Journal of Food and Nutrition Sciences
2026, Vol. 14, No. 5, pp. 335–340
https://doi.org/10.11648/j.jfns.20261405.17

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Research Article

Dietary Exposure to Genotoxic Mycotoxins in Maize (*Zea mays* L.) Grains Consumed in Cotonou Municipality (Benin): the Case of Aflatoxin B1

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Abstract

Aflatoxin B1 (AFB1) is a genotoxic and carcinogenic mycotoxin produced mainly by *Aspergillus flavus*, *A. parasiticus* and *A. nomius*, moulds that contaminate cereals, including maize (*Zea mays* L.), which is widely consumed in Benin. The aim of this study was to assess the level of dietary exposure to AFB1 in maize sold and consumed in the municipality of Cotonou. Thirty-eight samples of maize grain collected from markets in Cotonou were analysed by high-performance liquid chromatography with fluorescence detection. AFB1 dietary exposure was estimated based on the measured concentrations and maize consumption data. The results of the analyses show a wide range of AFB1 contamination levels in the samples, ranging from 1 to 104 µg/kg. Of the samples analysed, 81.57% had levels exceeding the regulatory limit set for AFB1. Average maize consumption was estimated at 136.22 kg/person/year, or 0.37 kg/person/day. AFB1 daily exposure was estimated to 0.07 µg/kg body weight/day. These results reveal high levels of AFB1 contamination in maize in the municipality of Cotonou and highlight chronic dietary exposure that may pose a health risk to consumers. Strengthening measures for monitoring, quality control and post-harvest management of maize appears necessary in order to reduce the population's exposure to this mycotoxin.

Keywords

Aflatoxins, AFB1, Maize, Dietary Exposure, Food Intoxication, Moulds, *Aspergillus*, Benin

1. Introduction

Maize (*Zea mays* L.) is one of the most widely produced and consumed cereals in the world and constitutes a staple food for populations in sub-Saharan Africa [1]. In Benin, it plays a predominant role in the dietary habits of several regions. Often poorly preserved, it constitutes a potential source of exposure to food contaminants.

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Received: 7 September 2026; **Accepted:** 18 September 2026; **Published:** 29 September 2026

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Aflatoxins are mycotoxins produced by certain fungal species and capable of contaminating cereals before or after harvest [2]. Aflatoxin B1 (AFB1), mainly produced by *Aspergillus flavus* and *A. parasiticus*, is the most abundant and the most toxic of the aflatoxins [3]. Its presence is favoured by hot and humid climatic conditions, as well as by inadequate practices in the harvesting, drying and storage of cereal grains. AFB1 contamination of maize is a major public health challenge in Africa. Indeed, several studies have highlighted high levels of AFB1 in marketed cereals, thereby exposing the population to this mycotoxin [4, 5]. Chronic exposure to AFB1 represents a major public health concern. Following ingestion, this mycotoxin is metabolised in the liver into a highly reactive epoxide capable of forming adducts with DNA, inducing genetic mutations and genotoxic effects that may trigger a carcinogenic process [6].

Beyond its health implications, aflatoxin contamination of maize also poses a major economic challenge [7]. Regulatory requirements applied in national and international markets lead to the rejection of contaminated batches, resulting in significant economic losses and restricting trade in agricultural products [4]. Aflatoxins thus pose a dual threat to public health and the competitiveness of the cereal sectors.

In this context, assessing dietary exposure to AFB1 through maize consumption appears essential to characterise the genotoxic risk to which populations are exposed and to provide scientific data useful for the implementation of prevention and risk management strategies.

2. Materials and Methods

2.1. Study Area

The study was conducted in the municipality of Cotonou, located in southern Benin. Covering an area of approximately 79 km²; it is the most densely populated. Economic capital of Benin, this municipality has a population of over 670.000, spread across thirteen districts [8]. The municipality of Cotonou is the main national hub for the marketing of foodstuffs. The markets in this municipality are supplied daily with maize from Benin’s main production areas, as well as from other West- African countries. Owing to its strategic role in the marketing of cereals, this locality is a particularly relevant area for assessing consumer exposure to chemical contaminants of fungal origin, notably AFB1.

The municipality of Cotonou is characterised by a sub-equatorial climate marked by two rainy seasons alternating with two dry seasons. Average annual temperatures range between 25 and 30 ℃, whilst relative humidity generally remains above 80% for much of the year [8]. Owing to the importance of maize in the diet and the conditions favourable to the growth of toxin-producing moulds in the municipality of Cotonou, the latter was selected to assess maize contamination by aflatoxin B1 and the population’s dietary exposure.

2.2. Dietary Survey

The maize consumption data used to assess exposure to aflatoxin B1 were obtained from a household survey conducted in the municipality of Cotonou between June and August 2021. The aim of this survey was to characterise maize consumption patterns. Particular attention was paid to the ways in which maize is acquired, preserved and stored. Data were collected from heads of households through semi-structured interviews conducted door-to-door using a digital questionnaire administered via the KoBoCollect app. A total of 714 households were surveyed in the municipality of Cotonou, following a sampling plan based on the Schwartz method [9]. The information gathered covered the frequency of maize consumption, the quantities consumed, and the methods of procurement and preservation.

2.3. Maize Grains Sampling

Thirty-eight samples of maize intended for human consumption were collected in the municipality of Cotonou in accordance with Regulation (EU) No 178/2010 on the methods of sampling and analysis used for the official control of mycotoxins in foodstuffs (Table 1).

Table 1. *Sampling plan for maize grains.*

Foodstuff sampled	Sampling location	Quantity (g)	Number of sample (n)
	Zongo Ehuzu	1000	2
	Jonquet	1000	2
	Gbeto	1000	2
Maize grains	Missèbo	1000	3
	Gbediga	1000	3
	Houéyiho	1000	2
	Gbégamey 2	1000	3

Foodstuff sampled	Sampling location	Quantity (g)	Number of sample (n)
	Vodjè Centre	1000	2
	Cadjehoun 1	1000	2
	Cadjehoun 2	1000	3
	Fidjrossè Centre	1000	2
	Aibatin 1	1000	2
	Agla Centre	1000	3
	Aibatin 2	1000	2
	Gbédégbé	1000	3
	Missité	1000	2
Total		16.000	38

2.4. Aflatoxin B1 Analysis

The determination of aflatoxin B1 (AFB1) was carried out in accordance with standard EN ISO 16050: 2011. The analytical performance of the method, including the limits of detection and quantification, trueness, precision and recovery rate, are presented in Table 2. Following extraction and purification on an immunoaffinity column, AFB1 was quantified by high performance liquid chromatography (HPLC) with fluorometric detection.

Table 2. *Method validation conditions.*

	Limit of Detection (µg/kg)	Quantification limit (µg/kg)	Accuracy (%)	Repeatability (%)	Recovery rate (%)	Standard deviation (%)
AFB1	0.00564	0.0188	6.01	1.80	93	3.01
Criteria			Must be less than 6%	Must be less than 6%	$80 \leq TR < 120$	Must be less than10%

2.5. Assessment of Dietary Exposure

Dietary exposure to AFB1 was estimated based on the concentrations measured in maize samples and dietary intake data from the household survey. The Estimated Daily Intake (EDI) was calculated using the following equation [10]:

$$EJE = \frac{C \times Q}{P}$$

where C represents the concentration of AFB1 in maize (µg/kg), Q the average daily intake of maize (kg/day), P the average reference body weight (kg).

2.6. Statistical Analysis of the Data

Statistical analyses were carried out using Python 3.12 and the SciPy library. The data were first recoded in Microsoft Excel 2021 (Microsoft Corp., Redmond, WA, USA).

3. Results

3.1. Maize Consumption Habits

Data from the household survey enabled us to estimate the frequency and quantities of maize consumption per day and per person in the municipality of Cotonou. Analysis of the Table 3 shows that over 99% of the population in the municipality of Cotonou consumes maize. Furthermore, Table 4 shows an estimated average maize consumption of 136.22 kg/person/year, corresponding to an average daily consumption of 0.37~~3~~ kg/person/day.

Table 3. *Maize consumption in Cotonou.*

Cereal	Affirmative	Negative	Total	H_0	H_1	P-value	Conclusion
Maize and its derivatives	345	0	345	99% or more	Less than 99%	1.000	H_0 validated

3.2. Aflatoxin B1 Levels in Maize

The AFB1 concentrations observed in the maize samples analysed are presented in Table 4. AFB1 levels ranged from 1 to 104 µg/kg, with a mean concentration of 21.27 µg/kg and a median of 12.1 µg/kg. 81.6% of samples had levels exceeding the maximum limit of 2 µg/kg set for AFB1 by Regulation (EU) 2023/915 in cereals intended for human consumption (Table 4).

Table 4. *Mean and median aflatoxin B1 levels in maize.*

	Minimum (µg/kg)	Maximum (µg/kg)	Mean (µg/kg)	Median (µg/kg)	Total
Maize	1	104	21.27	12.1	38

3.3. Level of Dietary Exposure to Aflatoxin B1 in Maize

Dietary exposure to AFB1 was estimated based on the median concentration observed in maize samples and the average daily maize consumption of the population in the municipality of Cotonou. The parameters used to estimate dietary exposure are presented in Table 5. The median AFB1 concentration used for the calculation was 12.1 µg/kg, whilst the average maize consumption was estimated at 136.22 kg/person/year, or 0.373 kg/person/day. The estimated daily exposure to AFB1 was calculated as 0.07 µg/kg body weight/day.

Table 5. *Exposure to aflatoxin B1 in the municipality of Cotonou.*

Product	Median AFB1 concentration (µg/kg)	Average quantity consumed per person per year (kg/year)	Average quantity consumed per person per day (kg/day)	Daily exposure
Maize	12.1	136.22	0.37	0.07

4. Discussion

Maize is one of the main cereals consumed in Benin and forms an essential part of the daily diet of households in the municipality of Cotonou. The results of the dietary survey revealed an average consumption of 136.22 kg/person/year, or 0.373 kg/person/day, confirming the importance of this cereal in local dietary habits. This high consumption is consistent with national data indicating that maize forms the basis of many traditional Beninese dishes and is one of the main sources of energy intake for the population [8-11]. In this context, even moderate levels of contamination can lead to significant chronic exposure to mycotoxins among consumers.

The analyses carried out in this study revealed significant contamination of maize samples with AFB1. The concentrations observed ranged from 1 to 104 µg/kg, with a median concentration of 12.1 µg/kg. Furthermore, 81.6% of the samples had levels exceeding the maximum limit of 2 µg/kg set by Regulation (EU) 2023/915 for cereals intended for human consumption. These results confirm that maize remains one of the main foodstuffs at risk of aflatoxin contamination in tropical regions [12].

The results obtained are consistent with those reported in several sub-Saharan African countries, where maize is both a staple food and a major source of aflatoxin exposure. In Kenya, [13] reported the presence of aflatoxins in 97% of the maize flour samples analysed, 16% of which exceeded the national regulatory limit of 10 ppb. Similarly, [14] observed contamination rates in Kenya and Tanzania ranging from 0 to 100% depending on the regions studied, with concentrations reaching as high as 6.075 µg/kg in some maize samples. These findings confirm the extent of aflatoxin contamination in maize across sub-Saharan Africa and highlight the significant influence of agro-ecological conditions on the occurrence of this contamination.

The high levels of contamination observed in this study can be attributed to several factors. In Benin's tropical climate, high temperatures and high humidity favour the growth of *Aspergillus flavus* and *A. parasiticus*, the main producers of aflatoxin B1. In addition to these environmental factors, post-harvest practices are often poorly managed, notably incomplete drying of the grains, unsuitable storage conditions, inadequate ventilation in storage facilities and prolonged periods of storage. Multiple handling stages during marketing can also contribute to increased contamination. These factors have been identified as major determinants of aflatoxin accumulation in maize throughout the value chain [12].

The estimated daily exposure to AFB1 was calculated at 0.07 µg/kg body weight/day, indicating chronic consumer exposure to this mycotoxin. Due to its genotoxic and carcinogenic nature, no tolerable daily intake has been established for AFB1 by international risk assessment bodies. The currently recommended approach is based on the ALARA (*As Low As Reasonably Achievable*) principle, which advocates keeping exposure as low as reasonably possible [15].

The potential health consequences of this exposure are a cause for concern. Following ingestion, AFB1 is metabolised in the liver to aflatoxin B1-8, 9-epoxide, a highly reactive metabolite capable of forming adducts with DNA and inducing genetic mutations. Chronic exposure is associated with an increased risk of hepatocellular carcinoma, particularly in individuals who are chronic carriers of the hepatitis B virus, in whom a synergistic effect between viral infection and AFB1 has been widely demonstrated. Recent health risk assessments also highlight that maize is among the foodstuffs contributing most significantly to dietary exposure to AFB1 in tropical regions [15, 16].

Beyond its health implications, this contamination also represents a significant economic challenge. Frequent exceedances of regulatory limits can lead to the withdrawal or rejection of maize consignments from national and international markets, reducing the competitiveness of the cereal sectors and affecting the incomes of producers and traders. Strengthening good agricultural practices, improving drying and storage techniques, and implementing regular monitoring programmes appear essential to reduce maize contamination and limit population exposure to aflatoxin B1 [12].

This study does, however, have certain limitations. Sampling was carried out solely in the Littoral department and does not cover the whole of the country. Furthermore, the exposure assessment is based on the concentrations measured in maize and does not take into account cumulative exposure from other foods that may contain aflatoxins. Despite these limitations, this study provides up-to-date data on the contamination of maize and dietary exposure to AFB1 in Benin, and helps to strengthen the knowledge required for the management of health risks associated with mycotoxins.

5. Conclusions

This study has documented the contamination of maize sold in the municipality of Cotonou with AFB1 and assessed consumers' dietary exposure to this mycotoxin. The results reveal a high prevalence of contamination, with concentrations ranging from 1 to 104 µg/kg and a high proportion of samples exceeding the maximum regulatory limit set for cereals intended for human consumption. The high consumption of maize observed in the study population results in chronic exposure to AFB1, which is likely to constitute a public health concern. These results confirm that maize represents a significant source of dietary exposure to aflatoxins and highlight the need to strengthen measures to control contamination throughout the production, storage and marketing chain. Improvements in post-harvest practices, combined with the implementation of regular monitoring programmes, appear essential to reduce population exposure and improve food safety. This study provides recent data on maize contamination and dietary exposure to AFB1 in Benin.

Abbreviations

AFB1	Aflatoxin B1
EU	European Union
HPLC	High Performance Liquid Chromatography
EDI	Estimated Daily Intake
USA	United States of America
ALARA	As Low as Reasonably Achievable

Author Contributions

Mouhamed Nazif Moutawakilou El-Hadji Alassane: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing

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Yemissi Sandra Gaelle Amoussou: Formal Analysis, Investigation, Methodology

Lamine Baba-Moussa: Conceptualization, Supervision, Validation, Visualization

Farid Baba-Moussa: Conceptualization, Supervision, Validation, Visualization

Conflicts of Interest

The authors declare no conflicts of interest.

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