

# The surveillance programme for mycotoxins in food in Norway 2016 - Mycotoxins from *Fusarium* and ergot in wheat and rye



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## Summary

Several mycotoxins, with emphasis on trichothecenes and ergot alkaloids as well as on the fungus *Claviceps purpurea* were analysed in samples of wheat and rye for human consumption from mills and food stores collected during 2016. The purpose of the programme was to survey the mycotoxin status of Norwegian whole and milled wheat and rye and *C. purpurea* in whole wheat and rye in 2016.

Trichothecenes, consisting of deoxynivalenol (DON), 3- and 15-acetylated-DON, DON-3-glucoside, nivalenol, T-2 toxin, HT-2 toxin, T-2 triol and T-2 tetraol, were generally low in levels. Except for one of 43 samples (2 %) of milled wheat exceeding the maximum limit of DON concentration in cereal flour (750 µg DON/kg) according to legislation, the remaining 42 samples milled wheat had insignificant trichothecene levels. Also the other samples consisting of whole wheat (51 samples), whole rye (22 samples) and milled rye (35 samples) had insignificant trichothecene levels.

Ergot alkaloids were detected only in a few samples slightly above the level of detection, and with maximum concentration in a sample of whole rye (638 µg ergotamine/kg). *C. purpurea* was detected in most samples of whole rye of which six samples above the maximum limit in EU (500 mg/kg). However, *C. purpurea* was hardly present in whole wheat. No correlation was found between ergot alkaloids and *C. purpurea*. No ergot alkaloids were found in the six samples that had *C. purpurea* above maximum EU-level.

Enniatin B and enniatin B1 were detected in most samples, with the highest concentrations in rye, but were nevertheless below concentrations of health concern. Zearalenone was hardly detected, and its  $\alpha$ - and  $\beta$ -zearalenol metabolites were not detected in the cereal samples.

In conclusion, except for a single sample of milled wheat where DON was slightly above its maximum concentration, there were low and insignificant mycotoxin concentrations in most of the samples of wheat and rye. Six samples of rye showed *C. purpurea* exceeding the maximum level with no correlation between *C. purpurea* and ergot alkaloids.

## Introduction

The surveillance programme for mycotoxins in foods is a collaboration between the Norwegian Food Safety Authority (NFSA) and the Norwegian Veterinary Institute (NVI) where NFSA decides the extent of the surveillance programme based on scientific advices from NVI. NFSA is responsible for sampling and NVI for analysing and reporting of the results.

Genus *Fusarium* is the most important mycotoxin-producing fungi primarily infecting cereals in the field during the growing season. It produces important mycotoxins like the trichothecenes deoxynivalenol (DON), HT-2 toxin and T-2 toxin, as well as zearalenone and fumonisins. DON has been surveyed in Norwegian cereals for about two decades and has been found to occur in Norwegian cereals at high concentrations, particularly in oats and wheat. DON is a health risk in cereals if contaminations above certain levels are ingested by animals and humans [1]. Prominent effects of DON exposure include gastrointestinal disorders such as reductions of feed intake and growth rate, that have been well documented in pigs [1]. HT-2 and T-2 are usually present at significant concentrations only in oats and products containing oats. The toxic effects of HT-2 and T-2 are similar to DON but more potent. They can cause gastrointestinal lesions as well as immune suppression [1]. The oestrogenic mycotoxin zearalenone is produced by the same *Fusarium* species as DON. The little data available so far have shown this toxin to be at insignificant concentrations in Norwegian cereals [1]. Fumonisins, primarily produced by *Fusarium* species infecting maize, cause various toxic effects in different animal species and are classified as possible human carcinogens[1].

Enniatins and beauvericin, have ionophoric and cytotoxic properties but are rather low in bioavailability from oral exposure. Their animal and human toxic effects are considered low and rather unknown [1].

Other *Fusarium* mycotoxins like moniliformin and 15-hydroxy-culmorin, have low toxic potency or unknown toxic profiles based on few available data.

Ergot alkaloids are emerging mycotoxins of considerable interest in EU and occurrence data are demanded [2]. They show moderately acute toxicity, but have neurotoxic properties, inhibit blood circulation and interfere with hormone levels. Among our cereal species, their producer, *Claviceps purpurea* is mainly found in rye.

## Aims

The aims of the programme are to provide reliable documentation on the occurrence of important mycotoxins in cereals for human consumption, with special emphasis on trichothecenes and ergot alkaloids and to examine the correlation between the content of ergot alkaloids and *Claviceps purpurea* in the grain.

## Materials and methods

In 2016, the surveillance programme for mycotoxins in foods consisted of: a) 51 samples of whole wheat, 43 samples of milled wheat, 22 samples of whole rye and 35 samples of milled rye. The numbers deviated slightly from the original sample plan of respectively 50, 40, 30 and 30. However, the total number of analysed samples (151) was similar to the planned number of samples (150).

The samples collected by NFSA were according to a specific plan for sampling involving the various NFSA regions. They were collected at mills in grain production areas, or at food stores in Hamar and Oslo, and sent to NVI between March and December. The sampling followed the EU Regulation 401/2006, in achieving representative samples. The sampling procedures took into account the size/volume of selected lot, like distribution pattern of the substances in the grain, number of incremental samples necessary, sampling tools, size of final samples etc.

Most of the samples were analysed for a range of mycotoxins and metabolites at NVI in Oslo. However, the initial 22 samples received were analysed at Premier Analytical Services in United Kingdom for a lower repertoire of mycotoxins, consisting of trichothecenes, zearalenone and ergot alkaloids.

## Quantitative determination of *Claviceps purpurea*

The method calculated *Claviceps purpurea* sclerotia in gram per kg cereal according to the recommendation of EFSA. The sample was weighed first, before being spread over a large light surface for visual inspection. Detected sclerotia of *C. purpurea* were picked out and weighed separately.

Margin of error for analysis must be considered: There is always uncertainty associated with visual inspection, primarily in relation to the risk of underestimation. However, careful examination can spot without difficulty the black sclerotia from the grains, and they appear larger than the normal grains. All sclerotia were assessed by experts or by DNA sequencing for morphological verification. The results are therefore considered highly specific, without the risk of false positives and with minimal risk of underestimation.

## Chemical analysis

*Analytical method at Premier Analytical Services in UK*

The trichothecene mycotoxins DON, 3-acetyl-DON, 15-acetyl DON, nivalenol, T-2 toxin and HT-2 toxin were determined by gas chromatography with mass spectrometry (GC/MS). Zearalenone was analysed using immunoaffinity columns for clean-up followed by determination by HPLC with fluorescence detection. Ergot alkaloids were determined using electrospray ionization and tandem mass spectrometry (MS/MS) combined with liquid chromatography (LC).

Analytes and limit of detections: DON, 3-acetyl-DON, 15-acetyl-DON, T-2 toxin, HT-2 toxin and nivalenol: all 10 µg/kg; zearalenone: 4 µg/kg; ergosine, ergotamine, ergocornine, alpha-ergocryptine and ergocristine: all 2 µg/kg.

#### *Analytical method at NVI*

The novel multi-mycotoxin liquid chromatography–high-resolution mass spectrometry (LC-HRMS/MS) method was applied for the simultaneous determination of selected mycotoxins in the various cereal samples. The developed LC-HRMS/MS multi-mycotoxin method was validated in order to ensure the quality and reliability of collected data. The performance parameters linearity, selectivity, limit of detection (LODs) and limit of quantifications (LOQs) were validated. In addition, spike recovery experiments were performed. The evaluation of matrix effects was performed by utilizing the signal suppression or enhancement (SSE) approach based on a relative difference of the slope of calibration curves constructed with and without matrix extract. The matrix effects were observed for all selected mycotoxins, varying from 64 to 148 %. Reasonable levels of signal suppression or signal enhancement was achieved for only 30 % of targeted mycotoxins. Therefore, in order to control the observed matrix effects, calibrations were based on matrix-assisted standards. With the intention of further improving the accuracy of the method, we introduced stable-isotope labelled internal standards for seven of the most important mycotoxins including DON, 3-acetyl-DON, nivalenol, HT-2, T-2, zearalenone and ochratoxin A.

In order to improve the extraction methodology with respect to polar and nonpolar compounds, a two-step extraction was carried out (MeCN:H<sub>2</sub>O:HCOOH, 80:19.9:0.1, v/v/v and MeCN:H<sub>2</sub>O:HCOOH, 20:79.9:0.1, v/v/v). LC-HRMS analyses were performed on a Q-Exactive™ Hybrid Quadrupole-Orbitrap mass spectrometer equipped with a heated electrospray ion source (HESI-II) and coupled to an UHPLC Dionex Ultimate 3000 system (Thermo FisherScientific).

#### *Analytes and limit of detections*

DON: 14 µg/kg, 3-acetyl-DON: 6 µg/kg, 15-acetyl-DON: 40 µg/kg, DON-3-glucoside: 26 µg/kg, T-2 toxin: 7 µg/kg, HT-2 toxin: 14 µg/kg, T-2 triol: 80 µg/kg, T-2 tetraol: 16 µg/kg, nivalenol: 100 µg/kg, zearalenone: 36 µg/kg, alpha-zearalenol: 15 µg/kg, beta-zearalenol: 11 µg/kg, ergonovine: 50 µg/kg, ergosine: 80 µg/kg, ergotamine: 80 µg/kg, ergocornine: 80 µg/kg, alpha-ergocryptin: 80 µg/kg, ergocristine: 80 µg/kg, fumonisin B1: 80 µg/kg, fumonisin B2: 80 µg/kg, enniatin A: 13 µg/kg, enniatin A1: 14 µg/kg, enniatin B: 25 µg/kg, enniatin B1: 27 µg/kg, beauvericin: 14 µg/kg, moniliformin: 20 µg/kg, 5-acetamidobutenolid: 64 µg/kg, 2-amino-14,16-dimetyloctadecan-3-ol (2-AOD-3-ol): 200 µg/kg, 15-hydroxyculmorin: 11 µg/kg, neosolaniol: 15 µg/kg, fusarin X: 18 µg/kg, alternariol: 48 µg/kg, alternariol methylether: 46 µg/kg, sterigmaotcystein 12 µg/kg.

## Results and discussion

### Trichothecenes

In general, the trichothecenes, consisting of deoxynivalenol (DON), 3- and 15-acetylated-DON, DON-3-glucoside, nivalenol, T-2 toxin, HT-2 toxin, T-2 triol and T-2 tetraol were found at low levels in wheat and rye (Table 1). However, one sample (2 %) of milled wheat showed DON concentration slightly above its maximum limit in cereal flour (750 µg DON/kg) according to legislation. Insignificant trichothecene concentrations were found in all other samples. Metabolites of DON were hardly found. Traces of HT-2 toxin were detected in a few samples of whole and milled wheat but no T-2 or HT-2 toxin were detected in rye.

In comparison with data for DON in Norwegian wheat analysed at NVI during 1990-2011, the present mean DON concentrations in wheat were at the lower end of the yearly means of DON during these years [1]. The finding of mostly non-detectable concentrations of T-2, HT-2 and nivalenol in wheat are consistent with previous results in wheat analysed at NVI during these years. Few data are available for metabolites of DON in Norwegian cereals. In a study by Uhlig et al [3] of several mycotoxins in Norwegian cereal from 2011, a year with considerable DON concentrations, 15 % DON-3-glucoside and 4 % 3-acetyl-DON compared with the wheat concentration of DON were found. These results and the present results indicate that the

levels of DON-metabolites are far lower than the level of DON itself. T-2 triol and tetraol were not detected in wheat by Uhlig et al [3], but T-2 tetraol in oats and barley were found at similar concentrations as T-2 and HT-2. Few data are available for trichothecenes in rye in Norway. However, VKM [1] presented some data analysed at NVI, indicating that rye has so far generally not been the cereal species most contaminated with DON, T-2 and HT-2.

Table 1. Concentrations (µg/kg) of deoxynivalenol (DON), 3-actetyl-DON (3-ADON), 15-actetyl-DON (15-ADON), DON-3-glucoside (DON-3-G), HT-2 toxin, T-2 toxin, sum HT-2 + T-2 toxin, zearalenone (ZEA), sum enniatins (Σ ENN), moniliformin (MON) and 15-hydroxy-culmorin (CUL) in whole wheat, milled wheat, whole rye and milled rye sampled in Norway during 2016.

	DON	3-ADON	15-ADON	DON-3-G	HT-2	T-2	HT2+T-2	ZEA	Σ ENN	MON	CUL
Whole wheat											
Number	51	51	51	44	51	51	51	51	44	44	44
Mean	52	<10	<40	<26	<14	<10	<20	<36	165	26	<12
Median	23	<10	<40	<26	<14	<10	<20	<36	116	<20	<12
Min-max	<14-609	<10	<40	<26-102	<14-23	<10	<20-26	<36-60	<79-594	<20-219	<12-92
St. deviation	92	1	5	15	3	1	3	8	136	43	15
% samples with detectable conc.	57	0	0	7	4	0	4	2	86	34	5
Milled wheat											
Number	43	43	43	36	43	43	43	43	36	35	36
Mean	34	<10	<40	<26	<14	<10	<20	<36	98	<20	<12
Median	<14	<10	<40	<26	<14	<10	<20	<36	<79	<20	<12
Min-max	<14-823	<10	<40-193	<26-44	<14-23	<10-11	<20-26	<36	<79-310	<20-102	<12-72
St. deviation	125	1	27	5	4	2	4	6	64	18	11
% samples with detectable conc.	28	0	2	3	9	5	14	0	81	20	3
Whole rye											
Number	22	22	22	18	22	22	22	22	18	18	18
Mean	14	<10	<40	<26	<14	<10	<20	<36	599	25	<12
Median	<14	<10	<40	<26	<14	<10	<20	<36	587	<20	<12
Min-max	<14-68	<10	<40	<26	<14	<10	<20	<36	<79-1819	<20-120	<12-18
St. deviation	14	1	6	0	1	1	0	7	432	33	3
% samples with detectable conc.	36	0	0	0	0	0	0	0	89	28	6
Milled rye											
Number	35	35	35	31	35	35	35	35	31	31	31
Mean	<14	<10	<40	<26	<14	<10	<20	<36	531	<20	<12
Median	<14	<10	<40	<26	<14	<10	<20	<36	723	<20	<12
Min-max	<14-23	<10	<40	<26	<14	<10	<20	<36	<79-1039	<20-88	<12
St. deviation	7	1	5	0	1	1	0	5	372	15	0
% samples with detectable conc.	29	0	0	0	0	0	0	6	90	13	0

### Other *Fusarium* mycotoxins

Except for trace concentration of zearalenone (ZEA) in a single sample of whole wheat, no ZEA or its metabolites α- and β-zearalenol were detected. ZEA has not been systematically surveyed in Norwegian cereal but few available data indicated detection at mostly low levels [1, 3].

Enniatins, particularly enniatin B and enniatin B1 were detected in most samples, with highest concentrations in rye. The enniatin level in wheat was within the range of Norwegian limited previous

data [1, 3]. We have no previous Norwegian data for comparison for enniatins in rye. The hazard characterisation of enniatins is currently inadequate and the concentrations detected so far suggest they are of little health concern. Moniliformin was present at low and insignificant concentrations in a few wheat and rye samples. Traces of 15-hydroxy-culmorin were detected in some wheat samples.

### Ergot and alkaloids

Ergot alkaloids were only present in single samples at slightly above the level of detection, and with maximum concentration in a sample of whole rye (638 µg ergotamine/kg) (Table 2). *C. purpurea* was present in most samples of whole rye, where six samples were above the maximum limit at 500 mg/kg in EU [4]. This limit was not yet implemented in Norway in 2016. The fungus was hardly present in whole wheat. No correlation was found between ergot alkaloids and *C. purpurea*, and no ergot alkaloids were found in the six samples with *C. purpurea* above maximum level according to EU regulations. The present results with the highest occurrence of *C. purpurea* and ergot alkaloids in rye were similar to a Norwegian pilot study of these agents in rye, wheat and barley in 2013 [5]. In the pilot study, however, the level of *C. purpurea* in rye was even higher with 6 of 8 samples exceeding 500 mg/kg, and maximum concentration was 13300 mg/kg. The pilot data were, however, too small to conclude any correlation between fungus and toxins.

Table 2. Concentrations of *Claviceps purpurea* sclerotia (mg/kg) in whole wheat and whole rye, and ergot alkaloids (µg/kg) in whole wheat, milled wheat, whole rye and milled rye sampled in Norway during 2016.

	<i>C. purpurea</i> sclerotia	Ergo-novine	Ergo-sine	Ergot-amine	Ergo-cornine	α-Ergo-cryptine	Ergo-cristine	Σ Ergot-alkaloids
Whole wheat								
Number	51	44	51	51	51	51	51	51
Mean	9	<50	<80	<80	<80	<80	<80	<450
Median	0	<50	<80	<80	<80	<80	<80	<450
Min-max	0-100	<50	<80-275	<80	<80	<80	<80-120	<450-540
St. deviation	20	0	36	14	14	14	21	90
% samples with detectable concentration	33	0	4	0	0	0	6	6
Milled wheat								
Number	n.d.	36	43	43	43	43	43	43
Mean		<50	<80	<80	<80	<80	<80	<450
Median		<50	<80	<80	<80	<80	<80	<450
Min-max		<50	<80	<80	<80	<80	<80	<450
St. deviation		0	14	15	14	15	15	82
% samples with detectable concentration		0	0	0	2	2	0	2
Whole rye								
Number	22	18	21	22	22	22	22	22
Mean	386	<50	<80	<80	<80	<80	<80	<450
Median	310	<50	<80	<80	<80	<80	<80	<450
Min-max	0-1400	<50	<80	<80-638	<80	<80	<80	<450-823
St. deviation	405	0	16	130	15	15	15	161
% samples with detectable concentration	95	0	0	5	0	0	0	5
Milled rye								
Number	n.d.	31	35	33	34	35	35	35
Mean		<50	<80	<80	<80	<80	<80	<450
Median		<50	<80	<80	<80	<80	<80	<450
Min-max		<50	<80	<80-322	<80	<80	<80	<450-507
St. deviation		0	12	51	13	13	12	87
% samples with detectable concentration		0	9	9	3	3	6	11

## Undetected compounds

The *Fusarium* compounds neosolaniol, fusarin X, 5-acetamidobutenolid, 2-Amino-14,16-dimetyloctadecan-3-ol (2-AOD-3-ol), alternariol and alternariol methylether, and the *Aspergillus* mycotoxin sterigmatocystin were not detected in the cereal samples.

## Conclusions

Except for a single sample of milled wheat with DON slightly above maximum concentration according to legislation, generally, there were low and insignificant mycotoxin concentrations found in most samples of wheat and rye for human consumption. There were six samples of rye that showed *C. purpurea* above the maximum level in grain intended for human consumption according to EU legislation.

## References

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## Appendix

Appendix Table 1. Concentrations of *Claviceps purpurea* (mg/kg) and various mycotoxins (µg/kg) in individual samples of whole wheat collected in Norway during 2016. C.pur=*Claviceps purpurea*, DON=deoxynivalenol, 3aDON=3-acetyl-DON, 15aDON=15-acetyl-DON, DON3G=DON-3-glucoside, T2=T-2 toxin, HT2=HT-2 toxin, T2tri=T-2 triol, T2tet=T-2 tetraol, NIV=niavalenol, ZEA=zearalenone, aZOL=alpha-zearalenol, bZOL=beta-zearalenol, E.nov=ergonovine, E.sin=ergosine, E.tam=ergotamine, E.cor=ergocornine, aE.cry=alpha-ergocryptine, E.cri=ergocristine, FB1=fumonisin B1, FB2= fumonisin B2, EA=enniatiin A, EA1=enniatiin A1, EB=enniatiin B, EB1=enniatiin B1, BEA=beauvericin, MON=moniliformin, CUL=15-hydroxy-culmorin.

Jnr	C. pur	DON	3a DON	15a DON	DON 3G	T2	HT2	T2 tri	T2 tetr	NIV	ZE A	a ZOL	b ZOL	E. nov	E. sin	E. tam	E. cor	aE. cry	E. cri	FB1	FB2	EA	EA1	EB	EB1	BEA	MO N	CUL
58-1	0.0	<10	<10	<10		<10	<10			<10	<4				<2	<2	<2	<2	<2									
61-2	100	18	<10	<10		<10	<10			<10	<4				17	<2	<2	2	32									
69-1	0	41	<10	<10		<10	<10			<10	<4				<2	<2	<2	<2	<2									
70-1	0	21	<10	<10		<10	<10			<10	<4				<2	<2	<2	<2	<2									
70-3	0	<10	<10	<10		<10	<10			<10	<4				<2	<2	<2	<2	<2									
71-1	0	29	<10	<10		<10	<10			<10	<4				<2	<2	<2	<2	<2									
82-1	0	<10	<10	<10		<10	<10			<10	<4				<2	<2	<2	<2	<2									
106-1	0	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	<25	<27	<14	<20	<11
118-1	0.0	119	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	114	<27	<14	<20	<11
142-2	0	<14	<6	<40	<26	<7	23	<80	<16	<100	<36	<15	<11	<50	275	<80	<80	<80	120	<80	<80	<13	<14	181	<27	<14	33	<11
143-1	0	<14	<6	<40	<26	<7	23	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	<14	41	<27	<14	<20	<11	
150-1	1	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	<14	41	<27	<14	<20	<11	
189-1	0	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	<14	41	<27	<14	<20	<11	
191-1	0	23	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	<14	<25	46	<14	<20	<11	
203-1	0	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	<14	41	<27	<14	<20	<11	
222-1	10	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	<14	273	46	<14	33	<11	
244-1	0	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	<14	41	<27	<14	<20	<11	
245-1	25	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	<14	<25	<27	<14	<20	<11	
246-1	0	23	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	<14	502	46	<14	208	<11	
247-1	0	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	<14	41	<27	<14	<20	<11	
248-1	10	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	<14	297	46	<14	33	<11	
257-1	0	80	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	<14	192	46	<14	33	<11	
270-1	0	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	23	41	46	23	<20	<11	
270-2	80	83	<6	<40	44	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	23	41	46	23	33	<11	
272-1	0	23	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	23	<25	<27	23	<20	<11	
277-1	5	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	23	41	46	23	<20	<11	
277-2	5	23	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	23	90	46	23	33	<11	
277-3	0	49	<6	<40	44	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	23	<25	46	23	<20	<11	
277-4	20	151	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	23	131	46	<14	33	<11	
279-1	45	23	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	47	400	141	23	<20	<11	
280-1	0	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	23	97	46	23	<20	<11	
280-5	0	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	23	41	46	23	<20	<11	
285-1	45	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	120	<80	<80	<13	23	41	46	23	33	<11
293-1	0	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	23	41	46	23	<20	<11	
296-1	13	86	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	23	88	46	23	33	<11	

296-2	0	53	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	41	46	23	<20	<11
296-4	16	106	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	204	46	23	33	<11
296-5	0	127	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	<25	46	<14	<20	<11
297-1	5	137	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	<25	46	<14	<20	<11
297-2	41	57	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	<25	<27	<14	<20	<11
297-3	0	60	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	<25	<27	<14	<20	<11
301-1	0	54	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	41	320	<14	33	<11
311-1	0	23	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	<25	<27	<14	<20	<11
311-2	13	146	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	41	143	<14	33	<11
311-3	0	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	<25	<27	<14	<20	<11
311-4	8	47	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	<25	46	<14	<20	<11
312-1	0	50	<6	<40	<26	<7	<14	<80	<16	<100	60	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	<25	104	<14	<20	<11
313-1	0	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	41	224	<14	<20	<11
322-1	0	175	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	41	163	<14	<20	92
324-1	0	66	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	41	309	<14	33	<11
328-1	0	609	<6	<40	102	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	41	324	<14	219	50

Appendix Table 2. Concentrations of various mycotoxins (µg/kg) in individual samples of milled wheat collected in Norway during 2016. DON=deoxynivalenol, 3aDON=3-acetyl-DON, 15aDON=15-acetyl-DON, DON3G=DON-3-glucoside, T2=T-2 toxin, HT2=HT-2 toxin, T2tri=T-2 triol, T2tetr=T-2 tetraol, NIV=nivalenol, ZEA=zearalenone, aZOL=alpha-zearalenol, bZOL=beta-zearalenol, E.nov=ergonovine, E.sin=ergosine, E.tam=ergotamine, E.cor=ergocornine, aE.cry=alpha-ergocryptine, E.cri=ergocristine, FB1=fumonisin B1, FB2= fumonisin B2, EA=enniatin A, EA1=enniatin A1, EB=enniatin B, EB1=enniatin B1, BEA=beauvericin, MON=moniliformin, CUL=15-hydroxy-culmorin.

Jnr	DON	3a DON	15a DON	DON 3G	T2	HT2	T2 tri	T2 tetr	NIV	ZEA	a ZOL	b ZOL	E. nov	E. sin	E. tam	E. cor	a-E. cry	E. cri	FB1	FB2	EA	EA1	EB	EB1	BEA	MON	CUL
15-1	27	<10	<10		<10	<10			<10	<4				5	<2	<2	<2	<2									
15-2	20	<10	<10		<10	11			<10	<4				<2	<2	<2	<2	<2									
15-3	16	<10	<10		<10	<10			<10	<4				<2	<2	<2	<2	<2									
16-1	55	<10	<10		<10	<10			<10	<4				<2	<2	<2	<2	<2									
17-2	<14	<10	<10		<10	<10			<10	<4				<2	<2	<2	<2	<2									
20-1	84	<10	<10		<10	<10			<10	<4				<2	<2	3	2	<2									
31-1	14	<10	<10		<10	<10			<10	<4				<2	<2	<2	<2	<2									
47-1	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	120	<80	<13	<14	41	<27	<14	33	<11
49-1	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	120	<80	<13	<14	41	<27	<14	<20	<11
64-1	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	41	<27	<14	<20	<11
66-1	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	41	<27	<14	<20	<11
67-1	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	41	<27	<14	33	<11
68-1	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	14	91	<14	<20	<11
69-1	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	41	<27	<14	<20	<11
70-1	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	41	<27	<14	<20	<11
72-1	23	<6	<40	44	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	41	<27	<14	<20	<11
73-1	<14	<6	<40	<26	11	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	<25	<27	<14	<20	<11
73-2	<14	<6	<40	<26	<7	<14	<80	<16	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	<25	<27	<14	<20	<11



Appendix Table 3. Concentrations of *Claviceps purpurea* (mg/kg) and various mycotoxins (µg/kg) in individual samples of whole rye collected in Norway during 2016. C.pur=*Claviceps purpurea*, DON=deoxynivalenol, 3aDON=3-acetyl-DON, 15aDON=15-acetyl-DON, DON3G=DON-3-glucoside, T2=T-2 toxin, HT2=HT-2 toxin, T2tri=T-2 triol, T2tet=T-2 tetraol, NIV=nivalenol, ZEA=zearalenone, aZOL=alpha-zearalenol, bZOL=beta-zearalenol, E.nov=ergonovine, E.sin=ergosine, E.tam=ergotamine, E.cor=ergocornine, aE.cry=alpha-ergocryptine, E.cri=ergocristine, FB1=fumonisin B1, FB2= fumonisin B2, EA=enniatiin A, , EA1=enniatiin A1, , EB=enniatiin B, EB1=enniatiin B1, BEA=beauvericin, MON=moniliformin, CUL=15-hydroxy-culmorin.

Jnr	C pur	DON	3a DON	15a DON	DON 3G	T2	HT2	T2 tri	T2 tet	NIV	ZEA	aZOL	bZOL	E. nov	E. sin	E. tam	E. cor	a-E. cry	E. cri	FB1	FB2	EA	EA1	EB	EB1	BEA	MON	CU L
61-1	1140	<10	<10	<10		<10	<10			<10	<4				<2	<2	<2	<2	<2									
70-2	80	25	<10	<10		<10	<10			<10	<4				<2	<2	<2	<2	<2									
71-2	1400	11	<10	<10		<10	<10			<10	<4				<2	<2	<2	<2	<2									
82-2	800	15	<10	<10		<10	<10			<10	<4				<2	<2	<2	<2	<2									
97-2	820	<14	<6	<40	<26	<7	<14	<80	87	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	120	<80	<13	<14	41	<27	<14	33	18
106-2	760	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	131	516	<14	<20	<11
118-2	480	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	638	<80	<80	<80	<80	<80	<13	23	134	575	<14	<20	<11
142-1	780	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	129	567	<14	33	<11
191-2	0	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	117	443	<14	<20	<11
222-2	320	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	59	275	1478	23	120	<11
247-2	21	23	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	41	304	<14	<20	<11
280-2	390	68	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	187	875	23	103	<11
280-3	300	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	143	534	23	<20	<11
280-4	90	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	121	414	23	<20	<11
285-2	110	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	122	433	23	<20	<11
290-1	40	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	116	392	<14	<20	<11
294-1	350	23	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50		<80	<80	<80	<80	<80	<80	<13	23	176	764	23	33	<11
296-3	75	23	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	120	493	<14	<20	<11
301-2	28	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	<25	<27	<14	<20	<11
311-5	483	23	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	106	431	<14	<20	<11
324-2	10	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	<25	46	<14	<20	<11
327-1	21	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	<25	<27	<14	<20	<11

Appendix Table 4. Concentrations of various mycotoxins (µg/kg) in individual samples of milled rye collected in Norway during 2016. DON=deoxynivalenol, 3aDON=3-acetyl-DON, 15aDON=15-acetyl-DON, DON3G=DON-3-glucoside, T2=T-2 toxin, HT2=HT-2 toxin, T2tri=T-2 triol, T2tetr=T-2 tetraol, NIV=nivalenol, ZEA=zearalenone, aZOL=alpha-zearalenol, bZOL=beta-zearalenol, E.nov=ergonovine, E.sin=ergosine, E.tam=ergotamine, E.cor=ergocornine, aE.cry=alpha-ergocryptine, E.cri=ergocristine, FB1=fumonisin B1, FB2= fumonisin B2, EA=enniatiin A, EA1=enniatiin A1, EB=enniatiin B, EB1=enniatiin B1, BEA=beauvericin, MON=moniliformin, CUL=15-hydroxy-culmorin.

Jnr	DON	3a DON	15a DON	DON 3G	T2	HT2	T2 tri	T2 tetr	NIV	ZEA	a ZOL	b ZOL	E. nov	E. sin	E. am	E. cor	a-E. cry	E. cri	FB1	FB2	EA	EA1	EB	EB1	BEA	MON	CUL
14-1	11	<10	<10		<10	<10			<10	<4				5	2	<2	<2	2									
17-1	17	<10	<10		<10	<10			<10	<4				<2	<2	<2	<2	<2									
20-2	<10	<10	<10		<10	<10			<10	<4				3	<2	2	2	<2									
30-1	10	<10	<10		<10	<10			<10	6				2	5	<2	<2	2									
47-2	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	120	<80	<13	<14	41	<27	<14	33	<11
57-1	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80		<80	<80	<80	<80	<80	<13	23	131	730	<14	<20	<11
65-1	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	134	729	<14	<20	<11
66-2	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	133	705	<14	<20	<11
74-2	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	110	582	<14	<20	<11
78-1	23	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	118	664	<14	<20	<11
88-1	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	152	781	<14	<20	<11
106-1	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	41	356	<14	<20	<11
129-1	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80		<80	<80	<80	<80	<13	23	131	720	<14	<20	<11
131-2	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	117	647	<14	<20	<11
137-2	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80		<80	<80	<80	<80	<80	<13	23	129	686	<14	<20	<11
140-3	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	127	644	<14	<20	<11
140-5	23	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	134	722	<14	33	<11
141-1	23	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	114	586	<14	<20	<11
142-2	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	146	724	<14	<20	<11
142-5	<14	<6	<40	<26	<7	<14	<80	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	23	134	651	<14	<20	<11
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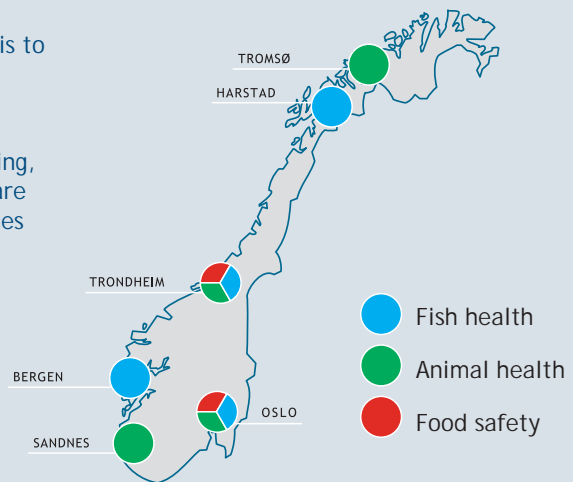
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