Preliminary survey of the occurrence of mycotoxins in cereals and estimated exposure in a northwestern region of Mexico

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ABSTRACT

Mycotoxins have several toxicological implications. In the present study, we evaluate the presence of aflatoxin B₁ (AFB₁), ochratoxin A (OTA), and fumonisin (FB₁) in paddy rice, polished rice, and maize from the fields and markets in Nayarit State (Mexico). The results indicated the presence of AFB₁ in 21.21% of paddy rice samples and 11.11% of market maize samples. OTA was present in only 3.03% (one sample) of paddy rice samples. FB₁ was detected in 87.50% and 88.88% of maize samples from field and market, respectively. The estimated human exposure was calculated for FB₁ using the probable daily intake (PDI), which suggested that FB₁ could contribute to the development of diseases through the consumption of contaminated maize. Positive samples indicated that some rice and maize samples were not suitable for human consumption. Further efforts are needed to continue monitoring mycotoxins and update national legislation on mycotoxins accordingly.

KEYWORDS

Co-occurrence of mycotoxins; probable daily intake; rice; maize
**Introduction**

Mycotoxins are secondary metabolites with varying organic structures and low-molecular weights that are produced by several filamentous fungi species, such as *Aspergillus*, *Penicillium*, and *Fusarium*. The most important mycotoxins are the aflatoxins (AFs), fumonisins (FBs), and ochratoxins (OTs) groups, as well as individual mycotoxins such as deoxynivalenol (DON) and zearalenone (ZEN) (Hojnik et al. 2017). These compounds can be present during the growth and storage of cereals and can be found both in the raw materials and in food and feed derived from them (Franco et al. 2019; Munkvold et al. 2019). The presence of these compounds has been reported in at least a quarter of the cereals produced for human consumption worldwide ([EFSA] European Food Safety Authority 2012; Trombete et al. 2013). In animals and humans, mycotoxins have been associated with adverse effects such as nephrotoxicity, hepatotoxicity, teratogenicity, and immunotoxicity (Mousavi Khaneghah et al. 2018; Szabó et al. 2018; Tao et al. 2018). Aflatoxin B1 (AFB1) has been considered the most potent known natural carcinogen to humans. It has been classified by the IARC as carcinogenic to humans (group 1) ([IARC] International Agency for Research on Cancer 1993), while ochratoxin A (OTA) and fumonisin B1 (FB1) have been classified as possibly carcinogenic to humans (group 2B) ([IARC] International Agency for Research on Cancer 1993, 2002).

Mycotoxins have been seen as a serious threat to public health and food safety around the world (WHO 2018). Due to the above, current European and North American regulations on mycotoxins have set limits for several dangerous mycotoxins in cereal foods such as corn (unprocessed, ground, or dry), rice, peanuts, pistachios, and almonds, among others, highlighting the importance of monitoring and controlling mycotoxin contamination in cereals intended for human and animal consumption.

In Mexico, there are only two Official Mexican Standards related to AFs limits in foodstuffs (12 and 20 µg/kg) ([NOM-187-SSA/SCFI-2002] Norma Oficial Mexicana 2002; [NOM-247-SSA1- 2008] Norma Oficial Mexicana 2008) and one for
aflatoxin M₁ (AFM₁) (0.5 µg/L) ([NOM-243- SSA1-2010] Norma Oficial Mexicana 2010). Nevertheless, the legislation is not comprehensive compared with that of the European Union – which has legislation for AFB₁ (0.1–12 µg/kg), total AFs (4–15 µg/kg), OTA (0.5–80 µg/kg), and FB₁ (2000–4000 µg/kg) – or the US FDA, which regulates AFs (20 µg/kg) and FBs (200–4000 µg/kg) ([FDA] Food and Drug Administration 2000, 2001; [EC] European Commission 2006, 2007, 2010a, 2010b).

Despite worldwide regulation, exposure to various mycotoxins in foods has been documented (Adetunji et al. 2017; Al Jabira et al. 2019; Foerster et al. 2020). Estimated exposure to mycotoxins can therefore be assessed by both the detection of the toxin in potentially contaminated foodstuffs, as well as by the evaluation of the dietary habits of a population. In this sense, the probable daily intake (PDI) expressed as ng/kg of body weight (bw) per day is a widely used tool for evaluating the dietary risk of mycotoxin intake ([WHO] World Health Organization 2002; [JECFA] Joint FAO/ WHO Expert Committee on Food Additives 2016). For toxic but non-carcinogenic effects caused by an agent, the PDI is compared with the tolerable daily intake (TDI) or provisional maximum tolerable daily intake (PMTDI) ([IARC] International Agency for Research on Cancer 2012). For genotoxic carcinogens such as AFs, the margin of exposure (MoE) method is used in which the estimated exposure is calculated by dividing the MoE and the calculated PDI; an MoE below 10,000 may indicate a public health concern ([EFSA] European Food Safety Authority 2013).

Mexico lacks information necessary to conduct exposure and risk assessments of AFB₁, OTA, and FB₁ in cereals intended for human consumption. Moreover, the Mexican authorities have shown little interest in updating the regulatory standards for mycotoxins. Therefore, the aim of this study was to determine the presence, exposure level, and risk assessment of AFB₁, OTA, and FB₁ in rice and maize intended for human consumption in a northwestern region of Mexico.
Materials and methods

Sampling

This study was conducted in two of the primary maize and rice producing municipalities of Nayarit State, Mexico. Santiago Ixcuintla is the leading region for rice production in Nayarit, and it is also a key region for the growth of irrigated and non-irrigated maize ([SEDER] Secretaría de Agricultura y Desarrollo Rural 2016; [INIFAP] Instituto Nacional de Investigación Forestal, Agrícola y Ganadera 2019). The second municipality studied was Tepic, where we focused specifically on its traditional Mexican markets.

Sample collection

Farmers and ‘ejido’ members from the village of Sauta in Santiago Ixcuintla were invited to participate through an informational meeting convened together with the ejido committee members. The purpose of the study and the procedures to obtain the samples were explained to them orally and in writing. Market sellers were invited to participate orally and in writing at their sales locations. Farmers and ejido members who agreed to participate in the study were interviewed to determine details of their agricultural practices, such as type of crop, harvest and destination of cereals, pests, and use of pesticides, among other data. Likewise, sellers were questioned about the types of cereals they sell, the presence of pests, and the use of pesticides.

Rice and maize samples from Santiago Ixcuintla were collected on harvest days (May and June 2018) according to the Official Mexican Standard ([NOM-247-SSA1- 2008] Norma Oficial Mexicana 2008) for the sampling of cereals. The plots were visited on the day of the harvest, as agreed by the farmers, and 7 and 10 points were sampled in trucks loaded with <30 tons and >30 tons, respectively. A total of 49 composite samples at 12 points each were collected, of which 33 were paddy rice (396 points) and 16 were maize (192 points). The samples were taken from open-box trucks, typically by using a T-handle double tube with twelve
zones of sampling (16-OH 72" brass open-handle, Seedburo®, Des Plaines, IL, USA).

At the same time, 10 polished rice samples and 9 maize samples were collected from the markets of Tepic, for a total of 19 collected samples.

**Chemicals and reagents**

AFB₁, OTA, and FB₁ standards were purchased from Merck-Sigma Aldrich (St. Louis, Missouri, USA). Formic acid and ammonium formate were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). MS-grade methanol and MS-grade acetonitrile were purchased from Merck (Darmstadt, Germany). Sample clean-up was performed with the Oasis PRiME HLB cartridge (code: 186,008,717), dSP tubes (Code: 186,008,081), and DisQuE products for QUEChERS (code: 186,006,813) from Waters Corporation (Milford, Massachusetts, USA).

**Sampling preparation**

The extraction of AFB₁, OTA, and FB₁ was carried out according to the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, following the manufacturer’s instructions. Briefly, maize and rice samples were ground in a conventional blender. For the extraction of mycotoxins, 2 g samples were mixed with 10 mL of an acetonitrile and formic acid (9:1) solution and were shaken for 1 h at room temperature. The extract was added into a tube with DisQuE Quechers salts composed of the following: trisodium citrate dihydrate (1 g), disodium hydrogen citrate sesquihydrate (0.5 g), NaCl (1 g), and MgSO₄ (4 g); the tube was stirred by hand for 1 min. Organic extract was obtained by centrifugation for 5 min at 1008 x g. A clean-up column Vac Oasis PRiME HLB (3 cc cartridge, 60 mg of sorbent per cartridge) was used for clean-up. After the extract was passed through the clean-up column, 1 mL of the cleaned extract was placed in a dispersive SPE tube (dSPE) and stirred for 1 min. The extract was centrifuged for 1 min at 1008 x g. A volume of 500 μL was collected and evaporated dry with nitrogen. The sample was reconstituted with 250 μL of acetonitrile:water (15:85)
Mycotoxin analysis by UPLC-MS/MS

Mycotoxin analysis was performed by ultra-high pressure liquid chromatography coupled to mass spectrometry (UPLC-MS/MS) using a ACQUITY UPLC® Class I system from Waters Corporation (Milford, Massachusetts, USA). A CORTECS® UPLC T3 column (1.6 μm, 2.1 × 100 mm from Waters Corporation, Milford, Massachusetts, USA) was used to analyse the target mycotoxins. The samples were maintained at 7 °C during the analyses. The mobile phases were: phase A) 0.5% of formic acid and 5 mM of ammonium formate and phase B) acetonitrile:methanol (50:50) with 0.5% formic acid and 5 mM of ammonium formate. The gradient was as follows: 0– 6 min 99% A, 6.0– 6.5 min 30% A, 6.5– 7.5 min 5% A, 7.5–9.7 min 1% A, 9.7–10 min 1% A, 10–11 min 99% A, 11 min 99% A. The flow rate was 0.4 mL/min, and the injection volume was 5 μL. The column was kept at 30 °C during the analyses.

The UPLC system was coupled to a Xevo® TQ-S MS/MS system from Waters Corporation (Milford, Massachusetts, USA). The positive polarity electrospray analysis mode (ESI+) was used and performed under the following parameters: capillary voltage, 3.0 kV; source temperature, 150 °C; desolvation temperature, 500 °C; desolvation gas flow, 800 L/h; and conical gas flow, 150 L/h. For operation in the MS/MS mode, argon was used as the collision gas with a pressure of 0.12 mL/ min. The Multiple Reaction Monitoring (MRM) for mycotoxins, the optimal parameters of mass spectrometry for AFB₁, OTA and FB₁ were 313.2, 404.2 and 722.4 m/z for precursor ions, respectively. The ions product were 241.1 and 285.1 m/z for AFB₁, 239.1 and 358.2 m/z for OTA, and 334.2 and 352.2 m/z for FB₁, first ion mentioned was used as Transition ion used for quantification each mycotoxin, respectively. Collision energy values were 35 and 22 V for AFB₁, 25 and 15 V for OTA, and 40 and 35 V for FB₁. The dwell time established were 0.003 s for all mycotoxins, while cone voltage values and holding time were 15, 20 and 30 V and 2.88, 4.16 and 3.22 min for AFB₁, OTA and FB₁, respectively. The massLynx (V4.1) software (Waters Corporation) was used for data acquisition. The analysis
was conducted in the certificated laboratory, Analytical and Metrological Services Unit of the Research and Assistance Centre for Technology and Design of the State of Jalisco (CIATEJ).

**Validation of the method**

The limits of detection (LOD) and quantification (LOQ) for mycotoxins were determined from a calibration curve. For AFB₁, the LOD = 1.20 μg/kg and LOQ = 1.80 μg/kg, in a linear range of 1.8–37.5 μg/kg ($R^2 = 0.9957$) and with a recovery percentage of 88.4–101.16%. For OTA, the LOD = 3.00 μg/kg and LOQ = 4.01 μg/kg, in a linear range of 4.005–40.005 μg/kg ($R^2 = 0.9996$) and with a recovery percentage of 95.23–107.45%. For FB₁, the LOD = 7.5 μg/kg and LOQ = 30.0 μg/kg, in a linear range of 30–75 μg/kg ($R^2 = 0.9938$) and a recovery percentage of 83.0–98.8%.

**Consumption data and characteristics of the study population**

A descriptive study was conducted in 150 participants from the state of Nayarit, Mexico. Participants were informed of the purposes of the study and signed an informed consent letter. A structured questionnaire was applied in the form of an interview to collect information about the participants’ general characteristics such as weight, height, age, diet, socioeconomic level, schooling, and harmful habits such as drug, alcohol, and tobacco consumption. The categories for rice and maize consumption frequency included in the questionnaire ranged from never, a few times a month, a few times a week, and up to six times a day. The population was categorized, according to 50th and 99th percentile of age.

**Human food consumption and estimated exposure to FB₁**

The estimated exposure was calculated based on measuring the PDI per unit of body weight, expressed as ng/kg of body weight (bw) per day ([WHO] World Health Organization 2002). Therefore, the estimated PDI of mycotoxins was made using the following formula:
The mycotoxin level in food is either the highest level or the mean mycotoxin concentration in rice and maize. The food consumption is the average consumption of rice and maize in Mexico, while the average body weight is the body weight of the study population.

The European Food Safety Authority (EFSA) recommends using the highest concentration levels of a chemical found in foods combined with the highest levels of food consumption in a deterministic approach (point estimate deterministic approach). When the resulting exposure is below the safety-concern threshold, a more refined analysis is not necessary ([EFSA] European Food Safety Authority 2010). However, the average or median concentration of a chemical in a food can be combined with food consumption data at the individual level to estimate the distribution of dietary exposure. This approach assumes that a given study subject randomly chooses their food combination and that, in the long term, the probability of exposure to a considered quantity of a chemical follows the general concentration distribution (deterministic approach based on the individual).

Food consumption was based on rice and maize consumption data obtained from the structured questionnaire administered to the study population and from the annual per capita consumption of rice and maize from the Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA) of Mexico ([SAGARPA] Secretaría de Agricultura y Desarrollo Rural 2017). Data for the average body weight were obtained from the sample population of this study (n = 150).

The human risk assessment based on TDI or PMTDI recommended by JECFA ([WHO] World Health Organization 2017) was made with the following formula:

\[ PDI = \frac{\text{Mycotoxin level in food} \left( \frac{ng}{kg} \right) \times \text{Food consumption} \left( \frac{kg}{\text{person} \text{ day}} \right)}{\text{Average body weight}(kg)} \]
\[
\frac{PDI}{TDI \text{ or } PMTDI} > 1
\]

Results greater than 1 indicate that the resulting exposure is above the threshold of safety concern ([EFSA] European Food Safety Authority 2010).

**Statistical analysis**

The general characteristics of the population and rice and tortilla consumption were analyzed for frequency, percentage, and means. The normality of the data was assessed by the Kolmogorov–Smirnov test. One-way analysis of variance (ANOVA) was used when data followed normal distribution, and the Mann-Whitney U test was employed when data were not normally distributed \((p < 0.05)\). The statistical analysis was conducted using the Stata version 14 software (Stata Corp LP, College Station, TX). Figure was created using the GraphPad Prism 6.01 (Graph Pad software, SanDiego, California, EUA).

**Results**

*Mycotoxins in rice and maize*

In the present study, AFB\(_1\), OTA, and FB\(_1\) were quantified in a total of 43 rice samples and 25 maize samples. Of these, 33 paddy rice and 16 maize samples were collected in the field at harvest time in Santiago Ixcuintla, Nayarit, and 10 polished rice and 9 maize samples were collected from the market in Tepic, Nayarit (Table 1).

AFB\(_1\) was detected in 21.21% of paddy rice samples, and the mean concentration was 17.43 \(\mu g/kg\) (2.27 to 47.07 \(\mu g/kg\)). In contrast, OTA was detected in one paddy rice sample (3.03%) at a concentration of 29.89 \(\mu g/kg\), and FB\(_1\) was not detected in any paddy rice samples. While, AFB\(_1\), OTA, and FB\(_1\) were not detected in polished rice. Regarding maize samples, AFB\(_1\) was below the LOQ in all of the field samples, and only one sample from the market was found to be contaminated with 21.46 \(\mu g/kg\) of AFB\(_1\). OTA was not found in any of the maize
samples studied. However, FB$_1$ was detected in both field maize and market maize. The mean level of FB$_1$ in the field samples was 1948.77 µg/kg (141.31 to 16,672.62 µg/kg), and the FB$_1$ mean in samples from the market was 234.42 µg/kg (79.22 to 606.68 µg/kg). In addition, co-occurrence of AFB$_1$ and FB$_1$ was observed in one maize sample from market (21.46 µg AFB$_1$/kg and 122.22 µg FB$_1$/kg).

Table 1. AFB$_1$, OTA, and FB$_1$ contamination in rice and maize sampled in Nayarit State (Mexico).

|                   | Field Paddy rice | Field Maize | Market Polished rice | Market Maize |
|-------------------|------------------|-------------|----------------------|--------------|
| AFB$_1$           | 7/33             | 0/16        | 1/10                 | 1/9          |
| OTA               | 1/33             | 0/16        | 0/10                 | 0/9          |
| FB$_1$            | 0/33             | 14/16       | 0/10                 | 8/9          |
| Frequency (%)     | 21.21            | 3.03        | 0                    | 11.11        |
| Range (µg/kg)     | 2.27-47.07       | —           | 141.31-16672.62      | —            |
| Mean (µg/kg)      | 17.43-29.89      | —           | 1948.77$^b$          | —            |
|                   |                   | —           | 21.46                | 234.42$^b$   |

$^a$Number of positive samples/Number of total samples. $^b$ Geometric means.

Analysis of FB$_1$ in maize from market samples showed its levels did not exceed the levels accepted by both regulations. Moreover, statistically significant differences were observed between the samples from field and market ($p = 0.005$), where FB$_1$ contamination in the field samples were 12.60 times higher than in the market samples (Figure 1).
Characteristics of the study population and consumption data

One hundred fifty participants were interviewed, 53.3% of which were women and 46.7% of which were men. The geometric mean ages were 31.2 years for women and 33.1 for men. Regarding weight and height, differences were observed by sex, with a greater weight (81.70 kg) and height (172.5 cm) observed in men with respect to women (64.87 Kg and 161.3 cm), as expected. With respect to body mass index (BMI), differences were observed by sex, with women (24.95 kg/m²) presenting normal weight and men presenting pre-obesity (27.45 kg/m²) (Table 2). The underweight group was not included in the obesity classification statistical analysis categorized by sex because only one woman and one man presented as underweight.
Table 2. General characteristics of the sample population.

|                  | Women 80 (53.33%) | Men 70 (46.67%) | p-value |
|------------------|--------------------|-----------------|---------|
| Age years, GM (CI 95%) | 31.27 (28.95-33.76) | 33.14 (30.80-35.65) | 0.191   |
| 19-32 years, n (%) | 45 (56.25)        | 34 (48.57)      | 0.347   |
| 33-62 years, n (%) | 35 (43.75)        | 36 (51.43)      |         |
| Weight kg GM (CI 95%) | 64.87 (62.41-67.43) | 81.70 (78.12-85.45) | <0.001 |
| Height cm (CI 95%) | 161.35 (160.06-162.65) | 172.56 (171.20-173.91) | <0.001 |
| BMI GM (kg/m²) (CI 95%) | 24.95 (23.96-25.98) | 27.45 (26.31-28.65) | <0.001 |
| Obesity classification*, n (%) | 44 (55.00) | 21 (30.00) | 0.003 |
| Normal weight (BMI 18.5–24.9) | 22 (27.50) | 23 (32.86) |         |
| Pre-obesity (BMI 25.0-29.9) | 13 (16.25) | 25 (35.71) |         |
| Obesity (BMI >30) | 13 (16.25) | 25 (35.71) |         |

GM: geometric mean. CI: confidence interval. BMI: body mass index. Age, weight, and BMI p values were obtained by Mann-Whitney U test (p < 0.05). Height differences were obtained by ANOVA (p < 0.05). *BMI classification by the WHO.

Rice and tortilla consumption data

The questionnaire on food consumption frequency was used to assess the intake of rice and maize tortillas. Differences by sex were not observed in rice and maize tortilla consumption (p > 0.05). Regarding the consumption of rice, women (56.25%) and men (55.71%) consumed a dish of rice 2–4 times per week. In the case of maize tortillas, 100% of the study population consumed maize as an ingredient of tortillas; 37.5 and 37.1% of women and men consumed maize tortillas 2–3 times a day. Taking into account that a rice dish has a net weight of 47 g and a maize tortilla is 30 g, according to Pérez Lizaur et al. (2008), 56% of the study population consumed 94 to 188 g of rice per week and 37.33% consumed 60 to 90 g of maize per day.

Exposure assessment and risk characterization for FB₁

The estimated health risk was not calculated for AFB₁ and OTA since the positive samples for both mycotoxins were detected in paddy rice from field, which does
not go directly to human consumption. Therefore, the estimated human exposure was calculated only for the FB$_1$ levels detected in our results. The PMTDI reported for FB$_1$ is 2000 ng/kg bw per day ([JECFA] Joint FAO/WHO Expert Committee on Food Additives 2016). Here, the levels of mycotoxins in food were recorded both in terms of the highest and the mean levels of mycotoxins found in maize in our study. In addition, food consumption was determined from data on the annual per capita consumption of maize (196.4 kg), data obtained from SAGARPA (NAP 2017–2030) ([SAGARPA] Secretaría de Agricultura y Desarrollo Rural 2017), and data obtained from our study population, based on tortilla consumption.

Table 3 shows assessments of dietary exposure to FB$_1$ based on maize and tortilla consumption. Regarding the highest and mean levels of maize consumption from field and market, the PDI/ PMTDI ratio was higher than 1; PDI values from the field were 60.62 and 12.95 times the PMTDI, respectively, and those from market were 2.21 and 1.03 times the PMTDI, respectively. In the case of tortilla consumption, the PDI values from the field were 10.14 and 2.17 times the PMTDI, respectively. Thus, the dietary exposure to FB$_1$ was higher in maize consumption than in tortilla consumption, but this difference could be due to the fact that Mexicans consume many maize derivatives, not only tortillas. In addition, our results for the estimated risk of FB$_1$ exposure based on maize consumption provided evidence of real exposure potential in the population of the studied zone during the sampling period. The levels of contamination by FB$_1$ and by other mycotoxins in maize in this region may exhibit seasonal variability linked to climate and other factors.

### Table 3. Dietary exposure to FB$_1$ from field and market maize and risk characterization based on PDI/PMTDI ratio.

| FB$_1$       | Highest level (ng/kg) | Mean level (ng/kg) | Consumption (kg) | Body weight (kg) | $^a$PDI (ng/kg bw/day) | $^b$PDI (ng/kg bw/day) | $^c$PDI/PMTDI | $^d$PDI/PMTDI |
|--------------|-----------------------|--------------------|------------------|------------------|------------------------|------------------------|----------------|----------------|
| **Maize consumption** |                       |                    |                  |                  |                        |                        |                |                |
| Field        | 16672620              | 3561630            | 0.538$^e$        | 73.99            | 121230.84              | 25897.51               | 60.62          | 12.95          |
| Market       | 6066680               | 282650             | 0.538$^e$        | 73.99            | 4411.32                | 2055.22                | 2.21           | 1.03           |
| **Tortilla consumption** |                       |                    |                  |                  |                        |                        |                |                |
| Field        | 16672620              | 3561630            | 0.09$^f$         | 73.99            | 20280.25               | 4332.30                | 10.14          | 2.17           |
With respect to the consumption of field and market maize, by participants categorized by age (Table 4), the estimated exposure was greater than the PMTDI value established by JECFA. In addition, dietary exposures calculated using both the highest and mean level of FB₁ were higher in the consumption of maize from field than from market for women and men (more than 50 times greater than the PMTDI), regardless of age categorization. In addition, the greater dietary exposure based on field maize consumption is due to the fact that in the field the FB₁ level was higher than in the market. Regarding the dietary exposure associated with the consumption of tortilla (Table 5), no risk was observed for the consumption of maize from market. However, in the case of field maize, women exceeded the PMTDI more than men in the age range of 19 to 33 years. For both the field and market maize consumption risk assessment, it is possible that the dietary exposure is higher in women than in men due to the low body weight of women compared to men (Table 2).

Table 4. Dietary exposure to FB₁ based on the consumption of field and market maize, categorized by age.

|       | Women          |          | Men          |
|-------|----------------|----------|--------------|
|       | Age (years)    | PDI      | PMTDI        | PDI      | PMTDI        |
| Field | 19-32          | 137722.55| 68.86        | 19-32    | 136154.67    | 68.08        |
|       | 33-62          | 134118.86| 67.06        | 33-62    | 113773.08    | 56.89        |
|       | 19-62          | 134154.67| 68.08        | 19-62    | 102865.48    | 51.43        |
|       | 33-62          | 136154.67| 68.08        | 33-62    | 107888.74    | 53.94        |
| Market| 29420.50       | 29850.67 | 29085.56     | 29420.50| 24304.38     | 21974.28     |
|       | 28650.67       | 29085.56 | 56.89        | 28650.67| 23047.35     | 53.94        |
|       | 2308.22        | 14.71    | 14.54        | 2308.22  | 12.15        | 11.52        |
|       | 1928.79        | 14.33    | 14.54        | 1928.79  | 10.99        | 11.52        |
|       | 1743.87        | 14.33    | 14.54        | 1743.87  | 10.99        | 11.52        |
|       | 1829.03        | 14.33    | 14.54        | 1829.03  | 10.99        | 11.52        |
|       | 1.17           | 2.51     | 2.44         | 1.17     | 2.44         | 2.48         |
|       | 1.14           | 2.44     | 2.48         | 1.14     | 2.44         | 2.48         |
|       | 1.15           | 2.44     | 2.48         | 1.15     | 2.44         | 2.48         |
Table 5. Dietary exposure to FB₁ based on the consumption of tortilla, categorized by age.

|                | Women                  | Men                  |
|----------------|------------------------|----------------------|
| **Field**      |                        |                      |
| **Age (years)**| 19-32 33-62 19-62 19-32| 33-62 19-62 19-62    |
| **PDI**        | 23039.09 22436.24 22776.80 19032.67 | 17207.98 18048.30 |
| **PDI/PMTDI**  | 11.52 11.22 11.39 9.52 | 8.60 9.02           |
| **PDI**        | 4921.64 4792.86 4865.61 4065.79 | 3675.99 3855.51    |
| **PDI/PMTDI**  | 2.46 2.40 2.43 2.03 | 1.84 1.93           |
| **Market**     |                        |                      |
| **PDI**        | 838.34 816.41 828.80 692.56 | 626.16 656.74      |
| **PDI/PMTDI**  | 0.42 0.41 0.41 0.35 | 0.31 0.33           |
| **PDI**        | 390.58 380.36 386.13 322.66 | 291.73 305.97      |
| **PDI/PMTDI**  | 0.20 0.19 0.19 0.16 | 0.15 0.15           |

*aAverage body weight of total study population. *bPDI: probable daily intake based on highest level. *cPDI: probable daily intake based on mean level. *dPMTDI: provisional maximum tolerable daily intake.
Discussion

AFs in rice were evaluated in only one previous study in Mexico, and 90.91% of the samples were found to be contaminated (16.9 μg/kg) (Suárez-Bonnet et al. 2013). In contrast, previous studies of Mexican maize showed evidence for the presence of AFs. AFB1 was present in 90% (<2.5 to 30 μg/kg) (Ellis et al. 1991) and 77.78% (3 to 10 μg/kg) of maize samples (Flores et al. 2006). In the case of derivatives such as tortillas, AFB1 was found in 64.6% of tortilla samples (3.0 to 140.3 μg/kg) (Castillo-Urueta et al. 2011) and in 61% and 27% (287.230 and 19.019 μg/kg) of samples from two rural communities (Zuki-Orozco et al. 2018). Only two previous studies examining OTA have been conducted in Mexico; the authors evaluated green coffee beans and reported contamination in 67% of the samples (30.1 μg/kg) (Robledo-Marenco et al. 2001). The second study was focused on animal consumption of sorghum; 40% of the samples were contaminated at levels ranging from 1 to 352 μg/kg (Flores et al. 2006). In the case of FB1, it was previously detected in 100% of maize samples (1000 to 8800 μg/kg) (Cortez-Rocha et al. 2003) as well as in two studies of hybrid maize (16.5 up to 606.0 μg/kg) (Figueroa-Gómez et al. 2006; Reyes-Velázquez et al. 2011). Furthermore, in two different studies carried out in maize flour and tortillas, FB1 was also found in 100% of samples (210 to 1800 μg/kg and 1 to 729 μg/kg, respectively) (Dombrink-Kurtzman et al. 2000; Dvorak et al. 2008), whereas in nixtamalized maize flour, it was detected in 62.5% of samples (1050 to 22,880 μg/kg) (Cortez-Rocha et al. 2005). The results of the present study provided similar evidence for the presence of mycotoxins.

Regarding contamination by mycotoxins relative to the limits set in legislation of the EU, USA (FDA), and Mexico, no samples of polished rice were contaminated with the studied mycotoxins. However, in samples of paddy rice (which does undergo a selection process or other physical treatment before human consumption), 15.15% of samples were naturally contaminated with AFB1 at levels above the EU regulation level for rice (5 μg/kg) ([EC] European Commission 2010a), and 6.06% of samples exceeded the limits set by USA regulations (20
µg/kg) ([FDA] Food and Drug Administration 2000) and Mexican regulations (20 µg/kg) ([NOM-247-SSA1- 2008] Norma Oficial Mexicana 2008). Similarly, Reddy et al. (2009) reported high levels of AFB₁ in samples of paddy and polished rice in India (0.1 to 308 µg/kg); 2% of samples had AFB₁ levels above the permissible limit in India (>30 µg/kg), which demonstrates that a high percentage of the samples exceed the levels set by legislation such as that of the EU and USA. Similar results were obtained with polished rice samples in studies conducted in Colombia (Martinez-Miranda et al. 2019), Pakistan (Iqbal et al. 2016; Majeed et al. 2018), France (Manizan et al. 2018), and Spain (Suárez-Bonnet et al. 2013). It is important to highlight that dehulling and polishing can contribute to a reduction of AFB₁ levels in rice (Lancova et al. 2008).

In the case of OTA, the single paddy sample that was found to be contaminated (29.89 µg/kg) in this study exceeded the EU regulatory limit for unprocessed cereals (5 µg/kg) ([EC] European Commission 2006, 2010b). With regards to USA and Mexico regulations, neither country regulates OTA levels in any food. In other studies, the presence of OTA was reported in polished rice samples at low levels that nonetheless exceeded the EU regulatory limit for cereals and cereal products (5 and 3 µg/kg). In broken rice from Myanmar, a single sample (0.5%) was contaminated with 46.5 µg/kg (Lim et al. 2015), and a Chinese rice sample (4.9%) was contaminated with 3.2 µg/kg (Lai et al. 2015). Relative to the EU regulations for baby foods (0.5 µg/kg), baby food from USA markets (10%) contained detectable OTA in a range of 1.3 to 1.4 µg/kg (Al-Taher et al. 2017).

Regarding regulatory limits for FB₁ in rice, the USA and Mexico do not address the presence of FB₁. Studies carried out in other countries have shown results in both ways. In Brazilian rice samples, FB₁ was not detected in the husk fraction (Moreira et al. 2020), and it was also not detected in rice samples imported to Canada in 2007 from the USA and Asian countries (Bansal et al. 2011).

In contrast, other authors have found FB₁-positive rice samples; Majeed et al. (2018) reported 42% of samples from Pakistan were positive, with a mean level of 42 µg/kg. In addition, in rice samples from Vietnam, FB₁ was detected in 5.4% of
samples at a maximum level of 675 µg/kg (Do et al. 2020) and in 8.1% at a range of 2.3 to 624 µg/kg (Huong et al. 2016).

Regarding maize, only one sample of maize from market in this study exceeded the acceptable level established by the EU regulation for AFB$_1$ (5 µg/kg) ([EC] European Commission 2010a). With respect to FB$_1$, maize samples (8%) had levels higher than the acceptable level for unprocessed maize according to UE regulation (4000 µg/kg) ([EC] European Commission 2007) and the acceptable level for maize-based foods intended for direct human consumption according USA regulation (4000 µg/kg) ([FDA] Food and Drug Administration 2001). Furthermore, 44% of samples exceeded the acceptable level for maize and maize-based foods intended for direct human consumption established by the EU regulation (1000 µg/kg) ([EC] European Commission 2007).

In general, Mexican studies on maize found AFB$_1$ and FB$_1$ levels above levels recommended by European legislation. Torres Espinosa et al. (1995) and García Aguirre et al. (2001) showed that the AFB$_1$ and FB$_1$ levels in Mexican maize were above the acceptable European levels. Similar results were reported in several studies of Mexican maize for human consumption and its derivatives (Desjardins et al. 1994; Dombrink-Kurtzman et al. 2000; Cortez-Rocha et al. 2003, 2005; Sánchez-Rangel et al. 2005; Figueroa-Gómez et al. 2006; Dvorak et al. 2008). Currently, Mexico has no established regulations for other mycotoxins such as OTA and FBs. The results found in this study, like previous ones, showed that positive samples frequently exceed the recommended levels established by international regulations. In this sense, lack of regulation does not mean that these mycotoxins are not present in Mexican crops and derivatives, but rather that it is necessary to work together with the Mexican government to develop new legislation regarding mycotoxins in Mexico.

In addition, our results show high PDI values representative of exposure and high risk for the effects associated with FB$_1$ due to consumption of maize from field and tortilla consumption. Previous studies have shown that human populations are not excluded from the risks of consuming food contaminated with mycotoxins, either
directly by consuming cereals and derivatives or indirectly by consuming food from animal sources. Four studies that assessed exposure to myco-toxins have been carried out previously in Mexico. Camarillo et al. (2018) reported that the population exceeded the toxicological reference values (TDI) for AFM₁ and AFM₂ in Oaxaca cheese consumption, with the children at highest risk, followed by adolescents and adult women. In addition, Sandoval et al. (2019) evaluated the estimated daily intake (EDI) values for AFB₁ and showed risk to human health resulting from the nixtamalized maize products consumed in Mexico. In contrast, Gong et al. (2008) conducted a study in Mexican women regarding consumption of maize products and found that the average daily intake of FB₁ was lower (0.4 μg/kg bw) than the maximum tolerable intake established by the WHO (2 μg/kg bw). Subsequently, Wall-Martínez et al. (2019) assessed the intake of AFs and FBs through the consumption of maize tortilla. The authors reported that intake of maize tortilla causes risk due to contamination with AFs. Moreover, differences between males and females were found because the male population had higher consumption of maize. However, the risk of FBs intake was low since its presence in maize tortillas was also low.

Similar to our results, Andrade et al. (2018) reported that in Brazil, heavy consumers of popcorns could be at potential risk of exposure to FBs (4600 to 26,780 ng/kg bw/day; more than twice the PMTDI). Despite the risk associated with our results relative to the highest level of FB₁, there is no risk level as high as that reported by Onyedum et al. (2020) in northern Nigeria, where it was reported that the maize consumers are at risk of exposure to FBs to a degree more than 3000 times the PMTDI (7,136,000 ng/kg bw/day). As in our results, Do et al. (2020) observed risk in individuals over 18 years of age consuming mainly maize in Ha Giang province in northern Vietnam (2200 to 3700 ng/kg bw/day, 1.1 to 1.9 times the PMTDI). Contrary to our results, Esposito et al. (2016) conducted a study of people affected by celiac disease; the results showed that Italian adults (18 to 65 years old) had lower intakes than the PMTDI, while children and adolescents had higher intakes than the PMTDI, which could be due to the fact that children weigh less than adults.
With respect to health and FB$_1$, exposure to FB$_1$ has been associated with oesophageal cancer and nephrotoxicity ([JECFA] Joint FAO/WHO Expert Committee on Food Additives 2001, 2007). Mexico has a high incidence of chronic kidney disease, and between 1990 and 2010, the incidence increased more than 300% (Lozano et al. 2013). In contrast, the incidence of oesophageal cancer has increased only slightly during the past decades from 1979 to 2008 (Gómez Urrutia et al. 2017). It is possible that exposure to FB$_1$ through food consumption could contribute significantly to the high incidence of oesophageal cancer and kidney disease in our country. Wild and Gong (2010) identified several reasons for the current inaction on mycotoxin risks in developing countries. Among them are lack of knowledge and poor communication with policy-makers about mycotoxins and their health risks.

**Conclusion**

Mycotoxin contamination is a problem that involves agriculture, health, and economics – fields that are often abandoned by governments resulting in policymaking that is not often based on current research. Thus, the present work contributes data regarding an assessment of exposure through the consumption of food contaminated with FB$_1$. Our data suggest that some maize samples are not suitable for human consumption, and the health of the Mexican population could be compromised due to long-term consumption of contaminated maize. Even though our study has limitations such as a lack of data on mycotoxin levels in rice and maize foodstuffs and small sample sizes from both field and local market, these results could contribute to new strategies for the control and prevention of mycotoxin contamination during crop harvest, transport, and storage, as well as provide the evidence needed to meaningfully update the regulation of mycotoxins in Mexico.

**Acknowledgments**

The authors thank the farmers and study population who participated in the study.

**Disclosure Statement**
The authors report no conflict of interest.

**Funding**

This work was supported by the Consejo Nacional de Ciencia y Tecnología (CONACYT) under Grant code SALUD-2017-2-289726.

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