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Co-occurrence of mycotoxins in corn samples from the Northern region of Paraná State, Brazil

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ABSTRACT

Natural mycoflora and co-occurrence of fumonisins and aflatoxins were evaluated in 300 freshly-harvested corn samples (2003 and 2004 crops) collected at two points of the production chain in the Northern region of Paraná State, Brazil. In the 2003 crop, fumonisins were detected in 100% of samples and the mean levels were 2.54 μg/g in the reception and 3.12 μg/g in the pre-drying samples. On the other hand, in the 2004 crop fumonisins were detected in 98.9% and 95% of the reception and pre-drying samples, respectively. The mean levels were 1.31 μg/g in the reception, and 1.36 μg/g in the pre-drying samples. Aflatoxins were not detected in 92% of the samples analysed. The maximum probable daily intake (PDI_M) estimated for the Brazilian population (0.95 μg/kg body weight/day) is below the tolerable daily intake of 2.0 μg/kg body weight/day for fumonisin B_1.

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1. Introduction

Mycotoxins are toxic secondary metabolites produced by fungi and contaminate agricultural staples along the food production chain from field, harvest, and transport to storage (CAST, 2003). The natural occurrence of mycotoxins in agricultural staples causes serious economic losses for commercial sectors including crop, livestock and poultry producers, as well as for food and feed processors. Losses may result from poor grain quality, low crop yields, crop downgrading and reduced animal performance (Pestka, Abouzied, & Sutikno, 1995). In addition, these compounds can cause acute and/or chronic intoxication symptoms in both humans and animals at low concentration levels (mg/kg to μg/kg range) (CAST, 2003).

The mycotoxins likely to be of greatest significance in Brazil and other tropical developing countries are fumonisins and aflatoxins. Aflatoxins are a group of mycotoxins produced mainly by *Aspergillus flavus* and *A. parasiticus* and the major naturally-occurring toxins are aflatoxin B_1 (AFB_1), AFB_2, AFG_1, and AFG_2 (CAST, 2003). They have been shown to cause mutagenic, teratogenic and hepatocarcinogenic effects (Wogan, 1969). The International Agency for Research on Cancer (IARC, 2002) has classified naturally-occurring mixtures of aflatoxins as carcinogenic to humans (Group 1).

Fumonisins are produced mainly by *Fusarium verticillioides*, a primary corn phytopathogen. Twenty-eight different fumonisins have been identified (Rheeder, Marasas, & Vismer, 2002), but only fumonisin B_1 (FB_1), FB_2, and FB_3 are detected as natural contaminants at significant levels in corn and corn-based products. Fumonisins induce several diseases in animals, most importantly leukoencephalomalacia in horses (Kellerman et al., 1990), pulmonary oedema in pigs (Harrison, Colvin, Green, Newman, & Cole, 1990) and hepatocarcinoma in rats (Gelderblom, Abel, & Smuts, 2001). They have also been associated with high incidences of human oesophageal cancer in Southern Africa (Rheeder et al., 1992) and China (Ueno et al., 1997). The International Agency for Research on Cancer (IARC, 2002) classified FB_1 as a Group 2B carcinogen (possible human carcinogen). The co-occurrence of AFB_1 with fumonisins, a cancer-promoting mycotoxin in corn (Ueno et al., 1993), is presumed to play an important role in the promotion of hepatocarcinogenesis and is evaluated as one of the possible risk factors for primary liver cancer (Ueno et al., 1997).

Fungal growth and mycotoxin production result from the interaction among the fungus, host and environment. The appropriate combination of these factors establishes colonisation of a deter-

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mined mycoflora in a substrate and subsequently the group and quantity of mycotoxin produced (Pitt, Basilico, Abarca, & López, 2000). It has been shown that water availability (water activity, a_w) and temperature play an important role in determining the extent of fumonisin and aflatoxin production (Lacey, Ramakrishna, Hamer, Magan, & Marfleet, 1991; Marín et al., 1999). The ideal temperature for growth and mycotoxin production ranges from 22 to 28 °C for *F. verticillioides* strains and 25 to 35 °C for *A. flavus* strains (Lacey et al., 1991). Marín et al. (1999) evaluated FB_1 production on irradiated corn by one isolate of *F. verticillioides* for a_w (0.89–0.97) and temperature (7–37 °C) and reported that no FB_1 was produced at 0.89–0.91a_w regardless of temperature. a_w values for aflatoxin production by *A. flavus* range from 0.95 to 0.99, with a minimum a_w value of 0.82 (ICMSF, 1996).

Natural occurrence of fumonisins and aflatoxins in corn and corn-based products is a worldwide problem (Abbas, Cartwright, Xie, & Shier, 2006; Binder, Tan, Chin, Handl, & Richard, 2007; Castells, Marín, Sanchis, & Ramos, 2008). Fumonisin and aflatoxin contamination in corn and corn-based products has been reported previously in Brazilian states (Kawashima & Valente Soares, 2006; Ono et al., 2001). Kawashima and Valente Soares (2006) analysed 74 samples of corn products from Pernambuco State, Brazil and detected FB_1 and AFB_1 in 94.6% and 6.8% of samples, respectively. Ono et al. (2001) detected fumonisins in 98% and aflatoxins in 11.3% of freshly-harvested corn samples (n = 150) from Paraná State, Brazil. Although corn is the major commodity contaminated by fumonisins, some occurrence has been reported in sorghum and rice (CAST, 2003). In addition to corn, peanuts (groundnuts) and cottonseed are the major crops affected by aflatoxins, but concentrations depend on the environmental conditions during the growing season (CAST, 2003).

Corn and corn-based product consumption by the Brazilian population has increased recently (IBGE, 2008). Taking into account that mycotoxin is a problem difficult to avoid and cannot be removed by industrial processing, monitoring the production chain is essential to assess the risks to which consumers are exposed. Therefore, the aim of this study was to evaluate the co-occurrence of fumonisins and aflatoxins in corn samples from the Northern region of Paraná State, Brazil, used as raw material by processing industries, at two points of the production chain regarded as critical control sites.

2. Material and methods

2.1. Sampling

A total of 300 freshly-harvested corn samples were collected at two points in the production chain from three processing industries, located in Apucarana and Andirá, Northern region of Paraná State, during the February–April 2003 crop and the March–May 2004 crop. Random sampling was performed at the industry delivery points (n = 90) and before the drying process (n = 60) for each crop. Samples collected at the industry delivery points and before the drying process were called reception and pre-drying samples, respectively. Corn kernels were taken randomly from 15 truck bin sites (approximately 650 g from each site) at the industry delivery points and pooled (10 kg), following the sampling protocol established by the Brazilian guidelines (Brasil, 1976). These guidelines establish sampling from 9 to 15 truck bin sites, totalling 5–10 kg depending on the truck bin size. The pre-drying samples were collected from the belt that takes the corn kernels to the drier, i.e., 650 g every 30 min and pooled (10 kg). After homogenisation, 1 kg of each corn sample (laboratory sample) was sent to the laboratory for analysis and maintained at 4 °C for a maximum of 7 days for microbiological analysis. For fumonisin and aflatoxin determination, 200 g of each corn sample were ground to 50 mesh and stored at −20 °C.

2.2. Mycoflora analysis

Two-hundred grams of each corn sample were ground to 50 mesh. Sub-samples (10 g) of ground corn were blended with 90 ml of sterile 0.1% peptone water (v/v), and serial dilutions were carried out with 9.0 ml of the same diluent to 10^{-6}. One millilitre of each dilution was transferred into a Petri dish and pour-plated with potato dextrose agar (PDA, pH 4.0), added to 50 μg/ml chloramphenicol and incubated at 25 °C for 6 days. After total fungal colony count, mould genera were identified, according to Singh, Frisvad, Thrane, and Mathur (1991).

2.3. Fumonisin analysis

Fumonisins B_1 and B_2 were analysed according to Shephard, Sydenham, Thiel, and Gelderblom (1990) with some modification (Ueno et al., 1993). Shephard et al. (1990) used 25 g sub-samples of ground corn mixed with 50 ml methanol:water (3:1, v/v) and Bond-Elut strong anion-exchange (SAX) cartridges for the sample extract clean-up procedure.

2.3.1. Extraction and clean-up

The corn kernels (200 g) were ground to 50 mesh in a laboratory mill, homogenised and sub-samples (10 g) of ground corn were mixed with 30 ml methanol:water (3:1, v/v). After standing for 10 min at room temperature, the suspension was shaken at 150 rpm for 30 min and centrifuged at 4500*g* for 10 min. The crude extract (1.0 ml) was applied to preconditioned Sep-Pak Accell Plus QMA cartridge (Waters Corporation, Milford, MA) with methanol (5 ml) followed by methanol:water (3:1, 5 ml). After washing the cartridge with methanol:water (3:1, 6 ml) followed by methanol (3 ml), fumonisins were eluted with 10 ml methanol containing 0.5% acetic acid. The eluate was evaporated to dryness under a stream of nitrogen at 45 °C.

2.3.2. HPLC analysis

The sample residue was dissolved in methanol–water (3:1, 800 μl) and a 200 μl aliquot dried under nitrogen. After derivatisation with 200 μl *O*-phthaldialdehyde reagent (40 mg OPA, 1 ml methanol, 5 ml 0.1 M sodium borate and 50 μl 2-mercaptoethanol), HPLC injections were made within 1 min. Fumonisins were analysed by a reversed-phase isocratic HPLC system (Shimadzu LC-10 AD pump and RF-10A XL fluorescence detector), using a Shim-pack CLC-ODS (M) column (4.6 × 250 mm, Shimadzu, Osaka, Japan). Excitation and emission wavelengths were 335 and 450 nm, respectively. The eluent was CH_3OH:0.1 M NaH_2PO_4 (80:20, v/v) adjusted to pH 3.3 with orthophosphoric acid. The flow rate was 1 ml/min. Analytical quality was assured by evaluating artificially spiked samples (three repetitions), during routine analyses. The peak identity assigned as FB_1 and FB_2 was confirmed by comparing test chromatograms with standards, with attention to retention, start and end time of peak elution. Samples that showed a peak at the fumonisin retention time were confirmed by adding the standard and reprocessing. The detection limits for FB_1 and FB_2 were 27.5 and 35.3 ng/g, respectively, defined as the minimum amount of toxin that could generate a chromatographic peak five times over the height/noise rate of the baseline. The recoveries of FB_1 and FB_2 from spiked corn in the range 100–400 ng/g FB_1 and 250–450 ng/g FB_2 averaged 95.6% (mean CV 8%) and 96.9% (mean CV 10%), respectively, based on duplicate spiking and duplicate analyses. Corn samples provided by the Agronomic Institute of Paraná (IAPAR) with non-detectable fumonisin levels were used for corn spiking.

2.4. Bright greenish-yellow fluorescence test

An aliquot of 100 g sample was ground and analysed under UV light (365 nm) by the corn processing industry's staff. The number of bright greenish-yellow fluorescence (BGYF) points was counted and samples with three or more points were considered suspect for aflatoxin contamination.

2.5. Aflatoxin determination by thin-layer chromatography (TLC)

The aflatoxin extraction was carried out according to Soares and Rodriguez-Amaya (1989). An aliquot of 50 g sample, previously ground to 20 mesh, was extracted with methanol containing 4% KCl, cleaned up with 30% ammonium sulphate and diatomaceous earth followed by two partitions with chloroform. Aliquots of 5 ml each of first and second chloroform extractions were combined and evaporated to dryness in an 80 °C water bath. The residue was dissolved in 200 µl chloroform and aflatoxins were analysed using silica gel G 60 plates (Merck, Darmstadt, Germany) and toluene:ethyl acetate:chloroform:formic acid (70:50:50:20, v/v/v/v) as developing solvent, according to Gimeno (1979). The mean recovery rates from spiked corn in the range 5–20 µg/kg were 97% for AFB_1, 93% for AFG_1, 93% for AFB_2 and 94% for AFG_2 (mean CV 8.3%) based on duplicate spiking and duplicate analyses. The detection limit was 4 µg/kg.

2.6. Statistical analysis

Differences in mean values of *Fusarium* sp., *Penicillium* sp. and total fungal colony count were evaluated statistically using ANOVA, followed by the Tukey multiple-comparison test ($p < 0.05$). Differences in mean fumonisin levels among the reception and pre-drying samples from the same crop and between the two crops were statistically evaluated using ANOVA, followed by the Tukey multiple-comparison test ($p < 0.05$) and *t*-test ($p < 0.05$), respectively. Statistical analysis was performed by Statistic software, Version 6.0 (Stat Soft, Inc., Tulsa, OK).

3. Results and discussion

The relative frequency of the main fungal genera in 240 freshly-harvested corn samples from 2003 ($n = 150$) and 2004 ($n = 90$) crops is shown in Fig. 1. *Fusarium* sp. was the prevalent genera (100%) for both crops, followed by *Penicillium* sp. which was detected in 97.3% (2003 crop) and 91.1% (2004 crop). *Aspergillus* sp. frequency was lower when compared to *Fusarium* sp. and *Penicillium* sp. and was detected in 37.3% (2003 crop) and 3.3% (2004 crop). Although *Aspergillus* and *Penicillium* were classified as storage fungi based on studies carried out in temperate climates (Christensen & Sauer, 1982), under warm, humid subtropical or tropical climates species of *Aspergillus* and *Penicillium* can infect grains in the field (Wilson & Abramson, 1992).

The high frequency of *Fusarium* sp. and *Penicillium* sp. (Fig. 1) is in accordance with the data reported by Ono et al. (2006) and Almeida et al. (2002). Ono et al. (2006) detected *Fusarium* sp. and *Penicillium* sp. in 100% and 91.6% of corn samples ($n = 24$) from the Northern region of Paraná State, Brazil. On the other hand, Almeida et al. (2002) analysed 90 corn samples from two regions of São Paulo State (Capão Bonito and Ribeirão Preto) and also detected predominance of these fungal genera, but at lower percentages. *Fusarium* sp. and *Penicillium* sp. were detected in 35% and 32% of samples from Capão Bonito and in 49% and 21% of samples from Ribeirão Preto, respectively.

Taking into account that there was no significant difference ($p < 0.05$) in the mean values of total mould and yeast count between reception and pre-drying samples (2003 crop) by the Tukey test, mycoflora analysis in samples from the 2004 crop was carried out only for the reception samples.

As shown in Table 1, *Fusarium* sp. was detected in the range from 10^2 to 10^5 CFU/g in the reception ($n = 90$) and pre-drying samples ($n = 60$) of 2003 crop, as well as in the reception samples ($n = 90$) of the 2004 crop. *Aspergillus* sp. was detected at a lower contamination level (10^2–10^4 CFU/g) in the reception samples, but in the range from 10^2 to 10^5 CFU/g in the pre-drying samples of the 2003 crop, and at a 10^2 range in the 2004 crop.

Fumonisin and aflatoxin levels, as well as the number of bright greenish-yellow fluorescence points in 300 corn samples from the Northern region of Paraná State, are shown in Table 2.

In the 2003 crop, fumonisins were detected in 100% of samples and the levels ranged from 0.08 to 15.32 µg/g (mean 2.54 µg/g) in the reception and from 0.11 to 18.78 µg/g (mean 3.12 µg/g) in the pre-drying samples. Aflatoxins were detected in 8.9% of the reception and 16.7% of the pre-drying samples, at levels ranging from 5.0 to 54.0 µg/kg (mean 24.1 µg/kg) and from 10 to 56 µg/kg (mean 23.4 µg/kg), respectively. On the other hand, in the 2004 crop fumonisins were detected in 98.9% and 95% of the reception and

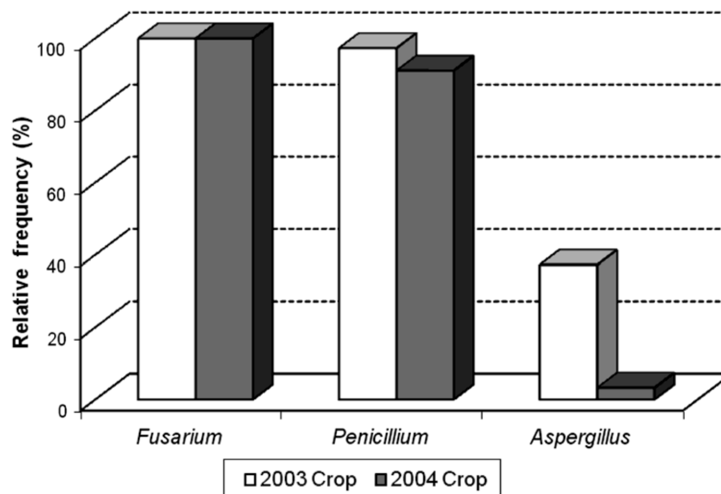

Fig. 1. Relative frequency of the main fungal genera in 240 freshly-harvested corn samples from the 2003 ($n = 150$) and 2004 ($n = 90$) crops from the Northern region of Paraná State.

Table 1
Profile of *Fusarium* sp., *Penicillium* sp., *Aspergillus* sp. and total fungal colony count in freshly-harvested corn samples from the Northern region of Paraná State (2003 and 2004 crops).

Crop	Sampling site	*Fusarium* sp. (CFU/g)		*Penicillium* sp. (CFU/g)		*Aspergillus* sp. (CFU/g)		Total count[c] (CFU/g)	
		Mean[b]	Range	Mean[b]	Range	Mean	Range	Mean[b]	Range
2003	Reception (n = 90)	2.4×10^4 a	1.0×10^2–2.5×10^5	9.3×10^4 a	1.0×10^2–7.0×10^4	1.0×10^3	1.0×10^2–1.5×10^4	1.1×10^5 a	2.3×10^3–1.5×10^6
	Pre-drying (n = 60)	2.4×10^4 a	1.5×10^2–2.0×10^5	1.0×10^4 a	1.0×10^2–8.0×10^4	6.8×10^3	1.0×10^2–1.0×10^5	6.6×10^4 a	2.0×10^3–4.9×10^5
2004	Reception (n = 90)	3.9×10^4 a	8.0×10^2–6.5×10^5	1.1×10^4 a	5.0×10^2–3.0×10^5	4.3×10^2	–	8.7×10^4 a	3.1×10^3–9.6×10^5

[b] Means followed by the same letter are not significantly different by the Tukey test ($p < 0.05$).
[c] Total mould and yeast count.

Table 2
Fumonisin and aflatoxin contamination in 300 freshly-harvested corn samples from the Northern region of Paraná State (2003 and 2004 crops).

Crop	Sampling site	Fumonisins			Aflatoxins				
		Positive samples (%)	Mean[c] (μg/g)	Range (μg/g)	Positive samples (%)	Mean (μg/kg)	Range (μg/kg)	BGYF[e] (%)	
								1 point	2 points
2003	Reception (n = 90)	100	2.54[aA]	0.08–15.32	8.9	24.1	5–54	28.9	1.1
	Pre-drying (n = 60)	100	3.12[aA]	0.11–18.78	16. 7	23.4	10–56	15	ND[f]
2004	Reception (n = 90)	98.9	1.31[bB]	0.07–18.16	1.1	40.0[d]	–	ND	ND
	Pre-drying (n = 60)	95	1.36[bB]	0.06–6.28	8.3	35.2	12–52	ND	ND

[c] Means followed by the same lowercase letter are not significantly different by the Tukey test ($p < 0.05$) for each crop. Means followed by the same uppercase letter are not significantly different by the t-test ($p < 0.05$) between crops.
[d] Detected in one sample.
[e] BGYF, bright greenish-yellow fluorescence.
[f] ND, not detected.

Table 3
Time elapsed, mean temperature and moisture content of corn samples from the Northern region of Paraná State before sampling at the industry delivery points and before the drying process (2003 and 2004 crops).

Site	2003 Crop				2004 Crop			
	Time elapsed (h)	Mean temperature (°C)	MC[a] (%)		Time elapsed (h)	Mean temperature (°C)	MC[a] (%)	
			Range	Mean			Range	Mean
Before sampling	0.85–2.25	34.5[b]	16.0–24.0	20.6	0.88–2.83	31.0[b]	14.3–20.2	17.0
Before the drying process	2.07–6.64	30.6[c]	16.5–27.7	20.7	1.62–6.16	30.6[c]	17.0–22.2	18.7

[a] MC, moisture content.
[b] In the truck bin.
[c] At the receiving pit.

pre-drying samples, respectively. The levels ranged from 0.07 to 18.16 μg/g (mean 1.31 μg/g) in the reception, and from 0.06 to 6.28 μg/g (mean 1.36 μg/g) in the pre-drying samples. Mean fumonisin levels showed no significant difference ($p < 0.05$) in the samples from the reception and pre-drying points by the Tukey test for both crops. However, fumonisin levels in samples from the 2004 crop were significantly lower than those from the 2003 crop ($p < 0.05$). Aflatoxins were detected in 1.1% of the reception (40 μg/kg) and in 8.3% of the pre-drying samples at levels ranging from 12 to 52 μg/kg (mean 35.2 μg/kg). The time elapsed, mean temperature and moisture content of the grains before sampling at the industry delivery points and before the drying process are shown in Table 3. Corn samples were unloaded and stored at the receiving pit for a short period of time, i.e., 2.07–6.64 h (2003 crop) and from 1.62 to 6.16 h (2004 crop) and the grain mean moisture content was 20.7% (2003 crop) and 18.7% (2004 crop) before the drying process (Table 3). Blandino, Reyneri, Vanara, and Ferreo (2004) reported a significant increase in fumonisins (77%) when corn kernels with 25% moisture content (a_w = 0.98) were kept for 7 days between harvesting and drying, indicating the importance of an efficient drying process in order to control mycotoxin contamination.

Fumonisin and aflatoxin levels (Table 2) were lower than those reported by Ono et al. (2001) and Abbas et al. (2006). Ono et al. (2001) evaluated the natural co-occurrence of fumonisins and aflatoxins in 150 freshly-harvested corn samples from the Central-Southern (n = 27), Central-Western (n = 86) and Northern (n = 37) regions of Paraná State, Brazil. Fumonisins were detected in 147 (98%) samples at levels ranging from 0.096 to 22.6 μg/g, while aflatoxins were detected in 17 (11.3%) samples. All the aflatoxin positive samples (range 38.0–460.0 μg/kg) came from the Central-Western region and were co-contaminated with fumonisins. Abbas et al. (2006) detected fumonisins and aflatoxins in 100% of corn samples (n = 65) from the USA, at levels ranging from 22 to 86 μg/g and from 21 to 699 μg/kg, respectively.

The corn-milling industry has widely used the presence of bright greenish-yellow fluorescence (BGYF) under an ultraviolet (UV) light as a presumptive indicator of aflatoxin. As shown in Table 2, in 28.9% and 1.1% of the reception samples one and two BGYF points were detected, respectively, whereas 15% of the pre-drying samples showed one BGYF point (2003 crop). Samples from the 2004 crop showed no BGYF point. According to the corn processing industry criteria, all the samples were adequate for human and

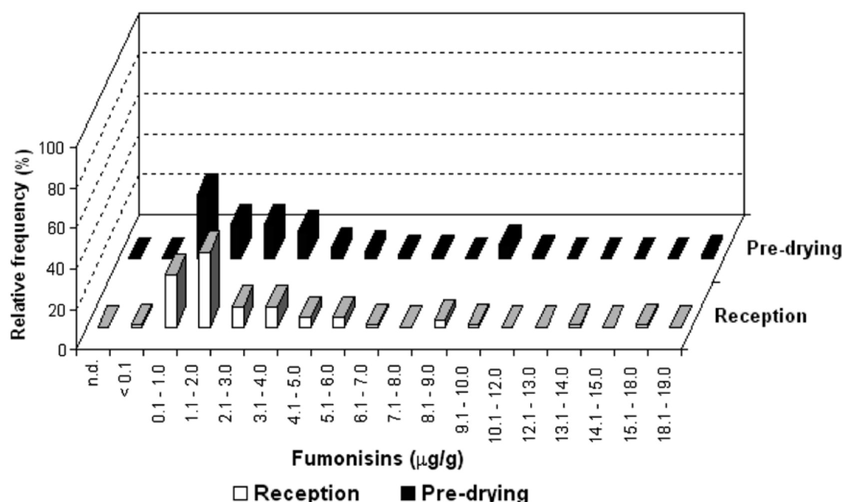

Fig. 2. Distribution of fumonisin ($FB_1 + FB_2$) levels in corn samples from the reception ($n = 90$) and pre-drying ($n = 60$) points from the Northern region of Paraná State (2003 crop).

animal consumption. These results were not in accordance with TLC analysis which detected aflatoxins above the maximum tolerated limit (20 μg/kg) in seven samples, considering both crops. The BGYF method is not quantitative, nor is it a direct detection of aflatoxin itself because the fluorescence is produced by the oxidative action of peroxidases in living plant tissue on kojic acid, which is formed with aflatoxin by *A. flavus* (Marsh et al., 1969). Out of 300 samples analysed, 13 (4.3%) did not show fluorescent points under UV light, although aflatoxins were detected by TLC. and 25 samples (8.3%) showed one or two BGYF points, although aflatoxins were not detected by TLC (data not shown). These results suggest the use of the BGYF test only as a screening method for detecting suspect lots of grains that should be tested further for aflatoxin by more sensitive methods. Glória, Fonseca, and Souza (1998) reported 14 false-negative results out of 286 corn samples analysed by the BGYF test, when compared to TLC whose contamination levels were >20 μg/kg. Thompson and Henke (2000) reported no relationship between the number and weight of fluorescing corn kernels and aflatoxin levels. Interfering compounds in corn matrix, when excited by UV light, can absorb and emit fluorescent light with wavelengths similar to aflatoxins, yielding false-positive results. In addition, the mycotoxin can occupy an inner region of the kernel, impairing detection by this method, in intact corn kernels (Shotwell, Goulden, & Hesseltine, 1974), causing false-negative results. Shotwell and Hesseltine (1981) recommended grinding the sample as an alternative, to reduce false-negative results.

Determination of the exposure degree is one of the most important parameters of the risk assessment of chemical compounds. Although the legal limits for fumonisins have not been established, the Food and Drug Administration (FDA) recommends a maximum limit of 2.0 and 4.0 μg/g for corn bran and pasta production, respectively, intended for human consumption (Avantaggiato, Quaranta, Desiderio, & Visconti, 2003).

Taking into account the results obtained in this study and the food consumption estimates in Brazil (IBGE, 2008), which reports

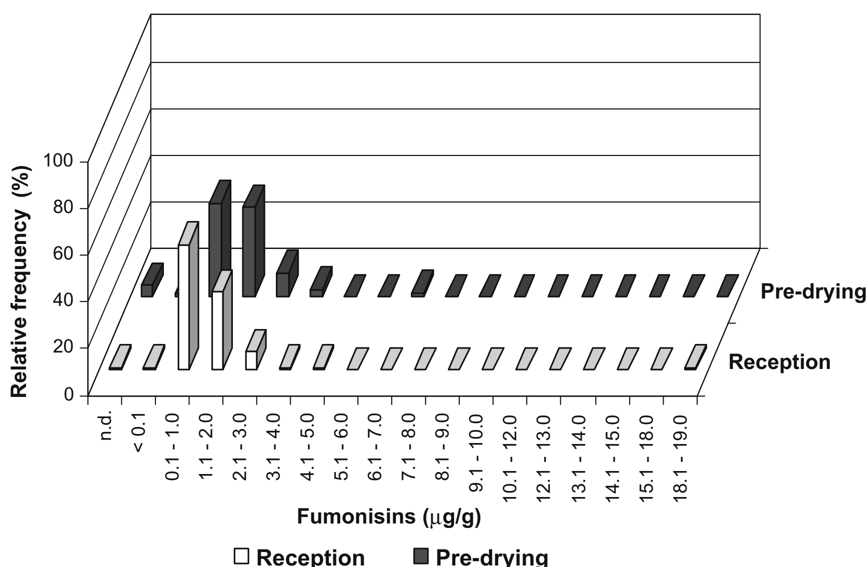

Fig. 3. Distribution of fumonisin ($FB_1 + FB_2$) levels in corn samples from the reception ($n = 90$) and pre-drying ($n = 60$) points from the Northern region of Paraná State (2004 crop).

that the average consumption of corn-based products is 21.38 g per day per person, the probable daily intake for fumonisins can be calculated. Considering the highest mean fumonisin levels (Table 2) which were detected in the pre-drying samples (3.12 μg/g) the total fumonisin consumption would be 66.7 μg/g, which would lead to a maximum probable daily intake (PDI_M) value of 0.95 μg fumonisin/kg body weight (bw)/day (as considered for 70-kg bw individuals). Moreover, considering 4.0 μg/g as the maximum fumonisin level recommended for pasta production, the maximum probable daily intake (PDI_M) would be 1.2 μg fumonisin/kg bw/day, i.e., 125 samples (83.3%) from the 2003 crop (Fig. 2) and 147 samples (98%) from the 2004 crop (Fig. 3) were safe for human consumption. The PDI_M values estimated for the Brazilian population are below the tolerable daily intake (TDI), equal to 2.0 μg/kg bw/day for FB_1, as proposed by the Joint FAO/WHO Expert Committee on Food Additives (WHO, 2002).

In Brazil the maximum limit established for the sum of aflatoxins ($B_1 + B_2 + G_1 + G_2$) in corn and corn-based products is 20 μg/kg according to the Resolution GMC No. 25/02 for MERCOSUL (ANVISA, 2008). In 287 of the 300 samples analysed (95.7%) aflatoxin levels were below the maximum limit established by the Brazilian guidelines. Moreover, aflatoxins were not detected in 92% of the samples.

In summary, only 8% of the corn samples ($n = 300$) from the Northern region of Paraná State showed co-contamination by fumonisins and aflatoxins and low levels of these mycotoxins were detected in most samples used as raw material by processing industries. Nevertheless, constant monitoring throughout the corn production chain is necessary, in order to comply with trade requirements and to minimise health risks.

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