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Daily intake estimates of fumonisins in corn-based food products in the population of Parana, Brazil

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ABSTRACT

One hundred (100) samples of corn-based food products were collected between February 2007 and July 2010 to estimate the intake of fumonisins in the state of Paraná, Brazil. The identification of fumonisins was performed by high-performance liquid chromatography with fluorescence detection and post column o-phthaldialdehyde (OPA) derivatisation. The occurrences of fumonisins B_1 (FB_1) and B_2 (FB_2) were 82% and 51%, respectively, with concentrations ranging from 126 to 4348 μg/kg ($FB_1 + FB_2$). Among corn-based products, corn meal had a higher prevalence of fumonisin (96.6%), and corn grits had the highest level of contamination (3462 μg/kg for FB_1 and 886 μg/kg for FB_2). The average probable daily intake (APDI) and maximum probable daily intake (MPDI) estimated for the population of Parana, Brazil, were 120.58 and 256.07 ng/kg body weight/day, respectively, which is lower than the provisional maximum tolerable daily intake (PMTDI), 2000 ng/kg body weight/day.

1. Introduction

The United Nations Food and Agriculture Organization (FAO, 2005) estimated that 40% of the reduction in life expectancy in developing countries is related to the presence of mycotoxins in the food consumed by these populations. Human exposure to mycotoxins is a public health issue worldwide (Brera, Miraglia, & Colatosti, 1998). Fumonisins (FBs) are mycotoxins produced by fungi of the *Fusarium* genus, especially *F. verticillioides* and *F. proliferatum* (Shephard, Thiel, Stockenström, & Sydenham, 1996). Since the discovery of FBs in 1988 (Bezuidenhout et al., 1988), twenty-eight molecules have been described (Rheeder, Marasas, & Vismer, 2002). Those that are analogous to the B-series, fumonisin B_1 (FB_1), B_2 (FB_2) and B_3 (FB_3) are the most abundant and found in natural conditions (Shephard et al., 1996). FB_1 is the most toxic and is responsible for 70% of food contamination in the world (Thiel et al., 1991). This mycotoxin is associated with leukoencephalomalacia (LEM) in horses, pulmonary oedema in swine and hepatocarcinoma in rats, in addition to being related to an inhibition of sphingolipid synthesis and increased risk of oesophageal cancer in humans (Marasas, 1996). Given these factors, the International Agency for Research on Cancer (IARC, 2002, 301–366) described FB_1 as class 2B, toxins produced by *Fusarium* as possibly carcinogenic in humans.

Corn is one of the main cereals produced in Brazil. In the 2008/2009 crop, 51.9 million tons were produced. The state of Paraná was the main producer, accounting for 24% of Brazilian production (CONAB, 2009). This cereal has a high nutritional value and a significant role in human nutrition, being the main raw material of many Brazilian dishes such as dent corn, couscous, polenta and Brazilian tamales.

In Brazil, the concentration of FBs in corn-based food products commercialized for human consumption has been reported by several authors (Caldas & Silva, 2007; Machinski Jr. & Valente Soares, 2000; Moreno et al., 2009; Scaff & Scussel, 2004; Westhuizen et al., 2003) to be at levels exceeding the limits recommended by the European Commission Regulation N° 1126/2007, ranging from 4000 μg/kg for unprocessed corn and 200 μg/kg for corn-based foods for children (EC, 2007). The World Health Organization (WHO) recommends a provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg body weight/day for FB_1, FB_2 and FB_3, independently or combined, which was calculated according to the dose of no observable adverse effect level (NOAEL) of 0.2 μg/kg/day with a safety factor of 100 (WHO, 2002).

Scientific reports of the presence of fumonisins in food products contribute to the assessment of exposure to these compounds. Thus, considering the consumption of corn and the role of this cereal in the Brazilian diet, the objective of this study was to

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estimate exposure to FBs in corn-based food products commercialized in the state of Parana, Brazil.

2. Materials and methods

2.1. Reagents, Solvents and other materials

The standard solutions of FB_1 and FB_2 were obtained from Acros Organics (Geel, Belgium). Chromatographic-grade methanol (Honeywell Burdick & Jackson, Muskegon, MI) and acetonitrile (Fisher Scientific, Fair Lawn, NJ) were used. Acetone (F. Maia, Cotia, Brazil), chlorotrimethylsilane (Merck Schuchardt, Hohenbrunn, Germany), glacial acetic acid (Mallinckrodt Baker, Xalostoc, Mexico), sodium tetraborate decahydrate (Mallinckrodt Baker, Phillipsburg, NJ), toluene (CAQ, Diadema, Brazil), hydrochloric acid (Merck, Darmstadt, Germany), hydrochloric acid (HCl)1 N (Dinâmica, Diadema, Brazil) and sodium hydroxide (NaOH) 1 N were all of analytical grade. Brij® Solution 30% (w/v) was purchased from Sigma Aldrich (St. Louis, MO). Thiofluor® and o-phthaldialdehyde (OPA) were from Pickering Laboratories, Inc. (Mountain View, CA). Ultra pure water was obtained from the Milli-Q system (Millipore, Bedford, MA).

The mobile phases and samples were filtered using LCR membranes in modified polytetra-fluorethylene - PTFE (Millipore, Billerica, MA) and Millex-LCR with a modified PTFE membrane (Millipore, Billerica, MA), respectively. Nitrogen (99.9% purity) was purchased from White Martins (Rio de Janeiro, Brazil), and the anion exchange column Sep Pak Vac Accel Plus QMA 6cc, 500 mg, was obtained from Waters® (Wexford, Ireland).

Sodium phosphate buffer was prepared with 13.79 g anhydrous sodium phosphate (Quimibrás, Rio de Janeiro, Brazil) and 1000 ml of ultra pure water. The pH was adjusted to 3.35 with orthophosphoric acid (Merck, Darmstadt, Germany).

Stock solutions of 100 μg/ml of each toxin were prepared in acetonitrile-water (50:50, v/v) and stored in amber vials at −20 °C. The solutions used were 0.39, 0.781, 1.562, 3.125 and 6.25 μg/ml.

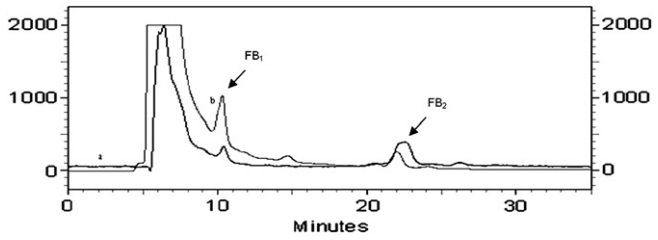

Fig. 2. Chromatograms of FB_1 and FB_2 in (a) standard solution (2.5 μg/ml) and (b) sample of naturally contaminated grits (FB_1 = 3462 μg/kg, and FB_2 = 886 μg/kg).

2.2. Sample collection

One hundred samples of corn-based products marketed in the State of Paraná, Brazil (Fig. 1), were collected between February 2007 and July 2010 in 20 municipalities. There were 29 corn meal, 28 grits, 17 popcorn, 15 corn flour and 11 corn flakes. The minimum weight of the samples was 500 g. The samples were crushed in a hammer mill, homogenised and stored at −20 °C until analysis.

2.3. Analytical quality control

All samples were analysed in duplicate on different days. Spiked samples at concentrations of 500 or 1000 μg/kg of $FB_1 + FB_2$ were used to evaluate the efficiency of the extraction process, with each set containing 9 samples. The results of each sample in the batch were corrected for the recovery of the spike. The limit of quantification was established as the lowest detectable concentration of the toxin present in an artificially enriched sample, with a variation coefficient lower than 10%.

2.4. Extraction and cleanup

The process of extracting FB_1 and FB_2 was performed according to the method of Shephard, Sydenham, Thiel, and Gelderblom

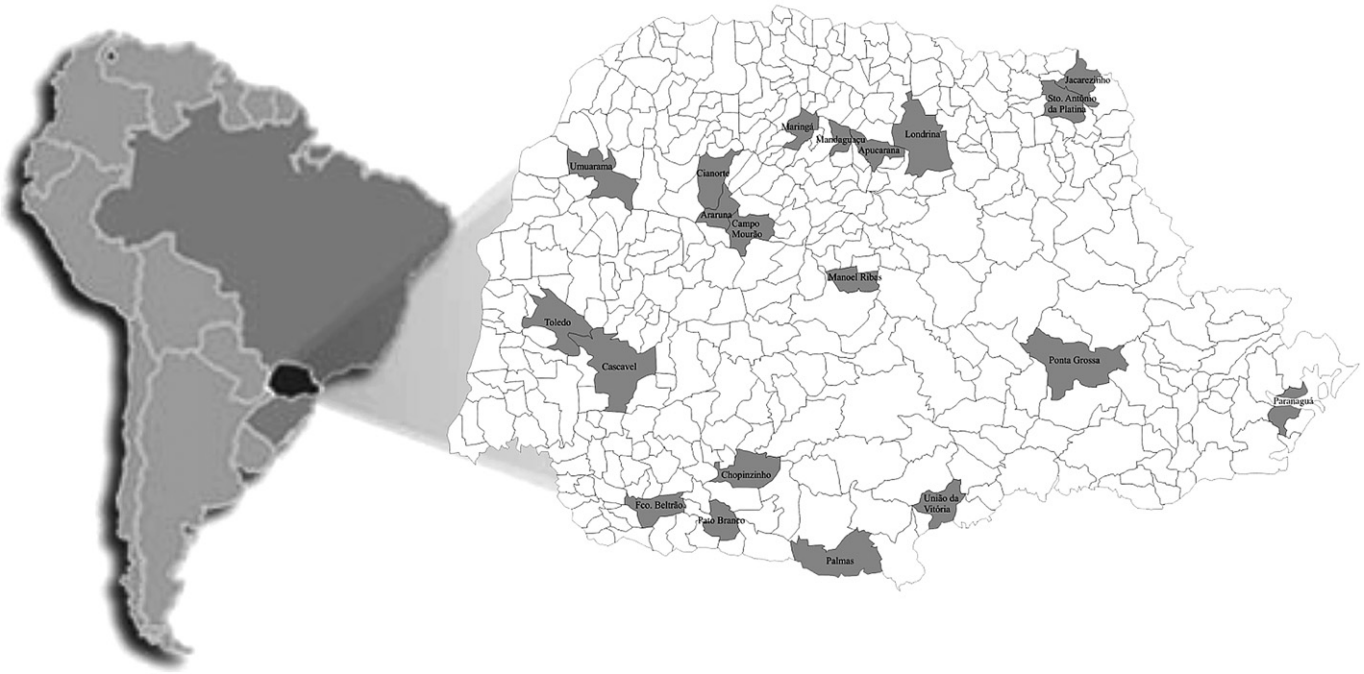

Fig. 1. Paraná state collection site of corn-based products from February 2007 to July 2010.

Table 1
Average recovery of fumonisins B_1 and B_2 from some corn matrices.

Product	Average recovery (%)	
	FB_1	FB_2
Corn meal ($n = 3$)	78.3	93.0
Corn grits ($n = 3$)	91.0	95.9
Popcorn ($n = 2$)	71.0	83.2
Corn flour ($n = 2$)	116.3	109.9
Corn flakes ($n = 2$)	95.2	114.2
Average	**84.6**	**102.9**

n – number of samples.

(1990) as modified by Camargos, Machinski Jr. and Valente Soares (1999). 50 g of the sample and 100 ml of methanol-water (3:1, v/v) were homogenised in blender (Waring Co., Torrington, CT) for 5 min. The mixture was centrifuged (EVLAB 025-M, Londrina, Brazil) for 10 min at 2500 g. The supernatant was filtered through an ordinary paper filter, and the pH was adjusted to 5.8–6.5 with NaOH 1 N or HCl 1 N.

The cleanup step was carried out in solid phase with a strong anion exchange column (Sep Pak Vac Accel Plus QMA, Waters®, Wexford, Ireland). The column was packed with 10 ml of methanol followed by 10 ml of methanol-water (3:1, v/v). Then 10 ml of the filtered extract was applied in the column and washed with 10 ml methanol-water (3:1, v/v) and 6 ml methanol. The FBs were eluted with 20 ml of a solution methanol-acetic acid (95:5, v/v). The eluate was evaporated under nitrogen flow at 60 °C with a sample concentrator (TECVAP TE-0194, Tecnal®, Piracicaba, Brazil). For chromatographic analysis, the samples were reconstituted with 1000 μl of acetonitrile-water (50:50, v/v) and filtered.

2.5. Chromatographic determination of fumonisins by high performance liquid chromatography with fluorescence detection

For quantification and determination, we used the high-efficiency liquid chromatograph Finnigan Surveyor Plus (Thermo Scientific ®, San Jose, USA) with a quaternary pump, an automatic injector, Finnigan Surveyor Autosampler Plus, and a post-column derivatization Vector PCX (Pickering Laboratories ®, Mountain View, USA) coupled to a fluorescence detector (Finnigan Surveyor) with an excitation wavelength of 330 nm and emission of 465 nm.

The separations were made in Spherisorb® C18 5 μm column (250 × 4.6 mm, Waters®, Wexford, Ireland). The derivation was performed with OPA reagent (0.315 g of OPA dissolved in 10.52 ml of methanol, 2.105 g Thiofluor ®, 3.157 ml of Brij® 35 and 4 g of sodium borate in 996 ml of ultra pure water).

The optimised chromatographic conditions were an isocratic mobile phase of methanol-phosphate buffer pH 3.35 (70:30), 0.5 ml/min flow; injection volume of 100 μl; and a 0.3 ml/min flow for the derivatization solution (OPA) at a 65 °C reaction temperature. The total running time was 30 min, with a retention time of 11 min for FB_1 and 22 min for FB_2 (Fig. 2).

2.6. Estimate of the average probable daily intake (APDI) and maximum probable daily intake (MPDI)

The estimate of the Average Probable Daily Intake (APDI) was calculated using the method of Herrman and Yunes (1999), and the estimate of the Maximum Probable Daily Intake (MPDI) was calculated according to Zimmer et al. (2008). The calculations were performed using the average levels of FBs and the maximum concentrations (90th percentile) found in the samples analysed, which were multiplied by the daily consumption of corn-based food (IBGE, 2011) and divided by 60 kg of body weight.

2.7. Statistical analysis

Data were analysed using the free software R 2.9.1.

3. Results and discussion

The process of extraction of fumonisins showed accuracy and efficient removal of interfering substances. Fig. 2 shows the chromatographic profiles of FB_1 and FB_2 in (a) the standard solution and (b) a sample of naturally contaminated grits. The average recovery rates were 84.6% for FB_1 and 102.9% for FB_2 (Table 1). The correlation coefficients obtained were 8.1% and 6.2% for FB_1 and FB_2, respectively. The calibration curve used for quantification of fumonisins was calculated by the method of least squares regression and was linear up to 6.25 μg/ml, with correlation coefficients (r^2) of 0.9999 and 0.9998 for FB_1 and FB_2, respectively. The limits of quantification (LOQ) were 78.1 μg/kg for FB_1 and 43.1 μg/kg for FB_2. The validation results show that the analytical method was applicable to this study.

The proportion of samples of corn-based products contaminated with fumonisins was high. We found that 96.6% of cornmeal samples were positive, along with 88.2% of popcorn, 81.8% of corn flakes, 73.3% of corn flour and 67.9% of grits. Table 2 shows the levels of contamination with FB_1 and FB_2 in the corn-based foods analysed. FB_1 and FB_2 were detected in 82% (82/100) and 51% (51/100) of the analysed products, respectively. The median and maximum concentrations (90th percentile) of $FB_1 + FB_2$ were 211 μg/kg and 1054 μg/kg, respectively. The levels of FBs in positive samples ranged from 126 to 4348 μg/kg ($FB_1 + FB_2$).

The highest proportion of fumonisin contamination was observed in cornmeal (96.6%), similar to the results of other authors. In Brazil, all cornmeal samples from the Federal District (Caldas & Silva, 2007) and from the city of Campinas (Machinski Jr. & Valente Soares, 2000) were contaminated with FBs, reaching

Table 2
Concentrations of fumonisins B_1 and B_2 found in corn-based food products commercialised from February 2007 to July 2010 in the state of Paraná, Brazil.

Samples	Frequency of positive samples (positive/total)		Fumonisin B_1 (positive samples)		Fumonisin B_2 (positive samples)		$FB_1 + FB_2$ median of all data (μg/kg)	$FB_1 + FB_2$ 90th percentile (μg/kg)
	FB_1	FB_2	Average (μg/kg)	Range (μg/kg)	Average (μg/kg)	Range (μg/kg)		
Corn meal	28/29	19/29	494	81–2322	158	45–333	297	1430
Corn grits	19/28	8/28	414	91–3462	171	45–886	129	787
Popcorn	15/17	10/17	338	89–1170	108	57–211	262	857
Corn flour	11/15	8/15	254	128–555	86	56–235	206	516
Corn flakes	9/11	6/11	341	120–840	87	50–151	311	796
Total	82/100	51/100	398	81–3462	131	45–886	211	1054

Limits of quantification (LOQ): 78.1 μg/kg FB_1 and 43.1 μg/kg FB_2.

Table 3
Average Probable Daily Intake (APDI) and Maximum Probable Daily Intake (MPDI) of fumonisins in corn-based food products consumed by the Brazilian population.

Corn-based products	Fumonisin levels $FB_1 + FB_2$		Consumption (g/person/day)[a]	APDI and MPDI for $FB_1 + FB_2$ (ng/kg body weight[b]/day)	
	Average (μg/kg)	90th percentile (μg/kg)		Average (μg/kg)	90th percentile (μg/kg)
Corn meal	601	1430	6.31	63.20	150.3
Corn grits	440	787	5.80	42.53	76.07
Popcorn	410	857	0.30	2.05	4.29
Corn flour	268	516	0.38	1.7	3.26
Corn flakes	399	796	1.67	11.10	22.15
Total	457	1054	14.46	120.58	256.07

LOQ: 78.1 μg/kg FB_1 and 43.1 μg/kg FB_2.
[a] Based on the Household Budget Survey 2008/2009 (IBGE, 2011).
[b] Body weight = 60 kg.

values of 6170 μg/kg and 2890 μg/kg, respectively. In Germany, Zimmer et al. (2008) found that 74.2% of the cornmeal samples were contaminated with FBs, ranging from 5 to 4766 μg/kg. In this study, the levels of FBs found were lower than in other regions of the Brazil and the world. This may be related to natural variation in the levels of toxins found in corn from one year to another (Ono et al., 1999).

Of the corn-based food products analysed, grits had the highest levels of contamination, reaching 3462 μg/kg for FB_1 and 886 μg/kg for FB_2. Scaff and Scussel (2004) found lower levels of FB_1, ranging from 297 to 2237 μg/kg, and only one sample was positive for FB_2 in the analysed samples of grits. The average levels of contamination with FB_1 and FB_2 in popcorn, flour and corn flakes were relatively lower than other foods analyzed. According to Kim, Scott, and Lau (2003), levels of contamination with fumonisin in corn flakes were low, although samples with high concentrations are occasionally found.

According to Brazilian legislation (Brasil, 2011), only two samples, one of cornmeal and one of grits, presented levels of mycotoxins above of the tolerable limits. However, seven samples of corn meal, one of grits and two of popcorn had higher levels than proposed by European legislation (EC, 2007).

The degree of exposure to chemical compounds is one of the most important parameters in the evaluation of risk (Moreno et al., 2009). According to data from the Household Budget Survey 2008/2009 (IBGE, 2011), cornmeal and corn grits are the most consumed corn-based products in Brazil, with daily intake levels of 6.31 g and 5.8 g, respectively. Table 3 presents the calculations of APDI and MPDI for fumonisins in the corn-based food products analysed.

According to our study, cornmeal was the corn-based product that most contributed to the intake of FBs in Brazil, a result similar to that found by Caldas and Silva (2007). The APDI and MPDI of FB_1 and FB_2 in the Brazilian population were 120.58 and 256.07 ng/kg body weight/day, respectively, representing 6.03% and 12.8% of the PMTDI. In the city of Campinas/SP, Brazil, FB_1 consumption was 1276 ng/kg body weight/day, 63.8% of the PMTDI (Machinski Jr. & Valente Soares, 2000). In another study conducted with samples of corn from the state of Paraná, Brazil, the APDI was 950 ng/kg body weight/day, representing 47.5% of PMTDI (Moreno et al., 2009). In the Federal District of Brazil, intake of FBs accounted for 24.1% of the PMTDI (Caldas & Silva, 2007). In Portugal (Silva, Lino, Pena, & Moltó, 2007) and Spain (Ariño, Estopañan, Juan, & Herrera, 2007), the APDIs were 65 and 3.8 ng/kg body weight/day, respectively, values that are lower than those obtained in our study.

The high frequency and high levels of fumonisin contamination in corn-based food products were correlated with the risk of developing oesophageal cancer (Thiel, Marasas, Sydenham, Shephard, & Gelderblom, 1992). In Africa with high rates of oesophageal cancer, the daily consumption of corn products was 456 g and the APDI was 8670 ng/kg body weight/day (Shephard et al., 2007). In Brazil, the state of Santa Catarina has a high incidence of oesophageal cancer, and Westhuizen et al. (2003) estimated APDI of 1600 ng/kg body weight/day from corn products collected in this state, which represents 80% of the PMTDI.

The state of Paraná in southern Brazil has the second highest incidence of esophageal cancer in the country, with an age-standardized incidence rate (ASIR) of 13.67 and 4.59 per 100,000 for male and female, respectively (INCA, 2011, 88–91). This state was the main producer of corn, accounting for 24% of Brazilian production (CONAB, 2009). The high esophageal cancer incidence and high corn consumption co-occur in the southern Brazil (Westhuizen et al., 2003). Additional epidemiological data on ASIR of esophageal cancer in different localities in Paraná and the consumption of corn-based food products are also required.

In this study, the APDI and MPDI of fumonisins estimated for the Brazilian population were below the PMTDI. However, high frequency of contamination with FBs in corn-based foods found demonstrates the need for monitoring these foods to minimise the risk of developing oesophageal cancer in the population of southern Brazil.

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