Dietary Exposure to Aflatoxins in Some Randomly Selected Foods and Cancer Risk Estimations of Cereals Consumed on a Ghanaian Market

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Aflatoxins have gained so much reputation among all mycotoxins due to their notoriety in causing countless adverse health effects on humans as well as animals. It continues to be a major concern in food safety globally. In this study, total and constitutive aflatoxins levels as well as the carcinogenic risks posed by 110 food and feed samples (55 cereals, 20 nuts and oils, 18 animal feed, and 18 fruits and vegetables) collected from the Ho Central market in the Volta region, Ghana, were assessed. Using high-performance liquid chromatography connected to a fluorescent detector (HPLC-FLD), levels of total aflatoxins (AFtotal) and aflatoxins constituents, namely, AFB1, AFB2, AFG1, and AFG2 were analyzed. By using the model prescribed by Joint FAO/WHO Expert Committee on Food Additives (JECFA), the risks posed by the food and feed samples were determined. The degrees of toxicity were in the ranges of 0.78–234.73 μg/kg, 0.47–21.6 μg/kg, 1.01–13.75 μg/kg, and 0.66–5.51 μg/kg, respectively, for AFB1, AFB2, AFG1, and AFG2. Out of the samples analyzed for AFtotal about 51 (46.4%) exceeded the limits of GSA and were in the range 10.63 ± 1.20–236.28 ± 4.2 μg/kg. While for EFSA, 71 (64.54%) exceeded and ranged between 4.72 ± 0.28 and 236.28 ± 4.2 μg/kg. Furthermore, estimated daily intake (EDI) of 27.10–283.70 ng/kg·bw/day, margin of exposure (MOE) of 1.409–14.76, average potency of 0–0.00396 ng aflatoxins/kg·bw/day, and cancer risks with a range of 0.107–1.122 cases/100,000 person/yr were observed. Taken together, it could be concluded that consuming cereals pose adverse effects on human health regardless of the age of the consumer.

1. Introduction

Mycotoxins are toxins that naturally contaminate food consumed by humans. These toxins originate from some fungi. When food becomes contaminated by these toxins, the untoward effect on health is enormous [1]. Mycotoxins such as aflatoxin, fumonisins, ochratoxin A (OTA), deoxynivalenol (DON), zearalenone (ZEA), and other trichothecenes are recognized to be produced by fungi genera such as Aspergillus, Penicillium, and Fusarium [2–4]. These are the most toxicologically prominent mycotoxins of significant concern in foods and animal feeds. Aspergillus flavus and A. parasiticus mostly produce aflatoxins, while A. nomius and Emericella venezuelensis rarely do. These fungal species are mostly found growing in agro-foodstuffs, namely, peanuts, maize grains, and cereals [5, 6], which eventually end up in the food chain for human and animal consumption [7]. Biochemically, they are composed of difuranocoumarin molecules synthesized through the polyketide pathway [8]. A. flavus is made up of two distinct
morphtypes—the morphtypes L and S, which are physiologically, morphologically, and genetically divergent [9,10]. Mehl et al. [11] explained that the L morphtype produces larger, numerous conidia, variable concentrations of aflatoxins, and fewer sclerotia, whereas the S morphtype produces abundant but small sclerotia and fewer conidia, but produces high aflatoxin levels.

Aflatoxins B₁ and B₂ are specifically produced by A. flavus, while Aspergillus parasiticus produces aflatoxins B₁, B₂, G₁, and G₂ moieties. Interestingly, Aspergillus species of agricultural importance belong to the section Flavi from aflatoxins [12] except in some rare conditions where other species from different genera (Emericella, Claviceps, Stachybotrys, and Alternaria) [12].

Of all the aflatoxins fractions, aflatoxin B₁ is known as the most carcinogenic [13] and has been aptly categorized as Group 1 carcinogen by the International Agency for Research on Cancer, and second in carcinogenicity to the most carcinogenic family of chemicals known (unnaturally derived polychlorinated biphenyl). They are the most copious and hence receives the most devotion in mammalian toxicology [14, 15]. Aflatoxin B₁ contamination has often been associated with A. flavus and has been caught up as the major causal agent of preharvest contamination of foodstuffs besides other aflatoxin-producing fungal species of which the exact dynamics is not fully understood [16]. Aflatoxin buildup in food and feeds is exacerbated by environmental conditions such as extreme heat, extraordinary humidity, and poor aeration in storage places. Insects and rodents also play roles in worsening the contamination of agroproducts with aflatoxin [17, 18].

Aflatoxins have been reported to work synchronously to exacerbate the risk of liver cancers, which is a very common form of cancers, globally [19, 20]. Epidemiological and animal studies have suggested that states of liver cancers are worsened by 3.3-fold by AFM1 in patients living with hepatitis B virus surface antigen-positive [21, 22]. Moreover, it has been established that in people with viral hepatitis, the liver cells are unable to detoxify aflatoxins [23], thereby increasing the chance of liver cancer. Therefore, it is not surprising to note that in many cases of individuals with liver cancer, most of them are exposed to aflatoxin. Majority of these data were reported in sub-Saharan Africa, Southeast Asia, and China. These occurrences are due to the accumulation of aflatoxin in foods together with hepatitis B virus infection [24]. Furthermore, the economic impact of mycotoxicosis is obvious [25].

Over the years, Ghana has not been spared from these and there has been ample evidence of cereals, grains, and spices contaminated by aflatoxin. The pervasiveness of aflatoxin in cereals, grains, and spices in Ghana is in the range of 0.61–1546 ppb as reported in some studies [6, 26–28]. For more than 40 years, several management tactics have been surveyed nationwide. Both pre- and postharvest strategies should have helped lessen the frequency and severity of aflatoxin contamination as suggested by [29, 30]; nonetheless, this problem of contamination still persists.

Most governmental authorities control and vary the levels of mycotoxins allowed in these animal feedstuffs and human foods because of their potential toxicity and public health consequences. Regulatory information for mycotoxins is usually posted on administration websites [31] as guides to aid producers and consumers. Besides enforcing regulations on limits of mycotoxins contents in foods, conducting health assessment on risk exposure levels in the population is imperative. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1997 and the margin of exposure (MOE) method suggested at the 64th JECFA meeting in 2005 introduced the low-dose deduction method. This method has since been endorsed for worldwide assessment of the risk of dietary acquaintance to mycotoxins.

In view of this, this study was designed to evaluate the quantity and health risks posed by aflatoxins via the consumption of assorted food (humans) and feed (animals) sold in the Ho central market, Volta Region, Ghana. The findings of this article would be useful in advising policymakers on food safety regarding fungal intoxication. The data could also provide proper health education and put emphasis on designing more effective mold and aflatoxin management strategies for Ghana. To the best of our knowledge, this study is novel in Ghana; therefore, it will serve as the basis for further studies in this subject area.

2. Materials and Methods

2.1. Study Area and Site. Ho municipality is the host of the capital town of the Volta region. The region is made up of 25 municipalities and districts. Ho municipality is the marketable hub of the region. According to the Ghana Statistical Service [32], the municipality has about 772 communities. The community members depend on the major market in Ho for selling and buying. Figure 1 represents the map of the Ho municipality, where this study was carried out.

2.2. Sample Collection. Food and feed samples analyzed in this study were randomly purchased from the Ho municipal market from February to September 2019. The samples were grouped into four categories (cereals, nuts and seeds, animal feed, fruits, and vegetables). Depending on the consistency of the samples, they were either bagged in 20 g or 20 mL in sterile zip-lock bags and sent immediately to the laboratory where they were frozen below -20°C until ready for analyses.

2.3. Solid-Phase Extraction of Aflatoxin from the Food and Feed Samples. Aflatoxin moieties, namely, AFB₁, AFB₂, AFG₁, and AFG₂, were extracted from the samples according to the European Committee for Standardization (CEN) official method EN14123 as described elsewhere [28]. Aflatoxin was extracted using 200 mL of methanol in water and 5 g of sodium chloride. Selectively, 100 mL of hexane was added to samples with 50% or more fat contents. The sample and extractant mixture were vortexed for about 2 minutes to obtain complete homogenate, followed by 1-minute centrifugation at 3500 rpm. Subsequently, 10 mL of the filtered extract was added to 60 mL of phosphate buffer saline (PBS) for solid-phase extraction using a preconditioned immunoaffinity column (R-Biopharm RHONE LTD EASI-
EXTRACT AFLATOXIN) specific for AFB$_1$, AFB$_2$, AFG$_1$, and AFG$_2$.

The filtrate-PBS mixture (~70 mL) was loaded onto the preconditioned column, and by gravitational force, the mixture was allowed to elute at a slower speed (~1 mL min$^{-1}$). The column was cleaned with 15 mL of double distilled water at a rate of 5 mL min$^{-1}$. Aflatoxins elution was performed in two steps: firstly with 0.5 mL of HPLC-grade methanol, and secondly, another 0.75 mL of the same grade of methanol into a 5-mL volumetric flask. Finally, the volume was made up to 5 mL by using deionized water. Shortly after that, 2 mL of the eluate was dispensed into HPLC vials for subsequent aflatoxin quantification [28].

2.4. HPLC Parameters. The following HPLC parameters were used to determine the aflatoxin fractions. An injection volume of 10 µL with a flow rate of 1 mL/min was used. Additionally, a column temperature of 35°C was used, while an excitation wavelength of 360 nm and an emission wavelength of 440 nm was used. Furthermore, the mobile phase made up of water, acetonitrile, and MeOH in the proportions of 65:15:20 (v/v/v) was used. The HPLC column specifications were Spherisorb ODS1-Excel (4.6 mm x 25 cm), 5 µm particle size, and 250 Å pore size. These parameters have been used in a previous study [28].

2.5. Calculation of Limit of Detection (LOD) and Limit of Quantification (LOQ). Limits of detection and limits of quantification of the HPLC were determined from a calibration curve using the aflatoxin standard 5 µg/kg. The LOD and LOQ were calculated by using the formulae: LOD = 3 × standard deviation/slope and LOQ = 3 × LOD.

2.6. Measurement Accuracy. To ensure measurement accuracy of the aflatoxin fractions, pure aflatoxin standard spiking was carried out. Using lower (5 ppb), middle (15 ppb), and upper (30 ppb) concentrations, three levels of spiking were performed. The volume of pure standards was calculated as
Volume of Pure Standard = \[\frac{\text{Sample weight (g) \times spike concentration (ppb)}}{\text{Concentration of standard (ug/ml)}}\] (1)

Spike volumes were then distributed on blanks, which were aflatoxin-free samples and analyzed for percentage recovery. Percentage recovery was calculated as

\[\left(\frac{\text{Concentration measured in spike} - \text{concentration measured in blank}}{\text{spiked amount}}\right) \times 100\%\]. (2)

2.7. Measurement Precision. An internal reference material (IRM) was used to ensure the precision of the method. Ten parallel extracts of the IRM were analyzed on the HPLC analyzer by the same technician to ensure repeatable analysis, whereas ten extractions of the IRM were made by a different technician on separate days to assess intermediate precision. The relative standard deviations were calculated using the following formula:

\[\left(\frac{\text{Standard deviation}}{\text{mean}}\right) \times 100\%\]. (3)

2.8. Assessment of Risk of Exposure to Total Aflatoxins in Humans via Consumption of Cereals

2.8.1. Estimation of Aflatoxins Exposure. Estimated daily intake (EDI) was computed by using the following: the daily intakes of the same samples, mean levels of aflatoxins obtained in cereal samples, and the average body weight in kilograms. The EDI for mean aflatoxin was calculated using the following formula, as used elsewhere [35] and expressed in \(\mu g/kg\) of body weight/day (\(\mu g/kg\cdot b.w/day\)):

\[\text{EDI} = \frac{\text{daily intake (food)} \times \text{mean level of aflatoxins}}{\text{average bodyweight}}\]. (4)

Daily intake of cereals in Ghana according to Galbete et al. [36] is approximately 0.0673 kg/day.

In this study, the age-weight categories according to EFSA [37] used are as follows: infants—2.9 (2.5–3.2) kg [38, 39], toddler—9.8 (7–12.6) kg [40, 41], children—26 (24–28) kg [42, 43], adolescents—46.25 (38.5–54) kg [44], and adults—60.7 kg [45].

2.8.2. Cancer Risk Characterization for Aflatoxins. Risk characterization of aflatoxins is based on the margin of exposure (MOEs), which is calculated as follows:

\[\text{MOE} = \frac{\text{Benchmark dose lower limit}}{\text{EDI (Exposure)}}\]. (5)

MOE lower than 100,000 raises a public health concern [15,46].

2.8.3. Estimating Risk of Liver Cancer due to Ingestion of Food Samples. The estimated liver cancer risk involves estimating the risk per 100,000 population. This was performed by multiplying the EDI value with the average hepatocellular carcinoma (HCC) potency figure from the individual potencies of HBsAg-positive and HBsAg-negative groups.

The JECFA estimated potency values for AFB1, which corresponded to 0.03 cancer/year/100,000 population ng/kg bw/day (uncertainty range: 0.05–0.5) in HBsAg-positive individuals and 0.01 cancer/year/100,000 population ng/kg bw/day (uncertainty range: 0.002–0.03) in HBsAg-negative persons were approved for this calculation.

Furthermore, in Ghana, the hepatitis B surface antigen positivity (HBsAg pos) rate of 10.2% for Ghanaians [47] was used and about 90% was inferred for hepatitis B surface antigen negativity (HBsAg neg) groups. Therefore, the average potency for cancer in Ghana was estimated using the following formula:

\[\text{Average Potency} = (0.03 \times 0.102) + (0.001 \times 0.900)\]

\[= (0.003958 \text{ cancers per year per 100,000 population per ng aflatoxin)/kgbw day}\]. (6)

Thus, the population risk was predicted with the following formula:

\[\text{Cancer Risk} = \text{Exposure (EDI)} \times \text{Average Potency}.\] (7)

2.9. Data Analysis. Excel for Microsoft Windows (version 10) was used to calculate the aflatoxin concentrations from the standards of aflatoxins. T-test was used to determine the differences in the means levels of aflatoxins. Descriptively,
Table 1: HPLC-FLD parameters for validating repeatability, precision, recovery, limit of detection (LOD), and limit of quantification (LOQ) for aflatoxins detection.

<table>
<thead>
<tr>
<th>Aflatoxin</th>
<th>Limits</th>
<th>Amount (μg/kg)</th>
<th>Repeatability (standard deviation)</th>
<th>Intermediate precision (reproducibility) (%)</th>
<th>Recovery percent (recovery of measurement procedure) (%)</th>
<th>Linearity of regression curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB1</td>
<td>LOD*</td>
<td>0.20</td>
<td>5.5%</td>
<td>13.2</td>
<td>107</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>LOQ**</td>
<td>0.60</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFB2</td>
<td>LOD</td>
<td>0.17</td>
<td>6.7%</td>
<td>13.4</td>
<td>87.2</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>LOQ</td>
<td>0.51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFG1</td>
<td>LOD</td>
<td>0.26</td>
<td>7.4%</td>
<td>13.7</td>
<td>113.4</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>LOQ</td>
<td>0.78</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFG2</td>
<td>LOD</td>
<td>0.36</td>
<td>12.1%</td>
<td>12.2</td>
<td>112.8</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>LOQ</td>
<td>1.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* LOD - Limit of detection: LOD for all aflatoxins should be less than 1 μg/kg. ** LOQ - Limit of quantification: LOQ for all aflatoxins should be less than 3 μg/kg.

Table 2: Summary of statistics of aflatoxins concentrations (μg/kg) in the various food categories sold in Ho central market.

<table>
<thead>
<tr>
<th>Food category</th>
<th>Aflatoxin</th>
<th>Mean</th>
<th>Median</th>
<th>Standard deviation</th>
<th>Variance</th>
<th>Skewness</th>
<th>Kurtosis</th>
<th>Sig. (2-tailed)</th>
<th>Percentiles 25</th>
<th>Percentiles 50</th>
<th>Percentiles 75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereals</td>
<td>AFB1</td>
<td>19.85</td>
<td>10.63</td>
<td>34.04</td>
<td>1159.04</td>
<td>3.54</td>
<td>13.81</td>
<td>0.00</td>
<td>1.96</td>
<td>10.63</td>
<td>22.47</td>
</tr>
<tr>
<td></td>
<td>AFB2</td>
<td>2.66</td>
<td>1.40</td>
<td>3.74</td>
<td>14.00</td>
<td>2.77</td>
<td>11.22</td>
<td>0.00</td>
<td>0.00</td>
<td>1.40</td>
<td>4.75</td>
</tr>
<tr>
<td></td>
<td>AFG1</td>
<td>1.45</td>
<td>0.00</td>
<td>2.44</td>
<td>5.95</td>
<td>2.76</td>
<td>10.96</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>2.51</td>
</tr>
<tr>
<td></td>
<td>AFG2</td>
<td>0.28</td>
<td>0.00</td>
<td>0.95</td>
<td>0.90</td>
<td>4.05</td>
<td>18.11</td>
<td>0.03</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>AFtotal</td>
<td>24.45</td>
<td>12.61</td>
<td>36.21</td>
<td>1311.37</td>
<td>3.01</td>
<td>10.36</td>
<td>0.00</td>
<td>2.73</td>
<td>12.61</td>
<td>32.75</td>
</tr>
<tr>
<td>Fats and oils</td>
<td>AFB1</td>
<td>16.13</td>
<td>11.80</td>
<td>16.10</td>
<td>259.11</td>
<td>0.31</td>
<td>0.32</td>
<td>0.00</td>
<td>0.55</td>
<td>11.80</td>
<td>25.90</td>
</tr>
<tr>
<td></td>
<td>AFB2</td>
<td>2.61</td>
<td>1.40</td>
<td>3.24</td>
<td>10.48</td>
<td>1.41</td>
<td>0.97</td>
<td>0.00</td>
<td>0.00</td>
<td>1.40</td>
<td>3.90</td>
</tr>
<tr>
<td></td>
<td>AFG1</td>
<td>1.02</td>
<td>0.00</td>
<td>2.08</td>
<td>4.34</td>
<td>2.36</td>
<td>5.23</td>
<td>0.04</td>
<td>0.00</td>
<td>0.00</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td>AFG2</td>
<td>0.50</td>
<td>0.00</td>
<td>1.36</td>
<td>1.87</td>
<td>3.09</td>
<td>9.97</td>
<td>0.12</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td></td>
<td>AFtotal</td>
<td>20.26</td>
<td>16.25</td>
<td>19.25</td>
<td>374.41</td>
<td>0.56</td>
<td>−1.06</td>
<td>0.00</td>
<td>0.55</td>
<td>16.25</td>
<td>36.75</td>
</tr>
</tbody>
</table>

Cereals (n = 55), Fats and oils (n = 20)

Table 3: Summary of statistics of aflatoxins concentrations (μg/kg) in the various food categories sold in Ho central market.

<table>
<thead>
<tr>
<th>Food category</th>
<th>Aflatoxin</th>
<th>Mean</th>
<th>Median</th>
<th>Standard deviation</th>
<th>Variance</th>
<th>Skewness</th>
<th>Kurtosis</th>
<th>Sig. (2-tailed)</th>
<th>Percentiles 25</th>
<th>Percentiles 50</th>
<th>Percentiles 75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal feed</td>
<td>AFB1</td>
<td>24.16</td>
<td>2.53</td>
<td>56.99</td>
<td>3248.17</td>
<td>3.37</td>
<td>12.13</td>
<td>0.09</td>
<td>0.86</td>
<td>2.53</td>
<td>16.78</td>
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<tr>
<td></td>
<td>AFB2</td>
<td>1.29</td>
<td>0.72</td>
<td>1.85</td>
<td>3.41</td>
<td>1.87</td>
<td>3.29</td>
<td>0.01</td>
<td>0.00</td>
<td>0.72</td>
<td>1.62</td>
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<tr>
<td></td>
<td>AFG1</td>
<td>0.37</td>
<td>0.00</td>
<td>0.54</td>
<td>0.29</td>
<td>0.83</td>
<td>−1.37</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
<td>1.03</td>
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<tr>
<td></td>
<td>AFG2</td>
<td>0.06</td>
<td>0.00</td>
<td>0.26</td>
<td>0.07</td>
<td>4.24</td>
<td>18.00</td>
<td>0.33</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td></td>
<td>AFtotal</td>
<td>25.86</td>
<td>4.50</td>
<td>57.59</td>
<td>3316.92</td>
<td>3.27</td>
<td>11.48</td>
<td>0.07</td>
<td>1.44</td>
<td>4.50</td>
<td>18.58</td>
</tr>
<tr>
<td>Fruits and vegetables</td>
<td>AFB1</td>
<td>4.15</td>
<td>2.13</td>
<td>5.23</td>
<td>27.30</td>
<td>1.94</td>
<td>3.09</td>
<td>0.00</td>
<td>0.86</td>
<td>2.13</td>
<td>5.24</td>
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<tr>
<td></td>
<td>AFB2</td>
<td>0.97</td>
<td>0.79</td>
<td>1.06</td>
<td>1.12</td>
<td>0.89</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.79</td>
<td>1.62</td>
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<td></td>
<td>AFG1</td>
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<td>0.00</td>
<td>0.92</td>
<td>0.86</td>
<td>2.45</td>
<td>7.39</td>
<td>0.02</td>
<td>0.00</td>
<td>0.00</td>
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<td></td>
<td>AFG2</td>
<td>0.60</td>
<td>0.00</td>
<td>0.27</td>
<td>0.07</td>
<td>4.24</td>
<td>18.00</td>
<td>0.33</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<td>AFtotal</td>
<td>5.68</td>
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<td>6.45</td>
<td>41.61</td>
<td>1.67</td>
<td>2.22</td>
<td>0.00</td>
<td>1.44</td>
<td>2.69</td>
<td>7.39</td>
</tr>
</tbody>
</table>

Animal feed (n = 18), Fruits and vegetables (n = 18)

Aflatoxin levels were presented as median, standard deviation, variance, and percentiles. Data were analyzed using SPSS v. 22 (Chicago, USA). Various formulae, stated herein, were used to calculate the limit of detection (LOD), limit of quantification, percentage recovery, estimated daily intake (EDI), margin of exposure (MOE), average potency, and cancer risk of aflatoxin exposures.

3. Results

3.1. Mycotoxico logical Findings of the Study Samples. From Table 1, it can be seen that the limit of detection (LOD) for AFB1, AFB2, AFG1, and AFG2 were 0.20 μg/kg, 0.17 μg/kg, 0.26 μg/kg, and 0.36 μg/kg, respectively. Good linearity ($R^2 > 0.990$) was obtained within the tested range in utmost
of the food samples tested. One cereal, nuts, oil, animal feed, fruits, and vegetable samples, analyzed to be certain for aflatoxin absence, were used in the validation procedure for the recovery analysis. The LOD for the aflatoxin fractions was 0.13–0.15, whereas the LOQ was 0.26–0.30 (Table 1). Repeatability values recorded ranged between 5.5 and 12.1%, Intermediate precision ranged between 12.2 and 13.7%, while recovery ranged between 87.2 and 113.4% (Table 1).

The sum of food samples tainted with the aflatoxin fractions (AFB1, AFB2, AFG1, AFG2) and AFTotal are presented in Tables 2–5. The degree of levels of contamination was presented in Tables 2 and 3. The level of occurrence of the AFTotal ranged between 0 and 179.49 μg/kg, 0 and 56.71 μg/kg, 0 and 236.28 μg/kg, and 0 and 21.66 μg/kg, respectively, for cereals, fats and oils, animal feed, and fruits and vegetables. The cereals recorded mean (24.45 μg/kg), median (12.61 μg/kg), and variance (1311.37 μg/kg), while the skewness and kurtosis were 3.01 and 10.36, respectively, and showed that the dataset of aflatoxins (AFTotal) obtained in this category was asymmetrical and heavy-tailed (Table 2). There were observed significant differences (p < 0.05) (Table 2). For fats and oils, toxin concentration values of mean (56.71 μg/kg), median (16.25 μg/kg), and variance (374.41 μg/kg) were recorded from the summary statistics, while 0.56 and −1.06 were recorded as skewness and kurtosis and implied moderate skewness and light-tailed. Toxin magnitude restrictions were adopted in this study. AFTotal and AFB1 (AFB1) were observed significant differences (p < 0.05) (Table 2). For animal feed, mean (25.86 μg/kg), median (4.50 μg/kg), and variance (3316.92 μg/kg) levels of aflatoxin were also recorded (Table 3). Lastly, for fruits and vegetables, the mean (5.68 μg/kg), median (2.69 μg/kg), and variance (41.61 μg/kg) were recorded. The dataset for fruits and vegetables was fairly symmetrical and light-tailed; 1.67 and 2.22 for skewness and kurtosis, respectively (Table 3). Both the European Food Safety Authority (EFSA) and Ghana Standards Authority (GSA) regulatory concentration limits for total aflatoxins (AFTotal) and Aflatoxin B1 (AFB1) (Table 4) were adopted in this study. Toxin magnitude restrictions approved by the Ghana Standards Authority dovetails with the European Food Safety Authority (EFSA).
3.2. Risk Assessment. The estimated daily intakes (EDI) of total aflatoxins for infants, toddlers, children, adolescents, and adults in the cereal samples were 283.70, 167.90, 63.28, 35.54, and 27.10 ng/kg bw/day, respectively. Margin of exposure (MOE) values recorded were 1.409, 2.382, 6.321, 11.25, and 14.76, respectively. The average potency of the aflatoxins was 0.00396 mg/kg bw/day and therefore the cancer risks were 0.0911, 0.054, 0.020, 0.011, and 0.011 respectively. Finally, the EDI for infants, toddlers, children, adolescents, and adults were 23.03, 13.63, 5.14, 2.89, and 2.20 ng/kg bw/day, respectively (Table 5). For AFB1, the EDIs for infants, toddlers, children, adolescents, and adults were 23.03, 13.63, 5.14, 2.89, and 2.20 ng/kg bw/day, respectively, whereas the MOE values noted down were 1.409, 2.382, 6.321, 11.25, and 14.76, respectively. The average potency of the aflatoxins was 0.00396 mg/kg bw/day and therefore produced a cancer risk of 1.122, 0.665, 0.250, 0.141, and 0.107 cases/100,000 people/yr, respectively (Table 5). For AFB1, approximately 62 (56.4%) of 110 samples exceeded and ranged between 4.72 ± 0.28 and 236.28 ± 4.2 μg/kg. For AFB1, about 51 (46.4%) exceeded the limits of GSA and were in the range of 10.63 ± 1.20–236.28 ± 4.2 μg/kg. While using the EFSA, as benchmark 71 (64.54%) of 110 samples exceeded and ranged between 4.72 ± 0.28 and 236.28 ± 4.2 μg/kg. For AFB1, approximately 62 (56.4%) of range 5.01 ± 1.7–236.28 ± 4.2 μg/kg exceeded the GSA tolerable limit, whereas 75 (68.2%) of range 2.2 ± 0.15–236.28 ± 4.2 μg/kg exceeded the limits of EFSA as shown in Table 4.

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Taken together, of the 110 samples analyzed for AFtotal, about 51 (46.4%) exceeded the limits of GSA and were in the range of 10.63 ± 1.20–236.28 ± 4.2 μg/kg. While using the EFSA, as benchmark 71 (64.54%) of 110 samples exceeded and ranged between 4.72 ± 0.28 and 236.28 ± 4.2 μg/kg. For AFB1, approximately 62 (56.4%) of range 5.01 ± 1.7–236.28 ± 4.2 μg/kg exceeded the GSA tolerable limit, whereas 75 (68.2%) of range 2.2 ± 0.15–236.28 ± 4.2 μg/kg exceeded the limits of EFSA as shown in Table 4.

### Table 6: Assessment of risk of cancer for aflatoxin B1 (AFB1) through cereals consumption.

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Age definition</th>
<th>Average body weight (kg)</th>
<th>Estimated daily intake (EDI) (ng/Kg bw/day)</th>
<th>Margin of exposure</th>
<th>Cancer risk (Cases/100,000 person/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>0–11 months</td>
<td>2.9</td>
<td>23.03</td>
<td>17.37</td>
<td>0.0911</td>
</tr>
<tr>
<td>Toddlers</td>
<td>12–35 months</td>
<td>9.8</td>
<td>13.63</td>
<td>29.35</td>
<td>0.054</td>
</tr>
<tr>
<td>Children</td>
<td>36 months–10 years</td>
<td>26</td>
<td>5.14</td>
<td>77.82</td>
<td>0.020</td>
</tr>
<tr>
<td>Adolescents</td>
<td>11–17 years</td>
<td>46.3</td>
<td>2.89</td>
<td>138.40</td>
<td>0.011</td>
</tr>
<tr>
<td>Adults</td>
<td>&gt;18 years</td>
<td>60.7</td>
<td>2.20</td>
<td>181.8</td>
<td>8.70x10⁻³</td>
</tr>
</tbody>
</table>

Mean of AFB1-19.85 μg/kg; daily intake of cereals for infants was halved (0.5x 0.0673 kg); Av. potency aflatoxins—0.00396

4. Discussion

Consumer health is severely obstructed and endangered by the infection of fungal and mycotoxins of crops and agricultural products. The most remarkable consequence, although often not well familiar and accepted, stems from clients who purchase and be contingent on unprocessed farm crops from the markets [49]. This is because there is no sufficient regulations on aflatoxins. These regulations are targeted at processed foods instead of raw farm produce. This is prevalent not only in Africa, of which Ghana is not an exception, but in other continents. Therefore, this study evaluated the aflatoxicological risks concomitant with the sale and ingesting of possible aflatoxin-contaminated human food and animal feed, in a domestic market in Ghana that serves neighboring countries like Togo and Benin with a vision of furnishing with reliable data to notify food safety policies and regulation as well as encourage public health. The concentrations of the different constitutive aflatoxins detected in the various foodstuffs and feeds were varied according to their class and their origin. Fungal contamination occurs during cultivation and storage in food and feed products as environmental conditions become favorable to toxigenic fungi.

The results obtained in this study for the cereals category were lower than the published findings of Sugri et al. [50] for the aflatoxin pervasiveness that ranged from 0.011 to 308 parts per billion (ppb) in maize samples (240) in some parts of Ghana, specifically, the Upper East and West regions. In Ghana, Kumi et al. [51] reported aflatoxin levels in homemade weanimix, which ranged from 7.9 to 500 ppb. The findings of their study suggested that out of the thirty-six (36) samples (from UNA-like communities), two (2) had greater concentrations of 460 and 500 ppb (μg/kg). James et al. [52] monitored aflatoxin pollution in maize grains from a total of 38 major market stores in Benin, Ghana, and Togo, and reported contaminated samples of 24–117.5 ng/g, 0.4–490.6 ng/g, and 0.7–108.8 ng/g in Benin, Ghana, and Togo, respectively.

However, the findings reported herein were higher than the findings obtained by Blankson and Mills-Robertson [53] and Blankson et al. [54] for total aflatoxins with the ranges 0.18 ± 0.01–44.42 ± 0.38 μg/kg and 0.18 ± 0.02–25.93 ± 0.29 μg/kg, respectively, in cereal-based foods for infants. Likewise, values of 35.46–86.06 μg/kg were also reported by Kortei et al. [28] for cereal-based foods from another market in Ghana.

For nuts and oils, Awuah and Kpodo [55] previously reported small concentrations of total aflatoxins in whole and unwhole kernels. Again, about 290 ppb of aflatoxin in peanut samples was obtained from a market in Accra [56]. Additionally, in the Northern part of Ghana, 1.0–7.45 μg/kg of total aflatoxin was reported [57]. Furthermore, in the Ejura-Sekyedumase District of Ghana, total aflatoxin in the range of 7.9–500 ppb in food samples used locally to prepare infant weaning foods has also been reported [38]. In these studies, it was observed that 83% of the food samples contained aflatoxin levels higher than the 20 ppb permissible limit. The aflatoxin contaminations were found to be higher in food samples stored at places with higher room temperature and higher humidity conditions [59].

In animal feed, there is a general paucity of data in Ghana. Notwithstanding, Ref. [60] reported a range of 0.02–22 ppb for total aflatoxins in poultry feed, which is approximately 12-fold lower than the values obtained in this research. As emphasized by Ref. [61], aflatoxicosis in animals...
brings about alterations in hemato-biochemical parameters, vital organ (liver and kidney) changes, and immunosuppression.

From the equitably greater concentrations of aflatoxins noticed in this study, we can assert that aflatoxin can persist in food irrespective of whether the fungi are viable or not [62]. There is a high probability of aflatoxin contamination regardless of how the cereal food was prepared. This is possible if the constituents used in the food were tainted prior to handling. A danger looms herein, when these highly prone food stuffs (groundnut and cereals) are the main ingredients commonly used for the formulation of local baby foods and other weaning foods for children. In some of the food samples, aflatoxin was not detected. This may be due to low levels of the toxin, below the detection limits of the equipment used in this study. Although not tested for in this present study, another possibility will be the coprevalence of some other fungal species and the subsequent production of mycotoxins in the food samples, which proposes a possible nonantagonistic metabolite interaction.

In a recent study, it was recounted that the level of aflatoxin infection is a result of pre- and postharvest handling, rather than the agronomical procedures used in crop production. However, the source of seed for planting and region of habitation were identified as contributing elements of aflatoxin infestation in Northern Ghana [63]. Additionally, Etwire and Marrey [63] observed that aflatoxin influx was high in the Upper West Region due to on-farm processing of cereals before storage.

Notwithstanding, interpolations such as consuming a variety of diets are recommended accumulation of mycotoxins, which are direct consequences of other alternative sources of food [1]. Since the availability of alternative sources of food will reduce the chances of mycotoxin accumulation, contaminated food will be eaten less often [64]. Alternative food sources are essential for improved health conditions [1]. However, this is a very difficult practice since mono-cropping is common in subsistence farms. Some of the challenges to implementing alternative crop production are environmental and soil factors, food insecurity, cultural traditions, and economic limitations confronting Africa [65]. Promoting the use of certified seeds and encouraging good postharvest practices are key to reduce mycotoxin contaminations.

Elucidated by Kepinska-Pacelik and Biel [66], hazard estimation is used to predict the carcinogenic effect of aflatoxin exposure. Analyzing for the margin of exposure values obtained in this study, infants, children, and adolescents are at risk of aflatoxins intoxication, with its attendant health concerns, especially in infants and children. Findings in this study collaborate those of Blankson and Mills-Robertson [53] as they reported similar levels of total aflatoxin EDI for infants and young children. Contrary to the findings of this study, Kortei et al [28] reported a low risk consumption of cereal-based food formula sold on a market in Ghana. Much higher EDIs have been reported in Korea with values of 1.2–5.8 ng/kg bw/day by WHO [67]. Again, the WHO [68] reported values of 91 ng/kg bw/day from China. A range of 0.03 to 1.28 ng/kg bw/day of AFB₁ has been validated as the dietary exposure of Europeans by members of the EU. From Australia and the United States (WHO), respectively, lesser EDI levels of 0.15 ng/kg bw/day and 0.26 ng/kg bw/day were reported. A recent dietary intake appraisal recorded an EDI of 0.072 ± 0.167 ng/kg bw/day for males and 0.077 ± 0.208 ng/kg bw/day for females, whereas estimates of 0.15 ± 0.126 ng/kg bw/day for children and 0.178 ± 0.208 ng/kg bw/day for adolescent were obtained from Spanish study participants [69].

It is worthy to note that any level of aflatoxin contamination is regarded as unsafe. Therefore, reduction to as low as reasonably achievable level is the recommendation of Joint FAO/WHO Expert Committee on Food Additives with regard to the safe levels of aflatoxins in foods following the momentous genotoxic carcinogenic likelihood of this toxin [3]. The current phenomenon of climate change has been involved in the escalated prevalence of mycotoxin in our foods. The growth of A. flavus is influenced by heat and dry conditions. Even in places where there are very few reports of mycotoxin contaminations, environmental changes could cause these molds to grow in conducive food samples. Environmental changes that led to dry and hot weather resulted in surges in maize contamination [70, 71]. Notwithstanding the foregoing, enzymatic biodegradation of mycotoxins in foods has been suggested as an option to reduce aflatoxins contaminations. Recently, mycosorb, a broad-spectrum mycotoxin binder and aluminosilicates, have been found to efficiently reduce mycotoxin contaminations [72].

5. Conclusion

In summary, this study observed that 46.4% of the food samples analyzed for total aflatoxin surpassed the GSA limits. Human health risk assessment for aflatoxin exposure through the dietary intake of cereals and cereal-based foods sold in the Ho central market consumed by infants, toddlers, children, adolescents, and adults posed a momentous adversarial health risk of cancer to humans since calculated values for MOE for all samples were far below 100,000. This study touches on a dreadful state of affairs that merit prompt action by stakeholders involved in ensuring food safety. Ghanaian public health authorities have to monitor ceaselessly to detect aflatoxins contamination and need to be suppressed to as low as reasonably achievable (ALARA) level.

Data Availability

The datasets used during the current study are available from the corresponding author on request.

Conflicts of Interest

The authors have no interests to declare.

Authors’ Contributions

Nii Korley Kortei, Theophilus Annan, and Adjoa Agyeman Boakye conceived and designed the study, contributed
reagents, performed the laboratory analyses, analyzed data, and drafted the initial manuscript. Theophilus Annan, Seidu A. Richard, Clement Okraku Tettey, and Enoch Annangyei performed experiments. Precious Agbemesei, Edward Ken Essuman, and Adjoa Agyeman Boakye analyzed and interpreted the data. The manuscript was edited and approved by all authors.

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References


