C O D E X   A L I M E N T A R I U S   C O M M I S S I O N   **E**

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World Health Organization

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Agenda Item 15 | **CX/CF 14/8/15**
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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON CONTAMINANTS IN FOODS

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

DISCUSSION PAPER ON AFLATOXINS IN CEREALS

(Prepared by the Electronic Working Group by chaired Brazil and co-chaired by the United States of America)

BACKGROUND

1. At the 23rd Session of the Codex Committee on Food Additives and Contaminants (CCFAC) (1991), a maximum level (ML) of 10 µg/kg total aflatoxins (B1 + B2 + G1 + G2) was proposed for all foods. However, as there was no consensus over the issue among the country members, the development of a ML for aflatoxins in foods was discontinued and the Committee decided to discuss the issue on a commodity-by-commodity basis.[1]

2. At the 6th Session of the Codex Committee on Contaminants in Foods (CCCF) (March 2012), the Committee agreed to the development of a discussion paper on aflatoxins in cereals through an Electronic Working Group chaired by Brazil and co-chaired by the United States of America for consideration and discussion at the 7th session to identify possible actions or new work on this issue. The Committee also agreed to initiate new work on the development of an annex to the *Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals* (CAC/RCP 51-2003) for the management of aflatoxins and ochratoxin A in sorghum.[2]

3. At its 7th Session (April 2013), the CCCF agreed that it would be necessary to have original occurrence data on aflatoxins in cereal grains in order to conduct a more sound evaluation of the current situation, the exposure levels and the impact on human health. The Committee agreed that the JECFA[3] Secretariat would put out a public call for data on aflatoxin contamination on cereals, which should be submitted to GEMS/Food. The Request for Data for Evaluation of Residues of Total Aflatoxin & Aflatoxin B1 Residues in Cereals and Cereal Products was issued on July 26, 2013, with a deadline for data submission of September 30, 2013. The EWG was re-established chaired by Brazil and co-chaired by the United States of America.[4] The List of Participants is presented in Appendix II.

4. This discussion paper is an update of the paper presented at the 7th CCCF session and includes the data submitted to GEMS/Food on rice, maize, sorghum and wheat grains, the main staple food worldwide. Although information on other cereals such as rye, oats and barley, processed food, and on cereals intended for use in animal feed were also provided, they were not included due to the limited time the EWG had to prepare the paper for discussion at the 8th CCCF.

5. The Committee is invited to consider the conclusions and recommendations of the discussion paper in order to determine the best way to proceed with this matter. For easy of reference, the conclusions and recommendations, including some points for discussion, are reproduced here below. The discussion paper providing the background information and the basis for the conclusions and recommendations are presented in Appendix I.

6. In considering these conclusions and recommendations, the Committee is kindly invited to take into consideration the *Annex for the prevention and reduction of aflatoxins and ochratoxin A contamination in sorghum* (CX/CF 14/8/10) and the conclusions on the revision of the *Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals* (CX/CF 14/8/14[5]) in order to have a consistent approach to management measures for the control of mycotoxins in cereals.

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1 ALINORM 92/12A, para. 118.
2 REP 12/CF, para. 175.
3 Joint FAO/WHO Expert Committee on Food Additives (JECFA).
4 REP13/CF, paras. 134-140.
5 Working documents for consideration by the 8th Session of the Codex Committee on Contaminants in Foods are available on the Codex website at: http://www.codexalimentarius.org/meetings-reports/en/ or by accessing the ftp-link: ftp://ftp.fao.org/codex/meetings/cccf/cccf8

CONCLUSIONS

7. Occurrence data on aflatoxins (AFs) on rice, maize, wheat and sorghum grain submitted to GEMSFoods referred to the analysis of 4536 samples, mostly rice samples (66.6%; 3021 samples). Rice was also the cereal with the highest AF incidence (17.7%) and level (total UB mean of 2.4 µg/kg). There is a large discrepancy on occurrence and concentration of AFs in rice among the cluster diets, ranging from 0.3 µg/kg in C09 (Asian countries) to 35.2 µg/kg in C13 (countries in Africa).

8. A preliminary risk assessment showed cancer risks from the exposure to the cereals ranging from 0.4 to 2.1 cancers/10^5 individuals, with the highest risk for C09, mainly from the consumption of rice. The AFs level in rice used for the assessment (2.4 µg/kg) is eight times higher than the AFs level in C09 (mean UB of 0.3 µg/kg).

9. It is important to emphasize that the exposure and the risk estimates shown in this paper are overestimates as they do not consider the impact of processing on AF levels, mainly after sorting, milling and maize nixtamalization. Cooking rice may reduce AFs content by up to 80%.

10. Very few data were provided for maize (588 samples), wheat (844 samples) and sorghum (81 samples), especially for clusters where these commodities have the highest impact on the dietary intake. For example, there is no data on sorghum submitted from African countries.

11. The impact of a hypothetical maximum level (ML) for AFs on cancer risk was only considered for rice. At a ML of 10 µg/kg, the cancer risk decreased by up to 63% compared to a no limit situation. Lower ML had no impact on cancer risk for all clusters, except C09 and C14 (Asian countries), for which a ML of 1 µg/kg would decrease the risk by 76-78%. The impact on the food supply would be about the same in both situations (3-4% of the samples rejected).

RECOMMENDATIONS

Consistent with the information provided in this discussion paper, the eWG recommends that:

- Country members should provide additional data to GEMS/Food on AFs, especially for wheat, maize and sorghum. Countries where these cereals are important in their diet are highly encouraged to submit data.
- Further work should include additional data on cereal grains and processed commodities provided to GEMS/Food.
- The Committee should decide whether it is appropriate to start ~~a~~ discussion on a ML for AFs in rice and associate sampling plan or wait until a larger database is available for considering whether MLs are needed for all cereals.
- The Committee should take into account the annex on aflatoxins that will be included in the *Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals* (CAC/RCP 51-2003) and the proposed revisions to the general part of the Code.

Points for discussion

Japan suggests that "*the CCCF should consider the establishment of an annex for aflatoxins in rice in the Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals. It should be given higher priority than establishment of a ML for rice*".

Currently, the Code has annexes for specific mycotoxins (fumonisins, ochratoxin A, zearalenone and trichothecenes) and the proposed revision (CX/CF 14/8/14) suggests the inclusion of an annex on aflatoxins. The Committee should consider the benefit of including a specific annex for rice, as it would probably require a complete change in the format of the Code. An *Annex for the prevention and reduction of aflatoxins and ochratoxin A contamination in sorghum* (*Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals*) is under discussion by the CCCF.

APPENDIX I

DISCUSSION PAPER ON AFLATOXINS IN CEREALS

INTRODUCTION

12. Aflatoxins (AFs) are considered the most important group of mycotoxins in the world's food supply and are produced in nature primarily by *Aspergillus flavus, Aspergillus nomius, Aspergillus parasiticus* and related species. AFs B1, B2, G1 and G2 are the four major naturally produced AFs. The B and G designations refer to the blue and green fluorescence colours produced under UV light (Pitt and Hocking, 2009).

13. *A. flavus* is often found in most food produced in tropical countries, having special affinity with maize, peanuts and cottonseed. Usually, *A. flavus* produces only B aflatoxins and yet is considered the main source of AFs. *A. parasiticus* produces both B and G aflatoxins and is commonly isolated from peanuts, being quite rare to find it in other foods (Frisvad et al., 2006). At least eight other *Aspergillus* species produce aflatoxins, but only two are of possible importance in foods: *A. nomius* and *A. minisclerotigenes*. Both resemble *A. flavus* in culture but *A. nomius* produces bullet shaped sclerotia, as distinct from the large spherical sclerotia produced by many *A. flavus* isolates, while *A. minisclerotigenes* produces small spherical sclerotia. Both of these species produce both B and G aflatoxins (Taniwaki & Pitt, 2013). *A. nomius* also produces aflatoxin B and G and are found mainly in tree nuts (Pitt and Hocking, 2009). Optimum conditions for AFs production by these species are 33°C and 0.99 aw (Sanchis and Magan, 2004). AFs could be produced by fungi either before and/or after harvesting of cereals, and is influenced by several environmental factors such as temperature, relative humidity, insect damage, drought and stress condition of the plants (Miraglia et al., 2009).

TOXICOLOGICAL ASPECTS

14. At its 49th Meeting (1998), JECFA evaluated toxicological data and evaluated the human dietary exposure to aflatoxins (B1, B2, G1 and G2; AFs) (FAO/WHO, 1998). The JECFA reviewed a wide range of studies, in both animals and humans, and concluded that AFs are human liver carcinogens, AFB1 being the most potent carcinogen. No tolerable daily intake was proposed since these compounds are genotoxic carcinogens.

15. The risks arising from exposure to AFs were evaluated through potency estimates for human liver cancer derived from epidemiological and toxicological studies. The potency of AFs was defined by the JECFA to be 30 times higher in carriers of hepatitis B virus ($HBsAg^+$; about 0.3 cancers/year/100,000 individuals) than in non-carries of hepatitis B virus ($HBsAg^-$; about 0.01 cancers/year/100,000 individuals). Thus, reduction of AFs intake in populations with a high prevalence of hepatitis B carriers will have a greater impact on reducing liver cancer rates than in populations with a low prevalence of carriers.

16. At its 64th meeting, the JECFA (FAO/WHO, 2005) decided that evaluations on compounds that are both genotoxic and carcinogenic, such as AFs, should be based on the estimation of Margins of Exposure (MOEs). The MOE is defined as the ratio between a toxicological threshold (such as the BMDL) and the intake. MOE lower than 10000 may indicate a public health concern (EFSA, 2005).

METHOD OF ANALYSIS

17. Methods typically used for AFs analysis include sample extraction with a mixture of water and organic solvents such as acetonitrile, methanol or acetone (Reiter et al., 2009) and sample clean-up using multifunctional (Fu et al., 2008; Garrido et al., 2012) or immunoaffinity columns (Daniel et al.,2011; Mazaheri, 2009; Mohammadi et al., 2012).

18. Detection and quantification methods include thin layer chromatography (TLC) (Hussain et al., 2011; Moreno et al., 2009) and high performance liquid chromatography (HPLC) with fluorescence (Almeida et al., 2012; Bansal et al., 2011; Ghali et al., 2010) or mass spectrometer detectors (Martos et al., 2010; Oueslati et al., 2012; Soleimany et al., 2012). Analysis using fluorescence detector (FD) usually needs pre/post column derivatization to enhance the fluorescent intensity of AFB1 and AFG1, thereby increasing sensitivity (Bakirdere et al., 2012). The enzyme-linked immunosorbent assay (ELISA) and nanogold or nanoeuropium probe-based immunochromatographic assay are practical alternatives for the determination of aflatoxins and have been used (Aydin et al., 2011; Donghong et al.2011; Karami-Osboo et al., 2012; Sun et al., 2011; Xin et al.,2013).

19. Limits of quantification of the methods vary considerably, depending on the aflatoxin analyzed and on the method chosen, ranging from 0.01 μg/kg (HPLC-FD) (Almeida et al., 2012) to 4.0 μg/kg (TLC) (Rocha et al., 2009). LC-MS/MS methods have LOQs ranging from 0.5 μg/kg (Soleimany et al., 2012) to 2.0 μg/kg (Oueslati et al., 2012).

AGRICULTURAL ASPECTS

20. The Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals (CAC/RCP 51-2003) was established in an attempt to control and manage mycotoxins contamination worldwide. The Code of Practice is currently under consideration for revision by the CCCF (CX/CF 14/8/14), with the suggestion of including a specific annex for aflatoxins. In addition to the general recommendations to reduce the presence of mycotoxins in cereal, this proposed Annex includes the possibility of using biological control, by applying atoxigenic isolates of *A. flavus* to reduce aflatoxin concentration in the crop.

21. *A. flavus* has no affinity with small grain cereal plants, members of the grass family Poaceae, and does not invade these plants before harvest (Pitt et al. 2013). Rice is grown under water in the early stages of development, so levels of *A. flavus* in rice growing soils are very low. In addition, the process of hulling rice creates heat, so freshly bagged, hulled rice has a very low fungal load. If these crops are harvested wet and then dried, infection by *A. flavus* and aflatoxin formation become more likely. If small grains do contain unacceptable levels of aflatoxin, this is more likely to result from poor storage (Pitt et al. 2013).

OCCURRENCE IN FOOD

22. Table 1 is an update of the table provided in the previous version of this discussion paper, and includes data on AFs concentration in cereals published in the literature up to July 2013. A total of 81 studies were found in the literature, related to the analysis of 17723 samples. Most studies were conducted on maize and rice, with upper level total means of 7.2 and 27.9 µg/kg, respectively. The studies on sorghum were mainly conducted in Africa, where human consumption of this cereal is the highest worldwide. Total AF mean levels in sorghum and wheat reached 26.6 µg/kg and 7.8 µg/kg, respectively (Table 1).

Table 1. Worldwide occurrence of total AFs in cereals obtained from published literature (from 2000 to July 2013).

	N[a]	Positive/analyzed samples (%)	Positive samples (µg/kg)		Total mean (µg/kg)[b]
			Mean ± SE	Range	Lower[c] – Upper[d] bound
Maize	**45**	**2404/10014 (24.0)**	**26.0 ± 5.7**	**0.01 – 48000**	**6.3 – 7.2**
Africa	17	835/2338 (35.7)	18.8 ± 6.6	0.01 – 48000	6.7 – 7.2
America[e]	10	479/4684 (10.2)	31.0 ± 4.4	0.1 – 1393	3.2 – 4.8
Asia	12	655/1134 (57.8)	35.6 ± 19.9	0.02 – 888.3	20.5 – 20.8
Europe[f]	6	435/1858 (23.4)	20.1[g] ± 5.5	0.01 – 820	4.8 – 5.0
Rice[h]	**34**	**1715/3310 (51.8)**	**53.6 ± 4.1**	**0.002 – 371.9**	**27.7 – 27.9**
Africa	6	64/99 (64.6)	28.9 ± 13.3	0.3 – 371.9	18.7 – 18.8
America	7	205/625 (32.8)	5.2 ± 7.6	0.002 – 176.3	1.7 – 2.3
Asia	18	1374/2388 (57.5)	64.3 ± 5.9	0.01 – 308	37.0 – 37.1
Europe	3	72/198 (36.4)	8.8 ± 3.1	0.05 – 21.4	3.2 – 3.5
Sorghum	**10**	**1426/2011 (70.9)**	**37.3 ± 18.3**	**0.01 – 1164**	**26.5 – 26.6**
Africa	8	248/393 (63.1)	82.6 ± 23.9	0.34 – 1164	52.1 – 52.2
America[e]	1	2/2 (100.0)	12.0 ± 0.07	11.9-12.0	12.0
Asia	2	1176/1616 (72.8)	27.8 ± 11.4	0.01 – 264	20.2 – 20.4
Wheat	**18**	**874/2388 (36.6)**	**18.0 ± 9.1**	**0.05 – 643.5**	**6.6 – 7.8**
Africa	6	66/206 (32.0)	4.9 ± 1.4	0.13 – 37.4	1.6 – 2.0
America	2	0/56 (0.0)	–	–	ND – 3.7
Asia	7	691/1721 (40.2)	14.4 ± 1.9	0.1 – 606	5.8 – 7.2
Europe	3	117/405 (28.9)	46.9 ± 51.4	0.05 – 643.5	13.5 – 13.9
Total	**81**	**6419/17723 (36.2%)**	**34.8±3.6**	**0.002 – 48000**	**12.6 – 13.4**

[a] Number of published studies found in the literature; [b] mean of all samples; [c] samples below LOD or LOQ were considered as zero; [d] samples below LOD or LOQ were considered as 0.5LOD or 0.5LOQ; [e] includes monitoring data from US FDA; [f] includes monitoring data collected by EFSA (2007); [g] mean of positive samples was not available in the EFSA report; [h] mostly rice collected on the market, but some studies may include rice samples with the husk; Africa: samples from Algeria, Benin, Togo, Burkina Faso, Cameroon, Côte d'Ivoire, Egypt, Kenya, Malawi, Morocco, Mozambique, Nigeria, South Africa, Tanzania, Tunisia, Uganda and Zambia; America: samples from Argentina, Brazil, Canada and United States of America;

Asia: samples from China, India, Iran, Japan, Korea, Malaysia, Pakistan, Qatar, Taiwan and Vietnam;

23. The GEMS/Food data on aflatoxin in cereals were extracted from the database and exported to Excel spreadsheets. First, we search for the crops corn, maize, rice, sorghum and wheat. For contaminant, we searched for aflatoxin total, aflatoxin B1, aflatoxin B1 and B2, aflatoxin B2, aflatoxin G1 and aflatoxin G2. All data were selected for WHO Regions and Countries, with sampling period from the year 2000. The data were first extracted on October 21, 2013, with updates on February after additional information was provided from USA and Canada.

24. Processed cereals were excluded based on the informed food codes (WHO food identifier, WHO food code and Local food identifier). Rice samples that included inedible portion (husk) or were cooked were also excluded. When no information on the portion analyzed was provided, it was considered to be as edible portion.

25. Data for some samples included information for the individual aflatoxins, sum of AFB1 and AFB2 and for total aflatoxins (up to 6 entries per sample). When the total aflatoxin value was not included, individual aflatoxin values reported as <LOQ or <LOD were considered as 0 or ½ LOQ/LOD for the lower bound (LB) or upper bound (UB) estimations of the means, respectively. When both LOD and LOQ were reported, ½ LOQ was used. Where LOD or LOQ was not reported, the value informed for other samples from the same laboratory or country was used.

26. Figure 1 shows a summary of the number of data on AFs contamination on maize, rice, sorghum and wheat grain provided by the countries that submitted data to GEMS/Food. AFs contamination data on 4536 samples were provided, most related to rice (66.6% of the samples). Singapore was the country that provided the largest data set, with information on all four crops (1028 samples).

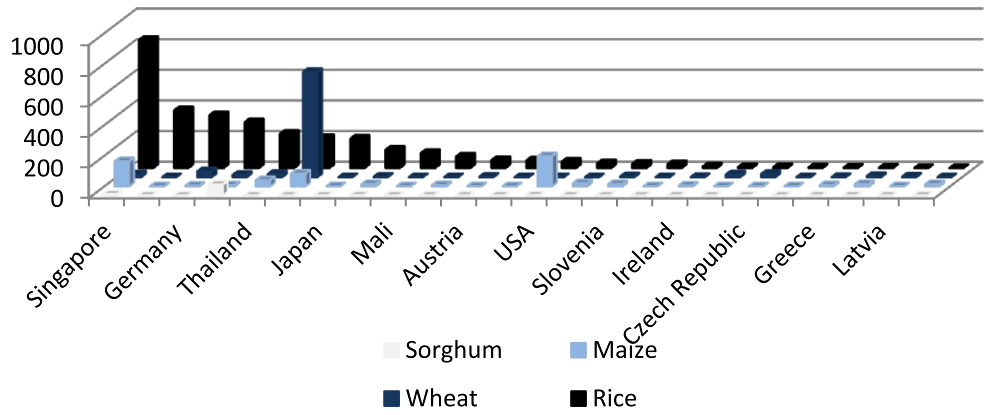

Figure 1. Number of samples submitted to the GEMS/Food database on AFs in maize, rice, sorghum and wheat grain

27. Table 2 gives the information on the incidence and concentration of AFs in cereal grains submitted to GEMS/Food grouped by continent. Rice was the commodity with the highest incidence (17.7%) and concentration (highest level of 347 µg/kg and upper bound total mean of 2.4 µg/kg) (Table 2). Only in a few cases, the type of rice analyzed (husked, polished, raw grain) was specified. It was not also specified whether the sample was analyzed before sorting or after sorting.

28. From the total of 588 maize samples analyzed, 5.6% were positive, with an UB total mean of 1.6 µg/kg. The incidence of AFs contamination in sorghum and wheat were 4.8 and 0.4%, respectively, with the highest level found in sorghum (8.6 µg/kg). Data on sorghum were provided by Singapore, Republic of Korea, USA and Slovakia (1 sample) (Figure 1 and Table 2).

Table 2. GEMS/Food data on AFs in cereals grouped by continent

	Positive/analyzed samples (%)	Positive samples (µg/kg)		Total mean (µg/kg)[a]
		Mean ± SD	Range	Lower[b] – Upper[c] bound
Maize	**33/588 (5.6)**	**13.0 ± 18.7**	**0.2-93.1**	**0.7-1.6**
America	20/279 (7.2)	18.3 ± 22.2	1.7-93.1	1.3-2.3
Asia	9/224 (4.0)	5.9 ± 6.3	0.2-14.8	0.2-0.6
Europe	4/85 (4.7)	2.1 ± 1.4	1.0-3.3	0.1-1.8
Rice	**536/3021 (17.7)**	**10.6 ± 36.3**	**0.002-347**	**1.9-2.4**
Africa	84/98 (85.7)	41 ± 71.3	0.2-347	35.1-35.2
America	223/615 (36.3)	8.8 ± 28.7	0.002-272.2	3.2-3.5
Asia	66/1553 (4.2)	0.4 ± 0.4	0.02-2.5	0.02-0.5
Europe	163/755 (21.6)	1.5 ± 2.5	0.04-17.0	0.3-1.0
Sorghum	**4/83 (4.8)**	**8.6 ± 5.4**	**0.6-12.0**	**0.4-0.6**
America	2/2 (100.0)	12 ± 0.07	11.9-12.0	12.0
Asia	2/80 (2.5)	5.2 ± 6.4	0.6-9.7	0.1-0.3
Europe	0/1(0.0)	ND	ND	ND-0.08
Wheat	**3/844 (0.4)**	**1.0 ± 0.7**	**0.1-1.4**	**0.003-0.6**
America	0/688	ND	ND	ND-0.5
Asia	0/54 (0.0)	ND	ND	ND-0.5
Europe	3/102 (2.9)	1.0 ± 0.7	0.1-1.4	0.03-1.4
Total	**576/4536 (12.7)**	**10.7 ± 35.3**	**0.002-347**	**1.4-1.9**

[a] mean of all samples; [b] samples below LOD or LOQ were considered as zero; [c] samples below LOD or LOQ were considered as 0.5LOD or 0.5LOQ.

Africa: samples from Mali; America: samples from Brazil, Canada and United States of America; Asia: samples from Japan, Philippines, Republic of Korea, Singapore, Thailand; Europe: samples from Austria, Belgium, Cyprus, Czech Republic, France, Germany, Greece, Ireland, Italy, Latvia, Portugal, Slovakia, Slovenia, Spain and Sweden.

29. Table 3 shows the AFs occurrence data grouped by the 17 GEMS/Food cluster diets (see cluster distribution on Figure 2). No data were provided by countries that are included in clusters C01, C02, C03, C04, C12, C14, C16 and C17. Singapore, not included by GEMS/Food in any cluster, was considered to be in C09.

30. The incident and concentration of AFs in rice are both vary widely depending on the cluster. The lowest total mean (UB) of AFs in rice was found for C06 and C09 (0.3 µg/kg) and the highest total mean (UB) was found for C13 (35.2 µg/kg).

Table 3. GEMS/Food data on AFs in cereals grouped by cluster diets.

	Positive/analyzed samples (%)	Positive samples (µg/kg)		Total mean (µg/kg)[a]
		Mean ± SD	Range	Lower[b] – Upper[c] bound
Maize	**33/588 (5.6)**	**13.0 ± 18.7**	**0.2-93.1**	**0.7-1.6**
C06	0/7 (0.0)	ND	ND	ND-0.3
C07	0/7 (0.0)	ND	ND	ND-0.8
C08	0/4 (0.0)	ND	ND	ND-0.5
C09	9/218 (4.1)	5.9 ± 6.3	0.2-14.8	0.2-0.6
C10	24/299 (8.0)	15.6 ± 21.1	1.0-93.1	1.3-2.3
C11	0/20 (0.0)	ND	ND	ND-3.5
C15	0/33 (0.0)	ND	ND	ND-0.7
Rice	**536/3021 (17.7)**	**10.6 ± 36.3**	**0.002-347**	**1.9-2.4**
C05	133/377 (35.3)	14.6 ± 36.0	0.08-272.2	5.1-5.5
C06	1/3 (33.3)	0.2	0.2	0.1-0.3
C07	25/75 (33.3)	3.6 ± 4.6	0.4-17.0	1.2-1.8
C08	90/400 (22.5)	1.1 ± 1.6	0.07-10.7	0.2-0.8
C09	53/1061 (5.0)	0.5 ± 0.5	0.07-2.5	0.02-0.3
C10	106/738 (14.4)	0.4 ± 0.9	0.002-7.1	0.1-0.9
C11	2/30 (6.7)	0.8 ± 0.2	0.7-0.9	0.1-3.3
C13	84/98 (85.7)	41.0 ± 71.3	0.2-347	35.1-35.2
C15	42/239 (17.6)	1.4 ± 2.0	0.04-8.7	0.2-1.0
Sorghum	**4/83 (4.8)**	**8.6 ± 5.4**	**0.6-12.0**	**0.4-0.6**
C09	0/9 (0.0)	ND	ND	ND-0.1
C10	4/73 (5.5)	8.6 ± 5.4	0.6-9.7	0.5-0.6
C15	0/1 (0.0)	ND	ND	ND-0.08
Wheat	**3/844 (0.4)**	**1.0 ± 0.7**	**0.1-1.4**	**0.003-0.6**
C06	0/1(0.0)	ND	ND	ND-0.3
C08	0/41 (0.0)	ND	ND	ND-1.1
C09	0/39 (0.0)	ND	ND	ND-0.6
C10	2/733 (0.3)	1.4	1.4	0.004-0.6
C15	1/30 (3.3)	0.1	0.1	0.004-1.2
Total	**576/4536 (12.7)**	**10.7 ± 35.3**	**0.002-347**	**1.4-1.9**

[a] mean of all samples; [b] samples below LOD or LOQ were considered as zero; [c] samples below LOD or LOQ were considered as 0.5LOD or 0.5LOQ. C05: samples from Brazil; C06: samples from Greece; C07: samples from France; C08: samples from Austria, Germany and Spain; C09: samples from Philippines, Singapore and Thailand; C10: samples from Canada, Cyprus, Italy, Japan, Latvia, Republic of Korea and United States of America; C11: samples from Belgium; C13: samples from Mali; C15: samples from Czech Republic, Ireland, Portugal, Slovakia, Slovenia and Sweden.

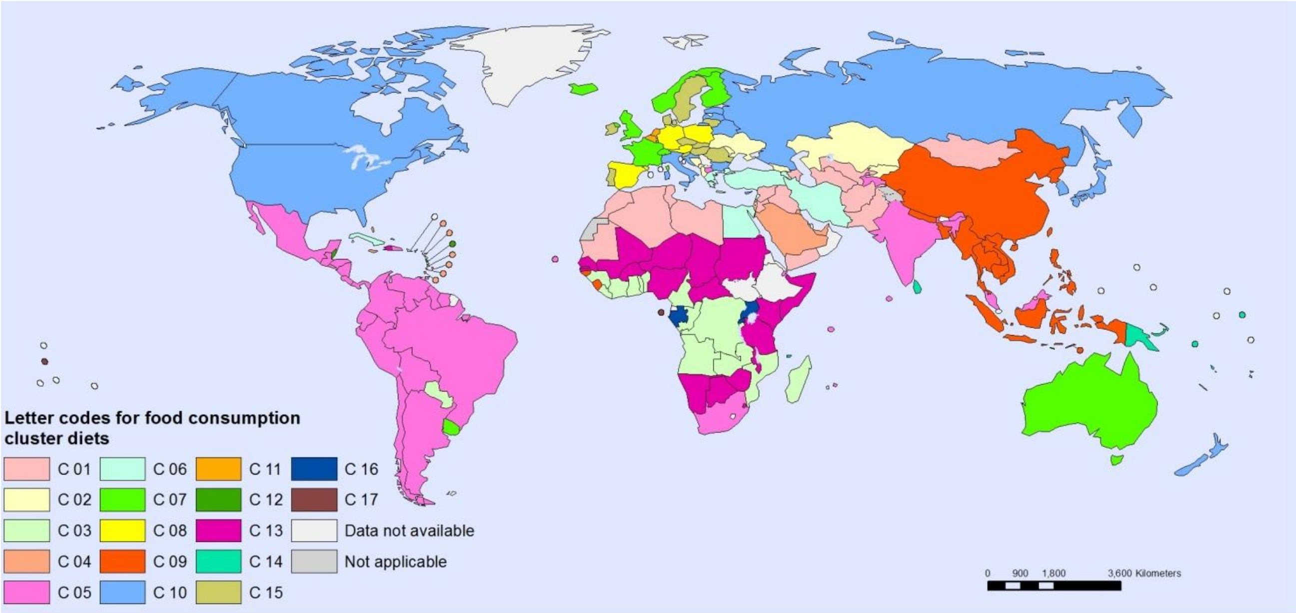

Figure 2. GEMS/Food 17 Consumption Cluster Diets (WHO, 2013).
Available at http://www.who.int/foodsafety/chem/gems/en/index1.html

HUMAN EXPOSURE AND RISK ASSESSMENT

31. A preliminary exposure assessment to aflatoxins through the consumption of rice, maize, sorghum and wheat was conducted using the GEMS/Food occurrence data and the 17 Cluster Diets. The total mean UB level of contamination for each cereal shown in Table 2 was used as concentration level (1.6 µg/kg for maize, 2.4 µg/kg for rice, 0.6 µg/kg for sorghum and 0.6 µg/kg for wheat).

32. Higher exposures (12.4 to 18.6 ng/kg bw/day**)** were found for C04, C05, C06, C09 and C14 (Tables 4a and 4b). Rice consumption was the cereal that most contributed to the total intake for C03, C05, C09, C12, C14 and C17. Wheat contributed the most to the total intake for C01, C02, C06, C07, C08, C11 and C15.

33. Preliminary risk assessments from the exposure to AFs were conducted using the JECFA procedure to estimate cancer risk (FAO/WHO, 1998) and the margin of exposures (MOE) approach (EFSA, 2007).

34. Cancer risks were estimated for each cluster using the Equations 1 and 2 (Tables 4a and 4b). The prevalence of hepatitis B virus ($HBsAg^+$) for each cluster was set based on the information given in Figure 3 (CDC, 2014), using the best agreement with Figure 2. The highest risk was found for C09 (2.1 cancers/year/10^5 individuals), mainly from the consumption of rice (86.6%), and the lowest for C07, C08, C11 and C16 (0.4-0.5 cancers/year/10^5 individuals).

$$P_{estimated} = [PHBsAg^+ \times \%\, pop.HBsAg^+] + [PHBsAg^- \times \%\, pop.HBsAg^-] \text{ Eq. 1}$$

$$Cancer\ risk = P_{estimated} \times Total\ intake \text{ Eq. 2}$$

Figure 3. Worldwide Prevalence of chronic hepatitis B virus infection among adults (CDC, 2014).
Available at http://wwwnc.cdc.gov/travel/yellowbook/2014/chapter-3-infectious-diseases-related-to-travel/hepatitis-b

35. The MOE, given by the ratio between the $BMDL_{10}$ (170 ng/kg bw/day) and the estimated exposure, is also shown in Tables 4a and 4b. A MOE below 10 000 may represent a public health concern (EFSA, 2007). The lowest MOE (highest risk) was found for C09 (9.1) and the highest (lowest risk) for C16 (49.2).

Table 4a. Aflatoxins intake (upper bound) through the consumption of maize, rice, sorghum and wheat for GEMS/Food Clusters C01 to C08 (ng/kg bw/day).

	AF (µg/kg)	CO1[a]	C02[b]	C03[c]	C04[a]	C05[a]	C06[a]	C07[a]	C08[a]
Maize	1.6	1.6	1.9	3.1	6.0	1.9	3.8	1.2	0.8
Rice	2.4	2.0	0.6	3.5	4.8	8.4	4.0	0.9	0.7
Sorghum	0.6	0.04	0.001	0.2	0.2	0.1	0.03	0.0	0.0
Wheat	0.6	4.2	3.8	0.4	3.1	1.9	4.8	2.8	2.7
Total	**1.9**	**7.8**	**6.3**	**7.2**	**14.0**	**12.4**	**12.5**	**4.9**	**4.2**
	Cancer risk[d]	0.8	0.7	0.8	1.5	1.3	1.3	0.5	0.4
	MOE[e]	21.7	27.1	23.7	12.1	13.8	13.5	34.8	40.5

[a] 3% $HBsAg^+$; [b] 6% $HBsAg^+$; [c] 8% $HBsAg^+$; [d] Cancers/ year/ 10^5 individuals, estimated according to FAO/WHO (1998);[e] based on a $BMDL_{10}$ in rodent of 170 ng/kg bw/day (EFSA, 2007).

Table 4b. Aflatoxins intake (upper bound) through the consumption of maize, rice, sorghum and wheat for GEMS/Food Clusters C09 to C17 (ng/kg bw/day).

	AF (µg/kg)	C09[b]	C10[a]	C11[a]	C12[b]	C13[c]	C14[b]	C15[a]	C16[c]	C17[b]
Maize	1.6	0.9	3.3	1.8	2.0	3.3	0.3	1.6	2.0	0.9
Rice	2.4	16.1	3.3	0.7	3.7	2.1	12.4	0.8	0.8	3.2
Sorghum	0.6	0.008	0.01	0.0	0.07	0.9	0.02	0.0	0.4	0.0
Wheat	0.6	1.6	2.6	2.4	1.8	0.6	1.2	3.0	0.3	1.4
Total	**1.9**	**18.6**	**9.2**	**4.9**	**7.6**	**7.0**	**14.0**	**5.4**	**3.5**	**5.6**
	Cancer risk[e]	2.1	1.0	0.5	0.9	0.8	1.6	0.6	0.4	0.6
	MOE[d]	9.1	18.6	35.0	22.3	24.4	12.2	31.4	49.2	30.6

[a] 3% $HBsAg^+$; [b] 6% $HBsAg^+$; [c] 8% $HBsAg^+$; [d] Cancers/ year/ 10^5 individuals, estimated according to FAO/WHO (1998);[e] based on a $BMDL_{10}$ in rodent of 170 ng/kg bw/day (EFSA, 2007).

RISK MANAGEMENT CONSIDERATIONS AND PUBLIC HEALTH CONCERNS

36. In the European Union, the maximum limit for AFs (AFB1+AFB2+AFG1+AFG2) is 4 µg/kg for all cereals and products derived from cereals, with the exception of maize and rice. As these cereals will be subjected to physical treatments before human consumption, an ML of 10 µg/kg has been established. For processed cereal-based foods and baby foods for infants, the ML is 0.1 µg/kg AFB1 (EC, 2006). In the United States, there is a general limit for AFs of 20 µg/kg for all foods (USFDA, 2000). In Brazil, there are MLs for AFs established for cereals and derived products (5 µg/kg, except maize), processed cereal-based foods and baby formulas for infants (1 µg/kg) and maize and its products (20 µg/kg) (ANVISA, 2011). In Iran, ML for AFs is 30 µg/kg for rice and corn, 15 µg/kg for wheat and 50 µg/kg for barley; for AFB1 the levels are 5 µg/kg for rice, wheat and corn and 10 µg/kg for barley (National Standard No. 5925).

37. Table 5 shows the impact of hypothetical MLs for AFs in rice (the cereal with the largest dataset submitted to GEMs/Food) on aflatoxin dietary intake and cancer risk for each cluster. As expected, the highest impact on risk was found for clusters for which rice contributed most to the total intake (Tables 4a and 4b). For C09 and C14, a ML of 40 µg/kg will decrease the risk by about 50% and a ML of 10 µg/kg will decrease the risk by about 63%. These clusters include mostly Asian countries (Figure 2), where rice consumption is high. Lower MLs will have almost no impact on the risk for all the clusters, except a ML of 1 µg/kg for C09 and C14.

38. Figure 4 shows the distribution of AFs in rice samples according to the concentration range. About 3% of the samples had higher than 10 µg/kg and 4% had levels higher than 1 µg/kg.

Table 5a. Effect of the implementation of MLs for rice on intake (ng/kg bw/day) and on cancer risk (cancers/year/10^5 individuals)

ML [f]	AFs, µg/kg [d]	CO1 [a]		C02 [b]		C03 [c]		C04 [a]		C05 [a]		C06 [a]		C07 [a]		C08 [a]	
		Intake [e]	Risk	Intake [e]	Risk	Intake [e]	Risk	Intake [e]	Risk	Intake [e]	Risk	Intake [e]	Risk	Intake [e]	Risk	Intake [e]	Risk
No limits	1.9	7.8	0.8	6.3	0.7	7.2	0.8	14.0	1.5	12.4	1.3	12.5	1.3	4.9	0.5	4.2	0.4
40	1.1	6.8	0.7	5.9	0.7	5.3	0.6	11.4	1.2	7.8	0.8	10.4	1.1	4.4	0.5	3.8	0.4
20	0.9	6.6	0.7	5.9	0.7	5.0	0.6	11.0	1.2	7.1	0.8	10.1	1.1	4.3	0.5	3.8	0.4
10	0.8	6.4	0.7	5.8	0.7	4.7	0.5	10.6	1.1	6.4	0.7	9.7	1.0	4.3	0.5	3.7	0.4
5	0.8	6.4	0.7	5.8	0.7	4.7	0.5	10.6	1.1	6.4	0.7	9.7	1.0	4.3	0.5	3.7	0.4
1	0.6	6.1	0.6	5.7	0.6	4.1	0.5	9.8	1.0	5.0	0.5	9.1	1.0	4.1	0.4	3.6	0.4

[a] 3% $HBsAg^+$; [b] 6% $HBsAg^+$; [c] 8% $HBsAg^+$; [d] total upper bound mean considering all cereals (Table 2); [e] upper bound intake; [f] considering setting the ML for rice only

Table 5b. Effect of the implementation of MLs for rice on intake (ng/kg bw/day) and on cancer risk (cancers/year/10^5 individuals)

ML [f]	AFs, µg/kg [d]	C09 [b]		C10 [a]		C11 [a]		C12 [b]		C13 [c]		C14 [b]		C15 [a]		C16 [c]		C17 [b]	
		Intake [e]	Risk	Intake [e]	Risk	Intake [e]	Risk	Intake [e]	Risk	Intake [e]	Risk	Intake [e]	Risk	Intake [e]	Risk	Intake [e]	Risk	Intake [e]	Risk
No limits	1.9	18.6	2.1	9.2	1.0	4.9	0.5	7.6	0.9	7.0	0.8	14.0	1.6	5.4	0.6	3.5	0.4	5.6	0.6
40	1.1	9.9	1.1	7.4	0.8	4.5	0.5	5.6	0.6	5.8	0.7	7.3	0.8	5.0	0.5	3.0	0.3	3.8	0.4
20	0.9	8.6	1.0	7.1	0.8	4.4	0.5	5.3	0.6	5.6	0.7	6.2	0.7	4.9	0.5	2.9	0.3	3.5	0.4
10	0.8	7.2	0.8	6.8	0.7	4.4	0.5	5.0	0.6	5.4	0.6	5.2	0.6	4.8	0.5	2.9	0.3	3.3	0.4
5	0.8	7.2	0.8	6.8	0.7	4.4	0.5	5.0	0.6	5.4	0.6	5.2	0.6	4.8	0.5	2.9	0.3	3.3	0.4
1	0.6	4.5	0.5	6.3	0.7	4.2	0.4	4.4	0.5	5.1	0.6	3.1	0.3	4.7	0.5	2.7	0.3	2.7	0.3

[a] 3% $HBsAg^+$; [b] 6% $HBsAg^+$; [c] 8% $HBsAg^+$; [d] total upper bound mean considering all cereals (Table 2); [e] upper bound intake; [f] considering setting the ML for rice only

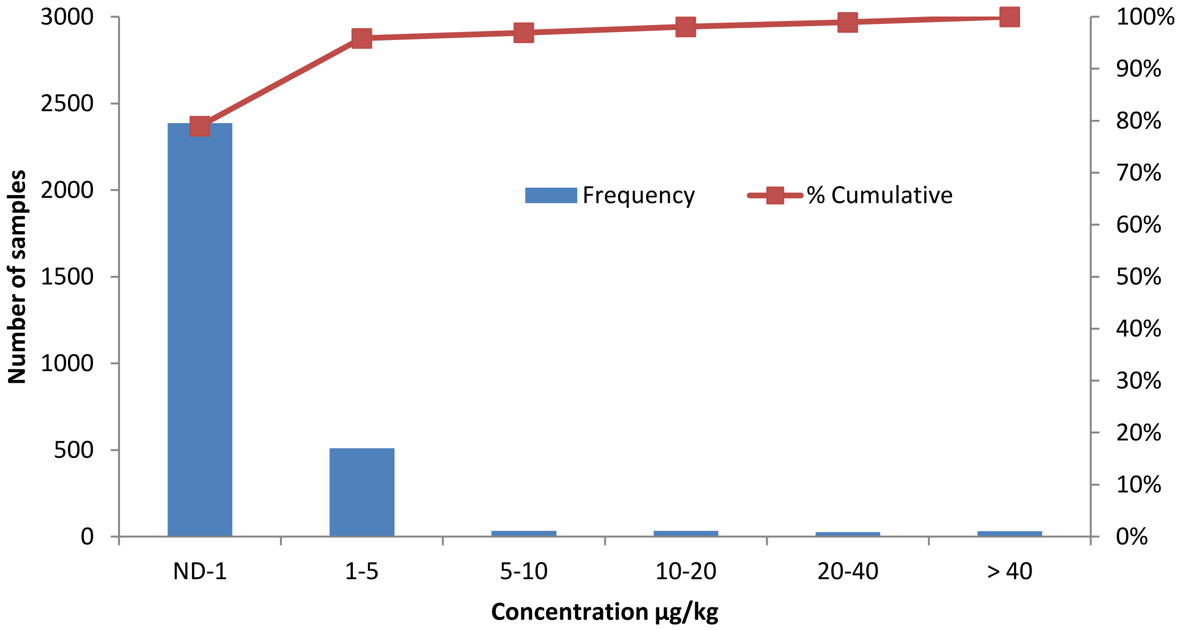

Figure 4. Distribution of AF levels in rice (GEMSFood data)

STABILITY DURING PROCESSING

39. AFs are relatively stable compounds that are not completely destroyed by most food processes, and therefore, cereal based foods ready for consumption may still be contaminated. Sorting, cleaning, milling and thermal processing (cooking, baking, roasting, flaking, extrusion) may reduce AFs content in food products. Figure 2 illustrates the time course of AF formation and reduction in maize in reference to the Food Safety Objective (Pitt et al, 2013).

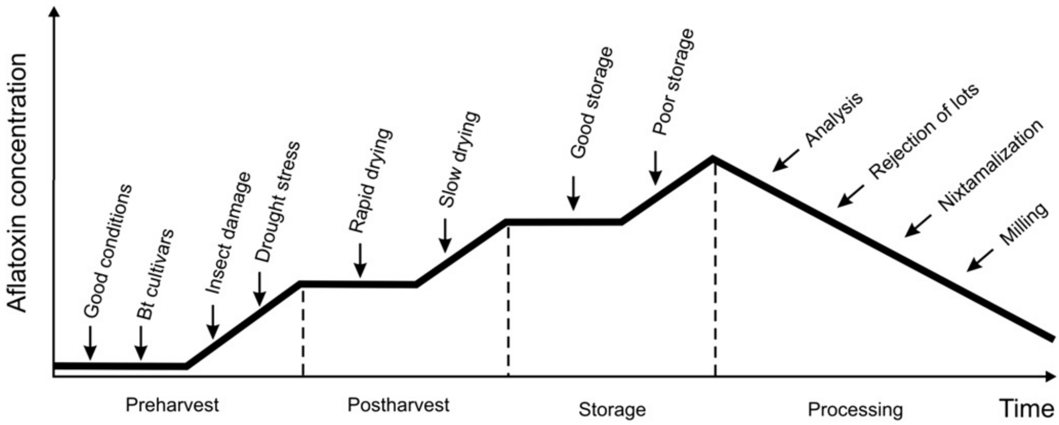

Figure 1. The time course of AF formation and reduction in maize, with reference to the Food Safety Objective (Pitt et al., 2013)

40. Sorting and cleaning usually remove the contaminated parts of the cereals, lowering AF concentration. Johansson et al. (2006) demonstrated that AFs are concentrated in the poor-quality grade components of shelled maize. About 60% of AF mass was found in damage kernels (DM), broken kernels and foreign materials (BCFM), representing only 5% of total mass. This study also found a correlation (0.964) between AF mass in the combined DM and BCFM components with AF concentration in the lot, indicating its potential value as a screening method to predict AF in a bulk lot of maize.

41. Pearson et al. (2004) tested a high-speed dual-wavelength sorter for removing maize contaminated with AFs. Reduction in AF content reached 82% in samples of yellow maize with initial AF level higher than 10 µg/kg, and 38% in samples contaminated with less than 10 µg/kg. The same approach was applied in white maize, reducing 46% of the AF content in the first sorting and 88% after a re-sorting (Pearson et al., 2010).

42. The same occurs in the milling process, where AFs may be redistributed and concentrated in certain fractions. Siwela et al. (2005) showed that AF concentration in maize meal was reduced by approximately 92% after dehulling of maize grains. During the production of polished rice (after dehulling and whitening process), AF reduction of 92-97% from the initial concentration in the raw grain was observed by Castells et al. (2007).

43. Several studies investigated the distribution of AFs during the maize wet -milling process (CRA, 2011). These studies demonstrated that AFs are mostly found in the aqueous phase of the process, due to their relatively high solubility in the water fraction. Therefore, starch, the fraction commonly used for food, is essentially aflatoxin-free.

44. The distribution of AFs in dry-milled maize fractions was evaluated by Castells et al. (2008). The authors found higher levels of AFs in the outer layers of the kernels, while processed products from the inner parts of grain, such as maize meal and flaking grits, had decreased mycotoxin levels. Pietri et al. (2009) found reductions of 8.0% (from a 5 µg/kg contaminated maize lot) and 57.0% (from a 120 µg/kg lot) of AF levels after cleaning steps. The subsequent removal of bran and germ led to a further decrease in contamination levels in the products destined for human consumption. In both papers, the most contaminated parts were those usually intended for animal feed production.

45. Hwang and Lee (2006) evaluated the reduction of AFB1 contamination in wheat after washing (10 to 30 min) and heating dry and wet wheat in an oven at various temperatures (50 to 200°C) during different periods of time (30 to 90 min). AFB1 reduction in all wheat samples was proportional to washing time (increased with longer time), ranging from 41.0 to 62.0%. AFB1 concentration decreased as temperature increased, with the most significant reduction at temperatures above 100°C. Reductions under wet heating were between 40.0 and 47.0% (100°C/30 min), up to 20% higher than what was found under dry conditions.

46. The effect of cooking (ordinary and under pressure) on AFB1 levels in polished rice was investigated by Park and Kim (2006). The ordinary cooking process reduced AF levels by 31-36%, while in the pressure-cooked rice the reduction of AF was considerably higher (78-88%). The Ames mutagenicity test showed reductions in the aflatoxin-induced toxicity of 19-29% for ordinary-cooked rice and 68-78% for pressure-cooked rice. Hussain and Luttfullah (2009) found the highest AFB1 reduction in rice cooked with excess water (87.5%), followed by ordinary (82.5%) and microwave (77.6%) cooking.

47. AF inactivation by extrusion cooking of maize flour was evaluated by Cazza niga et al. (2001). The effects of flour moisture, extrusion temperature and sodium metabisulphite addition were evaluated. The AFB1 reduction in maize flour ranged from 10.0% to 25.0%, with the greatest reduction when the additive was used. Extrusion of rice meal showed higher reductions of AFs content, ranging from 51.0% to 95.0%, depending on the AF and extrusion conditions (initial moisture content, barrel temperature and residence time) (Castells et al., 2006).

48. The reduction of AFB1 content in the process of nixtamalization and extrusion of maize during production of tortillas was investigated by Elias-Orozco et al. (2002). The traditional nixtamalization process reduced the levels of AFB1 by 94.0% and the extrusion process by 46.0%. However, when extrusion process was combined with treatment with calcium hydroxide, a 85% reduction of AFB1 was achieved.

49. Pérez-Flores et al. (2011) evaluated the effect of microwave heating during alkaline-cooking (calcium hydroxide) of AF (B1+B2) contaminated maize during the production of tortillas. The modified tortilla-making process caused a decrease of 68.0-84.0% in AF content and, after an extract acidification (as occurs during digestion), there was an increase of up to 3.0% in AF content in tortillas.

CONCLUSIONS

50. Occurrence data on aflatoxins (AFs) on rice, maize, wheat and sorghum grain submitted to GEMSFoods referred to the analysis of 4,536 samples, mostly rice samples (66.6%; 3021 samples). Rice was also the cereal with the highest AF incidence (17.7%) and level (total UB mean of 2.4 µg/kg). There is a large discrepancy on occurrence and concentration of AFs in rice among the cluster diets, ranging from 0.3 µg/kg in C09 (Asian countries) to 35.2 µg/kg in C13 (countries in Africa).

51. A preliminary risk assessment showed cancer risks from the exposure to the cereals ranging from 0.4 to 2.1 cancers/10^5 individuals, with the highest risk for C09, mainly from the consumption of rice. The AFs level in rice used for the assessment (2.4 µg/kg) is eight times higher than the AFs level in C09 (mean UB of 0.3 µg/kg).

52. It is important to emphasize that the exposure and the risk estimates shown in this paper are overestimates as they do not consider the impact of processing on AF levels, mainly after sorting, milling and maize nixtamalization. Cooking rice may reduce AFs content by up to 80%.

53. Very few data were provided for maize (588 samples), wheat (844 samples) and sorghum (81 samples), especially for clusters where these commodities have the highest impact on the dietary intake. For example, there is no data on sorghum submitted from African countries.

54. The impact of a hypothetical maximum level (ML) for AFs on cancer risk was only considered for rice. At a ML of 10 µg/kg, the cancer risk decreased by up to 63% compared to a no limit situation. Lower ML had no impact on cancer risk for all clusters, except C09 and C14 (Asian countries), for which a ML of 1 µg/kg would decrease the risk by 76-78%. The impact on the food supply would be about the same in both situations (3-4% of the samples rejected).

RECOMMENDATIONS

Consistent with the information provided in this discussion paper, the eWG recommends that:

- Country members should provide additional data to GEMS/Food on AFs, especially for wheat, maize and sorghum. Countries where these cereals are important in their diet are highly encouraged to submit data.
- Further work should include additional data on cereal grains and processed commodities provided to GEMS/Food.

- The Committee should decide whether it is appropriate to start ~~a~~ discussion on a ML for AFs in rice and associate sampling plan or wait until a larger database is available for considering whether MLs are needed for all cereals.
- The Committee should take into account the annex on aflatoxins that will be included in the *Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals* (CAC/RCP 51-2003) and the proposed revisions to the general part of the Code.

Points for discussion

Japan suggests that "*the CCCF should consider the establishment of an annex for aflatoxins in rice in the Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals. It should be given higher priority than establishment of a ML for rice*".

Currently, the Code has annexes for specific mycotoxins (fumonisins, ochratoxin A, zearalenone and trichothecenes) and the proposed revision (CX/CF 14/8/14) suggests the inclusion of an annex on aflatoxins. The Committee should consider the benefit of including a specific annex for rice, as it would probably require a complete change in the format of the Code. An *Annex for the prevention and reduction of aflatoxins and ochratoxin A contamination in sorghum* (*Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals*) is under discussion by the CCCF.

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