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Research Article

Aflatoxin Exposures and Risks in Maize-Based Foods Consumed by University Students

Isaac W Ofosu*, Gloria M Ankar-Brewoo, Herman E Lutterodt, Luke PM Tetteh, Edmund O Benefo and William O Ellis

Department of Food Science and Technology, Faculty of Biosciences, College of Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

***Corresponding Author:** Isaac W Ofosu, Department of Food Science and Technology, Faculty of Biosciences, College of Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

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Abstract

This study sought to monitor carcinogenic risk caused by aflatoxin contamination in maize-based foods eaten by University students in HBV infection belt. The detection of aflatoxins in the food samples (n = 100) was performed using a HPLC with Kobra Cell for the post-column derivatization. A food frequency questionnaire (n = 300) was used to study the consumption data of maize-based foods of students in the University communities. The data of the elements of exposure (total aflatoxins, mass of food consumed, exposure frequency, exposure duration, body weight and averaging time) were individually fitted to their distributions using the @ Risk software. The prevalence of total aflatoxin (B1, B2, G1 and G2) concentration, exposure and risk (studied as MoE and ELCR), which were determined based on regulatory models, presented a complete profile of the toxin. The result showed total aflatoxin contaminating rate of 85%, but the simulated (iterations=10^4) results showed a modal concentration of 0.7 ng/g and uncertainty as high as 80.73 ng/g at the 95th percentile. Again, the modal exposure which was 3.38 ng/kg(bw)-day, gave rise to high levels of risk (MoE < 6.00), significantly below the recommended threshold value (10^5). The modal ELCR value of 3 cases per 10^5 consumers, and also a regression coefficient (β) of 0.80 as due to food, was unacceptably high. High doses of aflatoxins which could lead to short term aflatoxicosis was recorded, though they appeared isolated. However, chronic doses of aflatoxins appeared most frequently, and this is what warrant serious public health concern in our quest for carcinovigilance.

Keywords: Aflatoxins; Maize-Based Foods; University Students; Exposures; Risks

Introduction

Many studies have documented exposures of aflatoxins, especially in maize-based foods, across sub-Saharan Africa [1-3]. However, a review of available literature shows that there is paucity of information with respect to risks of these carcinogenic mycotoxins. Subsequently, these risks result in high incidence of hepatocellular carcinoma (HCC), known to be exacerbated by hepatitis B infection [1-7]. University students belong to a larger population subgroup that have high risk factors for HCC [8,9]. This population group has high risk factors of hepatitis B infection stemming from alcohol abuse, multiple sexual partners and poor eating habits based largely on maize [10] maize was quickly adopted as the cornerstone of local cuisine, especially in sub-Saharan countries. Although maize provides macro- and micronutrients required for humans, it lacks adequate amounts of the essential amino acids lysine and tryptophan. For those consuming >50% of their daily energy from maize, pandemic protein malnutrition may exist. Severe protein and energy malnutrition increases susceptibility to life-threatening diseases such as tuberculosis and gastroenteritis. A nutritionally superior maize cultivar named quality protein maize (QPM. The recent advancement in quantitative probabilistic risks tools based on risk simulation, has provided greater opportunity to use risk analysis as a food safety management tool. This approach of risk determination provides a more complete profile of the scale of risk, as it presents the distribution (min, max, mean, mode, median and percentiles) of a risk value in a community. The outcome of such information will be fed into food policy and governance in the framework of ensuring food safety for the youth in higher education institutions.

Maize is one of the critical agricultural produce with production exceeding 370 million tons between 2017 and 2018 in the US alone in this period [11]. In Ghana, production stood at about 1.7 million tons in 2016 [12]. It has been reported that over 5 billion people in developing countries are at risk of exposures of aflatoxin resulting from poor handling of cereals, especially maize [13]. The exposures and risks of mycotoxins such as aflatoxin is quite a serious issue of importance and their production has been linked to the molds, *Aspergillus flavus* and *Aspergillus parasiticus*. These molds have been reported to contaminate at least 25% of agricultural crops with maize as a very good substrate [1,2]. Aflatoxin is of very important public health concern as a result of several health effects and subsequent deaths reported in many studies [14-18].

There are reports that infants and children are even more sensitive to the effects of mycotoxins in general due to their immature immune systems coupled with high ingestion of maize-based foods [7,19]. Students in educational institutions, especially in higher education, constitute a unique population subgroup of young adults. Majority of these students are still dependent on their equally stressed parents in a typical developing nation economy. In fact, there is an indirect evidence of maize-based foods constituting the major foods consumed by majority of university students [20]. Thus, these students constitute a critical population subgroup that are equally burdened with HCC, a leading cause of cancer deaths [18,21,22].

The devastating effects of mycotoxins is well-documented. Studies in sub-Saharan Africa have revealed that the mean age at diagnosis of HCC is significantly lower (46 years) relative to what prevails in northern Africa (58 years) [23]. It is believed that there could also be environmental or genetic factors that accelerate the disease endpoint of aflatoxin ingested in foods [24]. Acute aflatoxicosis has been shown to be responsible for the severe adverse health effects and the short term deaths of 125 Kenyans in 2004 [16]. There have also been report of probable daily ingestion of aflatoxins, in which over 5 billion people in developing countries continue to be exposed [25]. Studies have shown several fold increase in liver cancer risks in aflatoxin-exposed persons who are also hepatitis B virus (HBV) infected [17]. The mycotoxicosis pathway, starts with liver biotransformation of aflatoxins to aflatoxin-8,9-epoxide and the subsequent conjugation with DNA via nucleotide (guanine) resulting in the disease-end point (HCC) [1]. Stunted growth and immune suppression resulting from aflatoxin ingestion, and its adverse impact on the intestinal integrity and modulation of cytokine expressions have also been reported [1,8].

There has been inconsistency in the regularization of the carcinogenic effects of the various types of aflatoxins that have been isolated. However, in 2012, the International Agency for Research on Cancer (IARC) declared that aflatoxins B1, B2, G1, G2 and M1 are indeed group A human carcinogens [26]. Their judgement was based on the studies that confirmed that aflatoxin 8,9 epoxides, form DNA adduct to trigger the mechanism of carcinogenesis. The evidence for the production of aflatoxin 8,9-epoxide is indirect, however, results of metabolic studies have shown that the presence of aflatoxin 8,9-dihydro-8,9-diols can be isolated under certain conditions [22].

Exposures of aflatoxin have been quantified based on either the presence of hazards in food commodities usually consumed by humans, or through the presence of aflatoxin metabolites present as biomarkers. The aflatoxin-albumin biomarker has been used to quantify aflatoxin exposures across the sub-regions of Africa [5]. However, there are suggestions that some errors associated with this oral exposure pathway is significant, because it is doubtful whether aflatoxins are ingested only through the oral route. There is actually a reason to doubt the exposure to mycotoxins through ingestion alone, since dermal transfers and inhalation have been reported as other exposure routes [27]. Aflatoxin metabolites occur as biomarkers in blood and urine, and it is undoubtedly the method of choice for exposure studies in test animals and humans [28-30]. However, the oral route of exposure assessment is still being used. Another reason is that the biomarker approach is in its infancy and also respondents may decline to partake in this method which is invasive.

To study adverse health effects, risk characterization studies have been modelled within the framework of the margin of exposure (MoE) and estimated liver cancer risk (ELCR). MoE is defined as the benchmark dose lower bound (BMDL) for a regulatory standard of aflatoxin per exposure. Inasmuch as the MoE is lower than the 10^5 threshold, public health concern is implicated [7]. While the MoE approach are easily determined as BMDL per exposures [31], ELCR is computed as the product of exposures and potency factor [25]. Cumulative cancer risk approach of the quantitative risk assessment of aflatoxin is based on the fact that there is no safe concentration of aflatoxins in direct human consumption. However, countries such as US and those in the EU have set a safe limit of respectively 20 ng/g and 10 ng/g respectively [32].

To determine the cumulative risk, an average potency factor is required. Epidemiological evidence shows a strong positive correlation between chronic hepatitis B virus (HBV) infection and

dietary exposure to aflatoxins, and that these two major risk factors drive the multifactoral etiology of HCC. Coincidentally, these two risk factors co-exist in those countries with the highest incidences of HCC (Kew). Subsequently, the average potency, based on the prevalence of hepatitis B surface antigen (HBSAg) positive and negative groups, have been reported as 25% and 75% respectively [7,25]. It is therefore established that hepatitis B surface antigen positive ($HBSAg^+$) individuals present a potency of 0.3 cancers per year per 10^5 populations per ng AFB1/kg (bw)-day [7,25]. On the other hand, hepatitis B surface antigen negative ($HBSAg^-$) individuals, present a potency of 0.01 cancers per year per 10^5 populations per ng AFB1/kg (bw)-day. The age bracket between 16 to 39, of which University and other tertiary students are a subgroup, has been reported to be a high risk HBV infection group in Ghana [33]. Since it is very difficult or almost impossible to eliminate aflatoxins from raw foods, risk assessment is warranted in order to help food safety regulators and management experts, plan policies and governance that would help maintain food safety. This study sought to determine the exposures of students to aflatoxins in frequently consumed maize-based foods, and to determine the risks associated with them while in residence at the university.

Materials and Methods

Materials

The components of QuEChERS reagents: anhydrous MgSO4, NaCl and acetonitrile were all purchased from Sigma Aldrich (Darmstadt, Germany). Standard aflatoxin mix was procured from Romer Labs Division Holding GmbH (Getzersdorf, Austria).

Study area

The general study area was within the boundaries of Kwame Nkrumah University of Science and Technology (KNUST) with a student population of over 40,000 as at 2016 (University Relations Office, KNUST). However, since the study specifically targeted university students, the study area was extended to cover the immediate boundaries of the University campus where students reside in rented hostels. Specifically, sampling points included University communities such as Bomso, Ayigya, Kotei, Deduako and Ayeduase. Within the University campus, there are residential facilities, all of which have eateries. Specific sampling points included "Africa Hall", "Unity Hall, "Republic Hall", "Independence Hall", "Queen's Hall", "University Hall", "Chancellors' Hall" and "Brunei".

Food groups sampling and questionnaire for maize--based food consumption

The targeted foods for this study was based on maize. Specific food groups that were sampled included "Ga Kenkey", "Banku", "Fante Kenkey" and "Tuo zaafi". The questionnaire was structured to collect information such as biodata (weight, age, gender), weight of food consumed (sampled as amount purchased), exposure frequency (number of times food is consumed per week and thus, estimated for an academic year) and exposure duration (exposure duration for the total years students are required to stay in the University).

Skilled survey assistants were recruited from the Department of Food Science and Technology. They were further trained in the sampling of foods and the administration of questionnaire. In all, 300 respondents were randomly served with the questionnaire depending on the willingness of the respondents. Similarly, 100 individual food samples were collected for a period of 1 week during which the survey was launched. Sampling and data collection occurred from 8 am (breakfast) through lunch (1 pm) to supper (6 pm to 8 pm). The medium of communication was English, since the targeted respondents were all university students.

Sample preparation, extraction and clean-up

Sampled foods which were previously stored from the survey were separately homogenized in 500 mL distilled water in a Crompton Blender (Sierra 500, India) and stored in Ziploc bags (-2°C) pending further analyses. Aflatoxin was extracted using the "Quick Easy Cheap Effective Rugged Safe" (QuEChERS) method where 2 g of the stored samples was weighed into 15 mL centrifuge tubes and vortexed topped previously with 5 mL distilled water [34,35]. Acetic acid/acetonitrile (5 mL, 1% (v/v)) was added and vortexed, followed by anhydrous $MgSO_4$ (1.32 g) and NaCl (0.2 g) and further vortexed. The samples were then centrifuged at 4000 rpm for 5 min and 2 mL of the organic layer siphoned and cleaned-up. A final volume of 20 µL was then used for the HPLC analysis.

HPLC quantification of aflatoxins

HPLC quantification was done based on a slightly modified AOAC Official Method 2005.08 [36] where the Photochemical Reactor for Enhanced Detection (PHRED) was substituted for Kobra Cell for the post-column derivatization. A Cecil-Adept Binary Pump HPLC

coupled with Shimadzu 10A×L fluorescence detector (Ex: 360nm, Em: 440nm) and YMC C18 Column (150 × 4.60mm, 5um) was used. Methanol : water (40:60, v/v) was used as the mobile phase at a flow rate of 1 mL/min with column temperature maintained at 40°C. An amount of 119 mg of potassium bromide and 350 µL of 4 M nitric acid was added to 1 L of the mobile phase which was required for post column electrochemical derivatization with the Kobra Cell.

The retention times of the aflatoxin standards ware used to quantify each respective toxin using their calibration curves as described by Sirhan and co-workers [37]. Limit of Detection and Limit of Quantification of total aflatoxin were established at 0.5 ng/g and 1 ng/g respectively. The concentration of aflatoxin was calculated, using Equation 1, where "A" is the concentration of aflatoxin in the sample extract injected, "T", the final volume of sample after QuEChERS extraction and clean-up, "I", the volume of sample extract injected into the HPLC and "W", the mass of sample taken through the QuEChERS method.

Aflatoxin concentration (ng/g) concentration (ng/g)

$$A \times \frac{T}{I} \times W = A \times \frac{2000}{20} \times 2 \quad (1)$$

Data analysis and Risk assessment

The uncertainty and variability of the dataset of masses of food (per unit amount of money used in purchasing) were modelled in MS Excel spreadsheet (Microsoft, Redmond, WA, USA) using the Monte Carlo simulation in @Risk software (version 7.6, Palisade Corp., Newfield, NY, USA). The central tendencies were studied and the 95th percentile mass was used to represent the maximum/worst case scenario of mass of food per Ghana Cedis (GHS1= USD 0.2), that could be consumed in the study area. This unitized mass/cash system, served as the basis for calculating the various masses of maize-based foods consumed by the respondents during the survey. The dataset of the total aflatoxins (AFB1, AFB2, AFG1 and AFG2) was also fitted and the statistical distributions and the central tendencies of the total aflatoxin concentrations were recorded. Subsequently, distributions were fitted for the variables of the key elements of food consumption (Table 1), from which the probable daily intake (PDI), also called the exposure, was determined. These variables were integrated in Equation 2 and iterated at 10^5 in the Palisade@Risk software. The statistical distributions of the key elements of exposure and their central tendency values, in addition to the 5th and the 95th percentiles, were recorded.

$$\text{Exposure} = \frac{TC_{Afl} \times MF_{con} \times EF_{aca}}{BW} \times \int_1^6 \frac{ED_{Ty}}{AT_6} \quad (2)$$

Variables	Definition
$T_{CAf}l$	Total aflatoxin concentration
MF_{con}	Mass of maize-based food consumed
EF_{aca}	Exposure frequency during the academic year
ED_{Ty}	Exposure duration for the total number of years students stay in the University
BW	Body weight of respondents
AT_6	Averaging time of 6 years

Table 1: Variables used in the computation of the exposure assessment.

Risk characterization

In 2007, EFSA recommended that risk characterization for carcinogenic compounds such as aflatoxin B1 must be determined based on the MoE approach. This method uses a reference point, often taken from an animal or sometimes human study. Such threshold values correspond to doses that cause low but measurable (1–10%) increase in tumor formation above background levels in experimental animals [25]. The quantitative cancer risk approach proposed by IARC [26] was used to characterize the ELCR or the risk of HCC based on the average risk factor (potency factor) proposed for aflatoxins [7]. In order to study the adequacy of protection of regulatory standards, three different MoEs were determined (Equation 3) based on the BMDL of aflatoxin using three different thresholds. Among the three determinations, rodent aflatoxin threshold of a lower bound (10%), 170 ng/kg(bw)-day, was used [38]. A human threshold of a lower bound (10%), 870 ng/kg(bw)-day and also at a more sensitive (1%) level, 78 ng/kg(bw)-day) was also used [25]. In each case, the uncertainty and variability of the dataset of exposures of the study area (Equation 3) were modelled in MS Excel spreadsheet (Microsoft, Redmond, WA, USA) using Monte Carlo simulation in Palisade @Risk Software as before. The ELCR, on the other hand was computed as the product of the exposure of the study area and the average potency factor (Equation 4), and iterating at 10^5 in the Palisade @Risk Software.

$$\text{MoE} = \frac{\text{BMDL}}{\text{Exposure}} \quad (3)$$

$$\text{ELCR} = \text{Exposure} \times \text{Average potency} \quad (4)$$

The average potency factor used in this study was exactly the same as what was used in other studies; 0.0825 cancers per 10^5 consumers per year per ng total aflatoxin/kg(bw)-day [7,25].

Results and Discussion

Elements of exposure

The total samples collected from the study area amounted to 100 out of which 15 did not record detectable levels of any of the five aflatoxins. Thus, while 85% of the samples collected were contaminated with aflatoxins, about 15% of the samples collected recorded non-detectable aflatoxins. This observation is not surprising because some studies have reported no aflatoxins in their analysis, in which maize–based processed product (Cerelac) and rice were reported to have no detectable aflatoxins [39]. Together with another report that seasonal variation of aflatoxins occurs [40], it should be possible to study and predict these contaminations cycles which could make it possible to control the exposures. The current study (Table 2) in the area surveyed, showed that the total dietary aflatoxin was statistically distributed in "InvGauss" (20.517, 6.31398, -1.3294) to present cases from no-detectable aflatoxins in food samples to food samples containing as high as 138.29 ng/g total aflatoxins.

Element	Statistical distribution	Central Tendencies					Percentile	
		Min	Max	Mean	Mode	Median	5th	95th
Total AF (ng/g)	InvGauss (20.517, 6.31398, -1.3294)	0.0	138.29	19.20	0.70	6.68	0.09	80.73
MF_{cons} (g)	Expon (78.137, 284.74)	285	713	363	285	339	289	519
EF_{aca} (days)	Triang (-14.595, 356, 356)	30	356	233	356	248	68	347
ED_{Ty} (year)	Triang (1, 1, 5.4078)	1.0	5.40	2.50	1.11	2.30	1.11	4.40
Bw (kg)	BetaGeneral (2.5333, 4.7973, 43.225, 127.993)	43	128	72.50	67.61	71.30	51.80	97.53

Table 2: Elements of Exposure: Statistical distributions and central tendency metrics.

The mean and modal aflatoxin contaminations of respectively of 19.20 ng/g and 0.7 ng/g, in the cooked maize-based foods are similar to incidences of a reported mean total aflatoxins that ranged from 1.77-24.58 ng/g recorded in studies in Accra a few years back [41]. However, there are high aflatoxin endemic districts such as Ejura-Sekyedumasi, North Kwahu and Nkoranza in Ghana, with contamination levels of raw maize ranging from 31 to 4,832 ng/g in the raw maize samples [14,42]. Though cooking drains away some mycotoxins [19,43,44], there are genuine worries because of the proximity of the University to the aflatoxin endemic belt of the country, from where, presumably, food vendors in the study area, might source their maize. The fears are further heightened because processed maize dishes have been reported to have high levels of between 7.9 and 500 ng/g cases of aflatoxin contamination in one study in these maize sourcing districts [42]. Thus, the prevalence rate of 85% of aflatoxin contamination in the current study is not surprising since many of the maize could have been sourced from these endemic districts.

Relative to other studies, the levels of aflatoxin, as obtained in the study area, may not suggest a hopeless situation. In fact, extremely dangerous levels of aflatoxins (48,000 ng/g) had been reported in Kenya in 2005, where aflatoxin epidemic killed many people [45]. Again, in this current study, the most frequently (modal) recorded total aflatoxin content was 0.7 ng/g, though the median (50%) of the food samples collected, presented total aflatoxin level of contamination amounting to 6.68 ng/g. Though the modal value (0.7 ng/g) is low, the uncertainty is such that the 5th percentile could even be lower (0.09 ng/g) and the 95th percentile, as high as 80.73 ng/g. Low levels were in fact anticipated since there are reported drastic reductions of aflatoxins after cooking [21]. However, no matter how low the levels of aflatoxins ingested may be, there are certainly risk implications as far as these mycotoxins are carcinogens and the risk is not below the deminimus (10^{-5}) [1].

The mass of the maize-based foods consumed in the area ranged between a minimum of 285 g and a maximum of 713 g, and it followed a statistical distribution based on "Expon" (78.137, 284.74). The most frequently consumed mass of the maize-based food was 285 g which was far higher than the WHO Global Environment Monitoring System (GEMS) food consumption cluster diets data of 57 g as maize consumed per person per day in Ghana [46]. On the other hand, a higher mass of cooked, fermented maize dough (kenkey) eaten in Ghana, has been reported as 1000 g per day [25]. In this current study, the median (50%) food consumption, projected a mass of maize-based food consumed to be 339 g, though an uncertainty (95th percentile) could be as high as 519 g. The differences between the reported masses of kenkey eaten in the current study could be due to the limited sampling population which was restricted to only the University students on one campus.

Relatively, the other study could have covered typically maize-based food communities [25].

Presented in Table 3 is the probable daily intake (exposure) and the risk indicators obtained in the study area. There were some samples collected from the study area that did not show any detectable concentrations of aflatoxins (as presented in Table 2), thus, yielding no exposures to consumers. The observation of no detection of aflatoxins in processed food samples is surely a welcomed information because it shows that, there are still pockets of maize sourcing and or storage procedures that are safe. It is these indigenous safety procedures that must be investigated and maintained to control mycotoxins in foods. However, there were other samples that contained so much aflatoxins, to the extent that the exposures amounted to a maximum of 907,304 ng/kg(bw)-day, far above the value 850 ng/kg(bw)-day, reported in other studies in Ghana previously [25]. Again, the most frequently (modal) encountered exposure was 3.38 ng/kg(bw)-day, relative to a high value of 76.5 ng/kg(bw)-day reported as the mean total aflatoxin content of maize for infants and young children consumers in Nigeria [7].

Compared to the exposures of aflatoxin to adults at the 95th percentile who consume cooked rice, a popular staple food in Japan (1.2 ng/kg(bw)-day) at the 95th percentile [21], the values obtained in the current study at the 95th percentile (40,652 ng/kg(bw)-day) is extremely high. However, the incidence of high aflatoxin exposures in Japan might not be a serious problem because there is equally low incidence of HBV infection [1]. Therefore, such levels of aflatoxin as 1.2 ng/kg(bw)-day in Japan, will not result in serious risks because the HBV prevalence factor is low (1%). In fact, the estimated risk has been reported to be low (5.9×10^{-4} -6.7×10^{-4} HCC cases per 10^5 consumers), and more so at the 99.9th percentile [47]. However, in Ghana, where there is co-incidence of high exposures of aflatoxins and high incidences of HBV infection [48], thus, even very low level of aflatoxin contamination could lead to serious risks. The high exposure of 40,652 ng/kg(bw)-day in the current study area, is a clear indicator of how lazed the regulation and control of aflatoxin contamination is. Again, the modal exposures in the study area reported as 3.38 ng/kg(bw)-day, is low relative to endemic areas such as Nigeria, where the national mean total aflatoxin contamination in infants and children was recently reported as 76.5 ng/kg(bw)-day [7]. This suggests that the high prevalence of aflatoxin contamination in the study area is indeed, a reflection of the problem in the sub-region.

Risks

Table 3 reveals the risk prevailing in the current study area in terms of MoE and ELCR. In order to understand the effectiveness of MoE, three levels of MoE are presented. The highest MoE recorded (43,246), occurred when the human standard $BMDL_{10\%}$ was used, followed by the MoE when rodent standard $BMDL_{10\%}$ was used (11,603). These specific observations show that there are some pockets in the study area where maize-based foods were safe from aflatoxin contamination. However, these are isolated areas since the modal and median MoEs showed MoEs < 10^5. The reasons are that when the MoEs were all greater than the 10^5 threshold, then safety is guaranteed relative to when the MoEs < 10^5. It is obvious that if a stringent regulatory standard using human $BMD_{1\%}$ were used, an MoE of 3,216, which is obtained would mean greater public health attention relative to when a $BMDL_{10\%}$ of animal or human threshold were used. The records show that the prevailing central tendencies, as well as the 95th percentiles of MoEs in the study area, were all low (< 6.00), and significantly below the 10^5 regulatory threshold. The implication is that there is serious public health concern just like the other studies where the MoE for national adults stood at 0.2 [7]. The MoEs are however low, relative to the recently high MoEs of aflatoxin B1 exposures ranging between 10 and 69 in adults and children in Pakistan [19]. In spite of these worrying statistics, the modal exposures (3.38 ng/kg(bw)-day) may not be excessively high enough to cause acute aflatoxicosis, a situation which may result in definite short term deaths of susceptible consumers. On the other hand, these chronic probable daily exposures could lead to pathologies such as carcinogenesis and immune suppression and increase the burden of diseases in the long term [49].

The maximum ELCR of over 55,000 cases per 10^5 consumers (Table 3) obtained in the current study, again confirms the values obtained from the MoE analyses. This maximum value (55,000) is greater than the 70.1 cases per 10^5 consumers that was reported as the national prevalence in Ghana in 2008 [25]. The current study involved only University students exposed during a fraction of averaging time of 6 years, relative to 70 years that is normally used for long term studies. However, analysis of Equation 2 shows that whether the averaging time is 6 or 70 years, the probable daily intake (exposures) in the life time of a consumer will still remain the same. This is especially so if key policy drivers of dietary habits such as urbanization, food industry marketing and liberalization are not controlled [50]. Thus, the risk values reported here are likely to hold in the long term as well. The most frequently (modal) encountered risk in the study area registered 3 cases per 10^5 consumers (Table 3). Casually, this modal risk value obtained in the study area appears to be low, however it is still higher than the mean ELCR of aflatoxin B1 exposure in adults (0.070-0.122) and children (0.071-0.127) cases per 10^5 consumers in Pakistan [19]. It must be understood that no matter how low the risk obtained in the current study area may be,

	Central Tendencies					Percentile	
	Min	Max	Mean	Mode	Median	5th	95th
Exposure	0.0	907,304	9,522	3.38	2,630	18.00	40,652
$MoE_{rodent\ BMDL10\%}$	0.0	11,603.0	1.3×10^{-3}	4.26×10^{-3}	0.53×10^{-3}	1.27×10^{-3}	1.13
$MoE_{human\ BMDL10\%}$	0.0	43,246.0	0.40	0.22×10^{-3}	0.03×10^{-3}	6.37×10^{-3}	5.81
$MoE_{human\ BMDL1\%}$	0.0	3,216.0	0.01×10^{-3}	1.66×10^{-3}	0.25×10^{-3}	58.0×10^{-3}	0.52
ELCR per 10^5 people	0.0	55,581.0	786.0	3.0	217.0	2.0	3,375.0

Table 3: Exposure (ng/kg(bw)-day) and risk indices in study area.

it is still not acceptable since the risk uncertainty ranged between 2 to over 3,000 per 10^5 consumers (Table 3).

EFSA/WHO recommends the use of "*as low as reasonably achievable"* (ALARA) approach, which proposes that the level of aflatoxin exposure permitted must be limited to technically unavoidable amounts [51]. Though ALARA might work in certain situations, a limit of 9 ng/g aflatoxin in foods as the threshold to cause an increase of 1 HCC risk in 10^5 consumers have been suggested as a benchmark, though most countries have limits within 4 to 20 ng/g [1]. Such ranges of total aflatoxins may adequately protect consumers giving a covering with deminimus of between 10^{-5}-10^{-4} in a lifetime HCC risk [1]. Even though, the modal total aflatoxin contamination in the study area amounted to 0.7 ng/g, the uncertainty may go up to 80.3 ng/g (95th percentile) as shown in Table 2 and this is far in excess of what the ALARA approach should support. Thus, the exception may be in high aflatoxin endemic regions and communities where HBV infections are high. Admittedly, such high risks require serious safety management which is also problematic because such stringent control measures could put many maize-based industries out of business. Again, it has been suggested that higher aflatoxin levels greater than between 1 and 5 ng/g must occur before a risk greater than 1 case per 10^5 consumers is exceeded [1]. If so, then the modal aflatoxin contamination obtained in the current study (0.7 ng/g) is comparatively low. While this observation could suggest that the study area has low prevalence of aflatoxin contamination compared to many low aflatoxin prevalent countries, one should remember that lifetime or short term risk is computed based on exposure and average potencies (Equation 5). Thus, since the average potency is dependent on HBV infections, then, areas where contaminations are low can tolerate higher prevalence of aflatoxins relative to where HBV infections are high [15,23,52].

From table 4, the regression analysis show that aflatoxin contamination in the food product had the largest impact (β=0.80) on the risk, while other risk descriptors had lower impacts of values between β=-0.08 and β=0.17. This observation reinforces the conclusion of other studies that key risk contributors to low MoE and high cases of HCC per 10^5 consumers is the concentration of aflatoxins in stored raw food products [25]. Thus, one would be tempted to suggest the importance of stringent controls relating to aflatoxin contamination. This may sometimes prove to be challenging especially in situations of public hunger where consumers would make a decision between starving and safety issues. Again, the approach will certainly be accompanied by the economic costs of mitigation. Thus, other sources of control might be to pursue the possibility of exploring vegetables inhibitory effects to stabilize aflatoxin B1 8-9 epoxide which drives the carcinogenic process [6] in further studies. Another possibility is to probably control HBV infections since HCC risks is also dependent on the prevailing HBV infections in the communities [53].

Element	Coefficient
Total AF (ng/g)	0.80
MF_{cons} (g)	0.17
EF_{aca} (days)	0.15
ED_{ty} (year)	0.09
BW (kg)	-0.08

Table 4: Regression coefficients input to risk descriptors.

Conclusion

A very high aflatoxin contamination rate of 85% was recorded for maize-based foods that were sampled. Thus, the 15% of the food samples that did not record any aflatoxin contamination suggest further studies can reveal such sourcing or storage practices

in order to enhance aflatoxin controls. The most frequently(modal) aflatoxin contamination was 0.7 ng/g but it could be as high as 80.73 ng/g for the 95th percentile samples of heavily contaminated maize-based foods. Even though, the most frequent (modal) exposures stood at 3.38 ng/kg(bw)-day, it was too high for a community where the prevalence of HBV is also high. Such high levels of exposures precipitated significantly low MoE (< 6.00), below the recommended threshold (10^5). The modal ELCR value of 3 cases per 105 consumers clearly showed an unacceptably high risk, implying a serious public health concern is warranted. It was also clear from the studies that infected food had the highest impact (β=0.80) on the risk observed, suggesting stringent controls of agricultural produce as a measure. It is obvious such control measures might not be possible because of cost, thus, management and public health officials and risk managers must rise and act fast.

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Conflict of Interest

The Authors declare that there are not conflict of interest.

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Contribution of Authors

Isaac W. Ofosu, designed the study and wrote the final manuscript; Luke P.M. Tetteh, collected the data and drafted the initial manuscript; Gloria M. Ankar-Brewoo and Herman E. Lutterodt, made critical suggestions to the final manuscript and also corrected certain portions; Edmund O. Benefo, contributed significantly in writing the final manuscript; William O. Ellis worked on the final manuscript and made significant corrections prior to submission.

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