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## Aflatoxins in cereals: worldwide occurrence and dietary risk assessment

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### RESEARCH ARTICLE

#### Abstract

The worldwide occurrence of aflatoxins (AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, AFG<sub>2</sub>), genotoxic mycotoxins, in raw maize, rice, sorghum and wheat samples collected since the year 2000 was evaluated using published data and occurrence data from the GEMS/Food database (<https://extranet.who.int/gemsfood>). Dietary risk assessments were conducted using GEMS/Food total aflatoxin occurrence and food consumption data obtained from the 17 Cluster Diets. Risk characterisation arising from aflatoxin exposure was conducted using both cancer risk and margin of exposure (MOE) approaches. A total of 89 publications were retrieved from the literature, reporting data related to 18,097 samples, of which 37.6% were positive for at least one aflatoxin. The total upper bound (UB) mean for all samples analysed was 13.6 µg/kg, and was higher for rice (24.6 µg/kg) and sorghum (25.9 µg/kg). Of data related to the analysis of 4,536 samples reported to GEMS/Food database, 12.7% were positive for at least one aflatoxin. The total UB mean was 1.9 µg/kg, and was higher for rice (2.4 µg/kg) and maize (1.6 µg/kg). Total intakes ranged from 3.0 ng/kg bw/day (Cluster C11) to 17.1 ng/kg bw/day (Cluster C09). On average, the consumption of rice contributed to 41.6% of the total aflatoxin intake in all clusters, followed by wheat (35.4%), maize (21.2%) and sorghum (1.8%). The lowest cancer risk was found in cluster C11 (0.057 cancers/year/10<sup>5</sup> individuals), and the highest in cluster C09 (0.467 cancers/year/10<sup>5</sup> individuals). MOE ranged from 56 (C11) to 10 (C09), indicating a potential risk to consumers. These results highlight the need for continuous action by health authorities to decrease aflatoxin contamination in cereals, as they are staple foods in diets worldwide. These actions include the enforcement of code of practices at the national level and the establishment of maximum contamination levels by the Codex System.

**Keywords:** aflatoxins, cereal diets, dietary exposure, carcinogenicity, risks

### 1. Introduction

Cereals are staple foods in diets around the world. Wheat is the main cereal consumed in America and Asia accounting, respectively, for 14.1 and 24.3% of the total calorie intake in these regions. Rice is the main contributor to the total energy intake in Asia (28.5%) and wheat and maize contribute equally (30%) in Africa (FAO, 2014). The contamination of cereals with aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> (AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>) has been reported worldwide. Mean concentrations in positive maize samples in Argentina and Uganda were, respectively, 35.8 µg/kg (total aflatoxins; 264/3,192 samples) (Garrido *et al.*, 2012), and 19.5 µg/kg (total aflatoxins; 296/390 samples) (Kaaya and Kyamuhangire, 2006). The mean level of aflatoxins found in rice from Pakistan was 11.2 µg/kg (total aflatoxins;

185/413 samples) (Iqbal *et al.*, 2012), while in South Korea it was only 1.7 µg/kg (total aflatoxins; 6/160 samples) (Ok *et al.*, 2014). In Nigeria, 55% of the 168 sorghum samples were contaminated with AFB<sub>1</sub> with levels up to 1,164 µg/kg (Hussaini *et al.*, 2009), while in Turkey wheat samples reached levels up to 643.5 µg/kg (total aflatoxins; 24/41 samples) (Giray *et al.*, 2007).

Aflatoxins are human liver carcinogens, with AFB<sub>1</sub> shown to be genotoxic (IARC, 1993); as such, exposure should be as low as reasonably achievable (CAC, 1995). The complete elimination of aflatoxins from the food supply, however, is not possible, and worldwide strategies are needed to control and manage contamination (CAC, 2003). *Aspergillus flavus* and *Aspergillus parasiticus* infection and aflatoxin production in cereals are influenced by several

environmental factors such as temperature, humidity, insect damage and drought (Miraglia *et al.*, 2009). Furthermore, aflatoxins can also be produced after harvesting the grain (Pitt *et al.*, 2013), mainly during storage.

Several countries have established regulatory limits to control the presence of aflatoxins in cereals, including Brazil (Anvisa, 2011), European Union (EC, 2006), and the United States (USFDA, 2000). Internationally, maximum levels (ML) for aflatoxins in cereals are currently under discussion at the Codex Committee on Contaminants in Foods (FAO/WHO, 2014). Given the difficulty of eliminating aflatoxins from the food chain and considering the worldwide consumption of cereals, dietary risk assessments for aflatoxins are essential to help government authorities and the Codex Alimentarius to take actions aimed at reducing risk while still ensuring the food security.

In the context of food safety, risk assessment is a four-step conceptual framework that aims to estimate the risk of occurrence of adverse health effects after exposure to chemicals present in food. The hazard identification step is designed to identify the nature of the adverse health effects caused by human exposure to the contaminant, and the aim of the hazard characterisation step is to establish a quantitative relationship between exposure and the incidence of adverse effects. In the exposure assessment step the likely intake of contaminants through the diet is estimated, taking into account the concentration of the chemical in food, as well as consumption patterns. The risk characterisation step finalises the process, providing an estimation of the probability of occurrence of health outcomes in a population under defined exposure conditions (IPCS, 2009).

Dietary exposure assessments for aflatoxins have been conducted worldwide. In most studies, cereals accounted for over 90% of the total intake (Andrade *et al.*, 2013; Ding *et al.*, 2012; Li *et al.*, 2014; Park *et al.*, 2004; Yazdanpanah *et al.*, 2013). Risk characterisation for aflatoxins has been conducted using two different approaches. The first, developed by the FAO/WHO Joint Expert Committee on Food Additives (JECFA), estimates the cancer risk for a given population considering the incidence of the hepatitis B virus (HBsAg<sup>+</sup> individuals) and the carcinogenic potency of aflatoxins, which was defined for HBV carriers and non-carriers (FAO/WHO, 1998). More recently, the margin of exposure (MOE) approach has been used by the European Food Safety Authority (EFSA) and was recommended by JECFA to evaluate compounds that are both carcinogenic and genotoxic (EFSA, 2005; FAO/WHO, 2006). The MOE is the ratio of a toxicological threshold obtained from animal studies and the estimated human exposure (IPCS, 2009).

This study aimed to evaluate the current scenario on aflatoxin contamination in raw maize, rice, sorghum and

wheat commercialised worldwide, and to estimate the dietary exposure to aflatoxins and the potential health risks arising from this exposure. The first draft of this paper was the basis for the preparation of the Discussion Paper on Aflatoxins in Cereals presented at the 8<sup>th</sup> Session of the Codex Committee on Contaminants in Food (CX/CF 14/8/15; CAC, 2014a).

## 2. Materials and methods

### Aflatoxins occurrence: data obtained from the literature

Occurrence data on aflatoxins in raw maize, rice, sorghum and wheat were obtained from published studies related to samples collected from 2000 to 2014. The search was conducted in the Web of Science database and Google Scholar in September 2012, July 2013, and May 2014, using the following keywords: 'mycotoxin' and 'aflatoxin' alone, or in combination with 'maize', 'rice', 'sorghum' and 'wheat', using the logical operator AND. Papers related to samples that were inoculated with mycotoxin producing fungi in the laboratory were excluded. Only peer review papers were considered in the search, written in English or in other languages.

For each crop, the mean values estimated for all studies were calculated by weighting the reported mean of each study by the number of samples analysed in that study. When only the median value was reported in the study, this value was used to estimate the weighted mean. When only the concentration range was reported, the midrange was used in the calculation. The lower bound of the total mean (LB) was estimated considering samples below the limit of detection (LOD) or below the limit of quantification (LOQ) as zero. The upper bound (UB) was obtained considering samples below LOD or below LOQ as  $\frac{1}{2}$ LOD or  $\frac{1}{2}$ LOQ. Whenever the LOD or LOQ of the method used in the study were not reported, limits found in other studies that used a similar analytical method were used in the calculation of the UB mean. When both LOD and LOQ were reported, the latter was used in the estimation.

### Aflatoxins occurrence: data from the GEMS/Food database

The Global Environment Monitoring System/Food Contamination Monitoring and Assessment Programme (GEMS/Food) compile surveillance and monitoring data on food contamination submitted by national government authorities. In July 2013, the JECFA issued a specific public call for data on aflatoxin contamination in cereals, to be submitted to GEMS/Food (<https://extranet.who.int/gemsfood>). Data on total aflatoxin (AFB<sub>1</sub> + AFB<sub>2</sub> + AFG<sub>1</sub> + AFG<sub>2</sub>) in raw maize, rice, sorghum and wheat were extracted from the GEMS/Food database using an ADS WHO partner login, and exported to MS Excel (Microsoft,

Redmond, WA, USA) spreadsheets. Data were obtained for all WHO regions and countries, with the sampling period starting in 2000. Data were extracted on October 21, 2013 and on July 02, 2014.

The informed food codes (WHO food identifier, WHO food code and local food identifier) were used to identify processed commodities, which were not included in this study. Rice samples that included inedible portions (husk) or that were submitted to heat treatment (cooked) prior to analysis were also excluded. When the portion analysed was not mentioned, it was assumed that the analysis was performed in the cereal edible portion. Information regarding analytical quality assurance was also obtained from the GEMS/Food database.

For some samples, there were up to six entries in the database (individual aflatoxins, sum of AFB<sub>1</sub> and AFB<sub>2</sub>, and total aflatoxins), but only the total aflatoxins value was considered. When the total aflatoxins value was not included, it was estimated from the individual aflatoxin values. When values reported were below LOQ or LOD, they were considered as 0 or ½LOQ/LOD in the LB or UB estimations of the means, respectively. When both LOD and LOQ were reported, ½LOQ was used. Where LOD or LOQ was not reported, the value informed for other samples from the same laboratory or country was used.

#### Consumption of cereals: data from the 17 GEMS/Food Consumption Cluster Diets

The Food and Agriculture Organization of the United Nations (FAO) compiles country-level data on the production and trade of food commodities, producing

food balance sheets that provide data on the overall per capita supply of commodities within countries (FAO, 2014). GEMS/Food uses the FAO Food Supply Utilisation Account data to determine the food consumption patterns that are used in chronic dietary risk assessments conducted at the international level by FAO/WHO scientific panels, including the JECFA. The 17 GEMS/Food cluster diets were elaborated based on FAO Food Supply Utilisation Account data from 2002 to 2007 for 179 countries. Clusters were formed according to their consumption system profiles (combination of different food products and local factors such as availability, seasonality and socio-cultural habits) using statistical methods (Sy *et al.*, 2013). The average data were weighted by the population size to determine the average kg/person/cluster over a 5 year period. The countries included in each Cluster are shown in Figure 1. Body weight (bw) is 60 kg for all clusters, except cluster 09 (55 kg).

#### Dietary risk assessment

Total chronic intake of aflatoxins through the consumption of rice, maize, wheat and sorghum for each of the GEMS/Food Cluster Diets was estimated using the International Estimated Daily Intake (IEDI) 17 Cluster diets template, developed by the Dutch National Institute for Public Health and the Environment, in cooperation with the WHO, to conduct dietary intake by the FAO/WHO Joint Meeting on Pesticide Residues (FAO/WHO, 2013).

The IEDI 17 Cluster diets template estimates the dietary intake of aflatoxins, according to FAO/WHO recommendation (FAO/WHO, 2005), as shown in Equation 1:

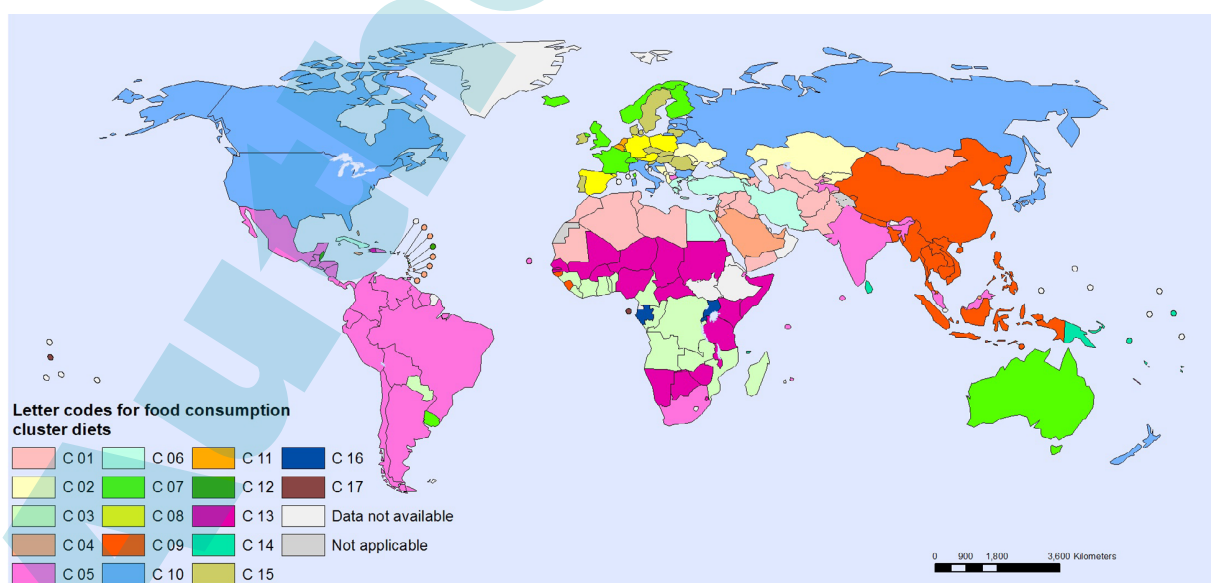


Figure 1. 17 GEMS/Food Consumption Cluster Diets (WHO, 2014).

$$Total\ intake = \sum \frac{(consumption \times concentration)}{body\ weight} \quad (1)$$

The Cluster diet consumption figures used in the intake estimation includes processed food. For maize, it includes flour, oil, beer, germ and starch; for rice, it includes polished and husked rice, flour, oil, beverages and starch; for sorghum, it includes beer and flour; and for wheat, it includes whole meal, flour, beverages, pasta, bread, starch, gluten, and mixed grain. The concentration used in the intake estimations was obtained from samples submitted to the GEMS/Food database (UB mean concentration).

Risk characterisation arising from aflatoxin exposure was conducted using both the cancer risk (FAO/WHO, 1998) and MOE approaches (EFSA, 2005). The cancer risk for each cluster was calculated by multiplying the carcinogenic potency ( $P_{cancer}$ ) by the total intake of AFs (Equation 2). The  $P_{cancer}$  considers both the carcinogenic potency of AFs for individuals with hepatitis B virus (PHBsAg<sup>+</sup> = 0.3 cancers/year/100,000 individuals/ng aflatoxin/bw/day) and for non-infected individuals (PHBsAg<sup>-</sup> = 0.01 cancers/year/100,000 individuals/ng aflatoxin/bw/day), as well as the percentage of carriers (HBsAg<sup>+</sup>) and non-carriers (HBsAg<sup>-</sup>) of hepatitis B virus in the population (Equation 3). The worldwide prevalence of chronic hepatitis B virus infection among adults published by CDC (2014) was used to estimate the prevalence of hepatitis B virus (HBsAg<sup>+</sup>) for each cluster.

$$Cancer\ risk = P_{cancer} \times total\ intake \quad (2)$$

$$P_{cancer} = (PHBsAg^+ \times \%pop.HBsAg^+) + (PHBsAg^- \times \%pop.HBsAg^-) \quad (3)$$

The MOE was given by the ratio between the benchmark dose level that caused a 10% increase in cancer incidence in rodents (BMDL10 = 170 ng/kg bw/day; 95% lower confidence limit) (EFSA, 2007) and the total intake (Equation 4). MOE values lower than 10,000 may indicate a public health concern (EFSA, 2005).

$$MOE = BMDL10 / total\ intake \quad (4)$$

### 3. Results

#### Aflatoxins occurrence: data from the literature

A total of 89 publications reporting data on aflatoxins contamination in raw cereal samples collected since 2000 were retrieved from the literature. The first such study was published in 2003, and the highest numbers of papers were found in 2011 and 2012 (15 and 14 papers, respectively). A summary of the published studies, grouped by continent, is shown in Table 1. Data covers samples collected in a wide range of countries. Most papers concerned maize (n=47) and rice (n=39), and 18 studies analysed two or more cereals of interest to this study. The majority of papers

Table 1. Summary of published data on aflatoxins in cereal samples collected from 2000 onwards.

Country	Cereal <sup>1</sup>	Reference
African continent		
Algeria	W	Riba <i>et al.</i> , 2010
Benin and Togo	M	Egal <i>et al.</i> , 2005
Burkina Faso	M	Probst <i>et al.</i> , 2014; Warth <i>et al.</i> , 2012
Cameroon	M	Abia <i>et al.</i> , 2013; Probst <i>et al.</i> , 2014
Egypt	M	Nogaim <i>et al.</i> , 2011
Ethiopia	S	Chala <i>et al.</i> , 2014; Probst <i>et al.</i> , 2014
Kenya	M, W	Daniel <i>et al.</i> , 2011; Muthomi <i>et al.</i> , 2008; Mwhia <i>et al.</i> 2008; Probst <i>et al.</i> , 2014
Ivory Coast	M, R	Probst <i>et al.</i> , 2014; Sangare-Tigori <i>et al.</i> , 2006
Lesotho	M	Mohale <i>et al.</i> , 2013; Probst <i>et al.</i> , 2007
Malawi	S	Matumba <i>et al.</i> , 2011; Probst <i>et al.</i> , 2014
Morocco	W	Zinedine <i>et al.</i> , 2006
Mozambique	M	Probst <i>et al.</i> , 2014; Warth <i>et al.</i> , 2012
Nigeria	M, R, S, W	Adejumo <i>et al.</i> , 2013; Ayejuyo <i>et al.</i> , 2011; Bandyopadhyay <i>et al.</i> , 2007; Bankole and Mabekoje, 2004; Hussaini <i>et al.</i> , 2009; Makun <i>et al.</i> , 2011
South Africa	M	Chilaka <i>et al.</i> , 2012; Shephard <i>et al.</i> , 2013
Tanzania	M	Kimanya <i>et al.</i> , 2008; Probst <i>et al.</i> , 2014
Tunisia	M, R, S, W	Ghali <i>et al.</i> , 2008, 2009, 2010; Oueslati <i>et al.</i> , 2012
Uganda	M	Kaaya and Kyamuhangire, 2006; Probst <i>et al.</i> , 2014
Zambia	M	Mukanga <i>et al.</i> , 2010; Probst <i>et al.</i> , 2014
D. Republic of Congo, Ghana, Mali, Rwanda, Senegal, Sierra-Leone, Somalia, Zimbabwe	M	Probst <i>et al.</i> , 2014

Table 1. Continued.

Country	Cereal <sup>1</sup>	Reference
American continent		
Argentina	M	Broggi <i>et al.</i> , 2007; Garrido <i>et al.</i> , 2012
Brazil	M, R	Almeida <i>et al.</i> , 2012; Carvalho <i>et al.</i> , 2010; Dors <i>et al.</i> , 2011, 2013; Moreno <i>et al.</i> , 2009; Nunes <i>et al.</i> , 2003; Oliveira <i>et al.</i> , 2010; Rocha <i>et al.</i> , 2009
Canada	M, R, W	Bansal <i>et al.</i> , 2011; Martos <i>et al.</i> , 2010
United States of America	M, R, W	Abbas <i>et al.</i> , 2006; Bruns <i>et al.</i> , 2007; Liao <i>et al.</i> , 2013
Asian continent		
China	M, R	Fu <i>et al.</i> , 2008; Gao <i>et al.</i> , 2011; Lai <i>et al.</i> , 2014; Liu <i>et al.</i> , 2006; Sun <i>et al.</i> , 2011; Zhu <i>et al.</i> , 2013
India	R, S, W	Ratnavathi <i>et al.</i> , 2012; Reddy <i>et al.</i> , 2009; Toteja <i>et al.</i> , 2006
Iran	M, R	Ghiasian <i>et al.</i> , 2011; Karami-Osboo <i>et al.</i> , 2012; Mazaheri, 2009; Mohammadi <i>et al.</i> , 2012; Sani <i>et al.</i> , 2014; Yazdanpanah <i>et al.</i> , 2013
Japan	M, R	Sugita-Konishi <i>et al.</i> , 2006
Korea	M, R	Kim <i>et al.</i> , 2013; Park <i>et al.</i> , 2004
Malaysia	R, W	Khayoon <i>et al.</i> , 2012; Rahman and Jinap, 2010; Reddy and Baharuddin, 2010; Soleimany <i>et al.</i> , 2011; Soleimany <i>et al.</i> , 2012
Pakistan	M, R, S, W	Ahsan <i>et al.</i> , 2010; Asghar <i>et al.</i> , 2014; Hussain <i>et al.</i> , 2011; Iqbal <i>et al.</i> , 2012; Khaton <i>et al.</i> , 2012; Lutfullah and Hussain, 2012; Shah <i>et al.</i> , 2010
Qatar	R, W	Abdulkadar <i>et al.</i> , 2004
South Korea	R	Ok <i>et al.</i> , 2014
Taiwan	R	Yu <i>et al.</i> , 2013
Vietnam	R	Nguyen <i>et al.</i> , 2007
European continent		
Austria	R	Reiter <i>et al.</i> , 2010
Germany	M, R	EFSA, 2007; Reinhold and Reinhardt, 2011
Italy	M, W	Covarelli <i>et al.</i> , 2011; EFSA, 2007; Pace <i>et al.</i> , 2012
Serbia	M, W	Jakic-Dimic <i>et al.</i> , 2009; Kos <i>et al.</i> , 2013
Turkey	M, R, W	Alptekin <i>et al.</i> , 2009; Aydin <i>et al.</i> , 2011; Giray <i>et al.</i> , 2007; Oruc <i>et al.</i> , 2006
Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Greece, Hungary, Ireland, Latvia, Luxembourg, Slovakia, Slovenia, Spain, Sweden	M	EFSA, 2007

<sup>1</sup> M = maize; R = rice; S = sorghum; W = wheat.

(56) reported method validation data. One study reported that the laboratory participated in proficiency testing, two in interlaboratory studies, and one reported the use of certified reference material for method validation. Thirty papers did not provide any analytical quality assurance information. Even though quality assurance information was not available in some studies, all data were included in the dataset in order to describe the occurrence scenario.

Table 2 summarises the published data on aflatoxin levels in cereals. A total of 18,097 samples were analysed in the studies, with maize accounting for 54.3% of the samples (9,819 samples), followed by rice (21.1%). About 41% of the samples were collected in Asia, of which 39.2% were rice samples. Maize was the main cereal analysed in American

countries, accounting for 85.6% of the samples for the region. Most of the analysed wheat samples were from Asian countries (72.1%). Sorghum was only analysed in samples from African and Asian countries.

Considering all samples analysed in the studies, 37.6% were positive for at least one aflatoxin (Table 2). Sorghum had the highest incidence of positive samples (68.9%), followed by rice (52.3%). Contaminated rice, sorghum and wheat samples were mostly from Asia (about 80%), while 40% of contaminated maize came from Africa. There was no positive wheat sample reported in the American continent and the lowest incidence of aflatoxins for the other commodities was also found in this continent.

**Table 2. Worldwide occurrence of total aflatoxin in cereals obtained from published literature (samples collected from 2000 onwards).**

	n <sup>1</sup>	Positive/analysed samples (%)	Positive samples (µg/kg)		Total mean <sup>2</sup>
			Mean ± SE <sup>1</sup>	Range	LB <sup>3</sup> - UB <sup>4</sup> (µg/kg)
Maize	47	2,496/9,819 (25.4)	28.2±5.5	0.01-48,000	7.2-8.1
Africa	20	997/2,771 (36.0)	25.9±6.2	0.01-48,000	9.3-9.7
America	9	409/4,056 (10.1)	30.8±4.5	0.1-1,393	3.1-4.9
Asia	12	655/1,134 (57.8)	35.6±19.9	0.02-888.3	20.5-20.8
Europe <sup>5</sup>	6	435/1,858 (23.4)	20.1 <sup>(6)</sup> ±5.5	0.01-820	4.8-5.0
Rice <sup>7</sup>	39	1,995/3,811 (52.3)	46.6±3.6	0.002-371.9	24.4-24.6
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	23	1,654/2,889 (57.3)	54.0±4.6	0.01-308	30.9-31.0
Europe	3	72/198 (36.4)	8.8±3.1	0.05-21.4	3.2-3.5
Sorghum	11	1,433/2,079 (68.9)	37.3±17.4	0.01-1,164	25.7-25.9
Africa	9	257/463 (55.5)	79.7±21.1	0.34-1,164	44.2-44.3
Asia	2	1,176/1,616 (72.8)	27.8±11.4	0.01-264	20.2-20.4
Wheat	18	874/2,388 (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	691/1,721 (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	89	6,798/18,097 (37.6)	34.2±3.4	0.002-48,000	12.9-13.6

<sup>1</sup> n = number of studies; SE = standard error.  
<sup>2</sup> Mean of all samples.  
<sup>3</sup> Lower bound: samples < LOD/LOQ = zero.  
<sup>4</sup> Upper bound: samples < LOD/LOQ = ½LOD/½LOQ.  
<sup>5</sup> Includes monitoring data collected by EFSA (2007).  
<sup>6</sup> Mean of positive samples not available in EFSA (2007).  
<sup>7</sup> Mostly rice collected on the market, but some studies may include rice samples with the husk.

The mean aflatoxin level found in positive samples, considering all cereals, was 34.2±3.4 µg/kg, with rice samples having the highest mean among the grains analysed (46.6±3.6 µg/kg). The highest aflatoxin level (48,000 µg/kg) was found in a sample collected in Kenya (Daniel *et al.*, 2011). Samples of maize and rice analysed from Asia had the highest mean of positive samples (35.6 µg/kg and 54.0 µg/kg, respectively), while Africa showed the highest mean level of contamination in sorghum (79.7 µg/kg), and Europe in wheat (46.9 µg/kg). The total UB mean of all samples analysed was 13.6 µg/kg. Sorghum samples had the highest total mean, with similar lower and UB levels (25.7 and 25.9 µg/kg).

#### Aflatoxins occurrence: data from the GEMS/Food database

Figure 2 shows the countries that submitted data on aflatoxins in raw maize, rice, sorghum and wheat to the GEMS/Food database, related to 4,536 samples collected since the year 2000. Singapore submitted the largest dataset

(1,028 samples), followed by Canada (967), the Republic of Korea (392), Germany (387), and Brazil (377). Most maize samples came from the USA (27.9%), rice from Singapore (27.8%), sorghum from Republic of Korea (85.5%) and wheat from Canada (81.5%). On average, 324 samples were collected for analysis each year, most of which in 2005 and 2011 (32% of all samples). The smallest number of samples was obtained in 2000 (0.9%), and 2004 (0.6%).

The GEMS/Food dataset was comprised mainly of samples collected by random sampling (78.5% of the samples), and 20.0% by target sampling. For 1.5% of the samples, the sampling method was not informed. Information on portions analysed was not available for 21 samples, none of which were contaminated with aflatoxins. Regarding analytical quality assurance of the laboratory, the GEMS/Food system allows one of four options to be checked: officially accredited methodology, internal quality assurance, proficiency testing, and unknown. For most of the samples (53.3%) officially accredited methodologies were used; for 19.3% the laboratory had internal quality



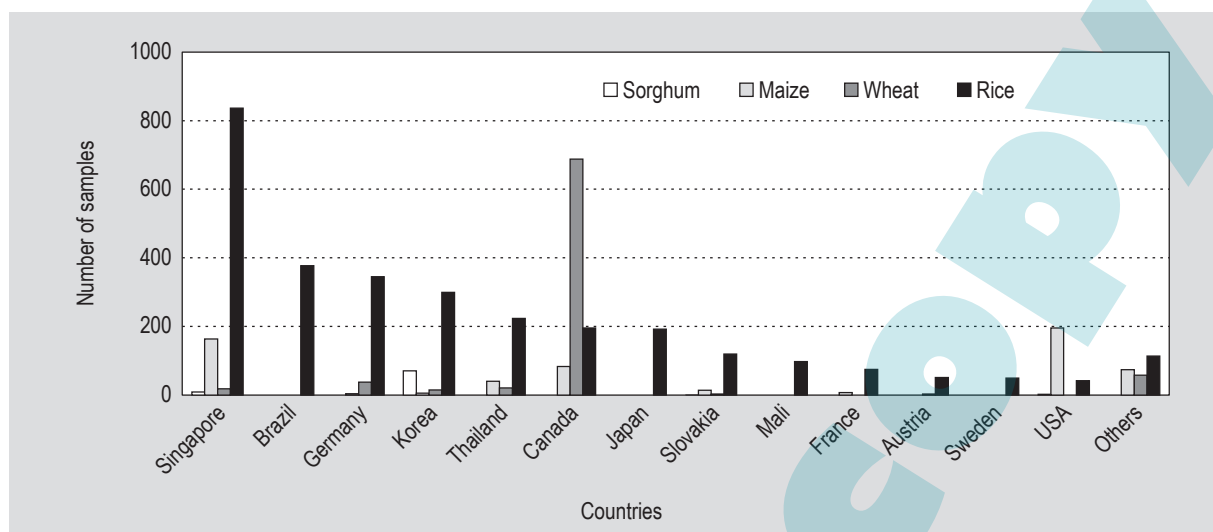


Figure 2. Number of samples submitted to the GEMS/Food database on aflatoxin in maize, rice, sorghum and wheat by country.

assurance, and for 17.9% the laboratory participated in proficiency testing. This information was unknown or was not provided for the remaining samples (9.5%). All samples were kept in the dataset, even those analysed by laboratories that have not provided quality assurance information.

Table 3 summarises the data submitted to GEMS/Food, grouped by continent. Considering all samples analysed, 12.7% were positive for aflatoxins, with a mean of  $10.7 \pm 35.3$   $\mu\text{g}/\text{kg}$ . Total LB and UB means were, respectively, 1.4  $\mu\text{g}/\text{kg}$  and 1.9  $\mu\text{g}/\text{kg}$ . Rice was the commodity with the largest number of records in the database (66.6%), and with the highest incidence of positive samples (17.7%), including the highest aflatoxin level (347  $\mu\text{g}/\text{kg}$  in a Mali sample). Rice also had the highest LB and UB values (1.9 and 2.4  $\mu\text{g}/\text{kg}$ , respectively). Wheat was the cereal with the lowest incidence and levels of aflatoxins (Table 3).

#### Consumption of cereals: data from the 17 GEMS/Food Cluster Diets

Consumption data for maize, rice, sorghum and wheat (including processed products) for the 17 clusters are summarised in Figure 3. Wheat is the cereal most consumed worldwide (daily mean of 205.8 g/person), and the most consumed in 11 of the 17 clusters, including C01, C02, and C06 (mainly countries in Northern Africa and Asia; Figure 1). Rice is the second cereal most consumed (91.3 g/person/day), and the main cereal consumed in clusters C05, C09, and C14 (mostly South American and Asian countries; Figure 1). Maize (mean of 48.9 g/person/day) is the main cereal consumed in clusters C03, C13, and C16 (mostly African countries; Figure 1). The mean worldwide consumption of sorghum is 11 g/person/day, with the highest consumption in clusters C13 (89.2 g/person/day), and C16 (35.4 g/person/day).

#### Dietary risk assessment of aflatoxins using GEMS/Food data

The UB total intakes of aflatoxins through the consumption of maize, rice, sorghum and wheat ranged from 3.0 ng/kg bw/day (Cluster C11) to 17.1 ng/kg bw/day (Cluster C09) (Table 4). LB intakes varied from 0.7 to 12.1 ng/kg bw/day (data not shown). As the concentration for each cereal in the intake calculation was constant (UB mean concentration for each crop; Table 3), only the consumption pattern had an impact on the total aflatoxin intake among the clusters.

On average, the consumption of rice contributed to 41.6% of the total intake in all clusters, followed by wheat (35.4%), maize (21.2%) and sorghum (1.8%). Figure 4 shows the impact of each cereal in total intake for each cluster. The highest impact of rice was mainly due to the highest contamination level (2.4  $\mu\text{g}/\text{kg}$ ), while for wheat, high consumption was the parameter that most affected intake, as the concentration was low (0.6  $\mu\text{g}/\text{kg}$ ). The consumption of rice contributed from 46.8 to 89.1% to total intake for eight clusters, including C05, C09, C14 and C17 (mostly Asian countries; Figure 1). Wheat consumption contributed the most to intake in seven clusters (42.9 to 71.3%; including C02, C07 and C11). Maize was the main contributor to total intake for clusters C13 and C16 (42.4–59.4%; mostly African countries; Figure 1). The contribution of sorghum to total intake reached a maximum of 13.4% in C13 (Figure 4).

Risk characterisation from the exposure to aflatoxins was estimated using the cancer risk and MOE approaches, and the results are shown in Table 4. The lowest cancer risk was found in cluster C11 (0.057 cancers/year/  $10^5$  individuals) and the highest in cluster C09 (0.467 cancers/year/ $10^5$  individuals). MOE ranged from 56 (C11) to 10 (C09).

**Table 3. GEMS/Food data on aflatoxins in cereals grouped by continent.**

	Positive/analysed samples (%)	Positive samples ( $\mu\text{g}/\text{kg}$ ) <sup>1</sup>		Total mean <sup>2</sup>
		Mean $\pm$ SD	Range	LB <sup>3</sup> – UB <sup>4</sup> ( $\mu\text{g}/\text{kg}$ )
Maize <sup>5</sup>	33/588 (5.6)	13.0 $\pm$ 18.7	0.2-93.1	0.7-1.6
America	20/279 (7.2)	18.3 $\pm$ 22.2	1.7-93.1	1.3-2.3
Asia	9/224 (4.0)	5.9 $\pm$ 6.3	0.2-14.8	0.2-0.6
Europe	4/85 (4.7)	2.1 $\pm$ 1.4	1.0-3.3	0.1-1.8
Rice	536/3,021 (17.7)	10.6 $\pm$ 36.3	0.002-347	1.9-2.4
Africa	84/98 (85.7)	41 $\pm$ 71.3	0.2-347	35.1-35.2
America	223/615 (36.3)	8.8 $\pm$ 28.7	0.002-272.2	3.2-3.5
Asia	66/1,553 (4.2)	0.4 $\pm$ 0.4	0.02-2.5	0.02-0.5
Europe	163/755 (21.6)	1.5 $\pm$ 2.5	0.04-17.0	0.3-1.0
Sorghum	4/83 (4.8)	8.6 $\pm$ 5.4	0.6-12.0	0.4-0.6
America	2/2 (100.0)	12 $\pm$ 0.07	11.9-12.0	12.0
Asia	2/80 (2.5)	5.2 $\pm$ 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 $\pm$ 0.7	0.1-1.4	0.003-0.6
America	0/688 (0.0)	ND	ND	ND-0.5
Asia	0/54 (0.0)	ND	ND	ND-0.5
Europe	3/102 (2.9)	1.0 $\pm$ 0.7	0.1-1.4	0.03-1.4
Total	576/4,536 (12.7)	10.7 $\pm$ 35.3	0.002-347	1.4-1.9

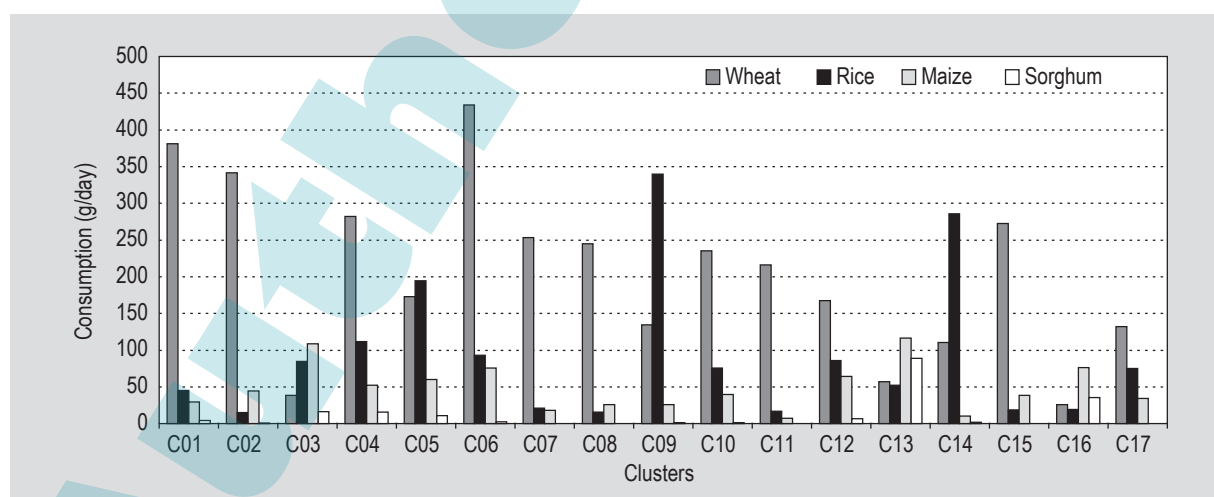
<sup>1</sup> ND = not detected; SD = standard deviation.

<sup>2</sup> Total mean = mean of all samples.

<sup>3</sup> Lower bound: samples < LOD/LOQ = zero.

<sup>4</sup> Upper bound: samples < LOD/LOQ =  $\frac{1}{2}$ LOD/ $\frac{1}{2}$ LOQ

<sup>5</sup> Africa: samples from Mali; America: samples from Brazil, Canada and USA; 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.



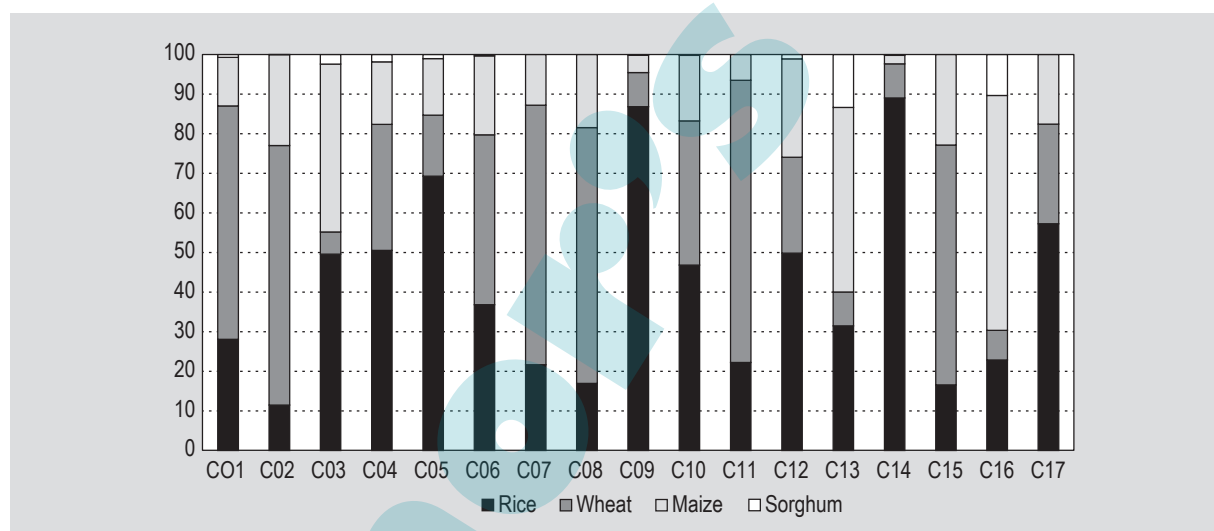
**Figure 3. Consumption of maize, rice, sorghum and wheat for the 17 Cluster diets, including consumption of processed cereals (WHO, 2014). For Clusters, see Figure 1.**

**Table 4. Upper bound of the aflatoxin intake, cancer risk and margin of exposure through the consumption of maize, rice, wheat and sorghum for GEMS/Food Clusters C01 to C17 (ng/kg bw/day).**

	Aflatoxins (µg/kg)	C01	C02	C03	C04	C05	C06	C07	C08	C09	C10	C11	C12	C13	C14	C15	C16	C17
HBsAg <sup>+</sup>		3%	6%	8%	3%	3%	3%	3%	3%	6%	3%	3%	6%	8%	6%	3%	8%	6%
Rice	2.4	1.8	0.6	3.4	4.5	7.8	3.7	0.8	0.6	14.8	3.0	0.7	3.4	2.1	11.4	0.7	0.8	3.0
Maize	1.6	0.8	1.2	2.9	1.4	1.6	2.0	0.5	0.7	0.8	1.1	0.2	1.7	3.1	0.3	1.0	2.0	0.9
Wheat	0.6	3.8	3.4	0.4	2.8	1.7	4.3	2.5	2.4	1.5	2.4	2.2	1.7	0.6	1.1	2.7	0.3	1.3
Sorghum	0.6	0.04	0.001	0.2	0.2	0.1	0.03	0.0	0.0	0.0	0.01	0.0	0.07	0.9	0.02	0.0	0.4	0.0
Total	1.9	6.5	5.2	6.8	8.8	11.2	10.1	3.9	3.8	17.1	6.4	3.0	6.9	6.7	12.8	4.5	3.4	5.2
Cancer risk <sup>1</sup>		0.121	0.143	0.227	0.165	0.21	0.189	0.072	0.071	0.467	0.121	0.057	0.189	0.222	0.352	0.084	0.114	0.144
MOE <sup>2</sup>		26.3	32.6	24.8	19.2	15.1	16.8	44.0	44.9	10.0	26.4	56.0	24.6	25.5	13.2	37.8	49.4	32.4

<sup>1</sup> Cancers/year/10<sup>5</sup> individuals, estimated according to FAO/WHO (1998).

<sup>2</sup> Based on a BMDL<sub>10</sub> in rodents of 170 ng/kg bw/day (EFSA, 2007).



**Figure 4. Impact of maize, rice, sorghum and wheat on the total aflatoxin intake for each cluster. For Clusters, see Figure 1.**

#### 4. Discussion

In this study, we reported data on aflatoxin contamination in maize, rice, wheat and sorghum grains obtained from the published literature and the GEMS/Food database. Literature data concerned samples collected in 64 countries; data from the GEMS/Food were submitted by 24 countries. No data on samples collected in Oceania countries were available in either dataset. Aflatoxin contamination data were mostly available for maize (54.2% of all samples analysed in the studies), while most of the data submitted to GEMS/Food were related to rice (66.6%). The interest in sorghum was lower in the literature in comparison with the other cereals, and the data provided to GEMS/Food were also very limited (83 samples), and did not include samples

collected in African countries, the highest consumers of sorghum worldwide. This dataset will probably increase in the next few years as a FAO/WHO project on mycotoxins in sorghum is being conducted, with samples collected in the four largest producing/exporting countries of this commodity (Burkina Faso, Ethiopia, Mali, and the Sudan) (CAC, 2012). Under this project, up to February 2014, a total of 20,908 of sorghum samples have been analysed, with 3.1% of samples positive for mycotoxins, mainly aflatoxins, fumonisins, and sterigmatocystin (CAC, 2014b). Data reported in the literature may include some monitoring data submitted to the GEMS/Food database, however it was not possible to trace it back. Nevertheless, the dietary risk assessment was conducted using only the GEMS/Food dataset.

With the exception of rice samples from Africa and American continents, the incidence of aflatoxins and the concentration were higher in the published data than in the GEMS/Food database, probably due to sampling differences in the two data sources. Research studies normally do not follow strict sampling plans, and may include samples involved in outbreaks of mycotoxin contamination, not reflecting the general scenario of a specific region or country. This was the case of a survey conducted in Kenya, where some samples were collected in households of patients involved in the aflatoxicosis outbreak (Daniel *et al.*, 2011). On the other hand, the data provided to the GEMS/Food by national authorities were mostly collected under monitoring programs (non-target sampling) and are more representative of mycotoxin contamination in a given country.

In general, higher incidence and concentration calculated from the literature lead to higher aflatoxin mean levels (for positive samples and for all samples) compared to GEMS/Food data. On the other hand, mean levels calculated from published data may be overestimated, as in some studies only the concentration range was reported, and the midrange was used in the estimation (Matumba *et al.*, 2011; Ratnavathi *et al.*, 2012; Reddy *et al.*, 2009; Reiter *et al.*, 2010; Riba *et al.*, 2010). The exclusion of the study that reported the highest value of aflatoxin contamination (maize sample – 48,000 µg/kg) did not have a significant impact on the mean values for this cereal.

UB and LB of total means did not differ greatly in both datasets, which show that LOQs and or LODs of the methods used for analysis were low. The method LOQs for aflatoxins in the published studies ranged from 0.03 µg/kg (high-performance liquid chromatography with fluorescence detection) (Reinhold and Reinhardt, 2011; Yazdanpanah *et al.*, 2013) to 4 µg/kg (thin layer chromatography) (Garrido *et al.*, 2012). Method LOQs provided to GEMS/Food were in the range of 0.05-8.7 µg/kg, although method description was not available in the database. It is important to emphasise that the uncertainties of the UB and LB estimations made using literature or GEMS/Food data could not be assessed due to the limitation of the information provided in both cases.

In this study, we used the UB mean concentration for each crop derived from all the data provided to GEMS/Food to estimate the total exposure. This is justifiable as the crops produced in one region may be in the international trade and consumed elsewhere. With the concentration level for each cereal remaining constant, only the consumption pattern had an effect on the total aflatoxin intake in each cluster. In four of the five clusters that showed the highest intake (8.8 to 17.1 ng/kg bw/day), rice was the cereal that most contributed to the total intake, indicating the

importance of controlling fungi infection and aflatoxin levels in this commodity.

Various studies published in the literature have estimated the dietary intake of aflatoxins (Table 5). In Malaysia (C05), the total UB intake of 58.0 ng/kg bw/day (from the consumption 38 foods, both raw and processed) (Chin *et al.*, 2012) was much higher than the intake for cluster C05 estimated in this study (11.2 ng/kg bw/day). On the other hand, the UB intake estimated for the total Brazilian population, also included in cluster C05, was considerably lower (6.8 ng/kg bw/day) (Andrade *et al.*, 2013), with rice contributing to 97.1% of the total intake.

The intakes obtained for C06, C07, C09 and C10 in this study were higher than the intakes found in countries belonging to these clusters. For example, the intake in France (C07), estimated through consumption of 212 foods (including rice and wheat products), was 0.9 ng/kg bw/day (Sirot *et al.*, 2013) while in China (C09) the intake of individual commodities reached 5.8 ng/kg bw/day (rice) (Ding *et al.*, 2012), as shown in Table 5. Most studies considered cereals in the intake estimations, but focused mainly on processed products, unlike the present study in which only contamination data on the raw commodity were considered. A case in point is the assessment performed in Japan, which only considered cooked rice (Sakuma *et al.*, 2013). Intakes found in the present study were also higher than the most recent risk assessment conducted by JECFA (Bendford *et al.*, 2010; FAO/WHO, 2008) (0.4-3.7 ng/kg bw/day), using the previous GEMS/Food Consumption Cluster Diets (13 Clusters). The only cereal considered in the JECFA assessment was maize (including processed products), in addition to peanuts, oilseeds, cocoa products, dried fruits, peanut oil, spices, tree nuts, dried figs, butter of Karité, and other nuts.

Chronic dietary risk characterisation for aflatoxins from the consumption of cereals was conducted in this study using two available approaches. One limitation to the cancer risk approach estimate is related to the prevalence rates of the hepatitis B virus, which were derived from the prevalence map made by the CDC (2014), and agreement with the GEMS/Cluster was not always possible. For example, Brazil (C05), Canada and the United States of America (C10) are considered by CDC as countries with low prevalence of hepatitis B virus (<2% HBsAg<sup>+</sup>). In this paper, a prevalence rate of 3% HBsAg<sup>+</sup> was used for C05 and C10, as they include countries with low-intermediate prevalence of hepatitis B virus (2-4% HBsAg<sup>+</sup>). Estimation made by the Brazilian Ministry of Health indicates that actual prevalence in the country is 0.37% (Brasil, 2010).

The total exposure to aflatoxins and the risk estimates shown in this paper may be overestimated, as they do not consider the impact of cereal processing on aflatoxin levels,

**Table 5. Dietary exposure and risk characterisation for aflatoxins estimated in the present study using GEMS/Food occurrence data and assessments reported in other studies.**

Country	Food analysed	Intake <sup>1</sup>	Cancer risk <sup>2</sup>	MOE <sup>3</sup>
Present work <sup>4</sup>	Maize, rice, sorghum and wheat	3.0-17.1	0.057-0.467	56-10
Africa – C03/C13 (Shephard, 2008) <sup>5</sup>	Beer, groundnuts, kenkey, maize, millet, peanut butter, rice, sorghum and yam chips	1.4-850	0.1-70.1	121.4-0.2
Brazil – C05 (Andrade <i>et al.</i> , 2013) <sup>6</sup>	Brazil nuts, maize products, other nuts, peanuts, peanut products and rice	6.6-6.8	0.0731-0.0753	25.8-25.0
China – C09 (Ding <i>et al.</i> , 2012) <sup>7</sup>	Maize and derived products, peanuts, peanut oil and rice	0.11-5.8	0.003-0.2	24.7-0.5
China – C09 (Li <i>et al.</i> , 2014)	Edible oils, maize, oats and other coarse grains, peanuts, rice, soybean and wheat <sup>8</sup>	8.3	. <sup>9</sup>	-
France – C07 (Sirot <i>et al.</i> , 2013) <sup>10</sup>	212 foods <sup>11</sup>	0.9	0.011	-
Iran – C06 (Yazdanpanah <i>et al.</i> , 2013)	Bread, peanuts, puffed maize snack, rice and wheat flour	3.6	-	-
Japan – C10 (Sakuma <i>et al.</i> , 2013) <sup>12</sup>	Cooked rice	1.2	0.0021	209
Japan – C10 (Sugita-Konishi <i>et al.</i> , 2010) <sup>13</sup>	24 foods <sup>14</sup>	0.003-0.004	0.00004-0.00005	-
Malaysia – C05 (Chin <i>et al.</i> , 2012) <sup>15</sup>	38 foods (raw and processed) <sup>16</sup>	28.8-58.0	0.72-1.45	-
New Zealand – C10 (Cressey and Reeve, 2013) <sup>17</sup>	Dried fruits, maize and derived products, peanut and derived products, snacks, spices, tree nuts and derived products	0.09 <sup>18</sup> 0.12 <sup>19</sup>	0.0015-0.0019 <sup>18</sup> 0.0018-0.0022 <sup>19</sup>	-
Republic of Korea – C10 (Park <i>et al.</i> , 2004) <sup>20</sup>	Barley and its products, maize and its products, <i>meju</i> and rice	1.2-5.8	-	-
Worldwide – 13 Cluster Diets (FAO/WHO, 2008)	Butter of Karité, cocoa products, dried figs, dried fruits, groundnuts, maize, oilseeds, other nuts, peanut oil, spices and tree nuts	0.4-3.7	-	-

<sup>1</sup> ng/kg bw/day.

<sup>2</sup> Cancers/year/10<sup>5</sup> individuals.

<sup>3</sup> Margin of exposure; based on a BMDL<sub>10</sub> in rodent of 170 ng/kg bw/day, except for China and Japan (140 ng/kg bw/day).

<sup>4</sup> Lower-upper bound, 3-8% HBsAg<sup>+</sup>.

<sup>5</sup> Range of individual commodities from different African countries, 25% HBsAg<sup>+</sup>.

<sup>6</sup> Lower-upper bound, 0.37% HBsAg<sup>+</sup>.

<sup>7</sup> AFB<sub>1</sub> only, range of individual commodities.

<sup>8</sup> Including derived products of all foods.

<sup>9</sup> Not estimated.

<sup>10</sup> Upper bound, 1% HBsAg<sup>+</sup>.

<sup>11</sup> Selected based on pattern of consumption and main known contributors to aflatoxins exposure – includes rice and wheat products.

<sup>12</sup> AFB<sub>1</sub> only, 95<sup>th</sup> percentile.

<sup>13</sup> AFB<sub>1</sub> only, 95<sup>th</sup> percentile, lower-upper bound, 1% HBsAg<sup>+</sup>.

<sup>14</sup> Selected on the basis of knowledge on the occurrence of AFs – includes rice and wheat.

<sup>15</sup> Lower-upper bound, 5.24% HBsAg<sup>+</sup>.

<sup>16</sup> Selected based on knowledge on the occurrence of AFs – type of food not informed.

<sup>17</sup> 1.5% HBsAg<sup>+</sup>.

<sup>18</sup> Female.

<sup>19</sup> Male.

<sup>20</sup> AFB<sub>1</sub> only, lower-upper bound.

such as sorting, milling and cooking (Castells *et al.*, 2007; Hussain and Luttfullah, 2009; Hwang and Lee, 2006; Park and Kim, 2006; Pearson *et al.*, 2004; Siwela *et al.*, 2005). On the other hand, no other sources of aflatoxin exposure

were considered, such as peanuts and oil seeds, which were shown to contribute significantly to the total exposure estimated by the JECFA for the 13 Cluster Diets (FAO/WHO, 2008; Benford *et al.*, 2010).

This work clearly showed that aflatoxin in rice is a major concern due to its high concentration and consumption patterns in certain regions of the world. Currently, the Codex ML for aflatoxins are only established for almonds, Brazil nuts, hazelnuts, peanuts, pistachios, and dried figs (CAC, 1995), food commodities whose average consumption is much lower than for cereals (maximum of 18.8 g/person/day for peanuts in C13; WHO, 2014). The establishment of a ML for rice would remove the most contaminated samples from the market and would have a significant impact on exposure in various regions of the world. For example, if a hypothetical ML of aflatoxins in rice were set at 40 µg/kg, the cancer risk would decrease by up to 48% in comparison with a no limit situation. At MLs of 20 and 10 µg/kg, the risk would be reduced by up to 63%. Lower limits would not have a significant 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 and 77.8%, respectively. This lower level, however, would have a significant impact on the food supply (about 20% of the samples rejected), when compared with the higher MLs (up to 4% of the samples rejected).

The dietary risk assessment of aflatoxins in cereals conducted in this study used incidence data provided to the GEMS/Food up to July 2014, in response to a public call made by the JECFA and requested by the 7<sup>th</sup> Session of the CCCF (REP13/CF) to support the discussion on aflatoxins in cereals at the international level. However, only 24 countries responded to this call, yielding a database which is not representative of every region of the world. For example, no rice data were available for China, a country with a high rice consumption rate and that is part of Cluster C09, which had the highest total intake of aflatoxins. In spite of these limitations, the information provided in this paper is of most relevance as it shows rice as a major driver of mycotoxin exposure in most clusters. Furthermore, the study clearly indicates the need for additional data on aflatoxin contamination in cereals, mainly from countries for which these data are lacking, in support of a more sound risk assessment, and the establishment of ML by the Codex Alimentarius.

## 5. Conclusions

Occurrence data summarised in the present study showed that raw cereals are frequently contaminated with aflatoxins, a genotoxic mycotoxin. Rice was one of the most contaminated cereals, and presented the highest concentration in both literature and GEMS/Food datasets. The dietary risk assessment conducted in this paper indicated a health concern for all 17 GEMS/Food Clusters (MOE<50), with the consumption of rice, wheat and/or maize as the main contributors to aflatoxin intake. Even if the impact of cereal processing on contamination levels had been considered, the MOE would still be much lower

than that considered of low health concern for genotoxic compounds such as aflatoxins (>10,000).

Since cereals are staple foods worldwide, and the elimination of aflatoxins from the food supply is not possible, they should be constantly monitored and actions taken to maintain concentration as low as possible. Actions aimed at lowering the risk of aflatoxin exposure, while still ensuring the food supply, include the enforcement of codes of practices and the establishment of ML. Therefore, considering the results of this study, priority should be given to actions focusing on rice, wheat and maize.

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