

## Original Research Article

### ASSESSMENT OF THE RISK OF EXPOSURE TO AFLATOXINS FOUND IN MAIZE (*Zea mays L.*) PRODUCED IN COTE D'IVOIRE IN IVORIAN ADULTS

#### Abstract

**Aims :** The objective of this study is to estimate the health risk incurred by the Ivorian adult consumer as a result of exposure to AFB1 and the sum of aflatoxins (AFT) through the consumption of maize.

**Place and Duration of Study :** During this study 375 samples of maize (grains, cobs, spathes) were taken from February 2016 to January 2017 and the analyzes were carried out at the Biotechnology Laboratory, Agriculture and Development of Biological Resources of the Félix HOUPHOUËT-BOIGNY University.

**Methodology :** The detection of aflatoxins (AFB1, AFB2, AFG1, and AFG2) was performed by HPLC using the AOAC method. The risk analysis procedure was conducted according to the Codex Alimentarius risk analysis model. It consisted of a quantitative assessment. The quantitative estimation of the aflatoxin exposure was performed according to the deterministic approach.

**Results :** The results indicate the presence of aflatoxins B1, B2, G1 and G2 in all forms of maize (grains, cobs, spathes) and in the five regions visited. The average concentrations of aflatoxin B1 (AFB1) and the sum of aflatoxins (AFT) ranged from  $0.79 \pm 0.04 \mu\text{g/kg}$  to  $130.31 \pm 22.56 \mu\text{g/kg}$  and from  $2.63 \pm 2.35 \mu\text{g/kg}$  to  $169.13 \pm 40.39 \mu\text{g/kg}$ , respectively. Subsequently, the Estimated Daily Intakes (EDI) by consumption of aflatoxin-contaminated maize vary according to the level of contamination. They range from  $0.28 \pm 0.00 \text{ ng/kg body weight/day}$  to  $52.85 \pm 9.14 \text{ ng/kg body weight/day}$  for AFB1 exposure. They ranged from  $1.00 \pm 0.85 \text{ ng/kg body weight/day}$  to  $68.57 \pm 16.28 \text{ ng/kg body weight/day}$  for AFT exposure. Samples of maize grains and cobs from the Gbèkè, Poro and Hambol regions showed low daily doses below the reference values ( $2.028 \text{ ng/kg body weight/day}$  and  $4.056 \text{ ng/kg body weight/day}$ ). The opposite results are observed with samples (grains, ears and spathes) from Indenié-Djuablin and Gountougo.

**Conclusion :** The results of the daily doses of aflatoxin exposure of these regions show a need for monitoring their production. Thus, the implementation of efficient technical itineraries throughout the maize distribution chain, both during production, post-harvest processing, and conservation will be able to guarantee a better sanitary quality of this agricultural food product.

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**Key words :** Maize, risk, exposure, aflatoxins, adults and Côte d'Ivoire

## INTRODUCTION

Mycotoxins are products of the secondary metabolism of moulds (microscopic fungi) that can develop during storage in foodstuffs [1]. More than 300 secondary metabolites have been identified but only about thirty have real toxic properties of concern, including aflatoxins [1]. Aflatoxins are known mainly because of the intoxications (acute or chronic) that they cause in animals and humans following the ingestion of contaminated food [2-3]. Chronic effects are the most feared due to dietary habits and the persistence of these toxins [1]. Aflatoxins are a group of 18 structurally related compounds, four of which are the most commonly found forms in food (B1, B2, G1, G2) and M1 in milk [4]. Aflatoxins are produced by *Aspergillus* growing on foodstuffs such as maize [5].

In Côte d'Ivoire, of all food crops, maize (*Zea mays L.*) stands out because of its very wide cultivation area due to its ease of adaptation and high consumption [6]. It is the second most cultivated cereal after rice (*Oryza spp.*). Its annual national production increased from 760,000 tonnes in 2016 to 1,006,000 tonnes in 2018, for a total sown area of 386,633 ha [7]. Maize is the staple food of the population in the centre and north of the country. It is consumed in various forms and is used in the preparation of several food recipes and animal feed [8]. Its daily consumption is estimated at 28.4 g per capita and is mainly in the form of flour (92%) [9]. However, despite its socio-economic importance, maize faces a sometimes very delicate sanitary quality problem. Indeed, maize during different conditions of production, post-harvest processing and storage is exposed to mycotoxins, in particular aflatoxins, which could cause a serious health problem for consumers if the toxicity due to these toxins is proven. Numerous studies have shown the contamination of this cereal by aflatoxins, sometimes with values exceeding the reference limits [11-13]. However, in view of the dangers caused by aflatoxins and the high consumption of maize by the population, health risk assessment is essential to guarantee the health safety of consumers. The present study aims to estimate the health risk incurred by Ivorian adults following exposure to aflatoxins found in maize produced in Côte d'Ivoire.

## 2. Material and methods

### 2.1 Material

#### 2.1.1. Study sites

The samples were taken in the regions of Gbêkê (Centre), Poro (North), Hambol (Centre-North), Indénié-Djuablin (East) and Gontougo (North-East). The specificities of these regions were given by Bamba et al [14].

#### 2.1.2. Biological material

The biological material consists of maize (*Zea mays L.*) grains, cobs and spathes.

### 2.2 Method

#### 2.2.1 Sampling

The producers' storage sites according to regions (Poro, Hambol, Gontougo, Gbêkê, Indénié-Djuablin) were visited. Proportions of their stocks were taken from above, in the centre and at the

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lower sides of the storage structures. This sample collection took place from February 2016 to January 2017. As a result, 375 stocks (125 on grains, 125 on cobs and 125 on spathes, Table 1) were sampled, labelled, packed in sterile packaging and transported to the Biotechnology, Agriculture and Biological Resource Valorisation Laboratory of the Félix Houphouët-Boigny University for analysis (Table I).

**TABLE I : Number of samples taken by type of maize and by region**

| REGIONS          | GRAINS | COBS | SPATHES |
|------------------|--------|------|---------|
| Gbêkê            | 25     | 25   | 25      |
| Poro             | 25     | 25   | 25      |
| Hambol           | 25     | 25   | 25      |
| Indénié-Djuablin | 25     | 25   | 25      |
| Gontougo         | 25     | 25   | 25      |
| <b>TOTAL</b>     | 125    | 125  | 125     |

### 2.2.2. Extraction and quantification of aflatoxins from maize

Aflatoxins were solvent extracted and assayed using a high-performance liquid chromatograph (HPLC), fitted with a fluorescence detector, according to the AOAC method [15].

#### 2.2.2.1. Extraction and Purification of aflatoxins

In a 250 mL Erlenmeyer flask containing 25 g of maize ground, 100 ml of methanol-water (v/v, 80:20) were added. The mixture was homogenized by shaking for 2 minutes and then stored at room temperature in the dark for 12 hours. The homogenate was filtered through filter paper and 50 ml of the filtrate were added 40 ml of phosphotungstic acid-zinc sulfate-water mixture (w/w/v; 5/15/980) then stored at room temperature for 15 minutes. The mixture was filtered through filter paper and aflatoxins were extracted from the filtrate with 3 volumes of 10 ml of chloroform. The extracts were collected and evaporated to dryness using a rotary evaporator (Buchi, Rotavapor R-215) at 40°C. A dry extract were added 0.4 mL of hydrochloric acid and 4.6 mL of bidistilled water. The mixture was filtered through a Rezist filter and purified through an immunoaffinity column (RIDA aflatoxin, Biopharm, Germany) containing an antibody specific for aflatoxins (AFB1, AFB2, AFG1 and AFG2) at a flow rate of 2 mL.min<sup>-1</sup>.

#### 2.2.2.2. Quantification of aflatoxins concentrations

Quantification of aflatoxins contents was achieved with high performance liquid chromatography column, using a Shimadzu liquid chromatograph (Kyoto, Japan) fitted with fluorescence detector. The operating conditions are given in Table 2.

**Table 2 : Conditions of aflatoxins assay with HPLC**

| ITEM       | AFLATOXINS (B1, B2, G1 et G2)               |
|------------|---|
| Pre column | Shim-pack GVP-ODS 10 x 4.6 mm               |
| Column     | Shim-pack GVP-ODS, 250 mm x 4.6 mm          |
| Detector   | Fluorescence, $\lambda$ excitation : 365 nm |

|                                  |  |
|----------------------------------|--|
|                                  | λ émission : 435 nm                    |
| <b>Mobile phase</b>              | Acetonitrile/Water/Methanol (20/20/60) |
| <b>Injection volume</b>          | 20 µl                                  |
| <b>Flow rate</b>                 | 1 mL/min at isocratique                |
| <b>Temperature of the column</b> | 40 °C                                  |
| <b>Rinsing solvent</b>           | Methanol                               |
| <b>Duration of analysis</b>      | 15 minutes                             |

#### Validation of the aflatoxin determination method

| Designation                            | AFB1       | AFB2       | AFG1       | AFG2       |
|--|------------|------------|------------|------------|
| <b>Linearity (R<sup>2</sup>)</b>       | 0.99       | 0.98       | 0.98       | 0.99       |
| <b>Limit of detection (ng/kg)</b>      | 6.18       | 0.058      | 114.5      | 2.64       |
| <b>Limit of quantification (ng/kg)</b> | 6.50       | 0.108      | 124.9      | 2.94       |
| <b>Repeatability (CV, %)</b>           | 2.08±0.10  | 3.75±0.22  | 0.50±0.00  | 3.56±0.18  |
| <b>Reproducibility (CV, %)</b>         | 3.20±0.18  | 4.93±0.37  | 0.89±0.10  | 4.72±0.15  |
| <b>Extraction yield (EY, %)</b>        | 98.92±2.49 | 97.53±1.93 | 95.31±0.33 | 97.63±2.09 |

### 2.2.3. Assessment of the risk of exposure to aflatoxin B1 and total aflatoxins in adult Ivorians

The risk analysis procedure was conducted according to the Codex Alimentarius risk analysis model [16].

#### 2.2.3.1 Aflatoxins exposure assessment

The exposure assessment was carried out using a deterministic approach which consists of multiplying a fixed value of food consumption by a fixed value of contamination and dividing the consumption by the actual body weight of the individual [17-18]. According to the Ministry of Agriculture the daily consumption of maize by a 70 kg adult Ivorian is 28.4 g/capita/day [9]. The exposure was determined on the one hand from the maize consumption data, the maximum reference concentration and on the other hand from the actual concentrations of the said mycotoxins determined by analysis of our samples. The maximum tolerated concentrations for maize are 5 µg/kg and 10 µg/kg for aflatoxin B1 and total aflatoxin respectively [19-20].

The exposure to these mycotoxins was calculated from formula 1

$$EDI = ([AF_s] \times Q_n)$$

EDI: Exposure Daily Intake (ng/kg body weight/day) ;

[AF<sub>s</sub>] : Concentrations of aflatoxins found in maize µg/kg,

Q<sub>n</sub>: quantity of maize consumed by an adult Ivorian (kg/day) ;

P: average body mass (exposed individuals) in kg ;

#### 2.2.3.2 Risk characterisation

The risk was characterised at two levels: the determination of the hazard ratio (HR) and the assessment of the excess individual risk (ERI).

#### **2.2.3.2.1. Determination of the hazard ratio (HR)**

The hazard ratio was carried out by comparing the estimated doses of AFB1 and AFT with the values of the maximum reference concentrations [21]. The proportions obtained were compared to 1 [22].

$$\text{HR} = \text{EDI} / \text{MRLD}$$

HR: Hazard Ratio ;

EDI: Exposure Daily Intake (ng/kg body weight/day);

MRLD: Daily Exposure Dose for maximum reference limits (ng/kg body weight/day).

EDI/MRLD < 1 means that the exposed population is theoretically safe, i.e. the exposed population is not likely to develop health effects

EDI/MRLD > 1 means that the toxic effect may occur without it being possible to predict the probability of this event occurring.

#### **2.2.3.2.2. Calculation of the excess individual risk by the oral route (ERI)**

This is calculated by multiplying the daily dose received by the acceptable daily dose attributable to the substance for a given exposure and effect [23].

$$\text{ERI (oral)} = \text{EDI} \times \text{ERUo}$$

ERI: Excess Individual Risk

EDI: Exposure Daily Intake (ng/kg body weight/day),

ERUo: Excessive unit risk by the oral administration.

An excess risk of  $10^{-5}$  presents the additional probability, compared to an unexposed person, of developing cancer per 1000000 people exposed over a lifetime.

#### **2.2.4 Statistical analysis**

The tests were carried out in triplicate and the averages were calculated in order to assess the level of aflatoxin contamination of the samples. The homogeneity of the means was assessed from the Student-Newman-Keuls test at 5% risk using SPSS software version 20.0.

### **3. RESULTS AND DISCUSSION**

#### **3.1. RESULTS**

##### **3.1.1. Concentrations of Aflatoxin B1 and total**

Table 3 shows the concentrations of the various aflatoxins found in maize grains, cobs and spathes. Aflatoxin concentrations vary regardless of region and form of maize. Concerning aflatoxin B1, the levels are between  $0.79 \pm 0.04 \mu\text{g/kg}$  and  $20.92 \pm 4.63 \mu\text{g/kg}$ ;  $2.26 \pm 1.39 \mu\text{g/kg}$  and  $32.22 \pm 10.40 \mu\text{g/kg}$ ;  $12.73 \pm 6.25 \mu\text{g/kg}$  and  $130.31 \pm 22.56 \mu\text{g/kg}$  respectively for grains, cobs and spathes. Average concentrations of less than  $5 \mu\text{g/kg}$  are obtained in the regions of Gbêkê, Poro and Hambol for maize grains and cobs, while those obtained in the regions of Indénié-Djuablin and Gountougo are above this value whatever the spathes of maize.

Regarding total aflatoxins, the concentrations obtained vary from  $2.63 \pm 2.35 \mu\text{g/kg}$  to  $169.13 \pm 40.39 \mu\text{g/kg}$ . Concentrations of less than  $10 \mu\text{g/kg}$  are obtained on maize grains (Gbêkê, Poro and Hambol) and the cobs (Gbêkê and Poro).

**Table 3:** Aflatoxin concentrations according to the different types of maize and regions ( $\mu\text{g/kg}$ )

| Aflatoxin B1 concentration (AFB1)   |                        |                        |                         |         |         |
|-------------------------------------|------------------------|------------------------|-------------------------|---------|---------|
| Regions                             | Grains                 | Epis                   | Spathes                 | F-value | P-value |
| Gbêkê                               | $1.97 \pm 0.69^{aA}$   | $2.26 \pm 1.39^{aA}$   | $12.73 \pm 6.25^{aB}$   | 4.01    | 0.001   |
| Poro                                | $0.79 \pm 0.04^{aA}$   | $2.52 \pm 0.93^{aA}$   | $18.27 \pm 5.96^{bB}$   | 7.18    | 0.001   |
| Hambol                              | $1.31 \pm 0.36^{aA}$   | $4.73 \pm 1.33^{aA}$   | $19.91 \pm 8.48^{bB}$   | 15.91   | 0.001   |
| Indénié-Djuablin                    | $9.30 \pm 2.76^{bA}$   | $32.22 \pm 10.40^{bB}$ | $130.31 \pm 22.56^{dC}$ | 27.66   | 0.001   |
| Gontougo                            | $20.92 \pm 4.63^{cA}$  | $13.77 \pm 4.45^{cA}$  | $55.41 \pm 14.10^{cB}$  | 0.46    | 0.001   |
| F-value                             | 10.62                  | 6.20                   | 19.64                   | nd      | nd      |
| P-value                             | 0.001                  | 0.001                  | 0.001                   | nd      | nd      |
| Total aflatoxin concentration (AFT) |                        |                        |                         |         |         |
| Regions                             | Grains                 | Epis                   | Spathes                 | F-value | P-value |
| Gbêkê                               | $5.69 \pm 4.68^{aA}$   | $7.04 \pm 4.03^{aA}$   | $18.60 \pm 8.14^{aB}$   | 3.71    | 0.020   |
| Poro                                | $2.63 \pm 2.35^{aA}$   | $7.38 \pm 4.78^{aB}$   | $27.08 \pm 11.65^{bC}$  | 4.44    | 0.015   |
| Hambol                              | $4.01 \pm 4.00^{aA}$   | $14.64 \pm 11.20^{bB}$ | $50.67 \pm 19.57^{cC}$  | 13.73   | 0.001   |
| Indénié-Djuablin                    | $26.45 \pm 18.25^{bA}$ | $71.04 \pm 20.88^{dB}$ | $169.13 \pm 40.39^{eC}$ | 17.94   | 0.001   |
| Gontougo                            | $60.78 \pm 30.24^{cB}$ | $44.53 \pm 15.58^{cA}$ | $65.32 \pm 21.67^{dB}$  | 25.02   | 0.001   |
| F-value                             | 10.53                  | 6.16                   | 17.51                   | nd      | nd      |
| P-value                             | 0.001                  | 0.001                  | 0.001                   | nd      | nd      |

Means with the same letters are statistically identical. Lowercase and uppercase letters are representative of columns and rows respectively.

### 3.1.2 Exposure daily intake to aflatoxins (B1 et totales)

#### 3.1.2.1 Daily exposure doses obtained from the maximum reference limits (MRLD)

The daily exposure doses obtained from the maximum reference limits for aflatoxins (B1 and total) were calculated from the quantities of maize consumed per day by an Ivorian adult ( $28.4 \text{ g/day}$ ) weighing  $70 \text{ kg}$  and the maximum reference concentrations recommended by the European Commission. The calculated daily doses for the maximum concentrations are  $2.028 \text{ ng/kg body weight/day}$  and  $4.056 \text{ ng/kg body weight/day}$  for aflatoxin B1 and total aflatoxin respectively (Table 4).

#### 3.1.2.2 exposure daily intake obtained from the real concentrations

##### ➤ exposure daily intake for AFB1

The exposure daily intake to AFB1 (EDI) calculated from maize consumption ranged from  $0.42 \pm 0.14 \text{ ng/kg body weight/day}$  to  $8.42 \pm 1.85 \text{ ng/kg body weight/day}$ ;  $0.85 \pm 0.42 \text{ ng/kg body weight/day}$ .

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weight/day and  $13.00 \pm 4.14$  ng/kg body weight/day;  $5.14 \pm 2.42$  ng/kg body weight/day and  $52.85 \pm 9.14$  ng/kg body weight/day for maize grains, cobs and spathes samples respectively (Table 4). All samples taken in the Indenié-Djuablin and Gontougo regions showed the highest doses (EDI) above the maximum reference limit (2.028 ng/kg body weight/day). The opposite observations are made in the regions of Gbèkè, Poro and Hambol with doses below the MRLD. However, regardless of the region, the EDIs for AFB1 through consumption of maize spathes are above the MRDL.

➤ **exposure daily intake to AFT**

The exposure daily intake to AFT calculated from the consumption of maize ranged from  $1.00 \pm 0.85$  ng/kg body weight/day to  $24.57 \pm 12.14$  ng/kg body weight/day respectively; from  $2.74 \pm 1.57$  ng/kg body weight/day to  $28.71 \pm 8.42$  ng/kg body weight/day and from  $7.42 \pm 3.28$  ng/kg body weight/day to  $68.57 \pm 16.28$  ng/kg body weight/day for maize grains, cobs and spathes (Table 4). As for AFB1, all samples from Indenié-Djuablin and Gontougo expressed high doses exceeding the MRLD. Also, regardless of the region, the daily doses obtained from the maize spathes samples are higher than the MRLD value ( $4.056$  ng/kg body weight/day).

**Table 4:** Assessment of aflatoxin exposure in adult Ivorians based on consumption of maize collected in different regions (ng/kg body weight/day)

| Daily exposure doses to AFB1 |                   |                  |                   |
|------------------------------|-------------------|------------------|-------------------|
| Regions                      | Grains            | Cobs             | Spathes           |
| Gbèkè                        | $0.71 \pm 0.14$   | $0.85 \pm 0.42$  | $5.14 \pm 2.42$   |
| Poro                         | $0.28 \pm 0.00$   | $1.00 \pm 0.28$  | $7.28 \pm 2.28$   |
| Hambol                       | $0.42 \pm 0.14$   | $1.85 \pm 0.42$  | $22.42 \pm 5.71$  |
| Indenié-Djuablin             | $3.71 \pm 1.00$   | $13.00 \pm 4.14$ | $52.85 \pm 9.14$  |
| Gontougo                     | $8.42 \pm 1.85$   | $5.57 \pm 1.71$  | $8.00 \pm 3.42$   |
| MRLD <sub>AFB1</sub>         | 2.028             |                  |                   |
| Daily exposure doses to AFT  |                   |                  |                   |
| Regions                      | Grains            | Cobs             | Spathes           |
| Gbèkè                        | $2.28 \pm 1.85$   | $2.74 \pm 1.57$  | $7.42 \pm 3.28$   |
| Poro                         | $1.00 \pm 0.85$   | $2.85 \pm 1.85$  | $10.85 \pm 4.71$  |
| Hambol                       | $1.57 \pm 1.57$   | $5.85 \pm 4.42$  | $26.42 \pm 11.14$ |
| Indenié-Djuablin             | $10.71 \pm 7.28$  | $28.71 \pm 8.42$ | $68.57 \pm 16.28$ |
| Gontougo                     | $24.57 \pm 12.14$ | $18.00 \pm 6.28$ | $20.42 \pm 7.85$  |
| MRLD <sub>AFT</sub>          | 4.056             |                  |                   |

**3.1.3 Hazard Ratio (HR)**

**Comment [P.7]:** Please explain and discuss why is this result below the MRLD ?

**Comment [P.8]:** Please explain and discuss why do these results below and above the MRLD ?

**Comment [P.9]:** Please explain and discuss why does this result exceed MRLD ?

The results of the hazard ratio are shown in Table 5. The respective values obtained for AFB1 and AFT range from 0.14 to 26.42 and from 0.25 to 17.14. However, the risk of exposure to adverse effects of AFB1 and AFT by consuming maize grains and cobs from the Gbèkè, Poro and Hambol regions is low because the hazard ratios are lower than 1 (HR<1). The opposite observations are made with maize in spathes in these regions. In contrast to the Gbèkè, Poro and Hambol regions, the risks of exposure to the toxic effects of AFB1 and AFT become real through the consumption of maize from the Indénié-Djuablin and Gontougo regions. Indeed, the hazard ratios determined are greater than 1, whatever the type of maize (grains, cobs and spathes).

**Comment [P.10]:** Is the statement based on opinion or references ?

**Table 5:** Hazard ratios characterising the risks associated with the consumption of maize contaminated with aflatoxins

| Hazard ratio to AFB1 |        |      |         |
|----------------------|--------|------|---------|
| Regions              | Grains | Cobs | Spathes |
| Gbèkè                | 0.35   | 0.42 | 2.57    |
| Poro                 | 0.14   | 0.50 | 3.64    |
| Hambol               | 0.21   | 0.92 | 11.21   |
| Indénié-Djuablin     | 1.85   | 6.50 | 26.42   |
| Gontougo             | 4.21   | 2.78 | 4.00    |

  

| Hazard ratio to AFT |        |      |         |
|---------------------|--------|------|---------|
| Regions             | Grains | Cobs | Spathes |
| Gbèkè               | 0.57   | 0.68 | 0.42    |
| Poro                | 0.25   | 0.71 | 2.70    |
| Hambol              | 0.39   | 1.46 | 6.60    |
| Indénié-Djuablin    | 2.67   | 7.04 | 17.14   |
| Gontougo            | 6.14   | 4.50 | 5.10    |

HR < 1 means that the exposed population is theoretically safe, i.e. the exposed population is not likely to develop health effects

HR > 1 means that the toxic effect may occur without it being possible to predict the probability of this event occurring

**Comment [P.11]:** Are there any references that reveal the statement?

### 3.1.4 Excessive individual risk by the oral administration (ERI)

Of all the mycotoxins studied, aflatoxins and in particular AFB1 are considered as one of the most powerful natural genotoxic carcinogens. The International Agency for Research on Cancer (IARC) considers AFB1 as "carcinogenic to humans" (group 1) [24]. The FAO/WHO Expert Committee on Food Additives (JECFA) calculated an excess risk for Europe for aflatoxin exposure of 0.013.10-5ng/kg body weight/day [25]. In other words, a lifetime exposure to 1 ng aflatoxin/kg body weight/day leads to an increase in the incidence of liver cancer of 1 case per year per 10 million people. Therefore, the higher the exposure dose, the greater the likelihood of cancer. The daily exposure doses in our study indicate a risk of liver cancer of more than one case per 10000000 people. Indeed,



for doses between  $1.00 \pm 0.85$  ng/kg body weight/day and  $68.57 \pm 16.28$  ng/kg body weight/day the number of cancer cases ranges from 1 to 89 cases per 10,000,000 people (Table 6).

**Table 6:** Characterisation of the risk of cancer occurrence due to aflatoxin exposure

| Daily exposure (ng/kg.PC/d) | Cancer occurrence by ERU0 (relative to the population) |
|-----------------------------|--|
| <1 ng /kg body weight/day   | Less than 1 in 10000000                                |
| =1 ng /kg body weight/day   | 1 case in 10000000                                     |
| >1 ng                       | 2 to 89 cases for every 10000000                       |

### 3. 2 DISCUSSION

The exposure of populations to mycotoxins is frequent through cereals so the European Commission in order to protect consumers has set maximum allowable limits (MRL) for maize [19-20]. Considering the average weight of an Ivorian adult individual of 70 kg and the daily consumption of maize in Côte d'Ivoire estimated at 28.4g/person according to Beugré *and al* [9] the daily doses of exposure to aflatoxins (AFB1 and AFT) from the consumption of maize grains and cobs from the Gbèkè, Poro and Hambol regions are low. These doses remain below the doses estimated from the maximum reference concentrations (MRLD). These results show that the probability of exposure of the population to the toxic effects of these toxins is low. Therefore, maize from Gbèkè, Poro and Hambol would be safe for human consumption. However, taking into account the effects of chronic exposure to these mycotoxins, their presence, even at very low doses, could be a source of danger for the Ivorian consumer [26-27] The hazard of aflatoxins results from chronic exposure to low amounts over a long period of time. It is estimated that more than 5 billion people in developing countries are at risk of exposure to chronic aflatoxicosis [28-29]. The major target organ of AFB1 is the liver [24]. Thus, repeated and moderate ingestion of aflatoxin (aflatoxin B1 in particular) is associated with an increased risk of developing hepatocellular carcinoma, or liver cancer. Each year, 550,000 to 600,000 new cases of liver cancer are recorded worldwide, and about 25,200 to 155,000 of the cases are attributable to aflatoxin exposure [27]. Furthermore, given the synergistic effect between the hepatitis B virus and the development of liver cancer, people infected with hepatitis B and exposed to AFB1 may develop liver cancer [30]. Liu and Wu [31] reported in their study that people with chronic hepatitis B infection and exposed to aflatoxin B1 have a high risk of developing liver cancer tenfold or more. Other studies have also highlighted the role of AFB1 in the development of other types of cancer such as kidney, respiratory and gastrointestinal cancer [32-33]. In addition to its carcinogenicity, AFB1 is an immunosuppressive compound, decreasing resistance to parasitic, bacterial and fungal infections. Indeed, AFB1 modulates the cellular response by decreasing the activity of B and T lymphocytes and inflammatory cytokines. This contributes to promoting chronic infections to other pathologies [34-35] and constitutes a real risk for immunodeficient people. Indeed, the adducts formed by aflatoxins (AF-ALB) accentuate the damage to the immune system in human immunodeficiency virus (HIV)-positive individuals [36]. Also, one of the possible consequences of chronic aflatoxin exposure is the alteration

of the gastrointestinal system. Epidemiological studies have shown a correlation between the level of exposure to AFB1 and developmental delay in children [29]. In addition, the frequent cases of malnutrition in developing countries may aggravate the effects of aflatoxins. Protein malnutrition induces disruptions in liver oxidases that favour the accumulation of aflatoxins in the body [27].

Exposure to the toxic effects of aflatoxins (AFB1 and AFT) becomes real from the consumption of maize husk. The estimated safety ratios for spathe maize in all regions are greater than 1. The daily exposure doses obtained are higher than the reference limit doses. These results indicate that samples of maize husk would be unsuitable for human consumption. This situation is much more pronounced for maize from the Indenié-Djuablin and Gountougo regions. The hazard ratios determined are greater than 1 for all types of maize. Such data indicate that the consumption of maize from these regions would expose the population to aflatoxicosis risks. Thus, these results suggest a danger for high consumers of maize. Given the potential pathological risks associated with these toxins, this level of exposure would pose a real public health problem. As aflatoxins are toxic substances at low doses, the doses obtained in this study could be a source of acute toxicity. Exposure to aflatoxins in the population can lead to recurrent health problems linked to aflatoxicosis. Indeed, depending on the dose of aflatoxin ingested, there are two types of aflatoxicosis: acute aflatoxicosis and chronic aflatoxicosis [37]. Acute aflatoxicosis is associated with sporadic incidences of consumption of food heavily contaminated with aflatoxin, the effects of which are manifested by acute hepatitis [38]. As the target organ of aflatoxins is the liver, during hepatic metabolism, the appearance of a highly unstable epoxide derivative leads to its interaction with macromolecules in liver cells and to cell death when these interactions are too numerous []. Signs of acute aflatoxicosis include jaundice related to haemorrhagic necrosis of the liver and hepatic encephalopathy, which can be lethal in 25% of cases. Adults are generally more tolerant of aflatoxins than children, whose more rapid cell turnover increases their susceptibility [37]. The last recognised acute poisoning occurred from April to September 2004 in the central and eastern provinces of Kenya during which 341 cases were diagnosed leading to 123 deaths [39].

#### 4 CONCLUSION

Exposure to the aflatoxins studied depends on the study area and the type of maize. Indeed, the Estimated Daily Doses (EDI) for AFB1 and AFT in adults through the consumption of maize grains and cobs from Gbêkê, Poro and Hambol are lower than the different daily doses of the reference limits. The calculated risks are lower than 1. Therefore, the consumption of maize from these regions would expose the consumer to the adverse effects of these mycotoxins to a small extent. On the other hand, the calculated risks for all the samples from Indenié-Djuablin and Gountougo are higher than 1. Therefore, the consumption of maize from these regions would present a high health risk for the consumer. The results of the daily doses of aflatoxin exposure of these regions show a need for monitoring their production. Thus, the implementation of efficient technical itineraries throughout the maize distribution chain, both during production, post-harvest processing, and conservation will be able to guarantee a better sanitary quality of this agricultural food product.

**Comment [P.12]:** conclusion have not addressed explicitly and comprehensively about aflatoxin exposure to maize production in the ivory coast in general, but rather explanations in certain areas. The conclusions also do not answer the research objectives clearly and precisely

**Comment [P.13]:** Please state clearly the names of region do you mean in this statement

## REFERENCES

- 1 **AFSSA**. French Food Safety Agency: Evaluation of the risks related to the presence of mycotoxins in the human and animal food chains. Final report, 2009;308 p.
- 2 **Fandohan P., Gnonlonfin B., Hell K., Marasas W. F. O. & Wingfield M. J. (2005)**. Impact of indigenous storage systems and insect infestation on the contamination of maize with fumonisins. *African Journal of Biotechnology*, 2005 ;5 : 546-552p
- 3 **Kankolongo M. A., Hell K. & Nawa I. N.** Assessment for fungal, mycotoxin and insect spoilage in maize stored for human consumption in Zambia. *Journal of Sciences Food and Agricultural*,2009; 10 p.
- 4 **Hell K., Cardwell K. F., Setamou M. & Poehling H.-M.** The influence of storage practices on aflatoxin contamination in maize in four agroecological zones of Benin, west Africa. *Journal of Stored Products Research*, 2000; **36**: 365-382.
- 5 **Toffa D.** Étude de la contamination de certains aliments d'origine végétale de la République du Niger par les moisissures toxigènes, Thèse de Doctorat en Biologie. Université Mohammed V, Faculté des Sciences Rabat, 2015 ;252 p.
- 6 **Kouakou K., Akanvou L., Konan A. & Mahyao A. (2010)**. Stratégies paysannes de maintien et de gestion de la biodiversité du maïs (*Zea mays* L.) dans le département de Katiola, Côte d'Ivoire. *Journal of Applied Biosciences*,2010 ;**33**: 2100-2109
- 7 **FAOSTAT**. [Statistical databases on African countries "food commodities" trade](http://www.uneca.org/sites/default/files/uploads/ice17-report-secu-aliment-industrie-fr-ecawa_2020-04-final.pdf), production, consumption, and utilization. *FAO*, Rome, Italy, disponible sur : [http://www.uneca.org/sites/default/files/uploads/ice17-report-secu-aliment-industrie-fr-ecawa\\_2020-04-final.pdf](http://www.uneca.org/sites/default/files/uploads/ice17-report-secu-aliment-industrie-fr-ecawa_2020-04-final.pdf).
- 8 **N'da A., Akanvou L. & Kouakou K.** Gestion locale de la diversité variétale du maïs (*Zea mays* L.) en Côte d'Ivoire par les Tagouana au Centre-Nord de la Côte d'Ivoire. *International Journal of Biological and Chemical Sciences*,2013; **7**: 2058-2068.
- 9 **Beugre G. A., Yapo B. M., Blei S. H. & Gnagri D.** Effect of Fermentation Time on the Physico-Chemical Properties of Maize Flour. *International Journal of Research Studies in Biosciences*,2014 ; **2** : 30-38.
- 10 **Niamketchi L, Bieho HM, Sidibe D, Coulibaly A, Konan Y & Chatigre O.** Changes in aflatoxins contents of the maize (*Zea Mays* L.) stored in clay granaries with use of biopesticides from rural conditions and estimation of their Intake. *International Journal of Environmental & Agriculture Research*. 2016 ;2(5) :198-211.
- 11 **Ezoua P, Konan C, Coulibaly A, Sidibe D, Niamketchi L, Konan Y, Amane D, Chatigre O.K & Bieho HM.** Daily intake of aflatoxin b1 and ochratoxin a from maize grain (*Zea mays* L.) during the storage with *Lippia multiflora* (Verbenaceae) and *Hyptis suaveolens* (Lamiaceae) Leaves in Côte d'Ivoire. *Asian Journal of Advances in Agricultural Research*. 2017 ;3(4) :1-13.

**12 Fofana-Diomande. A, Kouakou.K.J.M, Aka -Diemeleou.C, Traore. K.S & Dembele.A.** Dietary exposure to carcinogenic mycotoxins in the department of Séguéla (North-West of Cote d'Ivoire): case of aflatoxin B1. *Int. J. Biol. Chem. Sci.* 2019 ;13(2) : 937-949 P

**13 Yao VG, Biego HM, Konan KC, Niamketchi L & Coulibaly A.** Evolution of mycotoxins during maize grains storage in triple bags containing plants biopesticides (*Lippia multiflora* and *Hyptis suaveolens*). *Asian Food Sciences Journal.* 2020 ;17(3) :22-33.

**14 Bamba S, Biego HMG, Coulibaly A, Nyamien YB, Konan Y, Sidibe D.** Determination of merchantability of maize (*zeamaysl.*) as epis, spathes and grains stocked in the production areas of cote d'ivoire. *Journal of Agriculture and EcologyResearch International*, 2019, 20(4): 1-10, DOI: 10.9734/JAERI/2019/v20i430120.

**15 AOAC.** Aflatoxins in corn, raw peanuts and peanut butter: immunoaffinity column (aflatest) method. AOAC International, Method 991.31. *Natural toxins*, 2005 ;chapter 49, p. 21.

**16 FAO.** Système de qualité et de sécurité sanitaire des aliments- Manuel de formation sur l'hygiène alimentaire et le système d'analyse des risques -points critiques pour leur maîtrise (HACCP). Organisation des Nations Unies pour l'Alimentation et l'agriculture, 2007 ;220p.

**17 Kroes R., Muller D., Lambe J., Verger P. & Visconti A.** Assessment of intake from the diet. *Food and Chemical Toxicology*, 2002,.40.:327-385.

**18 OMS.** Régime alimentaire, nutrition et prévention des maladies chroniques, Rapport d'une consultation OMS/FAO d'experts, Genève, OMS, Série de rapport technique, 2003 ; n° 916, 189p.

**19 EC.** European Commission, Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.Official Journal of the European Union,2006; 5-24p.

**20 EC.** European Commission: Regulation N° 165/2010, of 26 February 2010 amending Regulation N° 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. Official Journal of the European Union, 2010; L50, 11-12p.

**21 ASTEE.** Association scientifique et technique de l'eau et de l'environnement Guide pour l'évaluation du risque sanitaire dans l'étude d'impact d'une UIOM. 2003 ; 60p.

**22 Dagnogo K., Coulibaly A., Kaba V., Kallo V., Dongo A. C., Soro K., Biego G. M. H., Datte J. & Dembele A.** Détermination du niveau de contamination et de l'apport en résidus de tylosines (macrolides) des œufs de consommation dans le District d'Abidjan (Côte d'Ivoire). *Journal of Applied Biosciences.* 2018 ; 129 : 13067 – 13074.

**23 INERIS** Méthode d'évaluation quantitative des risques sanitaires relatifs aux substances chimiques. Evaluation des risques sanitaires des filières d'épandage des boues de stations d'épuration, 2007 ; 45p

**24 IARC, International Agency for Research on Cancer.** Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. *In. Monographs on the evaluation of carcinogenic risks to humans*, 2002;82: 171-74p, Lyon: IARC.

- 25 JECFA, Joint FAO/WHO Expert Committee on Food Additives.** Safety evaluation of certain food additives. Seventy-first report of the Joint FAO/WHO Expert Committee on Food Additives, Genève. 2001
- 26 Kouadio J. H., Lattanzio V. M., Ouattara D., Kouakou B., Visconti A.** Assessment of mycotoxins exposure in Côte d'Ivoire through multi-biomarker analysis and possible correlation with food consumption patterns. *Toxicologie Internationale*, 2014 ; 21 : 248-257.
- 27 El Khoury.R.,** Maitrise du risque aflatoxique: utilisation d'extraits naturels et mise en évidence de leurs mécanismes d'action. Thèse DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE. 2016 ;199p
- 28 Wu, F. & Khlangwiset, P.** Health economic impacts and cost-effectiveness of aflatoxin-reduction strategies in African. : case studies in biocontrol and post-harvest interventions. *Food Additives & Contaminants*. 2010 ; Part A, 27 : 496–509.
- 29 Wu, F., Groopman, J.D. & Pestka, J.J.** Public health impacts of foodborne mycotoxins. *Annual Review of Food Science and Technology*, 2014 ;5, 351–372.
- 30 Kirk G. D., Bah E., & Montesano R.** L'épidémiologie moléculaire des cancers du foie humain : Un aperçu de l'étiologie, la pathogenèse et la prévention de la Gambie. *Carcinogenèses*. 2006 ; 27 : 2070-2082p.
- 31 Liu Y., & Wu F.** La Charge Mondiale du carcinoma hépatocellulaire induite par l'Aflatoxine : Une évaluation des risques, *Environ Health Perspect*.2010 ; 118 (6) : 818-824.
- 32 Cui, A., Hua, H., Shao, T., Song, P., Kong, Q., Luo, T. & Jiang, Y.** Aflatoxin B1 induces Src phosphorylation and stimulates lung cancer cell migration. *Tumor Biology*. 2015 1–7.
- 33 Bbosa, G.S., Kitya, D. & Lubega, A.** Review of the Biological and Health Effects of Aflatoxins on Body Organs and Body Systems. In *Aflatoxin - Recent Advances and Future Prospects*, 2013 ; chapter 12. 265p.
- 34 Marin, S., Ramos, A.J., Cano-Sancho, G. & Sanchis, V.** Mycotoxins: Occurrence, toxicology, and exposure assessment. *Food and Chemical Toxicology*, 2013 ;60, 218–237p.
- 35 Meissonnier, G.M., Pinton, P., Laffitte, J., Cossalter, A.-M., Gong, Y.Y., Wild, C.P., Bertin, G., Galtier, P. & Oswald, I.P.** Immunotoxicity of aflatoxin B1 : impairment of the cell-mediated response to vaccine antigen and modulation of cytokine expression. *Toxicology and applied pharmacology*, 2008 ; 2 :1, 14-29p

**36 Jiang, Y., Jolly, P.E., Preko, P., Wang, J.-S., Ellis, W.O., Phillips, T.D. & Williams, J.H.**

Aflatoxin-related immune dysfunction in health and in human immunodeficiency virus disease. *Clinical & developmental immunology*, 2008.10

**37 Nathan M., Gijs K., Monique de Nijs., Marie-Luise R., Ria D & H. J. van der Fels-Klerx.** The

aflatoxin situation in Africa: Systematic literature review. Received. *Comprehensive reviews in food science and food safety*. 2021 ; 1–19p

**38 EL Mahgubi, A., Bailly, S., Tadrict, S., Querin, A., Ouadia, A., Oswald, I. P., & Bailly, J.D.**

Distribution and toxigenicity of *Aspergillus* section *Flavi* in spices marketed in Morocco. *Food Control*, 2013 ; 32(1) : 143–148p.

**39 CDC [Centers for Disease Control and Prevention](#):** Outbreak of aflatoxin poisoning-eastern and

central provinces, Kenya, January-July 2004, *Morbidity and Mortality Weekly Report*. 2004 ;3: 790-793p.

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