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Natural occurrence of deoxynivalenol in wheat from Paraná State, Brazil and estimated daily intake by wheat products

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ABSTRACT

The occurrence of deoxynivalenol (DON) was evaluated in 113 wheat samples from the northern and central/southwestern regions of Paraná State, Brazil during the 2008 and 2009 growing seasons, and this rate of occurrence was used to estimate the DON dietary exposure. The DON determination was carried out by an indirect competitive enzyme-linked immunosorbent assay. DON was detected in 66.4% samples at levels ranging from 206.3 to 4732.3 μg/kg (mean 1894.9 μg/kg). The estimated daily intake (EDI) of DON through bread and pasta was evaluated in the inhabitants of Londrina City in northern Paraná State, Brazil. The average intake of these inhabitants was 0.79 μg/kg body weight (b.w.) for bread and 0.35 μg/kg b.w. for pasta. The total EDI was 1.13 μg/kg, which is above the Provisional Tolerable Daily Maximum Intake (PTDMI) of 1 μg/kg b.w. To our knowledge, this is the first report on natural DON occurrence in wheat and DON dietary exposure estimation from Paraná, Brazil.

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the major staple foods worldwide, accounting for 20% of cultivated land worldwide. Annual global production is estimated at 500 million tons, and the main producers are Russia, Ukraine, the United States, China, India and France (International Grains Council, 2009).

Southern Brazil accounts for 94% of Brazilian wheat production (5.9 million tons), and the main producing states are Paraná (56%) and Rio Grande do Sul (33%, CONAB, 2012). However, current domestic production has been unable to supply the 10 million ton annual national demand for wheat grain. For this reason, Brazil must import wheat grain to meet domestic demand. Last year, Brazil imported approximately 5.7 million tons of wheat grain, mainly from Argentina (CONAB 2012). Such dependence requires careful monitoring and food safety control primarily because undesirable toxic metabolites produced by toxigenic fungi frequently contaminate this commodity in the initial food producing stage in the field.

Fusarium sp. is a phytopathogen that produces mycotoxins and causes *Fusarium* Head Blight (FHB). Among *Fusarium* species, *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Scw.) Petch] frequently contaminates wheat and is associated with trichothecene production. Trichothecenes are potent inhibitors of eukaryotic protein synthesis, interfering at the initiation, elongation, and termination stages. Deoxynivalenol (DON; 12,13-epoxy-3α,7α,15-trihydoxytrichotecec-9-en-8one) is a type B trichothecene, classified by the International Agency for Research on Cancer in group 3 as "not classifiable as to its carcinogenicity to humans" (IARC, 1993). Damage from FHB includes reduction in seed quality, contamination with several mycotoxins (mostly DON), reduced yield, and the formation of discolored and shriveled kernels. Typically, a salmon-pink to red fungal growth with sporodochia may be observed along the edge of the glumes or at the base of the spikelet (Tomczak, Wisniewska, Stepien, Kostecki, Chelkowski, & Golinski, 2002).

A worldwide incidence of *Fusarium* toxins has been reported in cereals and frequent contamination can be expected in grain-based products (Bensassi, Zaied, Abid, Hajlaoui, & Bacha, 2010; Calori-Domingues, Almeida, Tomiwaka, Gallo, Gloria & Dias, 2007; Pinto, Terminiello, Basilico, & Ritieni, 2008; Schollenberger et al., 2006).

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Therefore, effective monitoring should be undertaken and achieved through reliable and rapid analysis. Increased efforts have been made to develop analytical methods suitable for rapid mycotoxin screening, such as enzyme-linked immunosorbent assay (ELISA) kits, which have been shown to have certain advantages. These kits do not require specialised analysts, and provide rapid and sensitive detection; they can thus be used by small laboratories and under field conditions. Researchers have reported the use of ELISA-based methods for DON screening in beer (Kostelanska et al., 2009) and in wheat (Yoshizawa et al., 2004).

Dietary ingestion is the main route for human exposure to DON, as this toxin is frequently detected in agricultural commodities for human consumption such as wheat, rye, barley, oats, and other cereals (Sudakin, 2003). The Joint FAO/WHO Expert Committee on Food Additives established a Provisional Maximum Tolerable Daily Intake (PMTDI) of DON at 1 μg/kg body weight (b.w.)/day, although the committee acknowledged that considerable uncertainty exists in estimates of dietary intake (JECFA, 2001). In addition, experts on pollutant-risk assessment consider mycotoxins to be the most important chronic dietary risk factor, more important than synthetic contaminants, plant toxins, food additives or pesticide residues (Kuiper-Goodman, 1995).

The objective of this study was to evaluate the natural occurrence of DON in wheat from Paraná State, Brazil and the contribution of wheat in dietary DON exposure to consumers.

2. Material and methods

2.1. Safety note

Deoxynivalenol is cytotoxic and should be handled with extreme care. Mycotoxin-contaminated material should be decontaminated with an aqueous solution of sodium hypochlorite (5%).

2.2. Characterisation of the studied area

The State of Paraná is located in the southern Brazil and comprises an area of 199,570 km^2, bisected by the Tropic of Capricorn. The predominant climate is subtropical with humid temperate weather, having an average temperature of >22 °C during the hottest and <18 °C during the coldest months. The southwestern region (25°S, 53°W, altitude 510 m) has an annual average temperature of 19 °C and total rainfall of 2100 mm. The centre region (25°S, 50°W, altitude 975 m) is characterised by colder weather (18 °C) and average annual rainfall of 1580 mm. The northern region (23°S, 51°W, altitude 610 m) has an annual average temperature of 21 °C and rainfall of 1590 mm (IAPAR, 2012).

2.3. Sampling

A total of 113 wheat (*Triticum aestivum* L.) samples from Paraná State were collected in the southwestern, centre, and northern Paraná regions during the growing seasons of 2008 and 2009. The sampling protocol followed Brazilian guidelines (Brasil, 2001). After homogenisation, 1 kg of the wheat samples was sent to the laboratory in paper bags placed in coolbox, ground to a fineness of 20 mesh in a laboratory mill (A11-Ika, Germany) and stored at 4 °C for a maximum of 10 days until DON analysis.

2.4. DON analysis

The DON levels were determined by indirect competitive-ELISA (ic-ELISA) using an anti-DON.3 monoclonal antibody (mAb) produced by DON.3 hybridoma cell culture, as described by Kawamura (2005).

Measured ground wheat samples (5 g) were extracted with 40 ml of a methanol and water mixture (70:30, respectively, v/v) at 150 rpm for 30 min. After centrifugation at 800×*g* for 5 min, the supernatant was maintained at −20 °C overnight, then centrifuged (2250×*g*/5 min) again. Two aliquots of 400 μl were dried under nitrogen stream at 40 °C and stored at −20 °C until analysis. As described by Santos et al. (2011), ic-ELISA was conducted. Polystyrene microtiter plate wells (Corning, New York, USA) were coated with 100 μl of DON-HG-BSA (DON-hemiglutarate-bovine serum albumin, 2.0 μg/ml) in 0.2 M carbonate/bicarbonate buffer pH 9.6 (4 °C/overnight). The microplate was washed with PBST (PBS with 0.05% Tween 20, 5 times), and nonspecific binding was blocked with 200 μl PBS-ovalbumine 0.1% (37 °C/1 h). The microplate was then washed (5 times), 50 μl DON standard or diluted wheat extract samples and 50 μl anti-DON.3 mAb (1.25 mg/ml) were added, and the incubation was carried out at 37 °C for 1 h. After washing five times with PBST, 100 μl horseradish peroxidase-labelled goat anti-mouse IgG were added, incubated at 25 °C for 1 h, and washed as previously described. Then, 100 μl TMB (3,3′,5,5′-tetramethylbenzidine; Sigma, St. Louis, USA) of a substrate solution was added. After 20 min at 25 °C, the reaction was stopped by adding 50 μl 1 M H_2SO_4, and absorption was estimated at 450 nm (Expert Plus, Asys, Cambridge, United Kingdom). The average absorbance was calculated from individual absorbance measurements obtained from duplicate wells and the results were expressed as a percentage of binding:

$$\text{Binding}(\%) = A^+/A^- \times 100$$

where A^+ is the mean absorbance in the presence of DON standard or wheat extract sample and A^- is the mean absorbance in the absence of the same. The DON concentration in the samples was calculated by plotting the percentage of binding against the log of the DON amount. A method blank was prepared to verify that none of the solvents, reagents, or instrumentation added any detectable positive biases to the toxin concentrations.

The ic-ELISA was previously standardised and validated (Santos et al., 2011). The limit of detection (LOD) of DON was 22.1 ng/ml (corresponding to 177.1 μg/kg), calculated as the mean minus 3-fold the standard deviation of absorbance from three replicate wells of zero standard (0 ng DON/well), and the (LOD) was significantly lower than current regulatory limits for DON control in Brazil and the European Community. The DON recovery from the spiked wheat at 350, 750 and 1750 μg/kg DON averaged 108.4% (with a mean RSD of 13.9%), based on duplicate spiking and triplicate analysis. Wheat samples with non-detectable DON (<140 μg/kg by liquid chromatography–mass spectrometry, LC–MS) were used for wheat-spiking. The ic-ELISA showed a good correlation coefficient (r = 0.93) compared to LC–MS.

2.5. DON exposure estimation

The intake of wheat products in Londrina City, in northern Paraná State, Brazil was evaluated by applying a Food Frequency Questionnaire (FFQ) to a random sample of 260 individuals out of a total of 447,000 inhabitants. The FFQ was designed to collect semi-quantitative information about the intake of wheat products and general information creating individual profiles. A commercial unit of French bread (50 g) and a portion of pasta (100 g) were used as portion size references to facilitate information about the product portions consumed. Using these portion sizes, subjects were asked to express the frequency of wheat product consumption as twice a day, once a day, five to six times a week, three to four times a week, once or twice a week, twice a month, or no intake. Questions about age, height and weight status were asked to define

the individual profiles. Estimated Daily Intake (EDI, μg/kg body weight/day) was calculated according to the following formula:

$$\text{Estimate of DON intake}(\mu g/\text{kg b.w./day}) = \frac{\text{DON concentration} \times \text{Food intake}}{\text{Individual body weight}}$$

Data for bread and pasta contamination were estimated from the contamination evaluated for wheat grain. The extraction rate for ground wheat to yield flour was assumed to be approximately 70%. Bread and pasta content in wheat flour is around 60 and 70 g%, respectively (Carvalho & Romano, 1997). In this study, DON contamination obtained from 113 samples from Paraná State was evaluated, and values below the detection limit were assumed to be ½ LOD, according to the recommendation of IPCS/GEMS (1995) criteria adopted to estimate trichothecene contamination when values less than the LOD were observed. The following criteria were used: first, when all observations were over the LOD then the true mean was calculated; second, when the proportion of observations less than LOD was lower than or equal to 60%, the mean was calculated by replacing those observations with LOD/2; and third, when the proportion was over 60%, two estimates were informed, the first by replacing those observations with 0 and the second by replacing them with the LOD. In the present study, less than 60% of observations were lower than the LOD, and the mean was calculated by replacing those observations with LOD/2.

2.6. Statistical analysis

The DON levels in different wheat-producing regions, growing seasons, and EDI according to sex were compared by the Mann–Whitney test. The analyses of Kruskal–Wallis were used to evaluate differences among the EDI according to the categories of consumption and age. Differences were considered to be significant at $p < 0.05$.

3. Results and discussion

Table 1 shows the natural occurrence of DON in wheat grains analysed by ic-ELISA in 113 wheat samples from Paraná State, Brazil. DON was detected in 66.4% samples at levels ranging from 206.3 to 4732.3 μg/kg, with a mean of 1894.9 μg/kg. These levels (Table 1) were lower than those reported by Bensassi et al. (2010) and Pinto et al. (2008), but higher than those obtained by Calori-Domingues et al. (2007) and Schollenberger et al. (2006). Bensassi et al. (2010) reported levels ranging from 7200 to 54000 μg/kg in wheat samples from Tunisia (83% positivity; $n = 65$). In Argentinean wheat samples (78.9% positivity, $n = 19$), Pinto et al. (2008) detected levels ranging from 300 to 70000 μg/kg. Calori-Domingues et al. (2007) detected lower DON levels in Brazilian wheat (332 μg/kg) but with a higher incidence (94% positivity; $n = 50$), while in wheat ($n = 50$) imported from Argentina and Paraguay, these levels ranged from 30 to 349 μg/kg (46% positivity). In Germany, Schollenberger et al. (2006) detected DON in 95% of samples ($n = 41$), with a mean level in positive samples of 309 μg/kg.

As shown in Table 1, DON was detected in 75% samples from the central/southwestern region at levels ranging from 206.3 to 4651.3 μg/kg (mean 1689.8 μg/kg), and DON was detected in 61.6% samples from the northern region at levels ranging from 222.6 to 4732.3 μg/kg (mean 2031.4 μg/kg). No significant difference was observed ($p > 0.05$) by the Mann–Whitney Test in the mean DON levels between the two regions investigated. Paraná State accounts for approximately 56% of the national wheat production, and several municipalities from the northern region are among the main national producers.

Table 2 shows the natural occurrence of DON in the 2008 and 2009 growing seasons. According to the Mann–Whitney Test, no significant difference ($p > 0.05$) was found in mean DON levels from the two growing seasons. DON was detected in 61.9% of samples from the 2008 growing season, with levels ranging from 206.3 to 4651.3 μg/kg (mean of 1750.8 μg/kg), whereas DON was detected in 72% of samples from the 2009 season (2051.1 μg/kg), with levels ranging from 300.3 to 4732.3 μg/kg (Fig. 1). DON levels from the 2008 and 2009 growing seasons (Table 2) were higher than those observed in the 2006 and 2007 growing seasons (8 non-detected samples in 2006, and 1 positive sample in 2007 over 7 samples; unpublished data) due to an outbreak of FHB that occurred in these years in northern Paraná State. In 2009, cultivation was delayed because the region received atypical rainfall during April, the month that wheat cultivation usually begins in Paraná. From June to August of that year, the region received approximately 400 mm of rainfall, above the historical average of approximately 200 mm (IAPAR, 2009). A relationship between FHB outbreaks and wet conditions during flowering has been previously reported (Pan, Bonsignore, Rivas, Perera, & Bettucci, 2007). In a study conducted in Rio Grande do Sul State (Brazil), Del Ponte, Garda-Buffon, and Badiale-Furlong (2012) revealed higher DON levels in the growing seasons of 2007 and 2008 than in 2006. The authors emphasised that a higher prevalence of DON may be partially related to the higher risk of FHB epidemics during those years. Stanković et al. (2012) observed higher DON levels in wheat from Serbia in 2005 (605.5 μg/kg) when compared to the 2007 growing season (282.8 μg/kg) and suggested that rainfall at the time of flowering was the decisive factor.

The maximum DON level tolerated by the Commission of The European Communities (2007) for unprocessed durum wheat is set at 1750 μg/kg. In Brazil, a specific guideline for DON was recently published by the Health Surveillance Agency (ANVISA; Brasil, 2011). The limits in whole wheat grains and whole wheat flour are set at 2000 μg/kg and 1750 μg/kg to wheat flour. Under the maximum limits proposed by the Brazilian Health Surveillance Agency for whole wheat grains, 71 samples (62.8%) were safe for human consumption (Fig. 1). The limit will be decreased over time to allow grain producers and the industry to adapt to the

Table 1
Deoxynivalenol levels in wheat grains from northern and central/southwestern regions of Paraná State, Brazil (2008 and 2009 growing seasons) evaluated by ic-ELISA.

Region	DON (μg/kg)				
	n	Positive samples			
		n (%)	Mean[1]	Median	Range
Northern	73	45 (61.6)	2031.4[a]	1941.2	222.6–4732.3
Central/Southwestern	40	30 (75.0)	1689.8[a]	1501.1	206.3–4651.3
All samples	113	75 (66.4)	1894.9	1885.2	206.3–4732.3

[1] Values within the same column that have the same letter are not significantly different by the Mann–Whitney Test ($p > 0.05$).

Table 2
Deoxynivalenol levels in wheat samples grains by ic-ELISA from Paraná State, Brazil in 2008 and 2009 growing seasons.

Growing seasons	DON (μg/kg)					
	n	Positive samples				Total rainfall (mm)[2]
		n (%)	Mean[1]	Median	Range	
2008	63	39 (61.9)	1750.8[a]	1633.4	206.3–4651.3	701.0
2009	50	36 (72.0)	2051.1[a]	1928.9	300.3–4732.3	680.5

[1] Values within the same column that have the same letter are not significantly different by the Mann–Whitney Test ($p > 0.05$).
[2] Total rainfall data considering the months from planting to harvesting period (April–September). Source: Agricultural Research Institute of Paraná (IAPAR, 2012).

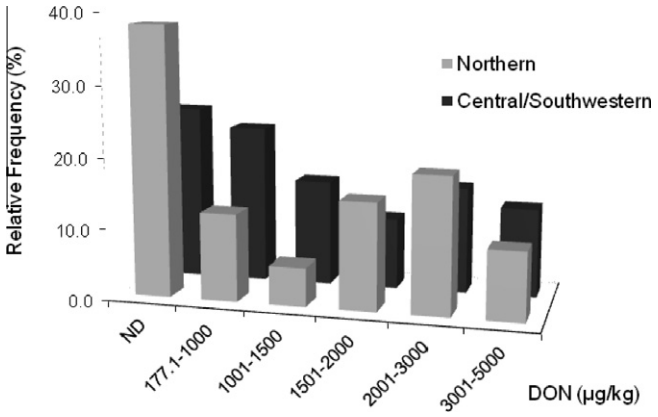

Fig. 1. Distribution of deoxynivalenol (DON) levels in wheat samples ($n = 113$) from Paraná State, Brazil (2008 and 2009 growing seasons) evaluated by ic-ELISA.

legislation without causing a shortage of wheat. From January 2016, DON limits for whole wheat grain will be set at 1000 μg/kg, and at 750 μg/kg for wheat flour (Brasil, 2011).

Determination of the exposure degree is one of the most important parameters for the assessment of risk from chemical compounds. In our study, the consumption of wheat products by individuals ($n = 260$) in Londrina, northern Paraná State, Brazil was surveyed. The individuals ranged from 8 to 76 years-old in age, with a mean age of 31.2 years-old. The individuals ranged in height from 1.32 to 1.94 m (mean 1.67 m) and in weight from 32 to 120 kg (mean 65 kg). Among these surveyed individuals, 89.2% and 91.3% consumed bread and pasta at least once a month, respectively. Londrina inhabitants consumed an average of 21.4 g and 39.3 g of pasta and bread per day, respectively. The Estimated Daily Intake (EDI) of DON from bread and pasta (Table 3) was calculated from the average concentration of DON in wheat grains, considering the samples below LOD to be LOD/2, extraction rate for ground wheat to yield flour, wheat flour content for bread and pasta production, the wheat product consumption, and the individual body weight of the surveyed individuals.

There are no indications for carcinogenic and/or mutagenic properties of DON. Thus, the evaluation can be based on a NOAEL (No Observed Adverse Effect Level) from the toxicity studies, such as chronic dietary studies with mice (0.1 mg/kg b.w./day) applying an uncertainty factor of 100. A Provisional Tolerable Daily Maximum Intake (PTDMI) of 1 μg/kg b.w. was determined. The PTDMI is temporary because DON belongs to the group of several trichothecenes with a common basic chemical structure that is produced by *Fusarium* sp. (e.g., T-2 toxin, HT-2 toxin, nivalenol). They are also believed to share common mechanisms of toxic action (Anonymous, 1999).

After accounting for the DON contamination in samples from Paraná State, the EDI of DON in Londrina was 0.79 μg/kg b.w./day for bread consumption and 0.35 μg/kg b.w./day for pasta consumption (Table 3). After calculating the EDI for these two foodstuffs, the mean value was 1.13 μg/kg/day, corresponding to 113% of the PTMDI, ranging from zero to 5.09 μg/kg b.w./day (Table 3).

There was no significant difference ($p > 0.05$) in the mean total EDI values for males (1.09 μg/kg b.w./day) and females (1.15 μg/kg b.w./day) (Table 3). A significant difference ($p < 0.05$) was observed in EDI between high (2.13 μg/kg b.w./day), medium (0.89 μg/kg b.w./day,) and low bread consumers (0.17 μg/kg b.w./day, Table 3). The EDI for high (2.46 μg/kg b.w./day) and medium (1.39 μg/kg b.w./day) was higher than for low pasta consumers

Table 3
Chronic dietary exposure assessment of deoxynivalenol in Londrina, PR, Brazil from the consumption of wheat-based products.

Source		Estimated daily intake (μg/kg b.w./day)			
		Total[1]		Bread[1]	Pasta[1]
		Mean	Range		
Gender	Male	1.09[a]	0–5.09	0.72[a]	0.38[a]
	Female	1.15[a]	0–3.95	0.83[a]	0.33[a]
Consumption[2]	HC			2.13[A]	2.46[A]
	MC			0.89[B]	1.39[A]
	LC			0.17[C]	0.32[B]
Age (years)	<20	1.35[α]	0–5.09	0.96[α,β]	0.39[α]
	21–30	1.08[α]	0–3.61	0.72[α,β]	0.36[α]
	31–40	1.27[α]	0.09–2.56	1.03[α]	0.25[α]
	41–50	1.02[α]	0–3.37	0.73[α,β]	0.31[α]
	>51	0.87[α]	0.04–2.52	0.51[β]	0.38[α]
Total		1.13	0–5.09	0.79	0.35

[1] Values within the same column that have no common superscript are significantly different by the Mann–Whitney or Kruskal–Wallis Test ($p < 0.05$).
[2] Individuals were divided into categories according to their consumption of bread and pasta. HC = High Consumers (indicated ingestion of the item twice a day; bread – 700 g/week; pasta 1400 g/week); MC = Medium Consumers (indicated to ingest bread between 175 and 350 g/week and pasta between 550 and 700 g/week); LC = Low Consumers (indicated to ingest bread between 12.5 and 75 g/week and pasta between 25 and 350 g/week).

(0.32 μg/kg b.w./day, $p < 0.05$, Table 3). As for age groupings, individuals older than 51 years old (0.51 μg/kg b.w./day) showed lower EDI for bread than the other groups ($p < 0.05$). The mean EDI by age groups for pasta (0.25–0.39 μg/kg b.w./day) and the total EDI (0.87–1.35 μg/kg b.w./day) showed no significant differences ($p > 0.05$, Table 3).

The DON levels in wheat products may be affected by some processing, such as sorting, milling, cooking, baking, frying, roasting and extrusion. In a preliminary study, 38 wheat grain and 16 wheat flour samples (2009 growing season) from Northern Paraná State were analysed by ic-ELISA (unpublished data). The mean DON levels in wheat flour samples (1801 μg/kg) were lower than those from wheat grain samples (2386 μg/kg). These data indicated a DON reduction rate of 25% after milling processing, which was similar to those reported by Visconti, Haidukowski, Pascale, and Silvestri (2004) and Trigo-Stockli, Deyoe, Satumbaga, and Pedersen (1996). DON is more prevalent in the outer parts of kernel, showing higher levels in bran (3400 μg/kg) when compared to whole wheat (2800 μg/kg) and flour (1500 μg/kg, Trigo-Stockli et al., 1996). The higher level of toxin in the bran may be attributed to the prevalence of the *F. graminearum* in the aleurone and pericarp tissues. For this reason, milling usually results in DON reduction in wheat flour. Visconti et al. (2004) observed a reduction of DON during durum wheat processing and spaghetti cooking. Neira, Pacin, Martinez, Molto, and Resnik (1997) reported 44% reduction in DON contamination with increased baking time of DON-contaminated products. These previous studies noted the effect of wheat processing on DON levels and consideration of these changes would provide a more realistic situation for DON occurrence in wheat-based products "as consumed" and for their corresponding intakes. Taking into account the DON reduction rate of 25%, the total EDI by Londrina inhabitants considering bread and pasta consumption would be 1.09 μg/kg b.w./day, similar to that previously calculated. To the best of our knowledge, ours is the first report about the estimated DON daily intake.

In the United Kingdom, Turner et al. (2008) detected DON in 296 out of 300 (98.7%) urine samples. Wholemeal bread was associated with the greatest percentage increase in urinary DON per unit of consumption, but white bread contributed approximately twice as much as wholemeal bread to the urinary DON levels because it was consumed in higher amounts. Schothorst and Van Egmond (2004) reported that wheat and wheat-derived products represent the major source of intake for DON and other trichothecenes.

This study provides information on deoxynivalenol contamination in wheat in the main production areas in Paraná, Brazil during the 2008 and 2009 growing seasons. In summary, 62.8% of wheat samples ($n = 113$) from the Paraná State showed DON levels below the maximum allowed level for human consumption (2000 μg/kg) by current Brazilian legislation (Brasil, 2011). As natural and unavoidable contaminants of important agricultural commodities, DON exerts great impact on human and animal health. Exposure assessment is a valuable tool in assessing risk to humans from mycotoxins in the food chain. Constant monitoring of the effect of wheat processing on mycotoxin levels is necessary to minimise health risks.

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References

Anonymous. (1999). *Opinion on Fusarium toxins. Part 1: Deoxynivalenol (DON) Scientific Committee on Food*. SCF/CS/CNTM/MYC/19 Final. 09/12/99. Annex VI to the minutes of the 119th Plenary meeting.

Bensassi, F., Zaied, C., Abid, S., Hajlaoui, M. R., & Bacha, H. (2010). Occurrence of deoxynivalenol in durum wheat in Tunisia. *Food Control, 21*, 281–285.

Brasil. (2001). Instrução Normativa no. 7, de 15 de agosto de 2001. Regulamento Técnico de Identidade e Qualidade do Trigo. Diário Oficial da União, 21 de agosto de 2001.

Brasil. (2011). Agência Nacional de Vigilância Sanitária. Resolução RDC no. 7, de 18 de fevereiro de 2011. Dispõe sobre limites máximos tolerados (LMT) para micotoxinas em alimentos. Diário Oficial da União, 22 de fevereiro de 2011.

Calori-Domingues, M. A., Almeida, R. R., Tomiwaka, M. M., Gallo, C. R., Gloria, E. M., & Dias, C. T. S. (2007). Ocorrência de desoxinivalenol em trigo nacional e importado utilizado no Brasil. *Ciência e Tecnologia de Alimentos, 27*(1), 181–185.

Carvalho D. & Romano, A. L. (1997). *Tratamento e tipificação de farinhas de trigo*. Curitiba: Granotec do Brasil, p. 52.

Commission of The European Communities. (2007). Commission Regulation (EC) No. 1126/2007 of 28 September 2007, amending Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products.

CONAB. (2012). *Companhia Nacional de Abastecimento. Indicadores agropecuários*. Available from: <www.conab.gov.com>.

Del Ponte, E. M., Garda-Buffon, J., & Badiale-Furlong, E. (2012). Deoxynivalenol and nivalenol in commercial wheat grain related to *Fusarium* head blight epidemics in southern Brazil. *Food Chemistry, 132*(2), 1087–1091.

IAPAR. (2009). *Instituto Agronômico do Paraná. Nota técnica: Excesso de chuvas causa perdas na safra de trigo*. Available from: <www.iapar.br>.

IAPAR. (2012). *Instituto Agronômico do Paraná. Cartas climáticas do Paraná*. Available from: <www.iapar.br>.

IARC. (1993). *International Agency For Research On Cancer. Monographs on the evaluation of carcinogenic risks to humans, some naturally occurring substances, food items and constituents. Heterocyclic aromatic amines and mycotoxins* (Vol. 56, pp. 397–444). Lyon, France: World Health Organization.

International Grains Council. (2009). *Grain market*, Report No. 393, 24 September 2009.

IPCS/GEMS. (1995). International Programme on Chemical Safety. *Food Euro workshop on reliable evaluation of low level contamination of food*, Appendix 5, Kulmbach, Germany, May 1995.

JECFA. (2001). Joint FAO/WHO Expert Committee Food Additives. *Safety evaluation of certain mycotoxins in food, prepared by the fifty-sixth meeting of the joint FAO/WHO expert committee on food additives*, WHO Food Additives Series 47 (WHO Geneva).

Kawamura, O. (2005). Production of monoclonal antibodies against deoxynivalenol and development of a high sensitive ELISA using enzyme amplification. *Technical Bulletin of Faculty of Agriculture, Kagawa University, 57*, 27–33.

Kostelanska, M., Hajslova, J., Zachariasova, M., Malachova, A., Kalachova, K., Poustka, J., et al. (2009). Occurrence of deoxynivalenol and its major conjugate, Deoxynivalenol-3-Glucoside, in beer and some brewing intermediates. *Journal of Agricultural and Food Chemistry, 57*, 3187–3194.

Kuiper-Goodman, T. (1995). Mycotoxins: Risk assessment and legislation. *Toxicology Letters, 82*(83), 853–859.

Neira, M. S., Pacin, A. M., Martinez, E. J., Molto, G., & Resnik, S. L. (1997). The effects of bakery processing on natural deoxynivalenol contamination. *International Journal of Food Microbiology, 37*, 21–25.

Pan, D., Bonsignore, F., Rivas, F., Perera, G., & Bettucci, L. (2007). Deoxynivalenol in barley samples from Uruguay. *International Journal of Food Microbiology, 114*, 149–152.

Pinto, V. E. F., Terminiello, L. A., Basilico, J. C., & Ritieni, A. (2008). Natural occurrence of nivalenol and mycotoxigenic potential of *Fusarium graminearum* strains in wheat affected by head blight in Argentina. *Brazilian Journal of Microbiology, 39*, 157–162.

Santos, J. S., Takabayashi, C. R., Ono, E. Y. S., Itano, E. N., Mallmann, C. A., Kawamura, O., et al. (2011). Immunoassay based on monoclonal antibodies versus LC–MS: Deoxynivalenol in wheat and flour in Southern Brazil. *Food Additives & Contaminants, 28*, 1083–1090.

Schollenberger, M., Muller, H. M., Rufle, M., Suchy, S., Plank, S., & Drochner, W. (2006). Natural occurrence of 16 *Fusarium* toxins in grains and feedstuffs of plant origin from Germany. *Mycopathologia, 161*, 43–52.

Schothorst, R. C., & Van Egmond, H. P. (2004). Report from SCOOP task 3.2.10 "collection of occurrence data of *Fusarium* toxins in food and assessment of dietary intake by the population of EU member states". *Toxicology Letters, 153*, 133–143.

Stanković, S., Lević, J., Ivanović, D., Krnjaja, V., Stanković, G., & Tančić, S. (2012). Fumonisin B1 and its co-occurrence with other fusariotoxins in naturally-contaminated wheat grain. *Food Control, 23*(2), 384–388.

Sudakin, D. L. (2003). Trichothecenes in the environment: Relevance to human health. *Toxicology Letters, 143*, 97–107.

Tomczak, M., Wisniewska, H., Stepien, L., Kostecki, M., Chelkowski, J., & Golinski, P. (2002). Deoxynivalenol, nivalenol and moniliformin in wheat samples with

head blight (scab) symptoms in Poland (1998–2000). *European Journal of Plant Pathology, 108*, 625–630.

Trigo-Stockli, D. M., Deyoe, C. W., Satumbaga, R. F., & Pedersen, J. R. (1996). Distribution of deoxynivalenol and zearalenone in milled fractions of wheat. *Cereal Chemistry, 73*, 388–391.

Turner, P. C., Rothwell, J. A., White, K. L. M., Gong, Y., Cade, J. E., & Wild, C. P. (2008). Urinary deoxynivalenol is correlated with cereal intake in individuals from the United Kingdom. *Environmental Health Perspectives, 16*(1), 21–25.

Visconti, A., Haidukowski, E. M., Pascale, M., & Silvestri, M. (2004). Reduction of deoxynivalenol during durum wheat processing and spaghetti cooking. *Toxicology Letters, 153*, 181–189.

Yoshizawa, T., Kohno, H., Ikeda, K., Shinoda, T., Yokohama, H., Morita, K., et al. (2004). A practical method for measuring deoxynivalenol, nivalenol and T-2 + HT-2 toxin in foods by an enzyme-linked immunosorbent assay using monoclonal antibodies. *Bioscience and Biotechnological Biochemistry, 68*, 2076–2085.