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Surveys of rice sold in Canada for aflatoxins, ochratoxin A and fumonisins

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Approximately 200 samples of rice (including white, brown, red, black, basmati and jasmine, as well as wild rice) from several different countries, including the United States, Canada, Pakistan, India and Thailand, were analysed for aflatoxins, ochratoxin A (OTA) and fumonisins by separate liquid chromatographic methods in two different years. The mean concentrations for aflatoxin B_1 (AFB_1) were 0.19 and 0.17 ng g^{-1} with respective positive incidences of 56% and 43% ($\geq$ the limit of detection (LOD) of 0.002 ng g^{-1}). Twenty-three samples analysed in the second year also contained aflatoxin B_2 (AFB_2) at levels $\geq$LOD of 0.002 ng g^{-1}. The five most contaminated samples in each year contained 1.44–7.14 ng AFB_1 g^{-1} (year 1) and 1.45–3.48 ng AFB_1 g^{-1} (year 2); they were mostly basmati rice from India and Pakistan and black and red rice from Thailand. The average concentrations of ochratoxin A (OTA) were 0.05 and 0.005 ng g^{-1} in year 1 and year 2, respectively; incidences of samples containing $\geq$LOD of 0.05 ng g^{-1} were 43% and 1%, respectively, in the 2 years. All positive OTA results were confirmed by LC-MS/MS. For fumonisins, concentrations of fumonisin B_1 (FB_1) averaged 4.5 ng g^{-1} in 15 positive samples ($\geq$0.7 ng g^{-1}) from year 1 ($n = 99$); fumonisin B_2 (FB_2) and fumonisin B_3 (FB_3) were also present ($\geq$1 ng g^{-1}). In the second year there was only one positive sample (14 ng g^{-1} FB_1) out of 100 analysed. All positive FB_1 results were confirmed by LC-MS/MS.

Keywords: chromatographic analysis; high-performance liquid chromatography (HPLC); liquid chromatography/mass spectrometry (LC/MS); aflatoxins; fumonisins; ochratoxin A; rice

Introduction

Aflatoxins are the most important human mycotoxins (World Health Organization (WHO) 1998). They are synthesized by certain *Aspergillus* species, in particular *A. flavus* and *A. parasiticus*. Chronic exposure leads to a high risk of developing liver cancer (Wang and Tang 2004). Aflatoxins may occur in peanuts, corn (maize) and cottonseed, as well as in many other agricultural commodities (Pittet 2001). Occurrence in rice has also been reported in a number of countries (Reddy et al. 2008; Gummert et al. 2009): Sri Lanka (Bandara et al. 1991), Bangladesh (Dawlatana et al. 2002), Japan (Tabata et al. 1993), China (Liu and Gao 2006; Wang and Liu 2007), Vietnam (Nguyen et al. 2007), Thailand (Shank et al. 1972), India (Pande et al. 1990; Toteja et al. 2006; Reddy et al. 2009), the Philippines (Sales and Yoshizawa 2005), Korea (Park et al. 2004, 2005a), United Arab Emirates (Osman et al. 1999), Turkey (Aydin et al. 2010), Tunisia (Ghali et al. 2008), Nigeria (Hussaini et al. 2007), Côte d'Ivoire (Sangare-Tigori et al. 2006b), Uruguay (Piñeiro et al. 1996), Brazil (Soares and Rodriguez-Amaya 1989; de Carvalho et al. 2010), and the United States (Abbas and Shier 2009), as well as in imported/marketed rice in the United Kingdom (Scudamore et al. 1998; Food Standards Agency (FSA) 2002), Austria (Reiter et al. 2010), Iran (Mazaheri 2009) and Sweden (Fredlund et al. 2009; Nordkvist et al. 2009). The study in Vietnam indicated that the rainy season was a major risk factor for occurrence of AFB_1 in rice (Nguyen et al. 2007).

Ochratoxin A (OTA) is a mycotoxin formed by certain species of *Aspergillus* and *Penicillium* (Bayman and Baker 2006; Clark and Snedeker 2006). It is carcinogenic, nephrotoxic, teratogenic, immunotoxic and hepatotoxic and has been classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans (Group 2B) (IARC 1993). OTA is found in grains and many other foodstuffs. Its occurrence in rice has been reported in Portugal (Pena et al. 2005; Juan et al. 2008a), Spain (Juan et al. 2005, 2008a; González et al. 2006), Turkey (Aydin et al. 2010), Egypt (Abd Alla 1996), Nigeria (Hussaini et al. 2007),

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Côte d'Ivoire (Sangare-Tigori et al. 2006a, 2006b), Morocco (Zinedine et al. 2007; Juan et al. 2008b), Tunisia (Zaied et al. 2009), Jordan (Salem and Ahmad 2010), Chile (Vega et al. 2009), Vietnam (Trung et al. 2001; Nguyen et al. 2007), Japan (Uchiyama et al. 1976; Goto et al. 2007), Korea (Park et al. 2005a, 2005b), and in rice bran used as animal feed in the UK (Scudamore et al. 1998).

Fumonisin B_1 (FB_1) is one of a group of mycotoxins produced mainly by *Fusarium verticillioides* (formerly known as *F. moniliforme*), a widespread fungal pathogen of corn. Also *Aspergillus niger* has now been found to form fumonisin B_2 (FB_2) (Frisvad et al. 2007). FB_1 can cause two diseases of farm animals: leukoencephalomalacia in horses and porcine pulmonary oedema (Marasas 2001; Jackson 2004). In humans fumonisins are associated with oesophageal cancer. FB_1 is carcinogenic, hepatotoxic and nephrotoxic in animals. The IARC designated FB_1 as a possible human carcinogen (Group 2B) (IARC 1993).

Fumonisins (mainly FB_1 and FB_2 – there are fewer data on the less common fumonisin B_3 (FB_3)) are frequently found in corn and corn-based foods (Shephard et al. 1996; Weidenbörner 2001). However, they have also been found in several other foodstuffs, of which rice is the most important from a consumption point of view (Reddy et al. 2008). Fumonisins have been reported in US rice with *Fusarium* sheath rot and scab disease (Abbas et al. 1998; Abbas and Shier 2009), and have also been detected in rice from Korea (Chung and Kim 1995; Kim et al. 1998; Park et al. 2005a), Japan (Kushiro et al. 2008), China (Trucksess 2000), Russia (Kononenko and Burkin 2008), Argentina (Lerda et al. 2005), Italy (Cirillo et al. 2003), and Germany (Zimmer et al. 2008).

There is only one previous study on analysis of rice for all three types of mycotoxins discussed in this paper: aflatoxins, OTA and fumonisins (Park et al. 2005a). The present paper describes analyses of rice available in Canada over 2-year periods in 2007–2009 for these three groups of mycotoxins.

Materials and methods

Samples and sample preparation

The sampling strategy was based on available market share data per country (Industry Canada 2006). The majority of rice consumed by Canadians is imported from the United States and Asian countries and therefore sampling was almost equally distributed between these areas. Samples of white (long, medium and short grain), quick-cook, brown, red, black, basmati, and jasmine rice, as well as wild rice, were collected by the Canadian Food Inspection Agency from retail stores across Canada.

For the first year of the study (2007), rice samples were made of one lot with a minimum amount of approximately 1 kg. For subsequent years, rice samples, with a minimum amount of approximately 1 kg from four portions of the same lot, were made into composites. They were thoroughly mixed and an approximate 1 kg subsample was obtained using a sample splitter (Retsch RT-25). This was ground with an Ultra Centrifugal ZM 200 Retsch Mill using a 0.5 mm ring sieve, resulting in a particle size of less than 200 µm. The sample was then mixed by blending for 30 min with a 1000 W Industrial Cuisinart Stand Mixer. About 400 g were frozen and analytical subsamples withdrawn as needed.

Aflatoxin determination

Aflatoxin B_1 (AFB_1), and in the second year also aflatoxin B_2 (AFB_2), were determined by an in-house-validated method (Tam et al. 2006), essentially the same as AOAC Official Method 2000.16 (AOAC International 2000). Briefly, 50 g of the test sample and 5 g NaCl were extracted with 250 ml methanol–water (8 + 2, v/v). The sample extract (10 ml) was diluted to 100 ml with water and cleaned-up by an immunoaffinity column (IAC) (VICAM AflaTest™ WB, Waters, Milford, MA, USA). Aflatoxins were eluted from the column with methanol, then evaporated and redissolved in 250 µl methanol–water (3.5 + 6.5, v/v). After derivatization with pyridinium hydrobromide perbromide (PBPB), they were determined by a Waters Alliance HPLC with fluorescence detection ($\varepsilon_{ex} = 360$ nm and $\varepsilon_{em} = 420$ nm). The LC column (reversed phase) was a Zorbax SB-C18, 5 µm, 4.6 × 250 mm, with guard column. The mobile phase was water–methanol–acetonitrile (54.5 + 27.3 + 18.2, v/v/v) at 1 ml min^{-1} and the injection volume was 50 µl.

AFB_1 was qualitatively confirmed in selected positive samples by the marked loss in peak area when PBPB derivatization was omitted. The method, which had previously been validated for barley cereal, was validated for rice by spiking ground brown rice with 0.25 ng g^{-1} for each aflatoxin (AFB_1, AFB_2); recoveries averaged 81% ± 5% and 83% ± 4%, respectively ($n = 4$). The limit of detection (LOD) and limit of quantitation (LOQ), determined by signal-to-noise ratios ($S/N = 3:1$ and 10 : 1, respectively) were 0.002 and 0.05 ng g^{-1} respectively for both AFB_1 and AFB_2.

Satisfactory *z*-scores for AFB_1, AFB_2, AFG_1 and AFG_2 as well as total aflatoxins were obtained in a Food Analysis Performance Assessment Scheme (FAPAS®) proficiency test on ground rice.

Ochratoxin A determination

OTA was determined by an in-house method previously validated for dry pasta (Ng et al. 2009), with

extraction and clean-up essentially the same as AOAC Official Method 2000.03 (Entwisle et al. 2000). The main difference was the transfer to a Waters Acquity Ultra Performance LC (UPLC®) instrument. Briefly, 25 g of test sample were extracted with 100 ml of acetonitrile–water (6 + 4, v/v). The extract was filtered through Whatman #4 filter paper and 5 ml diluted with 55 ml phosphate-buffered saline (PBS) solution. This was then filtered through a Whatman 934-AH microfibre glass filter under vacuum and 48 ml of the filtrate applied to an IAC containing antibodies specific for OTA (VICAM OchraTest™). Prior to use the columns were pre-rinsed with 3 × 1 ml deionized water, 3 × 1 ml methanol, then 20 ml PBS. OTA was eluted from the column into a silanized vial with 4 × 1 ml methanol, which was evaporated and the residue redissolved in 500 µl of methanol–water–acetic acid (30 + 70 + 1, v/v/v). The injection volume was 10 µl on a Acquity UPLC® BEH C18 1.7 µm, 2.1 × 50 mm column (Waters) at 35°C using a gradient of 0.083 M H_3PO_4-CH_3CN (1 + 1, v/v) and methanol, with fluorescence detection ($\varepsilon_{ex} = 330$ nm and $\varepsilon_{em} = 470$ nm).

The method was validated by spiking rice and obtaining recoveries of 86% ± 15%, 89% ± 6% and 88% ± 4% at 0.5, 3.0 and 5 ng g^{-1} spiking levels ($n = 5$), respectively. The LOD was 0.05 ng g^{-1} ($S/N = 3:1$) and the LOQ was 0.2 ng g^{-1} ($S/N = 10:1$).

For confirmation of OTA in positive rice samples, a Waters Acquity UPLC® coupled to a Waters Quattro Premier tandem mass spectrometer was used with the same extract (Tam et al. 2011). The UPLC was equipped with a Waters BEH C18 column (1.7 µm, 2.1 × 50 mm). The mobile phase consisted of a variable mixture of solutions A (water–formic acid, 99 : 1 v/v) and B (acetonitrile–formic acid, 99 : 1 v/v) at a flow rate of 0.3 ml min^{-1}. The linear gradient programme was set to provide 90% A at 0 min, 10% A at 7 min and 90% A from 8 to 10 min. The column temperature was 30°C and the injection volume was 20 µl. The mass spectrometer was operated in the positive electrospray ionization mode with argon as the collision gas at a flow rate of 0.3 ml min^{-1}. Multiple-reaction monitoring (MRM) mode was configured to monitor the following mass-to-charge ratio (m/z) transitions: both 404 → 239 (collision energy 25 eV) and 404 → 358 (collision energy 15 eV) for OTA. The second transition (m/z 404 → 358) was used for confirmation. The ion ratio for 404 → 239/404 → 358 was 1.9 (range ± 20%), which was determined from a standard and met the minimum requirement for the identification and confirmation of OTA in samples.

Fumonisins

Fumonisins B_1, B_2 and B_3 (FB_1, FB_2 and FB_3) were determined in rice by the method of Oh et al. (2009), with some modifications. A 10 g sample plus 1 g NaCl was submerged in 50 ml water for 30 min (Kushiro et al. 2008; Awaludin et al. 2009) and extraction was completed by blending (Polytron) for 3 min with 50 ml acetonitrile–methanol (1 + 1, v/v). After centrifuging at 2000 rpm for 10 min and filtering (Whatman #1), 40 ml PBS (pH 7) were added to a 10 ml aliquot of extract. A total of 10 ml of this solution was added to a VICAM FumoniTest™ IAC, previously conditioned with 5 ml PBS. The column was washed with 10 ml PBS, then the fumonisins were eluted with 2 × 0.75 ml methanol–water (8 + 2, v/v), which was evaporated to dryness under nitrogen at 60°C. The residue was dissolved in 500 µl acetonitrile–water (1 + 1, v/v) and filtered (Teflon (PTFE), 0.45 µm). LC pre-column derivatization for fluorescence determination was with *o*-phthaldialdehyde (OPA)/2-mercaptoethanol (ME) reagent. LC was modified by using injection volumes of 10 µl sample + 10 µl OPA/ME on a Zorbax Eclipse Plus C18 1.8 µm, 4.6 × 50 mm column (Agilent Technologies) at 35°C. Method validation by spiking triplicate blank samples of ground white rice showed recoveries for FB_1, FB_2 and FB_3 of 82% ± 5%, 78% ± 12% and 71% ± 18%, respectively, at 10 ng g^{-1}; 77% ± 3%, 65% ± 3% and 74% ± 4%, respectively, at 100 ng g^{-1}; and 78% ± 2%, 74% ± 3% and 84% ± 4%, respectively, at 500 ng g^{-1}. Spike recoveries were > 89% for arborio, basmati, brown, Thai and wild rice samples. The LODs ($S/N = 3:1$) were 0.7, 1 and 1 ng g^{-1} for FB_1, FB_2 and FB_3, respectively and LOQs ($S/N = 10:1$) were 2, 4 and 4 ng g^{-1}, respectively.

Confirmation of the identity of the fumonisins and some of the quantitations were done by LC-MS/MS. The LC was an Agilent 1200 HPLC system (Agilent Technologies); Phenomenex (Torrance, CA, USA) Gemini-NX C18, 3 µm, 2 × 150 mm at 15°C; injection volume 10 µl; mobile phase A: 0.1% formic acid in LC-MS-grade water; mobile phase B: 0.1% formic acid in acetonitrile–methanol (1 + 1, v/v); the gradient was 15% B from zero to 3 min (flow rate = 0.175 ml min^{-1}), ramped to 60% B from 3 to 8 min, held for 12 min, returned to 15% B at 21 min (flow rate - 0.250 ml min^{-1}), held for 4 min, then 15% B at 26 min (flow rate = 0.175 ml min^{-1}).

MS/MS instrumentation and conditions were the following: Waters Quattro-Ultima triple quadrupole tandem mass spectrometer (Milford, MA, USA) in positive electrospray ionization mode; the capillary voltage was 3.5 kV, cone voltage 35 V and source temperature 120°C. Desolvation temperature was 350°C, desolvation gas flow 700 l h^{-1}, cone gas flow 50 l h^{-1}, multiplier voltage 625 V, and collision cell pressure 3.0 × 10^{-3} mbar (Ar). Resolution for the first and the last quadrupole mass analysers was 1.0–1.2 mass units at base. For FB_1, MRM transitions were m/z 722 → 334 (collision energy = 37 eV), 722 → 352 (35 eV)

(quantitation ion transition) and 722 → 704 (27 eV); ion ratios for 722 → 352/722 → 334 and 722 → 352/722 → 704 were 1.05 (range ± 25%) and 1.15% ± 25%, respectively. For FB_2 and FB_3, MRM transitions were 706 → 336 (collision energy = 35 eV) (quantitation ion transition), 706 → 354 (35 eV) and 706 → 688 (30 eV); ion ratios for 706 → 336/706 → 354 were 3.6% ± 25% (FB_2 and FB_3) and for 706 → 336/706 → 688 were 3.6% ± 25% (FB_2) and 2.6 ± 25% (FB_3). The dwell time for each transition was set at 80 ms with an inter-channel delay time of 20 ms. The instrumental LOD was approximately 0.05 ng ml^{-1} for each fumonisin.

Results and discussion

Aflatoxins

In the first year (2008), only AFB_1 was analysed and the mean for AFB_1 was 0.19 ng g^{-1} ($n = 99$), with 56 of the samples positive (≥LOD). The mean AFB_1 of the positive samples was 0.34 ng g^{-1}. The highest AFB_1 contamination was in a sample of brown basmati rice from India (7.1 ng g^{-1}).

In the second year (2009), 100 composites of rice were analysed for AFB_1 and AFB_2. For AFB_1, the overall mean was 0.17 ng g^{-1}, and the mean was 0.39 ng g^{-1} for the 43% positive samples (≥LOD). Twenty-three samples analysed in the second year also contained AFB_2 at levels from 0.002 to 0.63 ng g^{-1}, usually together with AFB_1. Typically, the ratio of AFB_2/AFB_1 concentration was approximately 0.1 in a naturally contaminated sample. The normal distribution plot of the yearly distribution of AFB_1 in Canadian retail rice is illustrated in Figure 1.

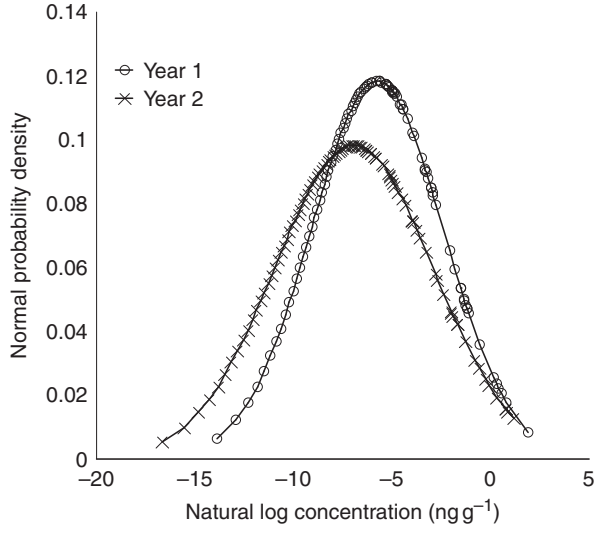

Figure 1. Yearly variation of AFB_1 in Canadian retail rice, expressed as a normal distribution plot. There were not enough positives in both years to conduct year-to-year comparisons for the other two mycotoxins.

It demonstrates that based on a *t*-test there is no significant difference at the 99% confidence level between the average and spread (distribution) of aflatoxin contamination in rice between the 2 years.

The five most contaminated samples in each year contained 1.44–7.14 ng g^{-1} of AFB_1 (year 1) and 1.45–3.48 ng g^{-1} of AFB_1 (year 2); they were mostly basmati rice from India and Pakistan, and black and red rice from Thailand.

The findings are comparable with surveys of rice imported into European countries: rice from the UK (Scudamore et al. 1998; FSA 2002), Austria (Reiter et al. 2010) and Sweden (Fredlund et al. 2009; Nordkvist et al. 2009) was found to be commonly contaminated with AFB_1.

Ochratoxin A

In the first year (2007), individual results for OTA were corrected by subtracting 0.13 ng g^{-1} due to a contamination problem with the reagent blank. The average amount of OTA detected in the 100 rice samples was 0.05 ng g^{-1}. Four rice samples had OTA concentrations greater than the LOQ (0.2 ng g^{-1}); three were from the United States (Calrose, brown and sushi rice) and one from India (brown basmati rice). The mean of positives was 0.11 ng g^{-1} for the 43 rice samples, with OTA levels detected at or above the LOD (0.05 ng g^{-1}).

A second survey (2008) included 99 rice samples. The overall mean for OTA was below the LOD. The only positive sample (≥LOD) was a black glutinous rice from Thailand, which contained 0.49 ng OTA g^{-1}. The distribution of OTA in rice is quite different between each survey year, but the overall contamination of rice remained well below 5 ng g^{-1}, the proposed Health Canada maximum limit (ML) for OTA in raw cereal grains, including rice, and below 0.5 ng g^{-1}, the proposed Health Canada ML for OTA in baby foods and processed cereal-based foods for infants and young children (Health Canada 2009).

Fumonisins

In the first survey (2008), 15/99 samples were positive (mean = 4.5 ng FB_1 g^{-1}). FB_2 and FB_3 were also found (up to 2 and 1 ng g^{-1}, respectively). Table 1 shows the 15 rice samples found positive by LC-fluorescence and which were confirmed by LC-MS/MS. For a few samples, because of its higher sensitivity and specificity, the LC-MS/MS quantitation is reported, especially when the concentration was above the LC-fluorescence result. A long grain brown rice, a black glutinous rice and a brown basmati rice had the highest FB_1 contamination (9–11 ng g^{-1}). Nine of the 15 positive rice samples originated from the United States. In the second survey (2009), there was only one

positive (14 ng $FB_1\,g^{-1}$) in the 100 rice samples analysed: a black sweet rice from Thailand. The finding was confirmed by LC-MS/MS.

Since fumonisins are known to be apparently unstable in rice flour (Kim et al. 2002), it was desirable to check whether they survived unchanged in ground rice at −30°C during the 15–24 weeks between surveys in the present study. It was found that fumonisins were stable on frozen storage of ground rice, so this does not explain the lower incidence in rice in the second survey. Different weather patterns and storage conditions are factors that could explain the lower incidence of fumonisins in the rice collected in 2009. We also evaluated the stability of fumonisins after spiking brown (25 days), Thai (18 h) and sticky rice (18 h). No significant loss of fumonisins was measured.

Seventeen retail heat processed rice samples (four rice cakes, five rice crisps, three adult breakfast cereals, four infant cereals and one rice pasta) were analysed, but no detectable FB_1, FB_2 and FB_3 contamination was found. FB_1 was found previously in rice cake and rice snacks in Korea (Kim et al. 1998), indicating stability to food processing.

Multitoxins

Although the rice surveys were for 2 years, only the 2008 rice survey included analysis of the three mycotoxins for a specific rice sample. Figure 2 illustrates the results for each mycotoxin. The concentration of FB_1 is generally the highest, especially in black rice. The black rice sample was positive for the three mycotoxins. Generally, basmati rice and brown rice contained AFB_1. Although the OTA concentration was highest in black rice, the concentration was low and often negligible in other rices analysed in this survey. Table 2 shows the overall incidences for each rice sample collected in 2008. Generally, the incidences are higher for AFB_1.

The arithmetic mean (positives and overall) of each mycotoxin are summarised in Table 3 for each collection year. Although the aflatoxin results are similar for both years, those of OTA and FB_1 are significantly different between each sampling year.

Table 4 shows the overall data (both years) for each mycotoxin. Although the highest incidence of positives was for AFB_1, the FB_1 concentrations are highest overall. The incidence of AFB_1 was more than twice that of OTA.

Rice can be contaminated with field fungi (*Fusarium* species), and be spoiled with storage fungi

Table 1. Samples with a positive fumonisin contamination in rice collected in 2008.[a]

	Concentration (ng g^{-1})			
Rice sample	FB_1	FB_2	FB_3	Fumonisins (total)
Enriched pre-cooked	1[b]	0	0	1
Wild mix	2	0	0	2
Long-grain brown	2	0	0	2
Long grain white	2	0	0	2
Organic white long grain	2	0	0	2
Medium grain Calrose	3[b]	0	0	3
Whole grain brown	3	0	0	3
Organic brown and wild	4	0	0	4
Wild rice and brown	4	0	0	4
Medium grain Calrose	5	0	0	5
Sticky	5	0	0	5
Organic brown basmati	5	1[b]	1[b]	7
Brown basmati natural	9	1[b]	1[b]	11
Black glutinous	9[b]	2[b]	1[b]	12
Long-grain brown	11	2	1[b]	14

Notes: [a]Only one positive (14 ng g^{-1}) out of 100 samples was found in the 2009 survey.
[b]LC-MS/MS result.
LC-fluorescence and LC-MS/MS data are reported.

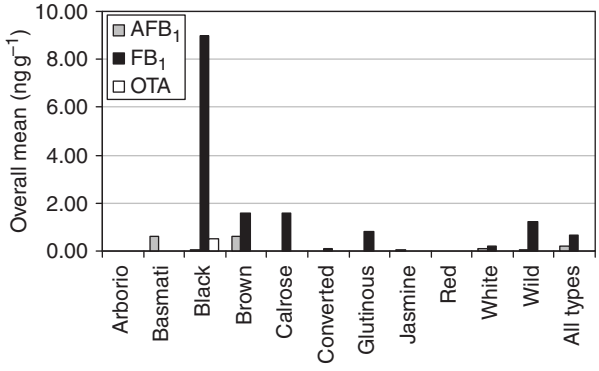

Figure 2. Overall means of AFB_1, FB_1 and OTA for each type of rice sample collected in 2008. Except where colour is indicated, rices are white.

Table 2. Overall incidences (≥LOD) of AFB_1, OTA and FB_1 for each type of rice sample collected in 2008.

	Incidence		
Rice type	AFB_1	OTA	FB_1
Arborio[a]	0/9	0/9	0/9
Basmati[a]	9/9	0/9	0/9
Black	1/1	1/1	1/1
Brown	11/19	0/19	5/19
Calrose[a]	2/5	0/5	2/5
Converted[a]	7/9	0/9	1/9
Glutinous[a]	1/6	0/6	1/6
Jasmine[a]	8/12	0/12	0/12
Red	1/2	0/2	0/2
White[a]	12/19	0/19	2/19
Wild[b]	4/8	0/8	3/8
All rices	56/99	1/99	15/99

Notes: [a]White rices.
[b]May be mixed with other types of rice.

Table 3. Natural occurrence of aflatoxins, ochratoxin A and fumonisins in rice for each sample collection year.

	2007 ($n=100$)			2008 ($n=99$)			2009 ($n=100$)		
	n (p)	Mean (p)	Mean (o)	n (p)	Mean (p)	Mean (o)	n (p)	Mean (p)	Mean (o)
AFB_1	–	–	–	56	0.34	0.19	43	0.39	0.17
AFB_2	–	–	–	–	–	–	23	0.08	0.02
OTA	43	0.11	0.05	1	0.49	0.005	–	–	–
FB_1	–	–	–	15	4.5	0.68	1	14	0.14
FB_2	–	–	–	4	1.5	0.06	1	2.9	0.03
FB_3	–	–	–	4	1.0	0.04	1	1.6	0.02

Notes: n (p), number of positives (≥LOD); mean (p), mean of positives ($ng\,g^{-1}$); mean (o), overall mean ($ng\,g^{-1}$). –, no data available.

Table 4. Two-year surveys of rice sold in Canada for aflatoxin B_1, ochratoxin A and fumonisin B_1.

All rice ($n=199$)	AFB_1	OTA	FB_1
Maximum concentration ($ng\,g^{-1}$)	7.1	0.49	14
Number of positives (≥LOD)	99	44	16
Mean of positives ($ng\,g^{-1}$)	0.36	0.12	5.1
Overall mean ($ng\,g^{-1}$)	0.18	0.03	0.41

(*Aspergillus* and *Penicillium*) (Park et al. 2005a). Based on the fairly consistent aflatoxin incidence found in rice (Table 3), contamination seems more likely to have occurred in storage. As FB_1 concentrations attain higher levels than the other mycotoxins, previous contamination with field fungi (*Fusarium*) is also indicated.

In conclusion, the survey results will contribute to future Canadian risk assessments for each of these mycotoxins in food.

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