

Occurrence and Human-Health Impacts of Mycotoxins in Somalia

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Supporting Information

ABSTRACT: Mycotoxins are secondary metabolites produced by various molds that contaminate many staple foods and cause a broad range of detrimental health effects in animals and humans through chronic exposure or acute toxicity. As such, the worldwide contamination of food and feed with mycotoxins is a significant problem, especially in sub-Saharan Africa. In this study, mycotoxin occurrence in staple foods consumed in Somalia was determined. A total of 140 samples (42 maize, 40 sorghum, and 58 wheat) were collected from a number of markets in Mogadishu, Somalia, and analyzed by a UPLC-MS/MS multimycotoxin method that could detect 77 toxins. All of the maize samples tested contained eight or more mycotoxins, with aflatoxin B_1 (AFB₁) and fumonisin B_1 (FB₁) levels reaching up to 908 and 17 322 μ g/kg, respectively, greatly exceeding the European Union limits and guidance values. The average probable daily intake of fumonisins (FB₁ and FB₂) was 16.70 μg per kilogram of body weight (kg bw) per day, representing 835% of the recommended provisional maximum tolerable daily intake value of 2 µg/(kg bw)/day. A risk characterization revealed a mean national margin of exposure of 0.62 for AFB₁ with an associated risk of developing primary liver cancer estimated at 75 cancers per year per 100 000 people for white-maize consumption alone. The results clearly indicate that aflatoxin and fumonisin exposure is a major public-health concern and that risk-management actions require prioritization in Somalia.

KEYWORDS: food safety, exposure assessment, mycotoxins, risk characterization, Somalia

1. INTRODUCTION

Mycotoxins are chemically diverse secondary metabolites produced by filamentous fungi that can contaminate food commodities in the field and during storage, transportation, and food processing, impacting both human and animal health as well as the feed in addition to the food industry and international trade.1 Although mycotoxin contamination of agricultural commodities is a global challenge and still occurs in the developed world, good agricultural practices and widely enforced legislation have greatly reduced mycotoxin exposure in these populations. However, the risk of mycotoxin contamination in food and feed in developing countries is increasing² because of environmental, agronomic, and socioeconomic factors.³ In most developing countries, such as those within sub-Saharan Africa (SSA), increased climatic stress and pest activity combined with poor storage conditions favor toxigenic fungal growth and thus the accumulation of mycotoxins in agricultural produce.^{2,4}

Maize and sorghum are two of the most important crops in the SSA region, with about 300 million people depending on maize in Africa alone.^{5,6} Susceptibility of cereals to mycotoxin contamination, particularly aflatoxins (AFs), fumonisins (FUMs), and deoxynivalenol (DON), have been widely described, and the high occurrence in Africa has been reported. 5-10 Consequently, the SSA region has suffered from many mycotoxin-poisoning incidences, some resulting in fatalities associated with acute exposure to AFs and FUMs. 7,11 Apart from acute toxicosis, chronic exposure to AFs has been associated with carcinogenicity, ¹² particularly in conjunction with chronic hepatitis B virus (HBV) infection. ^{13,14} Aflatoxin B₁ (AFB₁) has been suspected of having a causative role in 4.6 to 28.2% of all global hepatocellular-carcinoma (HCC) cases, ¹⁵ as strong synergy is observed between AFB1 and HBV, whereby toxin's potency may increase up to 30 times in individuals with HBV, 16 increasing liver-cancer risk substantially. 17 FUMs are also suspected of carcinogenicity 12 and have been associated with the elevated incidence of human esophageal cancer in many parts of Africa, Central America, and Asia. 18 As such, an estimated 40% of the reduction in life expectancy in developing countries is related to the presence of mycotoxins in the foods consumed by these populations. 19

What is more, substantial economic losses have been attributed to cereal contamination with mycotoxins; around

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one-third of foodstuffs (1.3 billion metric tons per annum) produced for the world's population are lost between the field and the consumer, ^{20,21} and this is especially severe in developing countries.⁵ In SSA, an estimated 40% of produce is lost; this produce is worth around \$4 billion and equivalent to the annual caloric requirement of at least 48 million people, ²² and there are additional export losses due to EU regulations, amounting to an additional \$670 million each year. 23 Consequently, the need for regulations imposing limits on the concentrations of mycotoxins in foods and feeds are generally recognized in many countries. Governmental and international institutions, including the World Health Organization (WHO), the European Commission (EC), and the U.S. Food and Drug Administration (FDA), have thus set specific mycotoxin regulations and established maximum tolerated levels of mycotoxins in foodstuffs, dairy products, and animal feedstuffs. 24-27 Currently, over 100 countries, 15 of which are African, have regulations that set maximum levels for the main foodstuffs affected to safeguard consumers from the harmful effects of the most significant mycotoxins, including AFs, FUMs, ochratoxin A (OTA), deoxynivalenol (DON), zearalenone (ZEA), trichothecenes and ergot alkaloids.²⁸ Nevertheless, food-safety-regulation and -management systems in most countries in SSA are either nonexistent or not effective enough to adequately protect both public health and the economic interests of developing economies.²⁹

Somalia has a turbulent recent history with a civil war followed by violent domestic conflicts since the collapse of the central government in 1991. Consequently, the problem of food safety has not been prioritized³⁰ because of food insecurity and famine resulting from natural disasters plaguing the region.³¹ As such, there are no regulations in place, and there is very little information regarding mycotoxin occurrence in food and the resulting exposure of the Somali population. The only available report on mycotoxin concentrations in maize focuses on AFB₁, fumonisin B₁ (FB₁), and DON only, which were assessed via ELISA.³² Consequently, the goal of this article was to assess multimycotoxin occurrence in staple foods for the first time in this geographical region. Generated data was employed to assess the possible impact of exposure to aflatoxins and fumonisins and could be used to assess the impact of other mycotoxins on the health of the Somali population. We hope the conclusions of the performed study encourage further research and bolster initiatives in the region aimed at providing safe food to the people of Somalia.

2. MATERIALS AND METHODS

2.1. Samples. A market survey of three Somali staple foods (i.e., maize, sorghum, and wheat) was performed utilizing a multianalyte LC-MS/MS approach. Approximately 80% of domestic cereal output in Somalia comes from the Bay, Bakool, and Lower and Middle Shabelle regions³³ around the larger inter-riverine area between the Shabelle and Juba river valleys of southern Somalia (Figure S1, Supporting Information). Maize, sorghum and wheat are harvested in the first monsoon season, Gu (long rains), which lasts between April and June and in the second rainy season, Deyr (short rains), that lasts from October to November. A set of 140 samples, which included 42 maize samples (21 white and 21 yellow), 40 sorghum samples (20 white and 20 red), and 58 wheat samples (25 locally grown and 33 imported), were collected from different local markets in Mogadishu, Somalia, between October 2014 and February 2015. Because the aim of the study was to perform a market survey to reflect real exposure of the Somali consumers living in Mogadishu, samples of 1 kg were bought

from local retailers and shipped to the United Kingdom. All samples were stored in a dark and dry place at 4 $^{\circ}\mathrm{C}$ until their analysis.

2.2. Sample Analysis. Sample extraction and analysis was performed using a previously validated multimycotoxin LC-MS/MS method.³⁴ Briefly, samples were homogenized to obtain a fine powder, extracted with a QuEChERS approach, and analyzed on an Acquity UPLC I-Class system coupled to a Xevo TQ-S triple quadrupole mass spectrometer (both from Waters, Milford, MA). The MS system was controlled by MassLynx software, and data was processed using TargetLynx software (Waters, Milford, MA). The method employed herein was initially validated for distiller's dried grain, maize, and wheat, and its performance was additionally assessed with white and red sorghum for the purpose of the current study. Mycotoxin quantitation was achieved using extracted, matrix-matched calibration with blank maize, sorghum, and wheat samples that had been spiked before extraction, covering seven different calibration ranges depending on the requirements and sensitivity. The blank samples (<LOD) of maize and barley were prescreened during the quoted method validation, whereas the sorghum blanks were selected during the initial method assessment for the matrix, performed herein. Because some analytes were present in all the samples, quantitation of those toxins was performed after external blank subtraction and recalibration. All included calibration points did not deviate more than 15% from the expected value (20% for the LOQ). The performance of the method in the latter matrix is presented in Table S1 (Supporting Information). When the mycotoxin concentration in a sample exceeded the linear range, the extracted sample was diluted with a blank extract and reanalyzed. The dilution integrity was assessed by diluting a high-concentration, extracted QC 10 times with blank matrix. The observed accuracy did not deviate more than 10% from the expected value (data not shown).

2.3. Point Estimates of Dietary Exposure. In the present study, a deterministic model based on an average consumers' exposure was applied as this was deemed to be the most relevant for long-term-exposure assessments by both the WHO³⁵ and the European Food Safety Authority (EFSA).³⁶ As an estimation, the degree of mean dietary exposure was expressed as the average probable daily intake (APDI) for maize. Because there is no nationwide data on demographic characteristics, maize-consumption patterns in the Somali population relied on data available for neighboring countries (a conservative approach). The average food consumption was based on the data available for Kenya and Ethiopia (2011–2013), adapted from FAOSTAT food-balance sheets³⁷ quoting 163 g per person per day for maize. Also, no data on average body weight is available for the Somali population; thus, an assumed body weight of 60 kg was used as outlined by the WHO.³⁵

The APDI of each mycotoxin was calculated according to the following equation:

$$APDI = (C \times K)/bw$$

where APDI is the probable daily intake (ng/(kg bw)/day) for each mycotoxin, C is the mean concentration of a mycotoxin in the food (ng/g), K is the average consumption of maize (g/person/day), and bw is the assumed body weight of 60 kg.

- **2.4.** Characterization of Risks from Consumption of Contaminated Grains. To assess the risks associated with exposure of the Somali population to mycotoxin-contaminated grains, the margin-of-exposure approach for AFB₁ (a genotoxic and carcinogenic compound) was adopted. Furthermore, because of the synergistic hepatocarcinogenic effects of AFB₁ and HBV, the population risk for aflatoxin-induced liver cancer was assessed.
- 2.4.1. Margin-of-Exposure Assessment. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) (WHO)³⁸ and the Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) of the EFSA recommend the application of the margin of exposure (MOE) for risk characterization and indication of the level of health concern of substances that are both genotoxic and carcinogenic, such as AFs.³⁹ As substances with both genotoxic and carcinogenic properties are not considered to have a threshold dose, risk even at low exposure levels cannot be excluded. This prevents the establishment of a tolerable-daily-intake amount or other health-based guidance values;

Table 1. Number of Mycotoxins Co-occurring in the Same Sample for the Different Types of Food Collected in Somalia

			percent (number) of samples								
food sample $(n)^a$	minimum number of mycotoxins ≥1	maximum number of mycotoxins	0 mycotoxins	1 mycotoxin	2-5 mycotoxins	6–9 mycotoxins	≥10 mycotoxins				
maize (42)	8	13	_	_	_	50 (21)	50 (21)				
white maize (21)	8	12	_	_	_	52 (11)	48 (10)				
yellow maize (21)	8	13	_	_	_	47 (10)	52 (11)				
sorghum (40)	2	5	7.5 (3)	22.5 (9)	70 (28)	_	_				
white sorghum (20)	1	5	15 (3)	25 (5)	60 (12)	_	_				
red sorghum (20)	1	4	_	20 (4)	80 (16)	_	_				
wheat (58)	1	1	_	98 (1)	_	_	_				
^a Total number of	^a Total number of samples tested.										

therefore, the MOE approach is used. To calculate the MOE, the benchmark-dose lower limit (BMDL₁₀), established by the CONTAM Panel for AFB₁ (a BMDL₁₀ value of 170 ng/(kg bw)/day was derived from animal data, and the lowest BMDL₁₀ and BMDL₁ values derived from human-epidemiological data were 870 and 78 ng/(kg bw)/day), 36 is divided by the aflatoxin APDI. The magnitude of the MOE gives an indication of the risk level (i.e., the smaller the MOE, the higher the potential risk posed by exposure to the compound of concern), with MOEs of 10 000 or higher (based on an animal study) being of low concern for public health. 36

2.4.2. Liver-Cancer-Risk Estimation. To assess the aflatoxin-related liver-cancer burden due to the consumption of contaminated grains, AFB₁ was used as it is the major proportion of total aflatoxins in the analyzed samples, ³⁶ and its ingestion is directly linked to the development of liver cancer. ¹⁵ The associated risk was characterized by estimating liver-cancer rates for average staple-grain consumers and expressed as the number of cancers per 100 000 people per year. The cancer rate was estimated by multiplying the APDI values by the average HCC potency based on individual potencies of the HBsAg+(0.3) and HBsAg-(0.01) groups ³⁶ and the respective prevalences of chronic HBV infection in Somalia (14.8 and 85.2%). ⁴⁰

cancer rate = $APDI \times average potency$

where average potency = $(0.3 \times 0.15) + (0.01 \times 0.85) = 0.0535$ cancers per year per 100 000 people per 1 ng/(kg bw)/day AFB₁. The values of 0.3 and 0.01 represent the potencies of HBsAg+ and HBsAg-, respectively, which were estimated by JECFA on the basis of reviewed animal and epidemiological studies.³⁶

3. RESULTS AND DISCUSSION

3.1. Mycotoxin Prevalence. Out of the 140 cereal samples collected in Somalia, 100, 93, and 98% of maize, sorghum and wheat, respectively, were contaminated with at least one mycotoxin (Table 1). Of these, 61% were contaminated with more than one mycotoxin. The largest number of mycotoxins detected in the same sample was found in maize, with 100% of the samples contaminated with at least 8 mycotoxins and 50% contaminated with at least 10 mycotoxins. The second most contaminated samples were from sorghum, with 70% of these samples being contaminated with two to five mycotoxins. One mycotoxin (tentoxin) was found in 98% of the wheat samples. A total of 23 mycotoxins (including 7 toxins regulated in the EU) were found to be contaminants across all the food types analyzed. Similar distributions and the co-occurrence of multiple mycotoxins were reported in maize 10,41-46 and sorghum 10,45 from SSA, reiterating the food-safety challenges due to mycotoxin contamination in staple foods in the region. The absence of major mycotoxins, including aflatoxins and

fumonisins, in wheat from Somalia agrees with the low incidences of these mycotoxins found in this crop in Nigeria.⁴⁷

3.1.1. EU-Regulated Mycotoxins. Currently, there are no national regulations in place that deal with mycotoxins in foodstuffs in Somalia. Therefore, the maximum levels permitted in the EU⁴⁸ for mycotoxins in cereals and derived products intended for direct human consumption were used as reference levels in the present study. AFs and FUMs were the most frequently detected EU-regulated mycotoxin in the analyzed food samples and occurred at high levels (Table 2). AFB₁, which is the most toxic aflatoxin, was found in all maize samples (both white and yellow) as well as in 50 and 35% of white- and redsorghum samples, respectively. AFB2 was also present in all the maize samples analyzed, and 33 and 5% of yellow-maize samples were contaminated with AFG1 and AFG2, respectively. The concentrations of AFB₁ were in the ranges of 25.5–908 and 0.6– 105 μ g/kg for maize and sorghum, respectively, and concentrations of total aflatoxins (AFB1, AFB2, AFG1, and AFG₂) were in the ranges of 28.3–1080 and 0.6–105 μ g/kg for those matrices, respectively. All the maize samples and 18% of the sorghum samples exceeded the EC maximum limits for AFB₁ $(2 \mu g/kg)$ and for total aflatoxins $(4 \mu g/kg)$ in cereals.⁴⁸ Levels of contamination up to 454 and 270 times the EU maximum limits for AFB₁ and total aflatoxins, respectively, were found in maize samples. Levels of AFB₁ in sorghum samples exceeded EU maximum limits by up to 52 times. Comparing different types of maize and sorghum, the levels of mycotoxins were lower in yellow maize and in white sorghum compared with in white maize and red sorghum. The prevalence and levels of aflatoxins that were found in the maize and sorghum samples from Somalia are similar to those previously reported in both food crops across SSA. 8,45,49-51 In some cases, aflatoxin levels in the maize samples reached 1000 μ g/kg, similar to the high concentrations that have been reported in Kenya, Nigeria, and Tanzania, which exceeded 1000 μ g/kg. 46,52,53 Such extreme high concentrations of aflatoxins in maize may have severe health implications for the consumers, considering the daily consumption of maize in Somalia. FUMs contaminated 100% of maize samples and 38% sorghum samples (Table 3). FB₁, the most prevalent and most toxic fumonisin, which has been classified as a group 2B possible human carcinogen, 12 contaminated all maize samples with concentration levels in the range of 843-17 322 and 1601-8113 μ g/kg for white and yellow maize, respectively. In addition, 75% of white-sorghum samples were found to contain FB₁ at concentration levels ranging from 13.5 to 160 μ g/kg. FB₂ was also found in all maize samples but with lower concentrations than those of FB₁. FB₂ concentrations were in the range of 450–

Table 2. Occurrence of Individual Aflatoxins (AFB₁, AFB₂, AFG₁, and AFG₂) and Total Aflatoxins in Food Samples Collected in Somalia^a

mycotoxin	food sample (n)	F, % (n')	median (range), $\mu g/kg$	$2^b \le X < 4^c \mu g/kg$	$2^b \le X < 4^c \mu g/kg$ $4^b \le X < 20 \mu g/kg$ $20 \le X < 50 \mu g/kg$	$20 \le X < 50 \ \mu \text{g/kg}$	$50 \le X < 100 \ \mu \text{g/kg}$	$50 \le X < 100 \ \mu \text{g/kg}$ $100 \le X < 500 \ \mu \text{g/kg}$ $X \ge 500 \ \mu \text{g/kg}$	$X \ge 500 \mu \text{g/kg}$
AFB_1	white maize (21)	100 (21)	492 (186–908)	I	I	I	I	12	6
	yellow maize (21)	100 (21)	162 (25.5–781)	I	I	3	4	12	2
	white sorghum (20)	50 (10)	0.9 (0.67–3.2)	10	I	I	I	I	I
	red sorghum (20)	35 (7)	1.1 (0.6–105)	4	2	I	I	1	I
	wheat (58)	pu	pu	Ι	I	I	I	I	I
AFB_2	white maize (21)	100 (21)	65 (30–173)	Ι	I	9	8	7	I
	yellow maize (21)	100 (21)	14.4 (2.7–58)	3	6	8	1	I	I
	white sorghum (20)	pu	pu	1	I	I	I	1	I
	red sorghum (20)	pu	pu	I	l	I	I	1	I
	wheat (58)	pu	pu	I	I	I	I	I	I
AFG_1	white maize (21)	pu	pu	I	I	I	I	I	I
	yellow maize (21)	33 (7)	3.2 (0.7–14.6)	S	2	I	I	I	I
	white sorghum (20)	pu	pu	I	I	I	I	I	I
	red sorghum (20)	pu	pu	1	I	I	I	1	I
	wheat (58)	pu	pu	Ι	I	I	I	I	I
AFG_2	white maize (21)	pu	pu	I	I	I	I	I	I
	yellow maize (21)	4.8 (1)	8.7 (8.7)	I	1	I	I	I	I
	white sorghum (20)	pu	pu	Ι	I	I	I	I	I
	red sorghum (20)	pu	pu	I	l	I	I	1	I
	wheat (58)	pu	pu	I	I	I	I	I	I
total AFs	white maize (21)	100 (21)	517 (225–1080)	I	I	I	I	7	14
	yellow maize (21)	100 (21)	202 (28.3–840)	I	1	3	3	13	2
	white sorghum (20)	50 (10)	0.9 (0.67–3.2)	10	I	I	I	I	I
	red sorghum (20)	35 (7)	1.1 (0.6–105)	4	2	I	I	1	I
	wheat (58)	pu	pu	I	I	I	I	I	I

 an , total number of collected samples; F, frequency (%); n', number of contaminated samples; nd, not detected (i.e., less than the limit of detection); X, number of samples falling into the concentration range. b EU limit for AFB₁ in cereals and derived products intended for direct human consumption.

Table 3. Occurrence of Fumonisins B_1 and B_2 and Total Fumonisins (FB₁ and FB₂) in Food Samples Collected in Somalia^a

	$X \ge 10000\mu\mathrm{g/kg}$	-1	I	I	I	I	I		I	I	I	1	1	I	I	I
	$5000 \le X < 10000\mu\text{g/kg}$	5	5	1	1	1	I		1	1	1	6	8	1	I	ĺ
	$2000 \le X < 5000 \ \mu \text{g/kg}$	11	13	I	I	I	2	2	I	I	I	6	12	I	l	1
ı	$1000^b \le X < 2000 \mu \text{g/kg}$	3	3	I	I	I	7	9	I	I	I	2	I	I	l	1
	$X < 1000^b \ \mu g/kg$	-1	I	15	I	I	12	13	4	I	I	I	I	15	I	I
	median (range), μg/kg	3656 (843–17 322)	3284 (1601–8113)	19.9 (13.5–160)	pu	pu	916 (450–3270)	883 (435–2309)	25.0 (16.6–37.6)	pu	pu	4577 (1293–20 592)	4146 (2036–10 168)	16.4 (13.5–197)	pu	pu pu
	F, % (n')	100 (21)	100 (21)	75 (15)	pu	pu	100 (21)	100 (21)	20 (4)	pu	pu	100 (21)	100 (21)	75 (15)	pu	
	food sample (n)	white maize (21)	yellow maize (21)	white sorghum (20)	red sorghum (20)	wheat (58)	white maize (21)	yellow maize (21)	white sorghum (20)	red sorghum (20)	wheat (58)	white maize (21)	yellow maize (21)	white sorghum (20)	red sorghum (20)	wheat (58)
	mycotoxin	FB_1					FB_2					total FUMs				

a, total number of collected samples; F, frequency (%); n', number of contaminated samples; nd, not detected (i.e., less than the limit of detection); X, number of samples falling into the concentration range. ^bEU limit for total fumonisins in cereals intended for direct human consumption 3270 and 435–2309 μ g/kg for white maize and yellow maize, respectively. FB2 also contaminated 20% of white-sorghum samples with concentration levels that ranged from 16.6 to 37.6 $\mu g/kg$. To put this into a food-safety context, all of the maize samples were above the acceptable EU regulatory limits of 1000 ug/kg set for total fumonisins (FB₁ and FB₂) in maize for direct consumption.⁴⁸ One sample of white maize was contaminated with total FUMs more than 20 times the EC limits. The mean concentration of FUMs (FB₁ and FB₂, 5577 μ g/kg) found in the white-maize samples also exceeded the EU regulatory limits for total fumonisins (FB₁ and FB₂) in unprocessed maize grains for human consumption. However, the levels of FUMs (FB1 and FB₂) found in sorghum did not exceed EU limits. The levels of FUMs in white maize were substantially higher than those in yellow maize with no FUMs detected in red sorghum and wheat. Current findings are in agreement with previous studies also reporting similar concentrations of FUMs in maize in Malawi, 8 Burkina Faso and Mozambique, 10 and Ghana. 54

Ochratoxin A (OTA) was detected in 33, 25, and 10% of the samples of white maize and white and red sorghum with the levels in the ranges of 4.1–42.6, 11.4–244, and 6.7–9.1 μ g/kg, respectively (Table 4). Contamination levels higher than the EU limit for OTA (3 μ g/kg)⁴⁸ were found in all the contaminated maize and sorghum samples. White sorghum had the highest concentrations of OTA, exceeding the EU limit by up to 81 times. No OTA was detected in the yellow-maize and wheat samples. OTA contamination of maize is becoming prevalent in SSA, ^{10,43,46} probably because of climatic change. This study adds to the accruing data on OTA prevalence in maize from Africa; the associated risks of nephrotoxicity should not be overlooked in the population.

3.1.2. Non-EU-Regulated Mycotoxins. A total of 16 non-EUregulated mycotoxins were detected in the analyzed food samples (Table 5). AFM₁, FB₃, meleagrin, and cyclopiazonic acid contaminated all maize samples, and other mycotoxins, except macrosporin and tentoxin, were found in at least one maize sample. OTB, skyrin, mycophenolic acid, and sterigmatocystin were found in 19, 14, 38, and 67% of white maize, whereas yellow maize was contaminated with beauvericin (24%), altenuene (10%), alternariol (14%), curvularin (10%), emodin (5%), equisetin (52%), and sterigmatocystin (27%). In white-sorghum samples, 20% were contaminated with OTB, whereas sterigmatocystin, meleagrin, curvularin, macrosporin were found in 100, 35, 10, and 60% of red-sorghum samples. In wheat samples, 98% were contaminated with tentoxin. AFM₁ has a potential carcinogenic hazard about 1 order of magnitude less than that of AFB₁ and is classified as a group 2B possible human carcinogen. 12 It is recognized as a secondary hydroxylated metabolite of AFB, and can be excreted into milk; however, it has been frequently detected in maize from different countries in SSA at similar levels as were found in the present study. 10,43,46 AFM₁ has also been reported in distiller's dried grain (DDG),³⁴ peanuts, maize, and rice.⁵⁵ The mean ratios of B aflatoxins to AFM_1 ($AFB_1/AFB_2/AFM_1$) in the maize samples in Somalia were 14:2:1 and 68:7:2 in white and yellow maize, respectively. The levels of cyclopiazonic acid and FB3 found in the maize samples in this study also agree well with previously reported levels in maize samples from various SSA countries. 10,41,50

3.2. Estimates of Exposure and Characterization of Risk Due to Consumption of Mycotoxin-Contaminated Maize. Because of the occurrence of AFs and FUMs in 100% of maize samples, their very high levels, and the high consumption

Table 4. Ochratoxin A (OTA) Occurrence in Food Samples Collected in Somalia^a

mycotoxin	food sample (n)	F, % (n')	median (range), $\mu \mathrm{g/kg}$	$X < 3^b \mu g/kg$	$3 \le X < 15 \ \mu \text{g/kg}$	$15 \le X < 30 \ \mu\text{g/kg}$	$30 \le X < 60 \ \mu\text{g/kg}$	$X \ge 60 \ \mu \text{g/kg}$
OTA	white maize (21)	33 (7)	7.5 (4.1-42.6)	_	5	_	2	_
	yellow maize (21)	nd	nd	_	_	_	_	_
	white sorghum (20)	25 (5)	87 (11.4–244)	_	1	_	1	3
	red sorghum (20)	10(2)	7.9 (6.7-9.1)	_	2	_	_	_
	wheat (58)	nd	nd	_	_	_	_	_

[&]quot;n, total number of collected samples; F, frequency (%); n, number of contaminated samples; n, not detected (i.e., less than the limit of detection); X, number of samples falling into the concentration range. b EU limit for OTA in cereals intended for direct human consumption.

Table 5. Occurrence of Other Non-EU-Regulated Mycotoxins in Cereals (Maize, Sorghum, and Wheat) and Derived Products Intended for Direct Human Consumption in Food Samples Collected in Somalia^a

	white maize		yellow maize		white sorghum		red so	orghum	wheat	
toxin	F, % (n')	median (range) μg/kg	F, % (n')	median (range) μg/kg	F, % (n')	median (range) μg/kg	F, % (n')	median (range) μg/kg	F, % (n')	median (range) μg/kg
aflatoxin M_1	100 (21)	38.1 (11.6–65)	100 (21)	5.7 (0.96–17.2)	nd	nd	nd	nd	nd	nd
fumonisin B_3	100 (21)	506 (247–1681)	100 (21)	365 (180–935)	nd	nd	nd	nd	nd	nd
ochratoxin B	19 (4)	4.3 (3.9–119)	nd	nd	20 (4)	19.6 (12.2–43)	nd	nd	nd	nd
cyclopiazonic acid	100 (21)	24.5 (6.3–122)	100 (21)	81 (9.9–393)	nd	nd	nd	nd	nd	nd
skyrin	14 (3)	24.4 (11.9–29.1)	nd	nd	nd	nd	nd	nd	nd	nd
meleagrin	100 (21)	2.4 (0.47–23.7)	100 (21)	3.3 (2.1–14)	nd	nd	35 (7)	0.5 (0.4-0.7)	nd	nd
mycophenolic acid	38 (8)	6.0 (3.1–91)	nd	nd	nd	nd	nd	nd	nd	nd
sterigmatocystin	67 (14)	18.7 (2.6–1567)	27 (6)	21 (9.7–75)	nd	nd	100 (20)	39.9 (6.9–69.1)	nd	nd
beauvericin	nd	nd	24 (5)	8.4 (6.7–14.6)	nd	nd	nd	nd	nd	nd
altenuene	nd	nd	10(2)	51 (48.5-54)	nd	nd	nd	nd	nd	nd
alternariol	nd	nd	14 (3)	50 (37.3-63)	nd	nd	nd	nd	nd	nd
curvularin	nd	nd	10 (2)	69 (40.8–98)	nd	nd	10 (2)	16.5 (32.2–64)	nd	nd
emodin	nd	nd	5 (1)	16.1 (16.1)	nd	nd	nd	nd	nd	nd
equisetin	nd	nd	52 (11)	10.4 (8.4–200)	nd	nd	nd	nd	nd	nd
macrosporin	nd	nd	nd	nd	nd	nd	60 (12)	33.5 (26.1–64)	nd	nd
tentoxin	nd	nd	nd	nd	nd	nd	nd	nd	98 (57)	10.4 (3.6–23.3)

[&]quot;n, total number of collected samples; F, frequency (%); n', number of contaminated samples; nd, not detected (i.e., less than the limit of detection).

of maize, the APDI values were calculated for that cereal only (Table 6). The estimated APDIs of AFB₁ in the Somali population were 1402 and 584 ng/(kg bw)/day for white maize and yellow maize, respectively, and APDIs of AFB2 were 212 and 61 ng/(kg bw)/day for white maize and yellow maize, respectively. The APDIs of AFG1 and AFG2 for yellow maize were 11.4 and 23.6 ng/(kg bw)/day, respectively. For total aflatoxins (AFB₁, AFB₂, AFG₁, and AFG₂), the APDIs were 1614 and 649 ng/(kg bw)/day for white and yellow maize, respectively. For the comparative purpose of this study, the estimated APDI for aflatoxins was compared with those from other continents as well as with those from countries within Africa. The exposure to total AFs for the Somali population was substantially higher than the estimated mean exposures to aflatoxins of populations in Europe (0.93-2.4 ng/(kg bw)/day), the United States (2.7 ng/(kg bw)/day), Asia (53 ng/(kg bw)/

Table 6. Exposure Assessment of EU-Regulated Mycotoxins in White- and Yellow-Maize Samples Collected in Somalia

mycotoxin	white-maize APDI ^a (ng/(kg bw)/day)	yellow-maize APDI (ng/(kg bw)/day)
AFB_1	1402	584
AFB_2	212	61
AFG_1	_	11.4
AFG_2	_	23.6
total AFs	1614	649
FB_1	11 893	10 769
FB_2	3257	3119
total FUMs	16 702	13 888

^aAverage probable daily intake.

Table 7. Estimated Liver-Cancer Prevalence in the Average Population Due to the Consumption of Aflatoxin B₁ Contaminated Maize and Risk Characterization Based on a Benchmark-Dose (BMD) and Margin-of-Exposure (MOE) Approach^a

food type	AFB ₁ APDI (ng/(kg bw)/day)	liver-cancer prevalence (cancers per year per 100 000 people)	MOE for rodent BMDL10 ^b	MOE for human BMDL10 ^c	MOE for human BMDL1 ^d
white maize	1402	75	0.12	0.62	0.06
yellow	584	31	0.29	1.49	0.13

"This is based on a model developed by the EFSA. 30,53 APDI, average probable daily intake; MOE, margin of exposure; BMD, benchmark dose; BMDL, BMD lower confidence level. BMD lower limit for 10% increased risk, based on 170 ng/(kg bw)/day. Human BMD lower limit for 10% increased risk, based on 78 ng/(kg bw)/day.

day), and Africa (1.4–850 ng/(kg bw)/day).⁵⁷ However, the results are similar to those from other studies on exposure estimates for Nigeria (113–6401 ng/(kg bw)/day).⁵⁶ and southern Guangxi province in China (11.7–2027 ng/(kg bw)/day).¹⁶

AFB₁ has been shown to be a potent liver carcinogen, causing HCC in humans and a variety of animal species. 12 Liver cancer is the third leading cause of cancer deaths in the world, with the highest rates in Africa and East and Southeast Asia. 15,58 The prevalence of HCC is 16-32 times higher in developing countries than in developed countries. More than a quarter of the 550 000-600 000 new HCC cases reported worldwide each year may be attributable to AF exposure. 15 Other studies have evaluated the relationship between the incidence of HCC and human exposure to aflatoxins in a number of African countries, including Kenya, Mozambique, and Swaziland. 14-16 With average dietary AFB₁ exposure estimated at APDIs of 1402 and 584 ng/(kg bw)/day through the consumption of white and yellow maize, 75 and 31 Somali individuals, respectively, per 100 000 people are at risk of developing primary liver cancer. It has been suggested that an AFB₁ contamination of 9 μ g/kg in foodstuffs would cause an increase of no more than 1 HCC case per 10 000 people. 59 HBV infections are a heavy burden in many developing countries, and small population surveys indicate that Somalia is one of the countries with an HBsAg+ prevalence rate of 14.8%, the highest in the eastern Mediterranean region.⁴⁰ A recent study found that liver cancer is the third most common cancer in Somalia. 60 Therefore, efforts, both national and international, must be directed to AF mitigation, and regulations must be put in place that can be enforced.

To assess the potential risk posed by exposure to AFB₁, the MOE of AFB₁ in Somalia was calculated, as shown in Table 7, and was based on the benchmark-dose lower confidence levels (BMDL10/1) from animal and epidemiological studies according to the EFSA.36 The calculated MOE values for AFB₁ ranged from 0.06 to 1.49 and were substantially lower than the 10 000 thresholds, indicating that dietary exposure to AFB₁ is a major public-health concern and should be considered a high priority for risk-management actions. Similar estimations were published for maize consumers in Nigeria, with MOEs ranging from 0.02 to 1.3,56 which is 700 times lower than the EU MOE range (88–483).³⁶ Because of the carcinogenic and genotoxic properties of aflatoxins even at very low exposure levels, most international agencies, including the JECFA and the EFSA, advise the as low as reasonably achievable (ALARA) principle to be used by risk managers to strictly limit the exposure to these toxins from all food sources to a minimum. 30 Therefore, this principle should be applied for Somalian maize and maize-based foods.

The levels of FUM exposure were estimated in the maize samples analyzed (Table 3). To assess the risk resulting from dietary exposure to FUMs in maize, the APDIs were compared

with provisional-maximum-tolerable-daily-intake (PMTDI) values, for which exceedance indicates the potential for health The Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended a PMTDI of 2 μ g/(kg bw)/ day for FB₁ and FB₂ separately or combined.³⁵ The average APDIs for FB₁ in the Somali population was 11.89 and 10.77 μ g/ (kg bw)/day for white maize and yellow maize, respectively, representing 595 and 539% of the PMTDI. The average APDIs for FB₂ in this population were 3.26 and 3.12 μ g/(kg bw)/day for white maize and yellow maize, respectively, representing 163 and 156% of the PMTDI. Total fumonisins (FB₁ and FB₂) that the Somali population were exposed to were 16.70 and 13.89 $\mu g/(kg \text{ bw})/day$ from white-maize and yellow-maize consumption, respectively, representing 835 and 694% of the PMTDI. These high exceedances of the PMTDI indicate potential health risks from fumonisin dietary exposure. Exposure to high fumonisin levels through the consumption of contaminated maize has been associated with the risk of developing liver lesions, which was observed in experimental animals, and human esophageal cancer, but its causality has not been confirmed. 18 In areas of South Africa and Brazil, where the APDIs for fumonisins were calculated to be 8.67 and 1.60 μ g/ (kg bw)/day, respectively, high rates of esophageal cancer have been reported. 58,62,63 A recent study that assessed the distribution of cancer cases in Somalia found that the most common type of cancer was esophageal cancer (32% of all cancer cases) and concluded that environmental risk factors and nutritional habits have a strong impact in this population. ⁶⁰ The current study points to exposure to high levels of fumonisins, which could be one of the risk factors contributing to esophageal-cancer incidences in this population. As with the case with aflatoxins, the levels of fumonisins found in the maize consumed in Somalia indicate a strong need for monitoring foods to reduce the risk of developing cancer.

Furthermore, the implications of aflatoxin and fumonisin coexposure for human health could be significantly high. Little research has been done to evaluate the effects of coexposure to these toxins. However, such coexposure is likely to result in additive or synergistic effects, particularly in terms of hepatotoxicity or hepatocarcinogenicity. Evidently, the Somali population is exposed to high levels of both AFs and FUMs, indicating high concern in terms of food safety. Nevertheless, a more complex dietary assessment could reveal even higher exposure levels if other commodities are to be included, such as nuts, which also have been reported to contain high levels of aflatoxins and other toxins. 41,55

The data presented herein indicate an urgent need to address mycotoxin contamination and exposure in the population of Somalia and also underline a need for further toxicological-data collection to estimate the full impact on Somali society. Further studies are critically needed to assess the risk of mycotoxin exposure in different age groups and in different foodstuffs in order to understand where the greatest interventions can be performed (i.e., pre- and postharvest) to reduce the human burden of mycotoxins in the diet. Somalia is a country where the mycotoxin problem has been totally neglected. Furthermore, from the data presented within this study, it can be seen that consumers should be urgently advised on maize consumption and that it is crucial to develop and implement mycotoxin regulations, mycotoxin-monitoring schemes, and mycotoxin-mitigation strategies to protect an entire nation from the catastrophic human-health consequences associated with such enormous levels of mycotoxin exposure.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.8b05141.

Map of Somalia with the main cereal-production regions highlighted and LC-MS/MS-method performance in sorghum (PDF)

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