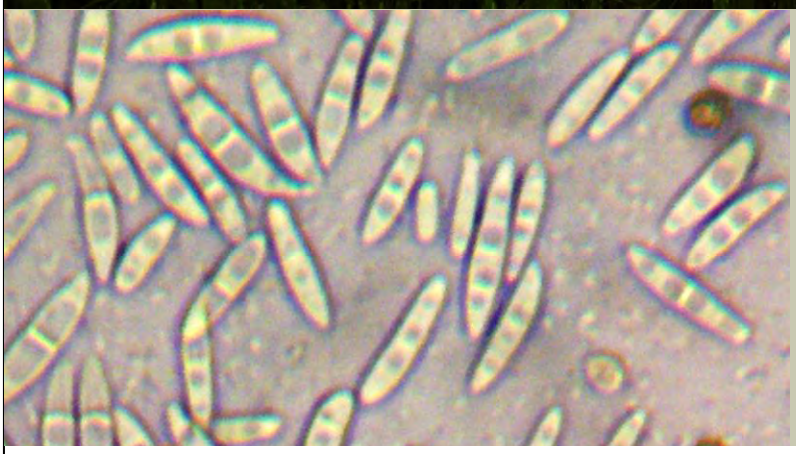

RGI
Regulatory Governance Initiative

MYCOTOXINS IN CANADA:

A PERSPECTIVE FOR 2013

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KEY IMPLICATIONS FOR DECISION MAKERS

For more than 80 years, mycotoxins in grains have been associated with domestic animal toxicosis in Canada. Starting in the late 1960s, as new toxins were described, they were measured in Canadian grains, often at unacceptable concentrations by the standards of today. Since 1981, increasing awareness of the impact of these compounds on human and animal health has led to increasingly stringent regulations in food and feed. A number of challenges exist now and are on the horizon in the management of mycotoxins in Canada. These include regulatory harmonization with Europe, climate variability, genetic changes in the populations of the fungi involved and other toxins not previously considered a problem in Canada including, but not limited to, ochratoxin A, deoxynivalenol glycoside and fumonisin.

- Research to improve tolerance to Fusarium Head Blight in wheat has had a moderate impact. Growers have generally been unwilling early adopters due to the yield penalty.
- The use of toxin or FHB prediction systems to avoid the unnecessary application of fungicides when the risk for deoxynivalenol at harvest is high has proven successful in the marketplace. The Canadian system (DONCAST) has proven itself worldwide. As an alternative, farmers can hire PhD consultant plant pathologists to help plan fungicide applications. Investments are needed to expand and improve these capabilities.
- In the case of *F. graminearum* disease in corn (Gibberella ear rot), breeding for tolerance is the better option. It is not clear that fungicides play a cost-effective role in protecting corn from this disease.
- The increased temperature will probably increase the prevalence of FHB associated with *F. avenaceum*. This results in the accumulation of moniliformin and enniatins. The European Food Safety Agency is reviewing the need to regulate these compounds.
- Warmer summers will increase the incidence and severity of *Fusarium* kernel rot and fumonisin.

RGI

The Regulatory Governance Initiative (RGI) at Carleton University builds on the proven track record of Carleton's *School of Public Policy and Administration* to develop regulatory capacity and competence through research, education, and dialogue. Its scope is regulatory policy, governance, and management. Its approach is holistic and problem-driven. The RGI assembles expertise from the humanities, social and natural sciences as needed.

- Genetic changes in *F. graminearum* that affect deoxynivalenol concentration in the kernels have occurred. These new populations are spreading with unpredictable long-term consequences. There is no capacity in Canada to effectively study and monitor these changes.
- There is no information on the occurrence of deoxynivalenol glucoside in Canadian cultivars of small grains. In Europe, there are a lot of data and regulatory interest.
- The quality of sampling through the value chain is the major barrier to the management of grains as regulatory limits go down and regulation of ochratoxin A expands.
- There is no capacity in Canada (or the US) to monitor many fungal metabolites in food. In contrast, a wide range of compounds are being monitored in food products in Europe. Some of these compounds are attracting regulatory interest in Europe including the mycotoxin glycosides, enniatins, and several *Alternaria* toxins.

EXECUTIVE SUMMARY

Small grains (wheat, barley, oats and triticale) are all affected by the crop disease Fusarium Head Blight. In Canada, FHB is mainly caused by *Fusarium graminearum* but also *F.avenaceum* and *F. culmorum*. There are now six genetic populations of *F. gramineaum* in the northern part of the USA and probably Canada. Wheat and oats can also be colonized by a number of saprophytic fungi that can also leave the trichothecene mycotoxin deoxynivalenol in the crop. In corn, *F. graminearum* causes the same disease with the common name Gibberella ear rot or pink ear rot. *Gibberella zeae* is the sexual state of *F. graminearum*. The disease occurs when it rains around anthesis for wheat and silk emergence for corn and the weather is reasonably warm. Wheat with FHB resulting from *F. avenaceum* infections can be contaminated by moniliformin and enniatins. The toxicity of these compounds is not clear.

Small grains, but particularly wheat can be colonized by weakly pathogenic *Fusarium* species, principally *F. sporotrichoides* but also *F. poae*, *F. acuminatum*. *F. sporotrichoides* produces T-2 and HT-2 toxins and another metabolite beauvaricin. T-2 and HT-2 are quite toxic and are more common in parts of Europe. There is an EU tolerance for the sum of these toxins in grains. In the US and Canada, T-2 and HT-2 toxins are a problem in grain that is abused (e.g. overwintered; Scott et al. 1980) or 'suspect' grain (Abramson et al. 1987). Additionally, small grains, but particularly oats can be contaminated by the metabolites of the saprophyte *Alternaria alternata.* Contamination usually results from wet weather before harvest. *A. alternata* toxins are fairly common in parts of Europe. There is very little known about these toxins. The EU has an interim tolerable daily intake for the mutagenic compounds made by this fungus.

In Ontario and Quebec, wheat and corn are grown in the same area, thus should there be an epidemic of Gibberella ear rot there is an increased risk of Fusarium Head Blight in the wheat crop in the subsequent year. As a result, farmers include soybeans in the crop rotation. However, the spores of the fungus can travel fairly long distances so the weather is the largest variable in assessing whether there will be an

epidemic. These diseases result in substantial reductions in yield but the larger consequence is the presence of toxins.

Nivalenol is more toxic than DON and to date nivalenol-producing strains of *F. graminearum* are uncommon in Canada. However, NIV- producing strains have been reported in Louisiana, New York and North Carolina (Gale et al. 2011a; Schmale et al. 2011). In addition, a NIV- producing sister species, *F. asiaticum* is also common in the southeast USA. This had hitherto only been known in Japan and China (Gale et al. 2011a). There is an EU (and Japanese) tolerance for NIV (EFSA 2013).

The indigenous chemotype of *F. graminearum* produces DON via the precursor 15 acetyl DON (15ADON, Miller et al. 1983a). In the 1980s, this dominated North and South America. The Asian chemotypes (3ADON pathway) of *F. graminearum* were introduced to Canada in the 1980s. By 2007, nearly 100% of the strains in Atlantic Canada were Asian as opposed to the native 15ADON producing strains (Ward et al. 2008). Although originally the two strains were genetically homogenous there continues to be a shift towards a population of genetically divergent strains (Mishra et al. 2009). When wheat is infected by strains of the 3ADON chemotype, somewhat more DON occurs in the kernel. It is not clear why. Strains that are crosses of the two ADON chemotypes but produce 15ADON are more virulent than either parent (Foroud et al. 2012). In addition, there is a so-called Northland population emergent in Minnesota that does not produce DON or NIV (Gale et al. 2007; 2011b). It appears that this strain makes un-described toxins.

TABLE 1: *FUSARIUM* SPECIES INVOLVED IN FUSARIUM HEAD BLIGHT.

Species	Pathogenicity	Toxins[1]
F. graminearum	high	3ADON, 15ADON, DON or NIV, ZEA
F. culmorum	high	3ADON, 15ADON, DON or NIV, ZEA
F. avenaceum	high	moniliform, enniatins
F. sporotrichioides	low, but common	T-2, HT-2, beauvericin
F. psuedograminearum	crown rot fungus that can spread to the head	DON
F. poae	low	DAS
F. acuminatum	low	T-2, enniatins
F. crookwellense	low	DON or NIV

F. verticilliodes and *F. proliferatum* produce fumonisin and can be recovered from virtually all maize kernels worldwide including those that are healthy (Miller 2008). Material concentrations of fumonisin only accumulate in stressed or senescing kernel tissue under warm conditions and dry conditions between silking and early grain fill (Miller et al. 1995). Maize genotypes containing the anti-insectan Bt protein have reduced amounts of fumonisins compared to non Bt genotypes (Schaafsma et al. 2002).

The accumulation of fumonisins in the cool corn-growing region of Southern Ontario has been shown to be related to rainfall where areas of drought had higher incidence of fumonisins (Miller et al.1995). In the drought year of 2012, 75% of the samples contained fumonisins and of those (30%) had values >1 μg/g (Limay-Rios, personal communication) with the higher values approaching the FDA requirements for sensitive animal species expressed on a dietary level. The risk for fumonisins to become more of a problem are suggested to increase due to the predicted climate shift of less rain in July and August and warmer temperatures (Rahman et al. 2012). Levels of ZEA and DON are also predicted to change concomitantly with the temperature and rainfall changes over the next decades (Miller et al. 2013)

When *F. graminearum* infects cereals and corn cultivars a percentage of DON is converted into a sugar conjugate called a glucoside. Further, when the process to make the food involves fermentation (bread, beer, where enzymes are added) additional glucoside is formed. These are not seen when analyzed by methods for DON. There are some data that indicate that when wheat products are consumed, DON glucoside is converted to DON. There is considerable interest in Europe to regulate DON glucoside (e.g. an ILIS-EU task force) and a lot of occurrence data. JECFA (2011) stated that DON glucoside will be added to the group TDI for DON and related compounds if its bioavailability is similar to that of DON; recent data has shown this is possible. There is currently little information on the percentage of DON glucoside in Canadian cultivars of small grains and the usual analytical methods fail to detect it.

The quality of sampling through the value chain is a major barrier to the management of mycotoxins in grains, especially as regulations change on a national and international level. Analytical methods need to improve and be developed for other metabolites of interest such as enniatins in wheat and corn as well as mycotoxin glucosides.

REPORT

FUSARIUM DISEASES OF SMALL GRAINS

The term Fusarium head blight of wheat and other small grains is a description of a disease that is caused by several species of the genus *Fusarium*. Although *F. graminearum* diseases of wheat and corn have been most studied, this species is not always the primary cause of disease in all regions where cereals are grown. Additionally, within regions, there is year-to-year variability in the species isolated.

Early colonists to New France and New England brought wheat cultivars from their home counties in England and France. Prior to that, Spanish cultivars were brought to Florida during the 15th century which spread south. In Canada in 1721 an 8000 ton crop was produced mainly in Quebec. By 1760, Ontario was the major wheat producer in Canada and remained so until the 1880s (Burton 1937).

In Quebec and Connecticut, scab was reported as a problem in the late 17th century (e.g. Ball 1930). The disease was recognized to be a major problem in the US and Canada by the 1890s (Goswami & Kistler 2004; Miller 1994). Systematic reports of wheat and corn disease including FHB began ca. 1900 in the US and Canada. By the 1930s, Canadian plant pathologists were well aware of the yield penalty the disease caused in, for example, Minnesota. From at least 1936, pathologists were aware that swine were affected when fed scabby wheat (Simmons 1941). Certainly compared to Minnesota where large scale cultivar screening programs for FHB tolerance began in the 1920s, there was much less interst in this disease in Canada. This is likely because wheat production was more important in western Canada where conditions are generally unfavourable for the disease. In eastern Canada, where the risk is much higher, the farms had shifted to corn, dairy and swine production (Reeds 1959). Awareness of FHB in the east changed dramatically in 1980 when Ontario grain was embargoed due to the presence of deoxynivalenol (Globe & Mail "Embargo set on wheat sales while tests made for toxin", September 10, 1980; Sutton 1982).

Wheat, corn, barley and some triticale cultivars are most affected by *Fusarium* toxins. Oats grown in eastern Canada have been reported to contain *Fusarium* mycotoxins albeit at low levels and frequencies (Tamburic-Ilincic 2010; Campbell et al. 2000). However, it is known that oats (Tamburic-Ilincic 2010) and rye are resistant or escape significant contamination (Miller 1994). Epidemics have become more

common in the past 25 years associated with the susceptibility of the cultivars being used and the weather (McMullen et al. 2012; Miller et al. 1985; Miller 1994).

Associating different *Fusarium* species with different climatological conditions is somewhat essentially arbitrary. For example, *F. graminearum* (teleomorph *Gibberalla zeae*) is more associated with cereals grown in "warmer" areas than *F. culmorum* (no known teleomorph). However, the influence of temperature relates to conditions that allow a sustained period of warm conditions (daytime temperatures >30°C). For example, although the Maritime Provinces are generally considered cool, *F. graminearum* is none the less the dominant head blight species.

F. avenaceum (teleomorph, *Gibberella avenacea*) was also common in wheat containing *Fusarium* Damaged Kernels from Ontario, Manitoba and Saskatchewan (Clear et al. 1990; Gräfenhan et al. 2013). In a study in PEI, the Fusaria were isolated from wheat kernels collected weekly from anthesis to harvest over four years in the same location. The frequency of occurrence of *F. avencaeum* and F. *sporotrichioides* was negatively correlated to temperature (see Miller 1994). *F. avenaceum* is more pathogenic than generally believed and the toxin moniliformin is accumulated in the kernels (Vogelgsang et al. 2008). Canadian strains of *F. avenaceum* produce enniatins (Blais et al. 1992). This cyclic peptide has been detected in Ontario and in a survey of export grain (Limay Rios, personal communication; Tittlemier et al. 2013b). There are few data from Canadian strains but in wheat in Europe, beauvericin is produced by *F. poae* and *F. sporotrichiodes* (Kokkonen et al. 2010; Vogelgsang et al. 2008). In corn, US strains of *F. proliferatum* are known to make beauvericin (Plattner & Nelson 1994).

The impact of temperature on the distribution of Fusaria recovered from heads was examined in three consecutive years in Ottawa. In 1991, the corn heat unit (CHU) value was 19% higher than the 30 year average. *F. graminearum* and *F. culmorum* were low but *F. sporotrichiodes* damage was higher. The following year, CHU value was 3% lower and rainfall was 18% higher. *F. sporotrichioides* was low and the cool temperature species *F. culmorum* was the highest of any year (Miller et al. 1998).

The distribution of the head blight species is broadly affected by pathogenicity where *F. graminearum>culmorum>avenaceum>crookwellense*. The regional and annual variation of the pathogenic species is most affected by temperature (from coldest to warmest): *F. culmorum>crookwellense>graminearum>avenaceum* (Miller 1994). As noted by Cook (1981), the

dominant head blight species is "determined by temperature more than any other factor". The less pathogenic species *F. poae* occurs under cooler conditions. As a generalization, only *F. graminearum*, and *F. culmorum* result in significant visual symptoms. Kernel damage resulting from *F. avenaceum* is harder to see compared to infections by *F. graminearum*.

Although increased rainfall promotes FHB, incidence is most affected by moisture at anthesis under warm conditions (Miller 1994; Schaafsma & Hooker 2007; Sutton 1982). The effect of water potential on growth and sporulation of perithecia or pycnidia on crop residue is similar for *G. zeae*, *F. culmorum* and *F. avenaceum* (Sung & Cook 1981). Climate models suggest that thirty years from now the Southern Ontario watershed will have slightly more rain in June and warmer temperatures (Rahman et al. 2012). This implies that FHB risk will remain a serious challenge.

Morphologically identical isolates of *F. graminarum* can produce either DON and zearalenone or nivalenol and zearalenone as the principal toxic metabolites that accumulate in grain. Although all strains produce zearalenone, this toxin was seldom seen in small grains including oats in Ontario. It occurs in greater frequency –but still low-, and, in low concentrations- in Quebec and the Maritimes (Campbell et al. 2000; 2002; Stratton et al. 1993).

As noted, *F. sporotrichiodes* is common on small grains and may facilitate the infection of the plant by *F. graminearum*. As with isolates from elsewhere, the principal toxins from Canadian strains are T-2 and HT-2 toxin (Davis et al. 1982; Greenhalgh et al. 1988). These are the most acutely toxic simple trichothecenes. T-2 toxin is only a problem in grain that is abused (e.g. overwintered; Scott et al. 1980) or ‘suspect’ grain (Abramson et al. 1987).

FUSARIUM DISEASES OF CORN

On the basis of symptoms, there are two general kinds of damage to corn ears caused by *Fusarium* species: Gibberella ear rot or pink ear rot and Fusarium ear rot/Fusarium kernel rot. The former is prevalent in north temperate climates especially in wet years and is mainly caused by *F. graminearum* (teleomorph: *G. zeae* (Miller 1994; Sutton 1982). The latter is associated with warm, dry years and insect damage and is caused by *F. subglutinans* (*Gibberella subglutinans*), *F. verticilliodes* (*Gibberella*

fujikuroi) and *F. proliferatum* (Miller 2001; 2008; Miller et al. 1995; Schaafsma et al. 2008; Vigier et al. 1997).

F. graminearum disease incidence and DON accumulation is affected by moisture at silk emergence and prevalence is increased with wet weather later in the season. Monitoring of the growth of *F. graminearum* in experimentally- infected ears showed the growth rate of the fungus was sensitive to temperature. A ten day period where the average "growing days [was] above 5°C" growth was virtually halted. An average value of ca. 15°C resulted in rapid growth (Miller et al. 1983b). Susceptibility of the corn hybrid, rotation and rainfall explain most of the variation in DON concentrations in the harvested crop (Hooker & Schaafsma 2005).

Bird and damage predisposes corn to infections by *F. graminearum* and *Fusarium* spores were found to be common on the bird species involved and on insects (Miller 1994). As is the case elsewhere (e.g. Folcher et al. 2012), European corn borer damage promotes the infection of Ontario corn by *F. graminearum* (Schaafsma et al. 2002). The effect of Bt corn on lowering DON by controlling European corn borer under permissive conditions for the fungus appears less dramatic than for fumonisins (see below; Folcher et al. 2010; Schaafsma et al. 2002).

Fusarium graminearum Chemotypes

Some strains of *F. graminearum* make deoxynivalenol (DON) by the 3 acetylated precursor, and others produce the 15 acetylated precursor. Historically, DON-producing strains with the 15 acetylated precursor dominated in North and South America. DON-producing strains with the 3 acetylated precursor were dominant in Europe and Asia (these are called chemotypes; Miller et al. 1991). The Asian and New World strains are genetically distinct (O'Donnell et al. 2000). Nivalenol-producing strains of *F. graminearum* that are common in parts of Europe, Japan and Australia exist in Canada (Tanaka et al. 1988). Nivalenol remains very uncommon so far in Canadian grains (Tittlemeier 2013a). However, NIV- producing strains have been reported in Louisiana, New York and North Carolina (Gale et al. 2011; Schmale et al.

2011). In addition, a NIV- producing sister species, *F. asiaticum* is also common in the southeast USA. This had hitherto only been known in Japan and China (Gale et al. 2011a). There is an EU (and

Japanese) tolerance for NIV.This is a concern because nivalenol is more toxic than DON. *F. culmorum* produces DON and zearalenone (Jennings et al. 2004; Miller et al. 1991 and references cited therein; Toth et al. 2004).

The Asian chemotypes were introduced to eastern Canada on cultivars from Europe to the Maritimes in the 1970s and 1980s. Another source of the strains was from breeding material brought in from China and Europe. By 2007, nearly 100% of the strains in Altantic Canada were Asian as opposed to the native 15ADON producing strains (Ward et al. 2008). The first 3ADON strains in the collection at Agriculture Canada in Ottawa were deposited in 1979 (Gilbert et al. 2002; Ouellet & Seifert 1993). Isolates from the 3ADON chemotype produce significantly more DON + 3ADON and are more fecund and have higher growth rates than isolates from the 15ADON chemotype (Ward et al. 2008). These conclusions have been confirmed in a number of field studies in wheat in Canada (Gilbert et al. 2010; Tamburic-Ilincic et al. 2008; von der Ohe et al. 2010) and in adjacent States of the USA (Schmale et al. 2011). Clear et al. (2012) reported studies that provide some information of the impact of this in practical terms. They inoculated a barley field with strains of the two chemotypes. Three years later, the prevalence of the two chemotypes changed such that the 3ADON chemotype dominated. Further, the highest DON and 3ADON concentrations were associated with the increased frequency of the 3ADON chemotype.

When the two chemotypes were recognized in 1983 (Miller et al. 1983; Miller et al. 1991), the genetics indicated that they were homogenous (O'Donnel et al. 2000). Although originally the two strains were genetically homogenous there continues to be a shift towards a population of genetically divergent strains (Gale et al. 2007; Miller et al. 1991; Mishra et al. 2009; O'Donnel et al. 2000). When wheat is infected by strains of the 3ADON chemotype, somewhat more DON occurs in the kernel. It is not clear why. Strains that are crosses of the two ADON chemotypes but produce 15ADON are more virulent than either parent (Foroud et al. 2012). In addition, there is a so-called Northland population emergent in Minnesota that does not produce DON or NIV (Gale et al. 2011b). It appears that this strain makes an un-described trichothecene.

Fusarium Kernel Rot

Corn is universally infected by *F. verticilliodes* or the related species *F. proliferatum*. The fungus occurs systemically in leaves, stems, roots and kernels and can be recovered from virtually all maize kernels

worldwide including those that are healthy (Foley 1962; Miller 2001; 2008). Studies of introduced and resident strains found that only inoculated ears were colonized by the introduced *F. verticillioides* and that there was limited spread of strains from plant to plant (Yates & Spark 2008). *F. verticilliodes* and *F. proliferatum* produce the fumonisin and fusarins. Ontario strains of both species produce predominantly fumoninin B1 (FB1). Ontario strains of *F. proliferatum* also produce the mycotoxin moniliformin (Miller et al. 1995). To date *F. verticilliodes* is much more common in kernels containing fumonisin than *F. proliferatum* (Miller et al. 1995, Schaafsma et al. 2008). In Argentina, some strains of *F. proliferatum* produce more fumonisin B2. The toxicity of FB2 is similar to that of FB1. Little is known about the factors that result in *F. proliferatum* becoming the dominant species in corn with kernel rot. It appears to be related to perhaps subtle differences in the temperature/moisture conditions (De La Campa et al. 2005).

As suggested by the field data discussed above (Miller et al. 1983b), *F. graminearum* only grows well below between 25 and 28° C, with growth virtually ceasing much above that range. In this temperature range, assuming that there is sufficient rain, this species fungus out-competes *F. verticillioides*. This latter species grows well at temperatures above 28° C (Miller 2001, his figure 2; Reid et al. 1999). Material concentrations of fumonisin can only accumulate in stressed or senescing kernel tissue under warm conditions and dry conditions between silking and early grain fill (Miller 2001; Miller et al. 1995; Reid et al. 1999). Shelby et al. (1994) found that fumonisin concentrations were inversely proportional to June rainfall. Since drought stress results in greater insect herbivory on maize, it is not possible to totally separate these variables among other complications (Miller 2001). However, there is a strong consistent relationship between insect damage and Fusarium kernel rot. Within a year or two of the availability of fumonisin analytical standards, a field survey demonstrated that the incidence of the European corn borer increased Fusarium kernel rot and fumonisin concentrations (Lew et al. 1991). This broad finding has been sustained over many studies in areas susceptible to chronic fumonisin contamination although the insect species can vary by location (Parsons & Munkvold 2009; 2010; Wu et al. 2011).

Maize genotypes containing the anti-insectan Bt protein have reduced amounts of fumonisin compared to non-Bt genotypes (De La Campa et al. 2005; Hammond et al. 2004). This also appears to be the case in Ontario (Schaafsma et al. 2002). De La Campa et al (2005) were able to integrate this information in a study of factors that affected fumonisin accumulation in maize. Insect damage and weather variables in

four periods around silking explained most of the variation in fumonisin concentrations at harvest. The first critical period for fumonisin accumulation was 4 to 10 days before silking when temperatures <15° C and >34 °C (permissive temperatures; Miller 2001) reduced fumonisin. Within permissive temperatures, some rainfall increased fumonisin after silking (De La Campa et al. 2005). Schaafsma et al. (2002) suggested that the previous crop might play a role in in fumonisin accumulation in Ontario.

In the cool corn-growing area of southern Ontario, fumonisin accumulation was limited to drought-stressed fields. Comparing three counties with similar temperatures, the three with the highest average FB1 concentrations (1.4 µg/g) had half the rainfall of the counties with the lowest average FB1 (0.4 µg/g). Only 9 of 100 samples from the 1993 corn crop contained fumonisin (Miller et al.1995). Hooker & Schaasma (2002) reported detectable levels of fumonisins in four of the subsequent seven years which is broadly similar to the data of Campbell et al. (2002). Schaafsma et al. (2008) reported little fumonisin in the 2006 crop. However, in the drought year of 2012, 75% of the samples contained fumonisin. Of these six (30%) had values >1 µg/g including values of 2.3, 3.2 and 4.2 µg/g (Limay Rios, personal communication).

Campbell et al. (2002) reported on FB concentrations in 461 feed corn samples collected in 1993 and 1998 from across Eastern Canada. Approximately, 29.1% of the samples were positive based on a detection limit of 0.1 ppm. Mean concentrations ranged from 0.31 ppm to 1.71 ppm over the course of the study (Campbell et al. 2002). Analysis by Hooker & Schaafsma (2005) of 856 randomly selected Ontario corn fields between the years of 1993 to 2000 showed a total incidence of 23% for fields contaminated with 1.0 ppm or greater. There was a large year-to-year variation, with the lowest incidence in 1993-1996. The highest incidence, 56% was observed in 1999. Mean concentrations varied between 1.0 ppm in 1994, and 2.3 ppm observed in 1995. The highest maximum concentration was seen in 1995 (7.0 ppm; Hooker & Schaafsma 2005).

These higher values come close to the FDA requirements for sensitive animal species expressed on a dietary level. Thirty years from now the Southern Ontario watershed is predicted to have less rain in July and August and warmer temperatures (Rahman et al. 2012). This suggests a continued increased risk for fumonisin.

The effect of climate on mycotoxin occurrence has already been seen in Ontario. In the period 1972-1981, the prevalence and concentrations of zearalenone in corn were quite high (Andrew et al. 1981;

Scott 1997). Sutton et al. (1980) found that zearalenone was associated with rainfall in August, but only moderately or weakly with rainfall in July, September and October, and occurred during the later part of the crop year. Studies from 1990-2000 report little zearalenone in corn (Campbell et al. 2002). This change was due to cooler mean daily temperatures measured in several sites in Southwestern Ontario in the period 1970-1980 (Environment Canada data for London airport; Hussell 2003, his figure 2). Another factor was the time to maturity was shortened during the same period (Tollenaar 1989) thus the crop escaped the conditions required for zearalenone production. The biosynthesis of zearalenone has a requirement for high oxygen tension. As noted, zearalenone is typically accumulated in corn in the late summer when the crop is drying. Oxygen tensions are higher than in living plants and later in the season it is cooler than in July. Oxygen solubility in water is increased in colder versus warmer water. In contrast, deoxynivalenol is produced under conditions of low oxygen tension. Deoxynivalenol is seen in corn kernels concurrent with development of the infection when the plant is living and little zearalenone is being produced (Miller 2001).

As noted, *F. subglutinans* is common in Ontario corn (Schaafsma et al. 2008; Vigier et al 1997). This fungus is now recognized to be two species but the taxonomy is not resolved. *F. subglutinans sensu latto* produces beauvericin, moniliformin and the toxin fusaproliferin. There are no data on the occurrence of this toxin in Canada. Strains that produce this metabolite occur in Iowa (Munkvold et al. 2009) and it has been detected in feed corn (Munkvold et al. 1998). There are no data on this toxin from Ontario corn samples.

THE TOXINS

A much more detailed and extensively referenced discussion of the toxins is found in Miller et al. (2013).

Deoxynivalenol (DON):

Toxicology:

Deoxynivalenol (DON; 2,13-epoxy-3α,7α,15-trihydroxy trichothec-9-ene-8-one) is also called vomitoxin. This has to do with the fact that deoxynivalenol was discovered by Yoshizawa & Morooka (1973) and unknowingly re-reported with the name vomitoxin by Vesonder et al. (1973).

In swine (and humans) DON toxicosis presents with vomiting, a decrease in food consumption and a decrease in body weight or weight gain (Arnold et al. 1986; Gaigé et al. 2013; Goyarts and Dänicke 2006; Khera et al. 1986; Khera et al. 1994; Prelusky et al. 1988; Robbana-Barnat et al. 1988; Sprando et al. 2005). DON is immunomodulatory and cytotoxic (Bensassi et al. 2009; Bensassi et al. 2012; Gouze et al. 2006; Königs et al. 2007; Ma et al. 2012; Pinton et al. 2008; Pestka et al. 1990).

The established provisional maximum tolerable daily intake limit (PMTDI) for DON is 1 µg/kg body weight/per day on the basis of the NOEL of 100 µg/kg bw per day in a Canadian 2-year feeding study of mice and a safety factor of 100 (JECFA, 2001). This was modified in 2010 to include both acetates (3, and, 15ADON) for a group PMTID (JECFA 2011). In 1993, IARC classified DON as a category 3, that is not classifiable as to its carcinogenicity to humans and no data have emerged to change this determination (JECFA 2001; 2011).

Since these determinations were made, there has been further research on DON that merits further comment.

DON has long been known to have a strong emetic effect in some animals (but not rodents) regardless of route of exposure (Goyarts & Dänicke 2006; Prelusky et al. 1988) and to induce anorexia as well as changes in organ weights (Robbana-Barnat et al. 1988; Tryphonas et al. 1984, 1986). A significant decrease in weight gain and feed consumption is observed in animals exposed to sub-chronic doses of DON (Arnold et al. 1986; Gaigé et al. 2013;Khera et al. 1986; 1994; Robbana-Barnat et al. 1988; Sprando et al. 2005;).

Mink has been shown to have a similar vomiting response to swine, the best studied species (Miller 2008; Wu et al. 2012). The emetic potency of DON, 15ADON, 3ADON, and NIV was 30, 40, 290, and 250 µg/kg bw, respectively (Wu et al. 2012). The doses that elicit a response in swine are similar to that required for humans (for a detailed explanation see Miller 2008).

There are several mechanisms of action for the anorexic effect, some of which have been elucidated only recently. Dosing swine by a continuous-exposure osmotic pump, implanted intraperitoneally, demonstrated that feed refusal could not be due to taste or learned responses (Prelusky 1997). A single dose of 0.25 mg/kg bw (i.v.) changed neurotransmitter concentrations in the hypothalamus, frontal cortex and cerebellum up to 8 days post-dosing. Norepinephrine increased in all three tissues, whereas

dopamine was decreased. In contrast, serotonin increased and then decreased in the hypothalamus. It was decreased in the frontal cortex and no change was seen in the cerebellum (Prelusky et al. 1992). A lower dose (10 mg/kg bw, i.v.) resulted in changes in cerebral spinal fluid neurotransmitters. It was shown that the known pathways in the brain did not explain this effect (Prelusky 1997).

Related to this, recent studies have identified the regions of the brain where changes in biochemistry take place. These have compared feeding activity, the amount of food consumed by mice and up-regulation of c-Fos. The cellular gene c-Fos is part of a family of transcription factors. In the brain it can be used to indicate areas of neuronal activity and cytokine formations (Dantzer et al. 2008). A number of experiments have shown that DON reduced feeding and modified satiety by interfering with brain networks dedicated to food intake regulation (Girardet et al. 2011a). The pathways that are affected are similar to those that are invoked when mammals (including humans) have infections or exposure to endotoxin and feel sick. DON exposure results in central inflammation indicated by the up-regulation of cytokines including interleukins- 1b and 6. Attempts to block these pathways did not change the feeding response. This means that although the cyctokine up-regulation observed are hallmarks of feeling sick, the detailed mechanism in the brain remains to be elucidated (Bonnet et al. 2012; Girardet et al. 2011b).

There are two mechanisms that do not involve neurotoxicity. Amuzie et al. (2010) showed that DON modified the concentrations of Insulin-Like Growth Factor Acid-Labile Subunit (IGFALS) in circulation in B63CF1 mice. The reduced circulating Insulin-like Growth Factor 1 which in mammals affects growth. The influence of DON on IGFALS was found to reflect the combined effects of reduced food intake as well as its physiological action involving suppressors of cytokine signaling (Flannery et al. 2013).

Additionally, DON has been shown to directly affect both the oral and systemic satiety receptor Peptide YY. Flannery et al. (2012) found that orolingual exposure to DON induced plasma PYY and CCK elevation in B6C3F1 mice. This produced a level of anorexia comparable to that for ip exposure.

DON glucosides:

When DON is formed in plants, varying proportions of the toxin in the kernel are glycosylated which makes the compound non-toxic to the plant. When this was discovered in Ottawa (Miller & Arnison 1986), the percentage of the glucoside of DON in wheat from the Central Experimental Farm was

modest. Study of this was renewed 20 years later by Berthiller et al. (2005) who reported that in cultivars of wheat in Austria, with the typical percentage ratio of DON glucoside to DON ca. 10%. However some cultivars approached 30%. A QTL (quantitative trait locus) for the property to make DON glucoside was identified (Lemmens et al. 2005) demonstrating that the property was heritable and hence its segregation is somewhat unpredictable. At least in Europe, cereal products and beer contain DON glucoside, normally at a low percentage of the DON present but sometimes nearly equal the amount of DON (Berthiller et al. 2009, 2013; De Boevre et al. 2012; Kostelanska et al. 2011a). This includes "American beers" (Varga et al. 2013). European bakeries have performed work on the effects of baking parameters on DON glucoside. Among other things when enzyme mixtures were added to dough ingredients, the DON glucoside concentration increased in fermented dough (Bergamini et al. 2010 Kostelanska et al. 2011a). This also happens during malting in beer making (Kostekanska et al. 2011b; Maul et al. 2012).

In summary, at the time of writing, there are no published data on DON glucoside in Canadian foods. In Europe, there are some cultivars that accumulate DON glucoside as a high percentage of DON. The distribution of DON glucoside accumulating cultivars in Canada is similarly unknown.

In the 2011 JECFA decision about DON discussed earlier, the committee said that if the DON glucosides were absorbed as DON, the concentration of DON glucoside would be added to the group PMTDI. The panel recommended that ADME data be generated and that occurrence data be obtained (JECFA 2011). An ILSI-EU task force was commissioned on the hazards of DON glucosides that suggested that work be done on the toxicology as soon as possible (Berthiller et al. 2013). Preliminary studies in Sprague Dawley rats demonstrated that DON glucoside was not metabolized to DON in the gut and hence was not bioavailable (Nagl et al. 2012). However, there was evidence that the bacterial flora of the rat was able to convert DON to DON glucoside (Berthiller et al. 2011). However, the rodent gut is not the same as that of a human (or pig) with respect to important parameters including pH and normal gut flora. Recently, Gratz et al. (2013) demonstrated that human colonic bacteria were able to hydrolyze DON glucoside and provided evidence of bioavailability in humans by analyzing urinary DON and metabolites. The in vitro aspects of this study were confirmed by another recent study by Dall'Erta et al. (2013).

Zearalenone (ZEA)

Zearalenone (ZEA) is an estrogenic mycotoxin. The provisional maximum tolerable daily intake (PMTDI) for zearalenone is 0.5 µg/kg bw based on the NOEL of a 15-day study in pigs and a safety factor of 100 (JECFA 2000). The EU Scientific Committee on Food (SCF) established a temporary TDI (t-TDI) of 0.2 µg/kg per day (EFSA 2011a).

Nivalenol

This toxin is more acutely toxic than DON. Many of the features of NIV toxicology are smilar to DON. Its emetic potential is ca. $1/10^{th}$ that of DON (Wu et al. 2012). In 2013, EFSA established a tolerable daily intake (TDI) of 1.2 µg/kg bw per day (EFSA 2013)

Fumonisin

Fumonisin B1 (FB1) has been classified as a Group 2B carcinogen; possibly carcinogenic to humans (IARC 2002). The established provisional maximum tolerable daily intake limit for the fumonisins (FB1, FB2, and FB3) from all sources is 2 µg/kg body weight/per day on the basis of the NOEL of 0.2 mg/kg bw per day and a safety factor of 100 (JECFA, 2001). This was reaffirmed in 2011 (JECFA 2011).

In 2001, it was believed that fumonisin was not teratogenic since no birth defects had been seen in serveral animal models including two non-human primates and fumonisin did not cross the placenta. Because of this, it was assumed that fumonisins did not cause birth defects. Unfortunately, a cluster of neurotube birth defects (NTDs) was seen in Texas in Mexican-American women in the year following a bad year for the horse disease caused by fumonisin, ELEM. This was associated with consumption of tortillas (Hendricks 1999). It was known that commercial tortilla making facilities hydrolyzed any fumonisin present which provided further assurance that fumonisin could not be the problem. It turned out that because the tortilla making facilities from which most of the women purchased their tortillas or masa were not the same as large commercial facilities in the US. The processes of some of these small companies left residual fumonisin in the product (De La Campa et al. 2004). It further emerged that the women concerned had low folate and consumed a lot of tortillas compared to the general population (Missmer et al. 2006).

It was known that in vitro, fumonisin blocked the folate receptor which presented a hypothesis for how fumonisin could cause NTP (which most often result from NTDs). Early studies with somates showed that indeed fumonisin resulted in folate-reversible NTDs (Marasas et al. 2004; Saddler et al. 2002). This was not confirmed in animals until 2005 in a rodent strain developed to study NTDs (Gelineau-van Waes et al. 2005). In strains of rodents more useful for regulation, fumonisin does cause NTDs but at a much lower rate for the same exposure (Gelineau-van Waes et al. 2009; Voss et al. 2006; 2009). NTDs on the US-Mexican border in Texas are now understood to be multifactorial and now include fumonisin (Suarez et al. 2012).

Since the re-consideration by JECFA in 2011, there are some data that deserve comment. Using biomarkers such as fumonisin in urine and alterations in sphingolipid ratios, human exposure has been documented in a number of populations in Africa, Mexico and Guatemala from the consumption of corn food which is often highly contaminated with fumonisin (Shephard et al. 2013). Some exposure has been reported from eating corn and eating maize based food in the USA (Riley et al. 2012).

T-2 / HT-2:

As noted above, T-2 and HT-2 toxins are generally minor contaminants in all but abused grain in Canada. These toxins have a much greater acute toxicity than DON. They are more common in European grains, especially in more northerly, wetter areas. Because worldwide exposure is so much less, these toxins are much less studied than DON, but, disregarding emesis, many of the features of T-2 and HT-2 toxicology are similar to DON. In 2001, JECFA established a group PMTDI of 60 ng/kg bw per day for T-2 and HT-2 toxins, alone or in combination. EFSA established a group tolerable daily intake (TDI) of 100 ng/kg b.w. for the sum of T-2 and HT-2 toxins (EFSA 2011b).

Enniatins & Beauvericin

There are limited data on the occurrence of these metabolites in Canadian grains and apparently none in food products. These are peptide metabolites that were first researched for their modest antibiotic activity (e.g. Brian 1951). Both compounds are phytotoxic and have anti-viral and antifungal properties (Jestoli 2008; Wang & Wu 2012). Enniatins remain under active study as possible drugs (Sy-Cordero et al. 2912).

Beauvaricin has has also been studied because it is produced by strains of the fungus *Beauvaria bassiana* which is registered by the US EPA and Health Canada as a biocontrol agent for various insects. Both compounds are potent ionophores and are highly toxic to insects (Grove & Pople 1980; Jestoli 2008).

When cell lines are exposed to either of these compounds, they are quite toxic because they are ionophores (Jestoli 2008; Wang & Wu 2012). Using in vitro models of bioavailability of enniatins and beauvaricins, as with all chemicals, the presence of fibre reduces the bioavailability. Both compounds have modest bioavailabilty (40%) when there are dietary fibres present (Meca et al. 2012a, b). In vitro bioavailability values for enniatins in bread, breakfast cereals and cookies were reported to be lower in high fibre materials and ca. 60% in white bread (Prosperini et al. 2013).

Mice given massive oral doses of beauvaricin did not result in any clinical findings (LD50> 100 mg/kg; Omura et al. cited in Jestola 2008). Oral doses between 6 to 50 mg/kg BW of enniatins in mice, rats, guinea pig and rabbits produced no toxic findings (Jestola 2008). Various species of domestic fowl were also tolerant to high oral doses of either beauvericin or one or more enniatins (Jestola 2008).

In mid-2013, the European Commission referred enniatins in food to EFSA for an opinion.

TOXINS FROM ALTERNARIA ALTERNATA

Alternaria alternata is one of the most common phylloplane fungi that is the non-parasitic flora and mycota of the leaf surface worldwide. The genus *Alternaria* comprises >300 species, and many are plant pathogens of economically-important plants (Seifert et al. 2011). Taxonomic studies of *A. alternata* suggest that there are several species (Andersen & Thrane 1996; Andersen et al. 2001). Many of the plant pathogens produce phytotoxins (Visconti & Sibilia 1994). *A. alternata* is a saprophyte on decaying plant material. In wheat left in the field or exposed to rain, the fungal growth results in blackened lemmae and tips of the kernels, sometimes called blackpoint. This is caused by a number of fungal saprophytes not just *A. alternata*. There are also plant pathogens that cause blackpoint (see Conner & Davidson 1988). These factors result in *A. alternata* being ubiquitous contaminants in surface sterilized seeds (Clear et al. 2005). If grain is stored wet, *A. alternata* becomes a major component of the mycoflora of the grain (Abramson et al. 1990; 1999). *A. alternata* makes a number of toxins including

alternariol, alternariol monomethyl ether, altenuene, altertoxins I, II and III and tenuazonic acid (Ostry 2008; Visconti & Sibilia 1994). Of these, alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid occur in cereals (Ostry 2008). These have very low acute toxicity (Visconti & Sibilia 1994). There are a lot of data on other toxicities of these compounds which include mutagenicity. A recent study of alternariol and alternariol monomethyl ether in a small number of samples of cereals and cereal products from Health Canada reported that most had one or both of these metabolites at <2 ppb. Of the 83 samples analysed, 70 were positive for AOH (≥ 63 ng/g, in soft wheat bran) and 64 contained AME (≥ 12 ng/g in a bran-based breakfast cereal). AOH and/or AME were found in 27/ 30 infant foods (≥ 4.4 ng/g and 9.0 ng/g, respectively in a sample of multigrain cereal (Scott et al. 2012).

There is no WHO risk assessment on these toxins. EFSA lists AOH and AME as mutagenic compounds with a suggested Threshold of Toxic Concern (TTC) of 2.5 ng/kg BW/day and recommended more toxicology data be generated. There do not appear to be Canadian data in cereals for the metabolite tenuazonic acid and a related compound (although they are very common in European grains; Müller & Korn 2013). The TTC value proposed for this non-genotoxic substance was 1500 ng/kg BW/day (EFSA 2011b).

MYCOTOXIN GLUCOSIDES AND ENNIATINS IN GRAINS & FOOD PRODUCTS: BRIEF SUMMARY OF AVAILABLE DATA

DON Glucosides

As noted above, Berthiller et al. (2005) reported typical percentage ratios of DON glucoside to DON ca. 10% with some cultivars approaching 30%. At the time of writing, there are much more data from both cereals in the field, a large numbers of food samples and beer in Europe and some data from China.

Levels of DON glucoside in cereal crops averaged 21% of DON in Danish wheat cultivars (6 harvest samples) and 13% (range 5-21%) in 23 cutltivars in FHB inoculation tests (Rasmussen et al. 2012). Samples of wheat, Durham wheat and corn from Germany and France contained 6-20 % of the DON value as DON glucoside (Desmarchelier & Seefleder 2011). Wheat (23 samples) from Austria, Germany and Slovakia and 54 corn samples contained an average of 15% DON glucoside compared to DON with a range of 5-46% (the highest was in a wheat cultivar; Berthiller et al. 2012). Samples of Durham wheat

from Italy (25 breeding lines) had an average of 10% of DON glucoside compared to DON, with a range from not detected to 30% (Dall'Asta et al. 2013). Wheat cultivars (9) from Belgium contained an average 12% of DON glucoside compared to DON with the values ranging from not detected to 25% (Audenaert et al. 2013). A small number of Chinese wheat cultivars had DON glucoside as a percentage of DON ranging from 7-56% (Feng-qin et al. 2011). A large survey of corn samples from 17 corn production regions in China had DON glucoside values ranging from 5-28% of the DON concentration as a percentage of DON, depending on year (Wei et al. 2012).

Flour: Samples of wheat flours from Brazil, England, Romania and Poland contained 6-15% of the DON value as DON glucoside; samples of barley and oat flour from Poland contained 24%, and 29%, respectively (Desmachier & Seedflder 2011). A large survey of corn flour from 17 corn production regions in China had DON glucoside values ranging from 11-29% of the DON concentration as a percentage of DON, depending on year (Wei et al. 2012).

Food products: Samples of white flour products (17), mixed flour products (36), breakfast cereals (7), snacks (34) and other flours (22) from the Czech Republic were analyzed for DON and DON glucoside. Of these, 16% of white flours were positive for DON and the proportion of DON glucoside averaged ~10%; 32% of mixed samples were positive for DON, and the DON glucoside averaged ~21%; 21% of snacks were positive for DON and the DON glucoside averaged 53%. Values of positive samples ranged from 13- 600 ppb. Breakfast cereals had only modest contamination (Malachova et al. 2011).

Beer: Among the first published data were from 166 beers from various EU countries as well as 10 from Canada and 2 from the USA. DON glucoside was present at ca. 40% of the DON values which were typically in the 10s of ppb range. Similar values were obtained from 374 beers from Germany, Austria and the Czech Republic (Kostelanska et al. 2009; Varga et al. 2013).

Samples of fibre-enriched bread, bran-enriched bread, cornflakes and oatmeal from the Belgium marketplace were anylzed for DON and DON glucoside (as well as 3 & 15ADON, zearalenone and zearalenone glucosides). For the breads and oatmeal, the DON glucoside concentration was equal to or greater than the DON concentration (De Boevre et al. 2012).

The International Life Sciences EU report on so called-masked mycotoxins concluded that the major impacts were on industries that involved fermentation of foods in the baking industry or where grains

are hydrolyzed during processing (and in malt and beer making). The addition of enzyme mixtures as bakery improvers gave a rise of up to145% of the level of D3G in fermented dough (Berthiller et al. 2012).

Enniatins

Samples of white flour products (17), mixed flour products (36), breakfast cereals (7), snacks (34) and other flours (22) from the Czech Republic were analyzed for enniatins A & A1 and ennitatins B & B1. Virtually all samples were positive for one or more enniatins. Breakfast cereals had the highest average concentration (595 ppb) followed by mixed flour products and flours (Malachova et al. 2011). Enniatins were found to decline ~30% during beer making and baking (Vaclavikova et al. 2013). Survey data for enniatins in infant food were collected in Spain. Samples of various cereals had the highest values (Serrano et al. 2012). Some 40% of 93 organic cereal samples (wheat, barley, rye and oat) from Italy were contaminated with ennitains. Total values depending on the grain ranged from 200-500 ppb (Juan et al. 2013). Total enniatins in pasta from Spain were all low (Serrano et al. 2013). Similar values were found in Portuguese samples with the highest concentrations found in breakfast cereals (210 ppb; Blesa et al. 2013). Enniatins have been shown to decline in concentration during the bread-making process. In beer, they are quantitatively transferred to the spent grain (Vaclavikova et al. 2013).

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