CODEX ALIMENTARIUS COMMISSION E

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World Health Organization

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PROPOSED DRAFT MAXIMUM LEVELS FOR ACETYLATES DERIVATIVES (DON) IN CEREALS AND CEREAL-BASED PRODUCTS

(Prepared by the Electronic Working Group led by Canada and Japan)

BACKGROUND

1. The 4th session of the Committee on Contaminants in Foods (CCCF) (March 2010) agreed to initiate work on maximum levels (MLs) for deoxynivalenol (DON) and its acetylated derivatives in cereals and cereal-based products for consideration by the 5th session of the Committee.[1] The 33rd session of the Commission approved this proposal as new work for the Committee.[2]

2. At the 7th session of the CCCF (April 2013), the Committee recalled its earlier decision, taken at its 5th session (March 2011) to proceed with the establishment of MLs for DON and that the extension of the MLs to acetylated derivatives would be considered at its 8th session.[3] The Committee agreed to re-establish the electronic working group, led by Canada and Japan, to prepare proposals for the extension of MLs for DON to its acetylated derivatives for consideration by the Committee.[4] The List of Participants of the EWG is presented in Appendix III.

3. In addition, the 7th Session of the CCCF agreed to forward the following MLs for DON in cereals and cereal-based products to Step 5 or 5/8 for adoption by the 36th Session of the Codex Alimentarius Commission (CAC)[5]:

a) For raw cereals (maize, wheat and barley): 2 mg/kg (Step 5)
b) For flour, semolina, meal and flakes derived from wheat, maize or barley: 1 mg/kg (Step 5)
c) For cereal-based foods for infants and young children: 0.2 mg/kg (Step 5/8)

4. It should be noted that at the 36th Session of the CAC (July 2013) noted that clarification was needed on whether the ML for cereal-based products for infants and young children should apply to products "as consumed" or to the "dry matter". The ML was adopted at Step 5 for further consideration by the CCCF[6].

5. The CAC also noted that while there was support for the adoption of the MLs for raw cereals and semi-processed products (flour, semolina, meal and flakes), there were also concerns expressed for either all or some of the proposed MLs, and in particular for raw cereal grains. Noting the concerns, the Commission decided to adopt the MLs for raw cereal grains (wheat barley, and maize) and for flour, semolina, meal, and flakes from wheat, barley, and maize at Step 5 and recommended that the CCCF give further consideration to the pending issues[7]. Comments in this regard from Codex Members were solicited via a Circular Letter (CL 2013/24-CF).

6. This document explores the possibility of extending the above MLs for DON in cereals and cereal-based products to also include 3-acetylated DON (3AcDON) and 15-acetylated DON (15AcDON). Conclusions are presented in Appendix I and background information providing the basis for the conclusions are contained in Appendix II.

7. The Committee is invited to consider the conclusions and recommendations put forward in Appendix I with a view to decide how to proceed further with the extension of the proposed maximum levels for DON to its acetylated derivatives. This issue should be considered in the context of the consideration of maximum levels for DON under Agenda Item 7 (see REP13/CF, Appendix III and CL 2013/24-CF).

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1 ALINORM 10/33/41, para. 110.
2 ALINORM 10/33/REP, Appendix VI.
3 REP11/CF, para. 41.
4 REP13/CF, para. 68.
5 REP13/CF, para. 64-66.
6 REP13/CAC, para. 80.
7 REP13/CAC, para. 100.

APPENDIX I

EXTENSION OF THE PROPOSED MAXIMUM LEVELS FOR DEOXYNIVALENOL IN CEREALS AND CEREAL-BASED PRODUCTS TO ACETYLATED DERIVATIVES

CONCLUSIONS AND RECOMMENDATIONS

8. JECFA's most recent review of the toxicology data for acetylated DON concluded that acetylated DON derivatives are considered equally toxic to DON. Therefore, based on the potential health hazard to humans from dietary exposure to acetylated DON, it is recommended that the CCCF extend the currently proposed MLs for DON to include the acetylated DON derivatives, 3AcDON and 15AcDON.

9. The available occurrence data for 3AcDON and 15AcDON in food commodities for which DON MLs are being proposed suggest that concentrations of acetylated DON compounds are generally only detected when DON concentrations are elevated and if detected, are present at much lower levels than DON. Acetylated DON concentrations typically account for a relatively small fraction (according to the data from Canada and Japan, 10% or less) of the overall DON concentration and would not be expected to have a significant impact on achieving the proposed MLs. As such, based on the low impact on the achievability of the proposed MLs and thus a low impact on trade, it is recommended that the CCCF extend the proposed MLs for DON to include the acetylated DON derivatives.

10. However, data on the occurrence of acetylated DON in commodities for which MLs are being proposed are still limited, particularly for semi-process cereal products and finished cereal-based food intended for infants and young children. Furthermore, the available data on the occurrence of acetylated DON derivatives are sourced from a limited number of countries and the results are not necessarily reflective of those that may occur on a global scale. Given that DON and acetylated DON contamination is highly dependent on climactic conditions, and that chemotypes producing acetylated DON have a different prevalence throughout the world, data from as many areas as possible becomes critical to determining whether the currently proposed MLs are suitable for application to the acetylated DON derivatives. The CCCF should encourage Codex Members from countries where data are lacking to submit additional occurrence data for acetylated DON compounds in relevant food products. Occurrence data for acetylated DON should be collected using validated analytical methods.

11. While current data suggests that acetylated DON concentrations represent a low proportion of the total DON levels in cereal-based foods, recent studies have demonstrated a potential shift in regional profiles of *Fusarium* head blight pathogens, including chemotypes that co-produce DON and acetylated DON compounds. Such shifts may result in future changes to the relative concentrations of DON and acetylated DON in cereal products. Prudently, the CCCF should recommend extending the DON MLs to include acetylated DON derivatives in order for the MLs to remain protective of human health if shifts in chemotypes were to significantly alter the current amounts of acetylated DON relative to DON generally present in cereal-based food commodities.

12. Some members of the e-WG indicated that it may be premature to extend the proposed MLs for DON to include the acetylated DON compounds. This opinion was generally based on the limited occurrence data available for acetylated DON and therefore a lack of global representation, as well as the absence of an internationally validated analytical method for acetylated DON, especially one that can be applied at the field and industry levels. Global representation of occurrence data is critical considering that profiles of DON-derivative-producing chemotypes can be different in various areas of the world.

13. Some members of the e-WG also noted that since acetylated DON concentrations account for a small fraction of the overall DON concentrations in cereal grains (and usually only detected when DON concentrations are elevated), implementing measures to control DON concentrations (i.e. MLs apply only to DON and not include the acetylated derivatives) is sufficient to ensure that overall DON concentrations remain protective of human health. These members recommend waiting to consider extending the MLs for DON to include the acetylated DON compounds until additional data demonstrate that acetylated DON concentrations have a significant impact on total DON concentrations in cereal and cereal products.

APPENDIX II

SUPPORTIVE INFORMATION ON EXTENSION OF THE PROPOSED MAXIMUM LEVELS FOR DEOXYNIVALENOL IN CEREALS AND CEREAL-BASED PRODUCTS TO ACETYLATED DERIVATIVES

INTRODUCTION

14. Trichothecenes are a class of mycotoxins grouped according to their chemical structure and the species of fungi that produce them. Type B trichothecenes, which include DON, 3AcDON and 15AcDON, are produced by various species of the *Fusarium* genus such as *F. graminearum* and *F. culmorum*. These fungi have gained attention on a global scale as they may occur in several different types of grains such as barley, maize, oats, rice and wheat, and can produce *Fusarium* head blight in temperate climates found worldwide. The severity of *Fusarium* head blight is generally dependant on the climactic conditions as high humidity and wet conditions promote mycotoxin production and thus potential *Fusarium* head blight epidemics.

15. Type B trichothecenes are classified by the strain-specific profiles that produce them (chemotype). Type 1 chemotypes are DON producers and these are further classified as Type 1A which produce DON and primarily 3AcDON (3AcDON chemotype), and Type 1B which produce DON and primarily 15AcDON (15AcDON chemotype) as a co-contaminant.

16. Studies from the 1980s demonstrated that the 3AcDON chemotype was generally found in areas with warmer climates such as in Europe, China, Australia and New Zealand, while the 15AcDON chemotype was predominant in cooler climate regions such as North America (Mirocha et al., 1989). However, there are indications that globalization of trade in horticultural and agricultural plants may have caused a shift in regional profiles of *Fusarium* head blight pathogens. Recent surveys have suggested that the 3AcDON chemotype of *F. graminearum* is replacing the 15AcDON chemotype in parts of North America (Guo et al., 2008; Ward et al., 2008; Von der ohe et al., 2010). In the United Kingdom, the Netherlands and Germany, there are indications that fungi species causing *Fusarium* head blight, historically dominated by *F. culmorum*, are shifting towards a higher proportion of *F. graminearum* (Jennings et al., 2007).

17. This discussion paper will consider the extension of the proposed maximum levels (ML) for deoxynivalenol (DON) previously developed by the CCCF (REP13/CF, para. 64-66), to include the acetylated DON derivatives, 3AcDON and 15AcDON.

TOXICOLOGY

18. At its seventy-second meeting, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) confirmed that the provisional maximum tolerable daily intake value for DON of 1 µg/kg bw, established at the fifty-sixth JECFA meeting, remained valid (FAO/WHO, 2011). In addition to previously reviewed data, JECFA also considered new toxicological studies and concluded that the No Observed Effect Level (NOEL)[8] based on decreased body weight gain from a 2-year feeding study in mice, from which the PMTDI was derived, remained appropriate.

19. JECFA also considered studies on the toxicity of acetylated DON (3AcDON and 15AcDON) at its seventy-second meeting. Results from new studies on absorption, distribution, metabolism and excretion (ADME) indicated that the toxicity of acetylated DON compounds is likely to arise from their conversion to DON. Furthermore, median lethal dose (LD_{50}) studies found that the toxicity of acetylated DON compounds in mouse is similar to that of DON. As a result, JECFA considered the toxicity of acetylated DON compounds to be equal to that of DON and decided to convert the PMTDI for DON into a group PMTDI of 1 µg/kg bw for DON and its acetylated derivatives.

20. At its seventy-second meeting, JECFA considered it appropriate to establish an acute reference dose (ARfD) for DON and its acetylated derivatives based on acute exposure to high doses having resulted in emesis in humans. Using the lowest $BMDL_{10}$ for emesis in pigs of 0.21 mg/kg bw/day and applying an uncertainty factor of 25, JECFA established a group ARfD of 8 µg/kg bw/day. However, it was noted that limited data from human case reports suggest that dietary exposures to DON up to 50 µg/kg bw/day are not likely to induce emesis.

21. Based on the latest opinion from JECFA that acetylated DON derivatives are to be considered equally toxic to DON, it would be appropriate from the perspective of human health to extend the proposed MLs for DON described above to include acetylated DON derivatives.

ANALYTICAL METHODS FOR ACETYLATED DON

22. Considerable research has been conducted on analytical methods for the determination of DON in the last decade. More recently, methods have been investigated for the determination of DON's acetylated derivatives. In its review of analytical methods, the 72nd JEFCA considered the use of mass spectrometry (MS) or tandem mass spectrometry (MS/MS) coupled with high performance liquid chromatography (LC-MS/MS) for DON to be the most important development. Certified standard solutions for DON, 3AcDON and 15AcDON are now commercially available.

23. Work on developing analytical methods for the detection of the acetylated derivatives, 3AcDON and 15AcDON, has been relatively limited. The acetylated DON compounds can be determined simultaneously with DON by LC-MS/MS. Alternatively, the acetylated derivatives have been determined by GC after suitable derivatization (JECFA, 2010). Recently, reviews have been published on analytical methods on DON and its derivatives (Ran et al. 2013). In this review, various analytical techniques currently available for the determination of DON and its derivatives are summarized including both advantage and disadvantage thereof.

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8 At its sixty-eighth meeting, JECFA decided to differentiate between NOAEL and NOEL; this NOEL would now be considered a NOAEL.

24. Validated methods, such as those adopted by the International Organization for Standardization (ISO), the AOAC International, or the European Committee for Standardization (CEN), are required for enforcement purposes. GSCTFF (CODEX STAN-193-1995) stipulates that in all cases, a validated method of analysis should be available with which a ML can be controlled. For DON in cereal grains and grain products, several validated methods are available. Although many analytical methods have been developed for DON derivatives in cereal grain and grain products, there are very few reports on validation studies for such methods. Recently, a first report on inter-laboratory validation study of an LC-MS/MS method for simultaneous determination of DON, 13AcDON and 15AcDON in wheat was published (Yoshinari et al., 2013).

OCCURRENCE OF ACETYLATED DON IN FOOD

Data submitted as part of the call for data for this discussion paper

25. Data on the co-occurrence of DON, 3AcDON, and 15AcDON in samples of wheat, barley, and maize, as well as in samples of semi processed products from these grains, submitted from various Members (Austria, Canada, and Japan) as part of the call for data for this discussion paper, are summarised in Table 1.

Table 1 – Summary of occurrence data for DON, 3AcDON, and 15AcDON in food products for which MLs are being proposed

Data submitting country	**N**	**% of samples < LOD**			**Mean concentration (µg/kg)***		
Food		**DON**	**3-AcDON**	**15-AcDON**	**DON**	**3-AcDON**	**15-AcDON**
Austria[a]							
Barley grain	1	100	100	100	<LOD	<LOD	<LOD
Maize grain	1	100	100	100	<LOD	<LOD	<LOD
Grain (not specified)	2	100	100	100	<LOD	<LOD	<LOD
Wheat grain	2	50	100	100	15	<LOD	<LOD
Maize flakes	9	89	100	100	35	<LOD	<LOD
Maize milling products	65	31	100	74	187	<LOD	68
Maize semolina and starch	6	40	100	100	70	<LOD	<LOD
Flour mix (wheat, barley, rye, oat)	9	89	100	100	46	<LOD	<LOD
Grain (not specified) milling products	19	42	100	84	118	<LOD	33
Wheat flour	95	25	100	99	125	<LOD	18
Wheat milling products	12	25	100	100	114	<LOD	<LOD
Cereal-based foods for infants	76	97	100	100	25	<LOD	<LOD
Foods for infants and small children	10	100	100	100	<LOD	<LOD	<LOD
Canada (Canadian Grain Commission)[b]							
Barley grain	46	65	100	100	132	<LOD	<LOD
Maize grain	9	0	100	89	983	<LOD	92
Wheat grain	448	13	100	100	292	<LOD	<LOD
Canada (OMAFRA)[c]							
Barley grain	87	3	98	48	2482	51	133
Maize grain	123	7	91	37	2304	60	195
Wheat grain	373	15	100	83	1954	<LOD	55
Japan[d]							
Barley grain (imported)	41	0	76	81	38	<1	<1
Wheat grain (imported)	150	2.7	71	65	54	<1	2
Maize grits (imported)	58	3.4	95	35	55	<1	7
Japan[e]							
Barley grain (domestically produced)	500	6	38	76	81	12	2
Wheat grain (domestically produced)	600	7	70	91	53	3	1

N; number of samples

[a]Limits of detection DON = 15 or 25 µg/kg; 3AcDON = 15 or 25 µg/kg; 15AcDON = 15 or 25 µg/kg

[b]Limits of detection DON, 3AcDON, 15AcDON = 50 µg/kg

[c]Limits of detection DON = 60 µg/kg; 3AcDON and 15AcDON = 50 µg/kg

[d]Limits of detection DON = 0.2 µg/kg; 3AcDON = 0.3 µg/kg; 15AcDON = 0.5 µg/kg

[e]Limits of detection DON = 0.6 to 5 µg/kg; 3AcDON = 0.5 to 6 µg/kg; 15AcDON = 0.4 to 3 µg/kg

*all mean concentrations calculated by assigning the LOD value to non-detected results

26. Results for DON and acetylated DON in samples of grains, semi-processed grain products, and finished foods for infants and young children, collected between 2007 and 2011, were submitted by Austria. Mean DON concentrations were generally low, up to 15 µg/kg in raw grains, 35 to 187 µg/kg in semi-processed grain products, and up to 25 µg/kg in finished cereal-based foods for infants and young children. Acetylated DON compounds were not detected in any raw grain samples. None of the semi-processed grain product samples or finished food samples had positive detections for 3AcDON, and 15AcDON was very rarely detected and only found in samples of milled maize, milled grains (not specified), and wheat flour. Mean 15AcDON in semi-processed grain products ranged from <LOD to 68 µg/kg. Based on calculated mean concentrations, 15AcDON in those foods accounted for approximately13 to 33% of the total DON concentration.

27. Data from Canada were independently submitted by the Canadian Grain Commission (CGC) and the Ontario Ministry of Agriculture, Food and Rural Affaires (OMAFRA). The CGC data were collected as part of their routine analysis of both domestic and exported grain shipments from 2010 to 2013. DON and its acetylated derivatives were analyzed as part of multi-Fusarium trichothecenes analysis using gas-chromatography/mass-spectrometry (GC-MS) with a derivatization step (Tittlemier et al., 2013). While DON was detected in most samples, the concentrations of acetylated DON were found to be below the limit of detection (LOD) in all samples of wheat, barley and maize, with the exception of one maize sample which had a concentration of 15AcDON above the LOD.

28. Occurrence data for DON and acetylated DON were generated by OMAFRA as part of a mycotoxin baseline study using field trial grain samples collected between 2006 and 2009. Samples for this particular survey were collected from field grains that were run through a combine where the lighter damaged kernels are expected to fall out. However, prior to analyzing the samples, there was no additional cleaning step such as that performed on grains intended for milling, and therefore, as expected, the incidence of DON is relatively elevated. Initial screening to detect the presence of various mycotoxins was performed using an enzyme-linked immunosorbent assay (ELISA) method. Some of the samples were then re-analyzed by either GC-MS or liquid chromatography coupled to tandem mass-spectrometry (LC-MS/MS). The results indicate that a high proportion of wheat, barley, and maize samples had detectable concentrations of DON and that a high proportion of barley and maize samples had detectable concentrations of 15AcDON. However, the mean acetylated DON concentrations are relatively low and represent only a minor fraction of the mean total DON (sum of DON and acetylated DON concentrations) concentrations (generally less than 15%).

29. Two dataset were submitted by Japan. The first on DON and acetylated DON in samples of wheat, barley, and maize grits imported into Japan from 2010 to 2012, which were analyzed using a procedure that includes sample extraction and cleanup, and detection by LC-MS/MS (Yoshinari et al., 2012 and 2013). The second on DON and acetylated DON in samples of wheat and barley produced in Japan from 2008 to 2012 and collected at county elevators or warehouses after sorting, and analyzed using a procedure that includes sample extraction, cleanup, and derivatization, and detection by GC-MS. Both methods have very low LODs in comparison to those from other datasets that were submitted. As a result, DON was detected in over 94% of barley, wheat and maize grit samples. However, even with the low LODs, acetylated DON was still not detected in a large proportion of the samples. The mean DON concentrations in wheat and barley grain samples ranged from 38 to 81 µg/kg. The mean 3AcDON and 15AcDON concentrations ranged from <1 to 12 µg/kg. Based on the calculated mean concentrations, acetylated DON derivatives account for roughly <5 to 15% of the total DON.

30. Occurrence data submitted by Japan indicate that the detection of ratio and mean concentrations of 3AcDON and 15AcDON in grain produced in Japan differ from those in imported grain. This reflects a potential difference in predominant chemotypes of *Fusarium* genus in various grain production areas.

31. The submitted data was also considered with respect to achieving the proposed MLs for DON when 3AcDON and 15AcDON are included. Results for each type of food for which an ML is proposed (raw wheat, barley, and maize; semi-processed products from wheat, barley, and maize; and cereal-based finished foods for infants) were assessed against the respective MLs when considering only the DON concentration and when considering the total DON concentration (total DON refers to the sum of DON, 3AcDON and 15AcDON) (Table 2).

Table 2 – Achievability of the MLs with and without the inclusion of acetylated DON derivatives

Country/Food	Proposed ML (mg/kg)	% of samples <ML	
		DON only	Total DON*
Austria			
Barley grain	2	100	100
Maize grain	2	100	100
Grain (not specified)	2	100	100
Wheat grain	2	100	100
Maize flakes	1	100	100
Maize milling products	1	97	97
Maize semolina and starch	1	100	100
Flour mix (wheat, barley, rye, oat)	1	100	100
Grain (not specified) milling products	1	100	100
Wheat flour	1	100	100
Wheat milling products	1	100	100
Cereal-based foods for infants	0.2	100	100
Foods for infants and small children	0.2	100	100
Japan (MHLW)			
Barley grain (imported)	2	100	100
Wheat grain (imported)	2	100	100
Maize grits	1	100	100
Japan (MAFF)			
Barley grain (domestically produced)	2	100	100
Wheat grain (domestically produced)	2	100	100

*Total DON refers to the sum of DON, 3AcDON and 15AcDON concentrations

32. The data submitted by the Canadian Grain Commission are not included in Table 2 since the information was provided in a manner that did not allow the concentrations of DON and the corresponding acetylated DON concentrations in each individual sample to be identified. However, with the exception of one maize sample, the concentration of acetylated DON in all grain samples from the CGC were below the LOD and samples that met the ML based on DON concentrations would likely have also met the ML when considering acetylated DON concentrations. The data submitted by OMAFRA were also not included in Table 2 since the grain were not necessarily intended for use as food or for human consumption, and therefore a comparison to the raw cereals ML for DON is not necessarily appropriate.

33. Extending the MLs to include the acetylated DON derivatives is not expected to significantly impact the achievability of the proposed MLs for DON. However, the data remains limited for semi-processed grain products and finished foods intended for infants. In addition, the data submitted are of limited global/geographical representation.

Data available in the GEMS/Food database

34. Data for DON and acetylated DON in relevant food products, currently available through the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (referred to as GEMS/Food), are summarised in Table 3. Results for acetylated DON derivatives in wheat, barley, maize, and their products were limited and much of them were still only available in aggregate form. Furthermore, the available data was sourced from a limited number of regions of the world. Nonetheless, the upper-end of the reported mean concentrations and maximum values suggest that acetylated DON is likely to occur in wheat, barley or maize products, at much lower concentrations compared to DON.

Table 3 – Summary of occurrence data for DON, 3AcDON, and 15AcDON in wheat, barley and maize and semi-process products of wheat, barley and maize available in the GEMS/Food database*

DON compound Food commodity	Total number of samples	% of non-detects	Concentration (µg/kg)	
			Range of means	Maximum
DON				
Barley	578	0 – 100	<4 - 153	619
Maize	539	0 – 56	19 – 1,056	8,850
Wheat	4205	0 – 100	<4 – 1,427	6,090
Maize meal/flour	79	0 – 100	<1 – 293	1,400
Wheat flour	1070	0 – 100	<4 - 993	50,000
3AcDON				
Barley	33	83 – 100	19 - 67	71
Maize	54	0 – 72	27 - 29	520
Wheat	20	0 – 100	<60 – 193	239
Maize flour	2	100	<67	<67
Wheat flour/germ	13	100	<67	<67
15AcDON				
Maize grain	173	0 – 64	51 – 236	0 – 1,320

*GEMS/Food data were sourced from Austria, Finland, France, Germany, Hungary, the Netherlands, New Zealand, Norway, Portugal, Singapore, Sweden, and the United Kingdom.

Data available from the scientific literature

35. At its seventy-second meeting, JECFA considered occurrence data for 3AcDON and 15AcDON in wheat, barley, maize, oats, rye, and products thereof for the first time (FAO/WHO, 2011). JECFA considered data submitted by China, France, Japan, and the United Kingdom, as well as published data from studies conducted in nine countries. Results for 3AcDON from 6980 samples and for 15AcDON from 4300 samples were considered. JECFA concluded that generally, acetylated derivatives are infrequently detected and concentrations are typically less than 10% of the reported DON concentrations. The highest reported mean concentrations of 3AcDON in wheat, barley and maize were 193 µg/kg, 19 µg/kg, and 27 µg/kg, respectively. For 15AcDON, the highest reported mean concentrations were 365 µg/kg, 0.3 µg/kg, and 236 µg/kg, in wheat, barley, and maize, respectively.

36. Edwards et al. (2009a; 2009b) analyzed various tricothecenes in samples of wheat (n=1624, from 2001 to 2005) and barley (n=446, from 2002 to 2005) from the United Kingdom. Ten 300 g subsamples were arbitrarily collected from various areas of the field and combined to generate a 3 kg sample. The sample was dried to a 15% moisture content. A 500 g sample was removed for visual inspection and the remaining was milled with a 1 mm screen. An aliquot of 200 g was analyzed for tricothecenes, including DON, 3AcDON and 15AcDON by GC-MS using a previously published protocol (Patel, 1996). The limit of quantitation (LOQ) was calculated to be 10 µg/kg. DON was quantified in 86% of wheat samples, while 3AcDON and 15AcDON were only quantified in 1.2% and 2.7% of the samples, respectively. The mean DON concentration in wheat was 230 µg/kg and the mean concentrations of both 3AcDON and 15AcDON were below 10 µg/kg. A regression analysis by the authors showed that in most samples of wheat, acetylated DON versions occur at a very low percentage of the DON concentrations (between 0.25 and 2.5% of the DON concentration) and that acetylated DON derivatives are generally only detected in samples of high DON content. However, anomalies were observed in a limited number of samples, where results of high DON concentrations showed no detectable levels of acetylated DON and where low DON concentrations showed high occurrence of acetylated DON. For barley, DON was quantified in 57% of the samples while 3AcDON and 15AcDON were quantified in only 1 sample; the sample with the highest DON level found (1416 µg/kg). The mean DON concentration in barley samples was 19 µg/kg and mean 3AcDON and 15AcDON concentrations were below the LOQ of 10 µg/kg.

37. Van Der Fels-Klerx et al. (2012) reported on mycotoxin contamination of cereal grains from national monitoring programs conducted in Finland, Sweden, Norway and the Netherlands over two decades. Results for DON concentrations in 4899 samples and 3AcDON in 1541 samples of mainly wheat, barley, maize, oats, and rye were reported. Detailed information on sample collection procedures and analytical methodologies were not provided. LODs varied between years and/or countries of data collection and the highest LOD that was reported most frequently (DON = 100 µg/kg; 3AcDON = 30 µg/kg) was selected for the purpose of calculating mean values. DON was detected in 45% of the samples and 3AcDON in only 13% of the samples. The mean DON concentration was found to be 257 µg/kg while the mean 3AcDON concentration was 22 µg/kg. Levels of 3AcDON in oats were found to be correlated to the levels of DON, however, the authors could not determine if a similar correlation exists for wheat.

38. Montes et al. (2012) collected 148 samples of wheat, maize, rice, and multigrain breakfast cereals from the Spanish market, for simultaneous analysis of various trichothecenes including DON, 3AcDON, and 15AcDON. Samples of 500 g were collected and ground to 1 mm particles. A 25 g subsample was homogenized in a water/acetonitrile solution, the filtrate was defatted with hexane and then purified. The purified sample was derivatised and analyzed by GC-MS. Limits of detection for DON, 3AcDON, and 15AcDON were 11.4, 14.7, and 12.6 µg/kg, respectively. DON was detected in 26% of samples, while none of the samples had 3AcDON or 15AcDON concentrations above their respective LODs. DON concentrations in positive samples only ranged from 32 to 468 µg/kg with a mean of 97 µg/kg.

39. The European Union's (EU) Scientific Co-operation (SCOOP) Task 3.2.10 collected occurrence data on trichothecenes in food from 12 EU member states (SCOOP, 2003). Samples consisted mostly of wheat and wheat flour, barley, oats, rye and rye flour, and maize. Results for DON in 11 022 samples were provided with 57% positives (above the LOD). Results for 3AcDON were provided for 3721 samples with 8% positives while results for 15AcDON for 1954 samples were provided with 20% positives. The majority of samples were analyzed by GC-MS. DON concentrations ranged from LOD to 50 000 µg/kg in wheat and wheat flour (n=6358), LOD to 619 in barley (n=781), and LOD to 8850 µg/kg (n=520). 3AcDON concentrations ranged from LOD to 239 µg/kg in wheat and wheat flour (n=1910), LOD to 101 µg/kg in barley (n=521), and LOD to 520 µg/kg in maize (n=271). Concentrations of 15AcDON ranged from LOD to 806 µg/kg in wheat and wheat flour (n=1041), LOD to 6 µg/kg in barley (n=58), and LOD to 1320 µg/kg in maize (n=340). Since the data is presented in aggregate form, it becomes difficult to determine the contribution of acetylated DON to the concentration of total DON. Nonetheless, based on the frequency of detection and the range of acetylated DON concentrations in grain samples, acetylated DON is generally expected to account for a relatively minor fraction of the total DON concentration.

40. Yoshizawa and Jin (1995) analyzed the trichothecenes content in samples of domestic wheat (n=17) and barley (n=17) obtained from prefectures of different locations in Japan. Grain samples from a previous collection, suspected to be positive for acetylated DON, were selected. Samples were extracted and analysed using a previously reported method (Luo et al., 1990, 1992). A subsample of 40 g was finely ground and extracted with a solution of acetonitrile/water. An aliquot of 80 ml was defatted with hexane and concentrated. The residue was dissolved in methanol and separated by silica column. Eluates were analyzed by GC-MS and high-performance liquid chromatography (HPLC). The LOD for trichothecenes was 5 µg/kg. DON was detected in 100% of the samples, while 3AcDON and 15AcDON were detected in 74% and 12% of all samples, respectively. DON concentrations ranged from 29 to 11 700 µg/kg in wheat samples and from 86 to 70 500 µg/kg in barley samples. Acetylated DON (3AcDON and 15AcDON combined) concentrations ranged from LOD to 920 µg/kg in wheat samples and from LOD to 18 700 in barley samples. Acetylated DON concentrations accounted for <1% to 26% of the total DON concentration, with mean contributions of approximately 6% for wheat and 13% for barley.

41. Van Asselt et al. (2012) measured various mycotoxins in 1 kg maize samples (n=43) collected from Dutch fields. A subsample of 100 g was dried at 35°C for a few days and stored at 4°C. Samples were freeze dried under pressure, milled to a particle size of < 1mm and stored again at 4°C. An aliquot of 2.5 g was added to 10 mL of extraction solvent (acetonitrile/water/ formic acid). Solutions were thoroughly mixed and centrifuged. The extracted sample was diluted in water, mixed and stored at 4°C until analysis. Trichothecene analysis was performed by LC-MS/MS. DON was detected in only 7% of samples, while 3AcDON and 15AcDON combined, were detected in 21% of samples. DON was quantified in only one sample (1154 µg/kg) in which the acetylated DON concentration (sum of 3AcDON and 15AcDON) was much lower (298 µg/kg). However, five samples in which DON was found to be below the LOQ had quantifiable acetylated DON concentrations (50 to 512 µg/kg). Although the study analyzed a limited number of samples of maize only, the results indicate that acetylated DON was more frequently detected and usually at higher concentrations than DON.

ACETYLATED DON CONCENTRATION AS A FUNCTION OF DON CONCENTRATION

42. While the data available in the scientific literature generally suggests that acetylated DON derivatives occur in grain foods as a low proportion of the DON concentration, the results are presented in aggregate or summarised form and do not allow for an accurate analysis of the co-occurrence of DON and its acetylated compounds. The data submitted by Members as individual results allows for a more refined analysis of the expected acetylated DON concentrations as a function of DON occurrence.

43. The OMAFRA study analysed raw cereal grains directly from the field without any prior cleaning step or removal of damaged kernels. As a result, DON and acetylated DON derivatives were detected in most samples. The results from the OMAFRA study should not be compared to the ML for raw cereal grains since the grains were not necessarily intended for use as food or for human consumption. Nonetheless, this data was useful in determining the mean acetylated DON concentration (3AcDON and 15AcDON) for the combined wheat, barley and maize samples, as a percentage of the total DON concentration, for different ranges of DON concentration (Figure 1a), when DON occurrence is high (close to the proposed 2 ppm ML for DON in cereal grains).

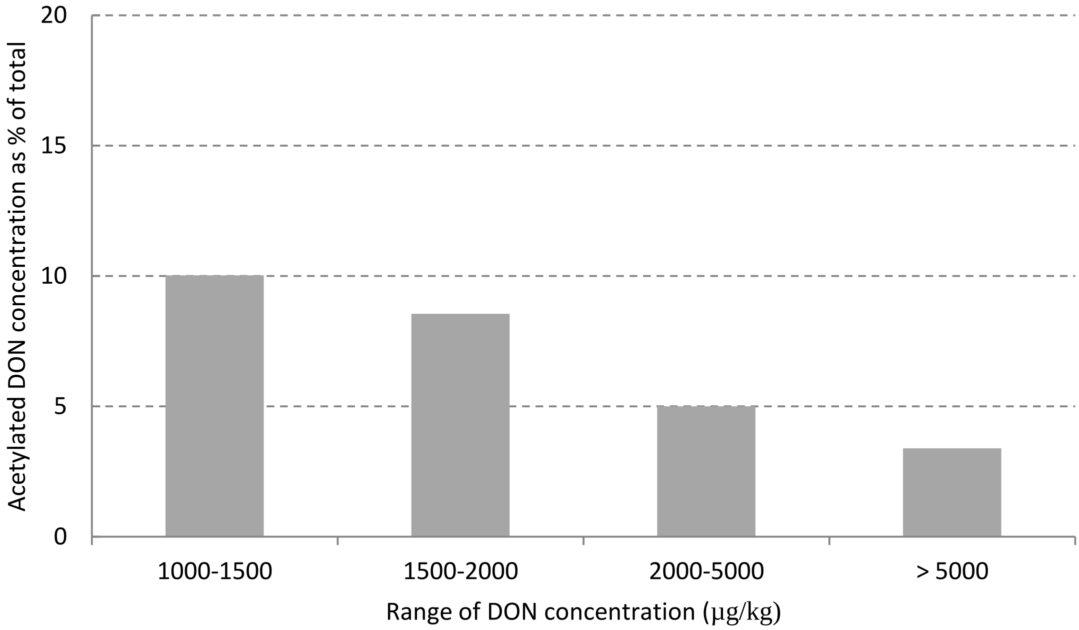

Figure 1a – Mean acetylated DON concentration (as a% of the total DON concentration) in wheat, barley and maize, as a function of DON concentrations from the OMAFRA (Canada) dataset.

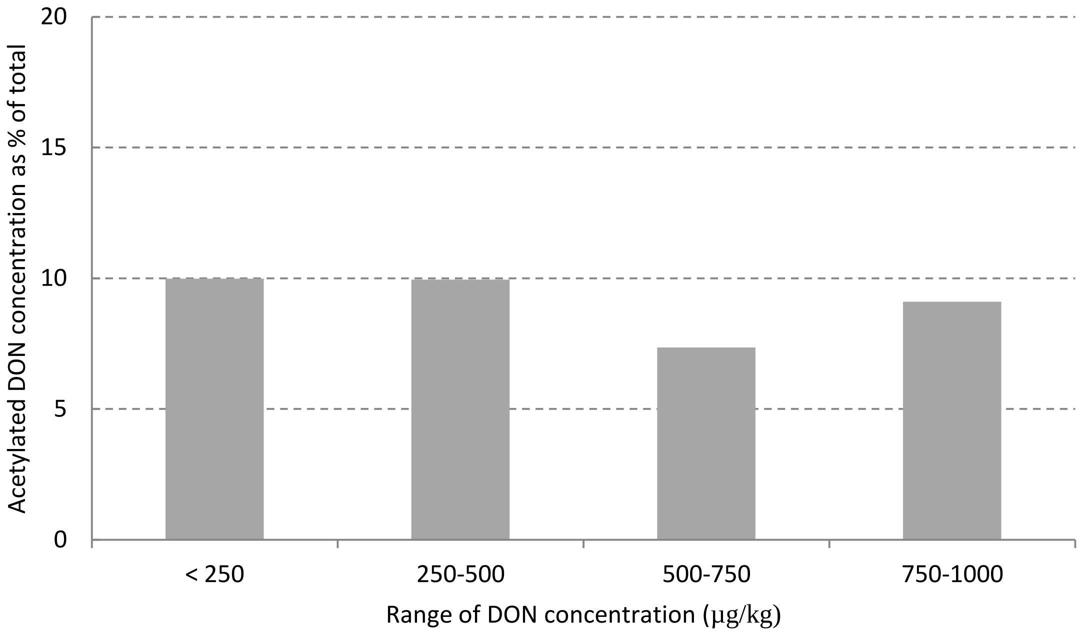

Figure 1b – Mean acetylated DON concentration (as a% of the total DON concentration) in wheat and barley, as a function of DON concentration from the Japan dataset.

44. A similar analysis was conducted using the wheat and barley data submitted by Japan but for lower DON concentration ranges as these samples were cleaned and analyzed using much lower LODs (Figure 1b). Overall, the results demonstrate that for both low and high incidence of DON, acetylated DON concentrations (3AcDON and 15AcDON combined) represent 10% or less of the total DON. Acetylated DON derivatives account for a low proportion of the total DON concentration, including at DON concentrations in the range of the proposed ML, and are therefore not expected to have a significant impact on achieving the proposed limit of 2 mg/kg.

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