Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Multi-Mycotoxins in Milled Grain Products and Grain-based Foods - April 1, 2015 to March 31, 2018

Food chemistry - Targeted surveys - Final report

Canada

Summary

Targeted surveys provide information on potential food hazards and enhance the Canadian Food Inspection Agency's (CFIA's) routine monitoring programs. These surveys provide evidence regarding the safety of the food supply, identify potential emerging hazards, and contribute new information and data to food categories where it may be limited or non-existent. They are often used by the CFIA to focus surveillance on potential areas of higher risk. Surveys can also help to identify trends and provide information about how industry complies with Canadian regulations.

The main objectives of this targeted survey were to expand baseline data on the presence and levels of mycotoxins in milled grain products and grain-based foods; and to compare these results to other data, where feasible. Mycotoxins are natural toxins released by moulds that infect agricultural crops before and after harvest. Their human health effects are varied; the health effects depend on the type and level of mycotoxin in the food. Canada does not have maximum levels for the mycotoxins in the products targeted in this survey, with the exception of ochratoxin A (OTA), for which Canada has proposed maximum levels in certain foods.

A total of 2240 samples of milled grain products and grain-based foods were analyzed for the presence of mycotoxins. Mycotoxins were detected in 1135 or (51%) of samples tested. A total of 22 different compounds were detected in the product types sampled by this survey. Aflatoxin G2, 3-acetyldeoxynivalenol (3-Ac-DON), and 15-acetyldeoxynivalenol (15-Ac-DON) were not detected in any of the samples. The compounds detected most frequently in this study was deoxynivalenol (DON) in a total of 887 samples (40%).

The levels of mycotoxins observed in this survey were evaluated by Health Canada who determined that none of the samples would pose an unacceptable human health concern, therefore there were no recalls resulting from this survey.

What are targeted surveys

Targeted surveys are used by the CFIA to focus its surveillance activities on areas of highest health risk. The information gained from these surveys provides support for the allocation and prioritization of the agency's activities to areas of greater concern. Originally started as a project under the Food Safety Action Plan (FSAP), targeted surveys have been embedded in the CFIA's regular surveillance activities since 2013. Targeted surveys are a valuable tool for generating information on certain hazards in foods, identifying and characterizing new and emerging hazards, informing trend analysis, prompting and refining health risk assessments, highlighting potential contamination issues, as well as assessing and promoting compliance with Canadian regulations.

Food safety is a shared responsibility. The CFIA works with federal, provincial, territorial and municipal governments and provides regulatory oversight of the food industry to promote safe handling of foods throughout the food production chain. The food industry and retail sectors in Canada are responsible for the food they produce and sell, while individual consumers are responsible for the safe handling of the food they have in their possession.

Why did we conduct this survey

Mycotoxins are natural toxins released by moulds that can grow on crops in the field or after harvest[1]. These toxins are released by moulds which can grow on agricultural products, such as on cereals (e.g. wheat, oats, and corn), legumes, nuts and fruit. The type of agricultural product, insect damage, and the climatic conditions (temperature, humidity) during growth, processing, and storage are some factors that can influence the types and levels of mycotoxins present in the foods available at the retail level[2]. The human health effects are varied; ranging from gastrointestinal distress to cancer, the health effects depend on the type and level of mycotoxin in the food.

Research has shown that of the hundreds of mycotoxins associated with food, a small fraction has the potential to adversely affect human health and pose a global health concern[2]. The Codex Alimentarius Commission is an international body established by the United Nations' Food and Agriculture Organization and the World Health Organization to develop harmonized international food standards, guidelines, and codes of practice to protect the health of the consumers and to ensure fair practices in the food trade. Codex has published a Code of Practice to reduce and prevent mycotoxin contamination in cereals (e.g., wheat, corn, oats, and barley)[2]. This Code of Practice acknowledges that the complete elimination of mycotoxins from foods is not possible but

it provides guidance on ways to control and manage the mycotoxin levels at the farm level and after harvest (for example, during processing, storage, and transport).

There are now more than 300 known mycotoxins of widely different chemical structures and differing modes of action - some target the kidney, liver, or immune system and some are carcinogenic. Common mycotoxins include aflatoxins, ochratoxin A, ergot alkaloids, fumonisins, trichothecenes (such as deoxynivalenol which is also known as vomitoxin) and zearalenone[Error! Bookmark not defined.].

Please see Appendix A for a list of the analytes included in the method. Please consult Appendix B for description of the health effects of the different myoctoxins.

What did we sample

A variety of domestic and imported milled grains (bran, flour, meal, starch, whole grains) and grain-based foods (baked goods, breads and bread products, cookies, baking mixes, crackers, pasta). The samples were collected over 3 fiscal years (2015-16 fiscal year - April 1, 2015 to March 31, 2016; 2016-17 fiscal year - April 1, 2016 to March 31, 2017; and 2017-18 fiscal year - April 1, 2017 to March 31, 2018). Samples of products were collected from local/regional retail locations located in 6 major cities across Canada. These cities encompassed 4 Canadian geographical areas:

- Atlantic (Halifax)
- Quebec (Montreal)
- Ontario (Toronto and Ottawa)
- West (Vancouver and Calgary)

The number of samples collected from these cities was in proportion to the relative population of the respective areas.

Table 1. Distribution of samples based on grain and origin

Grain	Products	Number of domestic samples	Number of imported[a] samples	Number of samples of unspecified[b] origin	Total number of samples
Amaranth	Flour, grain	3	13	36	52
Arrowroot	Starch/flour	0	4	34	38
Barley	Flakes, flour, pot/pearl	43	17	19	79
Buckwheat	Grain/kasha, baking mixes, flour	27	25	30	82
Corn	Flour, meal, starch	11	65	27	103
Corn-based foods	Baking mixes, English muffins, flour, pasta	0	7	2	9
Kamut	Flakes, flour, grains	24	19	36	79
Millet	Flakes, flour, grains, grits	8	49	28	85
Mixed grain foods	Baked goods, bread and bread products, baking mixes, flour, pasta	29	20	132	181
Mixed Grains	Grains	3	8	9	20
Oat	Bran, flour, grain, meal	99	23	87	209
Oat-based foods	Baking mixes	1	1	0	2
Quinoa	Flakes, flour, grains	3	37	52	92
Rice	Bran, flour, grains	17	231	98	346
Rice-based foods	Baking mixes, breakfast cereal, cakes, chips, crackers, paper, pasta	31	69	20	120
Rye	Flakes, flour, grains	37	3	35	75
Rye-based foods	Crackers	0	4	0	4
Sorghum	Baking mixes	1	0	0	1
Spelt	Flakes, flour, grains, groats, kernels,	43	19	19	81
Spelt-based foods	Cookie	0	0	1	1
Teff	Flour, grain	0	18	30	48
Teff-based foods	Pasta	3	0	0	3
Wheat	Bran, bulgur, cracked, couscous, cream, flour, freekeh, germ, gluten	79	37	84	200

Wheat-based foods	Baked goods, breads and bread products, cookies, crackers, pasta	85	63	182	330
Total	**n/a**	**547**	**732**	**961**	**2240**

[a] Imported from at least 32 countries

[b] Unspecified refers to those samples for which the country of origin could not be assigned from the product label or available sample information

How were samples analyzed and assessed

Samples were analyzed by an ISO/IEC 17025 accredited food testing laboratory under contract with the Government of Canada. See Appendix A for a list of the analytes included in the method. The results are based on the food products as sold and not necessarily as they would be consumed.

Health Canada has not established tolerances or standards for the majority of the mycotoxins in the grain products targeted in this survey. In 2009, Health Canada proposed maximum levels (ML) for OTA in a variety of foods. An ML of 3 ppb has been proposed for grains for direct consumption (barley, oats, rice and wheat) and derived cereal products (for example flour, bread, breakfast cereal), an ML of 7 ppb has been proposed for wheat bran and an ML of 0.5 ppb has been proposed for infant formulas and cereal-based foods[3]. These MLs as well as an industry guidance value for OTA in unprocessed cereal grains are still under consideration.

In the absence of applicable tolerances or standards, high levels of mycotoxins may be assessed by Health Canada's Bureau of Chemical Safety (BCS) on a case-by-case basis using the most current scientific data available.

What were the survey results

Multi-mycotoxins

A total of 2244 samples were analyzed for the presence of mycotoxins. The products sampled were separated into 16 types of milled grain products (bran, flakes, flour, grains, grits, groats, meal) and 9 types of grain-based foods (baked goods, bread products, baking mixes, cookies, crackers, pasta, snacks). Mycotoxins were detected in 1132 samples (50%). A total of 22 of 25 different mycotoxins were detected in the product types sampled in this survey. Aflatoxin G2, 3-Ac-DON, and 15-Ac-DON were not detected in any of the samples. Table 2 illustrates the number of samples with detectable levels of the various family of compounds for all of the

product types. Spelt-based foods (cookie) and oat-based foods (crackers, baking mixes) have the highest and lowest percentage of detectable mycotoxins, respectively.

Table 2. Summary of results of multi-mycotoxin testing in grain-based products

Product type	Number of samples	Number of samples (%) with detected mycotoxin (s)	Maximum number of mycotoxins per sample	Mycotoxin Family Detected							
				AF	CPA	EA	FUM	OTA	STG	TRI	ZEN
Amaranth	52	9 (17)	2				X		X	X	X
Arrowroot	38	2 (5)	1		X					X	
Barley	79	50 (63)	3		X	X	X	X	X	X	X
Buckwheat	82	23 (28)	3	X	X	X		X	X	X	X
Corn	103	78 (76)	5				X			X	X
Corn-based foods	9	7 (78)	4				X			X	X
Kamut	79	31 (39)	2			X	X	X	X	X	
Millet	85	14 (16)	3	X			X	X	X	X	
Mixed grain foods	181	153 (85)	5	X		X	X	X	X	X	X
Mixed Grains	20	5 (25)	2					X	X	X	
Oat	209	102 (49)	5			X	X	X	X	X	X
Oat-based foods	2	0 (0)	0								
Quinoa	92	10 (11)	2			X	X	X	X	X	
Rice	346	70 (20)	6	X	X	X	X	X	X	X	X
Rice-based foods	120	16 (13)	2	X		X	X		X	X	X
Rye	75	59 (79)	5			X	X	X	X	X	X
Rye-based foods	4	4 (100)	4								
Sorghum	1	1 (100)	1			X				X	
Spelt	81	43 (53)	4						X	X	X
Spelt-based foods	1	1 (100)	3		X			X		X	
Teff	48	2 (4)	2				X				
Teff-based foods	3	2 (67)	2					X	X		
Wheat	200	157 (78)	4		X	X	X	X	X	X	X
Wheat-based foods	329	291 (88)	5		X	X		X	X	X	X
Total	**2240**	**1135 (51)**	**6**	X	X	X	X	X	X	X	X

As can be seen from Table 2, up to 6 mycotoxins out of a possible 25 compounds were detected per sample. These may consist of multiple compounds from the same toxin family(3 forms of aflatoxin or 3 forms of fumonisin) or discrete mycotoxins (for example, sterigmatocystin).

Table 3. Frequency and levels of mycotoxins detected in the survey

Mycotoxin	Number of samples	Number (percentage) of positive samples	% of positive samples	Min (ppb)	Max (ppb)	Ave (ppb)
DON	2240	887	39	10	3900	181
Ergocristine	2240	367	16	6	951	61
Ergocryptine	2240	221	9.9	6	414	32
FB1	2240	98	4.4	4	1590	207
STG	2240	98	4.4	0.4	34	3.8
OTA	2240	97	4.3	0.8	36	3.1
FB2	2240	60	2.7	10	440	96
Ergosine	2240	58	2.6	9	296	41
FB3	2240	53	2.4	8	179	54
ZEN	2240	43	1.9	8	480	61
HT-2	2240	27	1.2	9	98	19
AFB1	2240	13	0.58	0.5	7.6	3.1
T-2	2240	12	0.54	9	240	37
β-ZOL	2240	12	0.54	8	120	48
CPA	2240	10	0.45	0.6	6.5	2.2
NIV	2240	7	0.31	10	47	27
α-ZOL	2240	6	0.27	9	270	62
DAS	2240	5	0.22	10	250	94
AFB2	2240	1	0.04	n/a	0.4	n/a
AFG1	2240	1	0.04	n/a	1.9	n/a
FUS-X	2240	1	0.04	n/a	23	n/a
NEO	2240	1	0.04	n/a	20	n/a
3-AcDON	2240	0	0	n/a	n/a	n/a
15-AcDON	2240	0	0	n/a	n/a	n/a
AFG2	2240	0	0	n/a	n/a	n/a

As can be seen in Table 3, deoxynivalenol was the most frequently detected toxin (887 or 40% samples). The least commonly detected mycotoxins were aflatoxin B2; aflatoxin G1, fusarenone-X, and neosilanol, which were each detected in only one sample each. The levels ranged from 0.40 ppb to 3900 ppb.

A number of studies have been published about impact of farming method (organic, conventional) on mycotoxin levels[4,5,6,7,8]. There is not a clear, consistent link between farming method and the prevalence or levels of mycotoxins. This is also the case in these targeted surveys. The milled grains and grain-based foods varied in the proportion of conventionally grown and organic products (based on label claims). The breakdown of results of multi-mycotoxin testing in conventionally grown and organic grain-based products is presented in Table 4. The percentage of organic products containing mycotoxins decreased in the order: kamut and spelt-based foods (100%) > amaranth (98%) > rye (87%) > quinoa (84%) > buckwheat (75%) > oat-based foods (50%) > millet (35%) > barley (27%) > mixed grains (25%) > corn and rice (18%) > wheat (14%) > spelt (10%) > mixed grain foods (7.7%) > arrowroot (5.3%) > rice-based foods (5.0%) > wheat-based foods (2.7%) > teff (2.1%)> corn-based foods, rye-based foods, sorghum and teff-based foods (0%). Of the 18 product types which included conventionally grown and organic products, 3 had similar detection levels (oat-based foods, rice, corn), 8 had higher detection rates in conventional products, and 7 were associated with higher detection rates in organic products.

There was also no clear trend observed with respect to number of mycotoxins per sample and growing method – 4 product types (barley, corn, millet, wheat) showed no difference, 6 had a higher number in organic products (amaranth, buckwheat, oat, quinoa, rye, and spelt) and 6 had a higher number in conventionally grown products (arrowroot, mixed grains, mixed grain foods, rice, teff, and wheat-based).

Table 4. Comparison of results of multi-mycotoxin testing in conventionally and organically produced grain-based products

Product type	Conventional			Organic		
	Number of samples	number of samples (%) with detected mycotoxin(s)	Maximum mycotoxins per sample	Number of samples	Number (%) of samples with detected mycotoxin(s)	Number of mycotoxins per sample
Amaranth	1	0 (0)	0	51	9 (18)	2
Arrowroot	36	2 (5)	1	2	0 (0)	0
Barley	57	37 (65)	3	22	13 (59)	3
Buckwheat	21	4 (19)	2	61	19 (31)	3
Corn	84	61 (73)	5	19	17 (89)	5
Corn-based foods	9	7 (78)	4	0	n/a	n/a
Kamut	n/a	n/a	n/a	79	31 (39)	2
Millet	55	7 (13)	3	30	7 (23)	3
Mixed grain foods	167	145 (87)	5	14	8 (57)	4
Mixed Grains	15	5 (33)	2	5	0 (0)	0
Oat	152	73 (48)	3	57	29 (51)	5
Oat-based foods	1	0 (0)	0	1	0 (0)	0
Quinoa	15	0 (0)	0	77	10 (13)	2
Rice	283	56 (20)	6	63	14 (22)	4
Rice-based foods	114	16 (14)	2	6	0 (0)	0
Rye	10	10 (100)	4	65	59 (79)	5
Rye-based foods	4	4 (100)	4	0	n/a	n/a
Sorghum	1	1 (100)	1	0	n/a	n/a
Spelt	9	3 (33)	2	1	40 (56)	4
Spelt-based foods	n/a	n/a	n/a	1	1 (100)	3
Teff	47	2 (4)	2	1	0 (0)	0
Teff-based foods	3	2 (67)	2	0	n/a	n/a
Wheat	173	139 (80)	4	27	18 (67)	4
Wheat-based foods	321	286 (89)	5	9	6 (67)	2
Total	**1578**	**860 (54)**	**6**	**595**	**285 (48)**	**5**

n/a = no samples fit this description so cannot report on prevalence of mycotoxins or on maximum number of mycotoxins per sample

What do the survey results mean

In comparison to previous survey years[9], the detection rates for mycotoxins in various types of grain-based foods were generally consistent, with the exception of sterigmatocystin, as noted in Table 4 below. This may be related to differences in product types, conditions during a specific growing year, source of the grains, and/or use of fungicides. Health Canada has not established maximum limits for any of the mycotoxins in products included in this survey, with the exception of OTA[3]. The compliance rate for grain based products for OTA (97.1 % in 2015-16 fiscal year, 99.8% in 2016-17 fiscal year and 97.5% in 2017-18 fiscal year was comparable to previous survey years (96.5%). As observed in previous surveys, DON was the most commonly observed mycotoxin.

Health Canada has determined that the levels of mycotoxins in the grain-based foods observed in the current survey are not expected to pose a concern to human health, therefore there were no recalls or follow up actions resulting from this survey.

Table 5. A comparison of Mycotoxin testing results in grain-based products between the current and previous CFIA survey years

CFIA Survey	Year	Analyte	Number of samples	Number of samples with detectable mycotoxins (%)	Maximum mycotoxin level (ppb)	Average[c] mycotoxin level (ppb)
CFIA	**2017**	**Aflatoxins B1, B2, and G1**	**746**	**4 (0.5)**	**4.6**	**4.3**
CFIA	**2016**	**Aflatoxins B1, B2, and G1**	**750**	**4 (0.5)**	**7.6**	**5.0**
CFIA	**2015**	**Aflatoxins B1, B2, and G1**	**744**	**6 (0.8)**	**1.4**	**0.9**
CFIA	2013 to 2015	Aflatoxins B1, B2, and G1	2235	57 (2.5)	17	3.6
CFIA	**2015 to 2018**	**3- and 15-acetyldeoxynivalenol**	**2240**	**0 (0)**	**-**	**-**
CFIA	2013 to 2015	3- and 15-acetyldeoxynivalenol	2235	8 (0.004)	53	35.25
CFIA	**2017**	**Deoxynivalenol**	**746**	**440 (59)**	**1000**	**160**
CFIA	**2016**	**Deoxynivalenol**	**750**	**244 (32)**	**1360**	**177**
CFIA	**2015**	**Deoxynivalenol**	**744**	**203 (27)**	**3900**	**232**
CFIA	2013 to 2015	Deoxynivalenol	2235	1044 (46)	2330	176
CFIA	**2017**	**Diacetoxyscirpenol**	**746**	**0 (0)**	**-**	**-**

CFIA Survey	Year	Analyte	Number of samples	Number of samples with detectable mycotoxins (%)	Maximum mycotoxin level (ppb)	Average[c] mycotoxin level (ppb)
CFIA	**2016**	**Diacetoxyscirpenol**	**750**	**1 (0.1)**	**10**	**n/a**
CFIA	**2015**	**Diacetoxyscirpenol**	**744**	**4 (0.5)**	**250**	**115**
CFIA	2013 to 2015	Diacetoxyscirpenol	2235	0 (0.0)	-	-
CFIA	**2017**	**Fusarenone-X**	**746**	**0 (0)**	**-**	**-**
CFIA	**2016**	**Fusarenone-X**	**751**	**1 (0.1)**	**23**	**23**
CFIA	**2015**	**Fusarenone-X**	**744**	**0 (0)**	**-**	**-**
CFIA	2013 to 2015	Fusarenone-X	2235	0 (0.0)	-	-
CFIA	**2017**	**Neosolaniol**	**746**	**1 (0.1)**	**20**	**N/A**
CFIA	**2016**	**Neosolaniol**	**750**	**0 (0)**	**-**	**-**
CFIA	**2015**	**Neosolaniol**	**744**	**0 (0)**	**-**	**-**
CFIA	2013 to 2015	Neosolaniol	2235	1 (0.0004)	30	30
CFIA	**2017**	**Nivalenol**	**746**	**6 (0.8)**	**47**	**28**
CFIA	**2016**	**Nivalenol**	**750**	**0 (0)**	**-**	**-**
CFIA	**2015**	**Nivalenol**	**744**	**1 (0.1)**	**17**	**n/a**
CFIA	2013 to 2015	Nivalenol	2235	4 (0.002)	98	42
CFIA	**2017**	**Ergot Alkaloids**	**746**	**232 (31)**	**1060**	**60**
CFIA	**2016**	**Ergot Alkaloids**	**750**	**63 (8.4)**	**1530**	**138**
CFIA	**2015**	**Ergot Alkaloids**	**744**	**86 (12)**	**1145**	**109**
CFIA	2013 to 2015	Ergot Alkaloids	2235	478 (21.4)	1078	62
CFIA	**2017**	**Fumonisins B_1, B_2 and B_3**	**746**	**21 (2.8)**	**864**	**90**
CFIA	**2016**	**Fumonisins B_1, B_2 and B_3**	**750**	**48 (6.4)**	**2209**	**430**
CFIA	**2015**	**Fumonisins B_1, B_2 and B_3**	**744**	**30 (4.0)**	**1142**	**214**
CFIA	2013 to 2015	Fumonisins B_1, B_2 and B_3	2235	233 (10)	2062	187
CFIA	2011	Fumonisins B_1 and B_2	274	161 (59)	4442	253
CFIA	**2017**	**HT-2 and T-2 toxin**	**746**	**2 (0.3)**	**335**	**174**
CFIA	**2016**	**HT-2 and T-2 toxin**	**750**	**19 (2.5)**	**85**	**21**
CFIA	**2015**	**HT-2 and T-2 toxin**	**744**	**12 (1.6)**	**32**	**17**
CFIA	2013 to 2015	HT-2 and T-2 toxin	2235	66 (2.9)	271	28
CFIA	**2017**	**Cyclopiazonic acid**	**746**	**4 (0.5)**	**6.5**	**2.5**

CFIA Survey	Year	Analyte	Number of samples	Number of samples with detectable mycotoxins (%)	Maximum mycotoxin level (ppb)	Average[c] mycotoxin level (ppb)
CFIA	**2016**	**Cyclopiazonic acid**	**750**	**1 (0.1)**	**2.5**	**N/A**
CFIA	**2015**	**Cyclopiazonic acid**	**744**	**5 (0.7)**	**3.1**	**1.9**
CFIA	2013 to 2015	Cyclopiazonic acid	2235	35 (1.5)	8.3	2.7
CFIA	**2017**	**Ochratoxin A**	**746**	**39 (5.2)**	**7**	**1.8**
CFIA	**2016**	**Ochratoxin A**	**750**	**20 (2.7)**	**20**	**2.7**
CFIA	**2015**	**Ochratoxin A**	**744**	**38 (5.1)**	**36**	**4.7**
CFIA	2013 to 2015	Ochratoxin A	2235	128 (5.7)	34	2.6
CFIA	**2017**	**Sterigmatocystin**	**746**	**26 (3.5)**	**12**	**2.8**
CFIA	**2016**	**Sterigmatocystin**	**750**	**40 (5.3)**	**34**	**3.0**
CFIA	**2015**	**Sterigmatocystin**	**744**	**32 (4.3)**	**28**	**5.7**
CFIA	2013 to 2015	Sterigmatocystin	2235	41 (1.8)	18	3.1
CFIA	**2017**	**Zearalenone, α-zearalenol and β-zearalenol**	**746**	**13 (1.7)**	**267**	**50**
CFIA	**2016**	**Zearalenone, α-zearalenol and β-zearalenol**	**750**	**23 (3.1)**	**145**	**39**
CFIA	**2015**	**Zearalenone, α-zearalenol and β-zearalenol**	**744**	**25 (3.4)**	**477**	**82**
CFIA	2013 to 2015	Zearalenone, α-zearalenol and β-zearalenol	2235	93 (4.0)	577	50

[c] Average of positive results only

Can I access the survey data

The data associated with this report will be accessible on the Open Government Portal.

Appendix A

Table A1. List of analytes in the multi-mycotoxin method with their limits of detection (LOD) and limits of quantitation (LOQ)

Compound	Abbreviation	Mycotoxin Family	LOD (ppb)	LOQ (ppb)
Aflatoxin B1	AFB1	Aflatoxins (AF)	0.5	5
Aflatoxin B2	AFB2	Aflatoxins (AF)	0.5	5
Aflatoxin G1	AFG1	Aflatoxins (AF)	0.7	5
Aflatoxin G2	AFG2	Aflatoxins (AF)	0.9	5
Cyclopiazonic acid	CPA	none	0.6	5
Ergocristine	EA	Ergot Alkaloids (EA)	5	50
Ergocryptine	EA	Ergot Alkaloids (EA)	6	50
Ergosine	EA	Ergot Alkaloids (EA)	9	50
Fumonisin B1	FB1	Fumonisins (FB)	4	50
Fumonisin B2	FB2	Fumonisins (FB)	10	50
Fumonisin B3	FB3	Fumonisins (FB)	8	50
Ochratoxin A	OTA	none	0.8	5
Sterigmatocystin	STG	none	0.4	5
Deoxynivalenol	DON	Trichothecenes (TRI)	10	50
3-acetyldeoxynivalenol	3-Ac-DON	Trichothecenes (TRI)	13	50
15-acetyldeoxynivalenol	15-Ac-DON	Trichothecenes (TRI)	13	50
Diacetoxyscirpenol	DAS	Trichothecenes (TRI)	10	50
Fusarenone-X	FUS-X	Trichothecenes (TRI)	17	50
Neosolaniol	NEO	Trichothecenes (TRI)	10	50
Nivalenol	NIV	Trichothecenes (TRI)	7	50
HT-2	HT-2	Trichothecenes (TRI)	8	50
T-2	T-2	Trichothecenes (TRI)	9	50
Zearalenone	ZEN	Zearalenone (ZEN)	7	50
α-zearalenol	α-ZOL	Zearalenone (ZEN)	7	50
β-Zearalenol	β-ZOL	Zearalenone (ZEN)	7	50

Appendix B

1 Aflatoxins

Aflatoxins are a family of naturally-occurring, toxic secondary metabolites produced by *Aspergillus flavus* and *A. parasiticus* fungi[10]. Aflatoxin-producing fungi may contaminate agricultural products (such as corn, nuts, spices, dried fruit) if grown, transported, stored, or processed under hot, humid conditions for prolonged periods of time, or with pest pressures resulting in bruising or cuts on the commodity[10,11]. Drought pressure on corn is also a major risk factor for the occurrence of aflatoxins in the field[10,11,12]. Due to the cooler Canadian climate, domestically-grown agricultural commodities (and products) are less likely to contain aflatoxins than those imported from warmer climates. Aflatoxins are not destroyed by heating, cooking or most other processing methods[13].

One aflatoxin form, aflatoxin B1, is among the most potent naturally-occurring liver carcinogens known[14]. The International Agency for Research on Cancer (IARC) classified aflatoxins to be carcinogenic to humans (Group 1 carcinogen)[15]. Chronic exposure to aflatoxins has also been associated with growth impairment in children living in developing countries where exposure to aflatoxins is relatively high. Aflatoxins have been shown to cause immune suppression in experimental animals[169,17,18,19].Short-term exposure to high levels of aflatoxins can cause illness in humans which is characterized by vomiting, abdominal pain, convulsions, coma and death. The illness is very rare in the developed world[20]. This study included aflatoxins B1, B2, G1 and G2.

2 Cyclopiazonic Acid

Cyclopiazonic acid (CPA) is produced by *Penicillium cyclopium, Penicillium* species (e.g. *P. commune* and *P. camembertii*), *Aspergillus flavus and A. versicolor*. Cyclopiazonic acid has been detected in corn, millet, peanuts, pulses, cheese, ham, sausage, hot dogs, tomato and milk[21].

There is little information available regarding potential human health effects associated with CPA. However, it has been linked to 'Kodua' poisoning in India resulting from ingestion of contaminated millet seeds. The symptoms included sleepiness, tremors and giddiness which lasted 1-3 days, followed by complete recovery[22]. Experimental animal studies indicate that CPA is toxic only when ingested in high concentrations. Repeat exposure to high doses of CPA show a range of effects such as neurotoxicity, liver and kidney damage, weight loss, diarrhea, dehydration, convulsions and death in several different species[23].

3 Ergot Alkaloids

Ergot alkaloids (EA) are formed by fungi of the *Claviceps* species, particularly *C. purpurea*. These fungi parasitize the seed heads of cereals, replacing individual grain kernels with discoloured fungal structures (dark purple or black) known as sclerotia or ergot bodies. The predominant ergot

alkaloids present in ergot bodies are ergometrine, ergotamine, ergosine, ergocristine, ergocryptine and ergocornine (only ergosine, ergocristine and ergocryptine were successfully included in the current multi-mycotoxin method). The type and levels of these alkaloids in ergot bodies vary considerably depending on the fungal strain, the host species, the weather conditions and geographic region. Wet weather and soil favour the growth of ergot bodies. These bodies are harvested with the cereals and can thus lead to contamination of cereal based food and feed products with ergot alkaloids. The cleaning methods used during grain processing usually remove the ergot bodies from the grain[24].

Long-term exposure to ergot alkaloids causes ergotism, also known as ergotoxicosis, ergot poisoning and Saint Anthony's Fire[25,26]. The symptoms can include fevers, hallucinations, swollen or rigid limbs, severe inflammation sometimes followed by loss of affected tissues and death[27]. Experimental animal studies indicate the ergot alkaloids act on a number of neurotransmitter receptors which with repeat dosing results in restricted blood flow, particularly of the limbs, weight loss and changes in the levels of some hormones in rats[28]. This study included ergosine, ergocristine and ergocryptine.

4 Fumonisins

Fusarium moniliforme and *Fusarium proliferatum* are plant pathogens common in grain-growing regions throughout the world. These pathogens can infect grain crops either in the field (pre-harvest) or during storage (post-harvest). The moulds proliferate if grains are grown in hot, dry weather followed by very humid conditions. Mould growth is also favoured by storage under wet conditions. The plant pathogens produce mycotoxins known as fumonisins (FUM). Corn is the grain most vulnerable to fumonisin contamination[29]. The levels of fumonisins can be quite high, even in the absence of visible signs of mould proliferation[30]. There are several forms of fumonisin: fumonisins B1, B2 and B3 are the most prevalent. While studies have focused on fumonisin B1, available data suggests that fumonisins B2 and B3 have a similar toxicological [31,32,33,34]. Fumonisins are heat-stable up to 150°C and are unaffected by mechanical forces (such as grinding), but can be reduced by alkaline treatment (a traditional means of preparing corn masa and other corn-based products such as tacos)[35].

Although fumonisin contamination is mainly observed in corn, some scientific studies have shown the presence of fumonisins in red wine[36], sorghum[37], white beans, wheat[31], barley[31], soybeans[31], figs[31], rice[38], black tea[31], and medicinal herbs[31].

The ingestion of foods containing fumonisins may be harmful to human health. Health effects which have been observed in specific populations where corn is a major component of the diet and where the climate may favor fumonisin proliferation include esophageal cancer in South Africa and China[30,39], neural tube defects in Central America and the southwestern US[34]. The

precise biological effects of fumonisins are complex and relate to their interference with cell metabolism[29]. Experimental animal studies have revealed that fumonisins induce liver and kidney damage in many species[40]. Fumonisin B1 has been classified by IARC as possibly carcinogenic to humans based on evidence in experimental animal studies[41]. This study included fumonisins B1, B2 and B3.

5 Ochratoxin A (OTA)

OTA is a naturally occurring metabolite of *Aspergillus* and *Penicillium* moulds. Under favourable moisture and temperature conditions, the fungi can grow on stored material and produce OTA[42]. OTA has been widely detected in cereal grains (wheat, corn, oat, and barley), green coffee, grape juice, beer, wines, cocoa, dried fruits, and nuts[43]. OTA is heat-stable and is only partially destroyed under normal cooking or processing conditions[44].

The International Agency for Research on Cancer (IARC) has classified OTA as a possible human carcinogen based largely on data from animal studies[45]. The mechanism by which OTA causes kidney tumours in rodents has yet to be fully explained. In animal studies, OTA has also been shown to have effects on the kidneys, the developing fetus, and the immune system. Health Canada completed a risk assessment for OTA, and as a result, has proposed maximum levels for OTA in various food commodities**Error! Bookmark not defined.** as well as an industry guidance value for OTA in unprocessed cereal grains**Error! Bookmark not defined.**.

6 Sterigmatocystin (STG)

Sterigmatocystin is a mycotoxin produced mainly by various *Aspergillus* species. It can also be produced by species such *as Bipolaris, Chaetomium*, and *Emiricella*. It has been detected in grains, corn, bread, cheese, spices, coffee beans, soybeans, and pistachio nuts. Wet, warm, conditions favour sterigmatocystin production[46].

The IARC has classified sterigmatocystin as a possible human carcinogen[47]. It also has properties capable of causing DNA mutations. It is acutely toxic to animals, with the liver and kidneys as its principle targets. This toxin is structurally similar to aflatoxin, however, tests in rats have shown that it is ten times less lethal following acute exposure to high doses and ten to a hundred times less effective at inducing liver cancer[46]. Its human health effects have not been well-studied.

7 Trichothecenes (TRI)

This large family of mycotoxins are typically found in cereal grains (notably wheat, barley, and corn), and have been detected in their derived products (flours, meals, bran, grits, cereals, and beer). These toxins are produced by various species of *Fusarium* mould in some crops prior to harvest. These toxins are observed in grains suffering from Fusarium head blight (FHB) in the field. Wet, warm weather conditions in the field will favour the development of FHB, and subsequently the production of trichothecenes[48]. The trichothecenes are heat-stable and are only partially destroyed under normal cooking or processing conditions[49]. The most widely commonly occurring trichothenece is DON.

The human health effects of nivalenol[50], fusarenone[51], 3-Ac-DON[48], 15-Ac-DON[48], neosolaniol (NEO)[48] and DAS[48] are not as well-studied as those of DON. DON is not known to be carcinogenic, but it has been associated with acute and chronic health effects. Outbreaks in Asia, attributed to the consumption of grains with high levels of DON, are associated with short-term human illness, involving nausea, vomiting, abdominal pain, headache and dizziness. In experimental animal studies, long-term exposures to low levels of DON are associated with decreased food intake, weight loss, and effects on the immune system[52].

T-2 and HT-2 toxins are formed when grain crops remain in the field at or after harvest for extended periods, especially in cold weather, or in grain that becomes wet during storage. They have been detected in wheat, corn, oats, barley, rice, beans, and soybeans and some cereal-based products. Oats are most likely to contain these toxins but they have been detected frequently at lower concentrations in barley. Wheat is only rarely contaminated with these toxins[53].

The human health effects associated with chronic exposure to HT-2 and T-2 are not known. In animals, these toxins inhibit DNA, RNA and protein synthesis and are cytotoxic. IARC considers HT-2/T-2 toxins not classifiable as to their carcinogenicity to humans based on the lack of available human carcinogenicity data and only limited evidence in experimental animals[54,55]. This study examined nivalenol, fusarenone, 3-Ac-DON, 15-Ac-DON, NEO, DAS, DON, HT-2 and T-2.

8 Zearalenone and related compounds

Zearalenone (ZEN) is a mycotoxin produced mainly by *Fusarium* species. It has been detected in wheat, barley, rice, corn, and other cereals. It is heat-resistant and can be found in finished grain-based products. ZEN is metabolised to α-zearalenol (α-ZOL) and β-zearalenol (β-ZOL)[56,57,588]

ZEN is not an acute toxin. ZEN is an estrogenic compound and its major metabolites are more potent estrogenic compounds. It causes infertility in sheep, cattle and pigs, and may lead to

earlier sexual maturation in some animals. In experimental animal studies, high oral doses of ZEN have also been shown to be genotoxic, toxic to the liver, and affect blood and the immune system[55,659]. IARC concluded that there is limited evidence of the carcinogenicity of ZEN[60]. ZEN has been considered a possible contributing agent in the outbreaks of early puberty in thousands of girls in Puerto Rico and may play a role in human breast and cervical cancer in highly exposed populations[56].

This study examined ZEN α-ZOL, and β-ZOL.

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