with specific kernel fractions (e.g. bran), the test portion will be biased if it is selected from any one location of the comminuted laboratory sample. The test portion must be prepared using a division method that avoids bias. This could be a rotary sample divider, a stationary gravity-fed divider such as a riffle divider, or manual sampling of a number of small increments (of equal mass) taken throughout the entire comminuted laboratory sample. The preferred procedure is always to take many increments randomly from the entire lab sample to obtain the test portion, rather than just the top. The same process should be done at this stage as the original sampling of the original lot to arrive at the aggregate sample. It's the same sort of sampling exercise, many small increments to achieve an aggregate. All mass reduction of bulk sample material should be done in the same way. It is suggested that three test portions be selected from each comminuted laboratory sample. The three test portions will be used for enforcement, appeal, and confirmation if needed. If testing is to be done in triplicate, perhaps there should be some details provided around how results are to be reported and applied with respect to error/variability?	**Canada**

ANALYTICAL METHODS

Table 3. Performance criteria for Total Aflatoxins.

COMMENTS	MEMBER/OBSERVER
RSDR debería variar dependiendo el rango de concentraciones de trabajo. Principalmente, en los niveles más bajos como los alimentos a base de cereales para lactantes y niños pequeños (para 1µg/kg, RSDR = 68%, según la ecuación de Horwitz, multiplicado por 3/2). Recuperación: Se observa que los valores correspondientes al % de recuperación son bastante inferiores a los recomendados: 1 a 15 µg/kg debería encontrarse entre 70 – 110% >15 µg/kg debería encontrarse entre 80-110%	**Argentina**
Table 3: If we are read this correctly, in a couple of cases, it looks like the ML is < 10 µg/kg (e.g., polished rice) and the recovery (%) range applies to the 10 µg/kg range (60 – 115 vs 40 – 120). If accurate, could this be clarified in the table? It would be helpful to define these acronyms in footnotes to the table. In Table 3, the calculation of the LOQ is based on the formula LOQ=2*LOD, while the general accepted guideline is typically that LOQ is 3 or 3.3 times LOD (i.e. a signal-to-noise ratio of 9:1 or 10:1 for LOQ, as opposed to 3:1 for the LOD). It is recommended that this guideline be applied in keeping with internationally accepted standards. With HPLC or LCMS methods, aflatoxins are detected individually, therefore performance of aflatoxins methods is set for individual aflatoxins. In Table 3, the performance criteria are set for total aflatoxins. There are no instructions as how to convert individual aflatoxin to total aflatoxin performance criteria. We recognize ML is based on total aflatoxins, but performance criteria could address the individual aflatoxins.	**Canada**
In the tables related to each matrix, a numeric value for the test portion has been set, while the weight of the test portion can vary depending on the validation of the method in each laboratory.	**Iran**
It must be clarified if the RSDR % has been estimated at the ML level or not. One needs the variability at this level for establishing a guard band and follow the recommendations set in ISO GUM for compliance. A guard band should be set using the measurement uncertainty estimated close to or at a given ML. In this regard, it seems that this document ignores the concept of guard band for compliance assessment. Proposed change: Clarify the concentration level (e.g. as close as possible to ML) at which the RSDR% is to be determined (e.g. as a footnote). The abbreviation 'RSDR%' should be defined.	**EURACHEM**