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Executive summary

Ergot alkaloids are toxins produced by members of the fungal family of Clavicipitaceae. In Europe rye ergot (*Claviceps purpurea)* is the predominant species, occurring mostly on rye, wheat, triticale and barley. In the European Union, a maximum limit of 1000 mg/kg of rye ergot sclerotia has been established for feed containing unground cereals. However, size, weight and composition of the sclerotia may vary considerably. In addition, sorting is impossible in processed feed materials. Moreover, sclerotia show significant differences in their total alkaloid content and show differences in the pattern of produced alkaloids that is determined by the individual fungal strain in a geographical region and the host plant. The aim of the work described in this report was to study the relationship between the presence of sclerotia and the content of ergot alkaloids and to study the differences in the pattern of the individual ergot alkaloids. The study was confined to cereals, intended for use as feed materials and to compound feeds. The samples were taken by the Netherlands Food and Consumer Product Safety Authority (NVWA) in the framework of the Dutch National Plan for Feed.

A total of 184 samples has been selected for this study, 136 unground cereals and 48 compound feeds, which were all chemically analysed. Of the unground cereals, 120 cereal samples were visually inspected, and sclerotia of 48 samples were chemically analysed. Visual inspection revealed that 67 samples (56%) contained sclerotia, with an average content (including blanks) of 317 mg/kg and a maximum of 6554 mg/kg. Ten samples exceeded the official limit of 1000 mg/kg: eight rye samples, one triticale and one wheat sample. Chemical analysis showed that 43% of the unground cereal samples contained ergot alkaloids (average contamination 93 µg/kg, maximum 1231 µg/kg). Rye was more heavily contaminated than wheat and triticale. Compound feeds were often contaminated (83%) and with an average ergot alkaloid concentration (77 µg/kg) that was not much lower than that of unground cereals. This was somewhat unexpected because cereals compose only part of the ingredients used in compound feeds.

The sclerotia samples contained ergot alkaloids in a concentration ranging from less than 1 µg/g to a maximum of 6003 µg/g, with an average concentration for all samples of 659 µg/g. In combination with literature data, the obtained results for the ergot alkaloid content of sclerotia, point to an average concentration of 800 µg/g for sclerotia collected in North-west and Central Europe. The previously proposed level (2000 µg/g) for average ergot alkaloid content of sclerotia may therefore be too high.

In less than 10% of the samples that according to visual inspection contained no sclerotia, (traces of) ergot alkaloids were detected. The maximum amount found (297 µg/kg) was well below the average concentration expected for a sample contaminated with rye ergot at the maximum allowed level. The amount of sclerotia in cereals was not a very reliable indicator of the actual ergot alkaloid content of the specific cereal samples. In a relatively high number of cases (20%) the ergot alkaloid content of cereals contaminated with (sometimes substantial) amounts of sclerotia, was very low or non-detectable. Visual screening followed by a chemical analysis of selected samples is a cost effective strategy for monitoring ergot alkaloids in unground products without causing additional risk.

Summary

Ergot alkaloids are toxins produced by members of the fungal family of Clavicipitaceae represented among others by *Claviceps purpurea, C. paspali*, and *C. fusiformis*, occurring predominantly on rye, wheat and barley, but also on rice, maize, sorghum, oats and millet. The fungal structures develop instead of kernels on grain ears or seeds on grass heads, being visible as large dark or purple sclerotia. These sclerotia contain different classes of alkaloids, the most prominent being the lysergic acid derivative ergometrine, and the ergopeptines ergotamine, ergosine, ergocristine, ergocryptine and ergocornine. Of interest are also their related C-8 epimers - the so called -inines. Intoxications induced by *Claviceps purpurea* have been known in Europe for many centuries. Ergot alkaloids exert toxic effects in all animal species. Typical clinical symptoms are vasoconstriction that may progress into vaso-occlusion and gangrenous changes, but also into abortions. The neurotoxic signs comprise feed refusal and dizziness but also convulsions.

In the European Union, a maximum limit of 1000 mg/kg of rye ergot *(Claviceps purpurea)* sclerotia has been established for feed containing unground cereals. However, size, weight and composition of the sclerotia may vary considerably. In addition, sorting is impossible in processed feed materials. Moreover, sclerotia show significant differences in their total alkaloid content and show differences in the pattern of produced alkaloids that is determined by the individual fungal strain in a geographical region and the host plant. The aim of the work described in this report was to study the relationship between the presence of sclerotia and the content of ergot alkaloids and to study the differences in the pattern of the individual ergot alkaloids. The study was confined to cereals, intended for use as feed materials and to compound feeds. The samples were taken by the Netherlands Food and Consumer Product Safety Authority (NVWA) in the framework of the Dutch National Plan for Feed. A total of 184 samples has been selected for this study, 136 unground cereals and 48 compound feeds, which were all chemically analysed. Of the unground cereals, 120 cereal samples were visually inspected, and sclerotia of 48 samples were chemically analysed.

Of the 120 samples of cereals that were visually inspected, 67 samples (56%) contained sclerotia, with an average content (including blanks) of 317 mg/kg and a maximum of 6554 mg/kg. Ten samples exceeded the official limit of 1000 mg/kg: eight rye samples, one triticale and one wheat sample.

Of the 184 samples of cereals and compound feeds that were chemically analysed, 98 (53%) contained ergot alkaloids in a concentration exceeding 10 µg/kg, with an average content (including blanks) of 89 µg/kg and a maximum of 1231 µg/kg. Compound feeds were most often contaminated (83%) and the average content was 77 µg/kg with a maximum of 583 µg/kg. The average contamination for the cereals was 93 µg/kg and 43% of the samples was contaminated with ergot alkaloids. Rye contained the highest amounts of ergot alkaloids, with respect to average concentration (134 µg/kg) and maximum (1231 µg/kg). Triticale and wheat, respectively, showed lower averages (33 and 54 µg/kg) and maximum amounts (297 and 529 µg/kg). Major ergot alkaloids detected were ergosine, ergotamine, ergocristine and ergocryptine. Unexpectedly, the average concentration of ergot alkaloids in compound feeds was in the same order of magnitude as the average concentration in cereals, although cereals compose only part of the ingredients used in compound feeds.

A total of 48 samples of sclerotia was chemically analysed. The composition of the sclerotia was highly variable with respect to ergot alkaloid concentrations as well as composition. In total 34 samples contained ergot alkaloids in a concentration exceeding 1 µg/g, with an average concentration for all samples of 659 µg/g and a maximum of 6003 µg/g. The six major ergot alkaloids (ergocornine, ergocristine, ergocryptine, ergometrine, ergosine and ergotamine) accounted for 508 µg/g, the corresponding epimeric -inines for 134 µg/g. Major ergot alkaloids detected were ergocryptine, ergocornine, ergosine and ergotamine. None of the individual ergot alkaloids contributed more than 20% to the total. Of the minor ergot alkaloids only agroclavine and chanoclavine-1 were found and they contributed 17 µg/kg to the total.

The current study and the available data from literature (Franzmann et al, 2010; Appelt and Ellner, 2009; Mainka et al, 2007a) on ergot alkaloid content of sclerotia, collected from various locations in North-west and Central Europe, point to an average concentration of 800 µg/g. The previously proposed level (2000 µg/g) for average ergot alkaloid content of sclerotia (Müller et al, 2009; Verstraete, 2010) may therefore be too high. In addition, the available data indicate that in sclerotia the average ratio between the sum of -ines and the sum of -inines is approximately 3:1.

Manual removal of sclerotia resulted in a reduction of the ergot alkaloid content by on average 70%. Remaining alkaloid content on average was highest in cleaned triticale (52.3%) and wheat (45.8%), while it was lowest in rye (23.7%). The remaining alkaloid content could be due to very small sclerotia particles ('ergot dust') or perhaps to non-recognised infestation of cereal grains.

In 9.2% of the samples that contained no sclerotia upon visual inspection, (traces of) ergot alkaloids were detected, with a maximum of 297 µg/kg. A weak correlation was found between the amount of sclerotia and the ergot alkaloid content of samples. This is in accordance with information available from the literature. In a relatively high number of cases (20%) the ergot alkaloid content of cereals contaminated with (sometimes substantial) amounts of sclerotia, was very low or non-detectable. The amount of sclerotia in cereals therefore is not a very reliable indicator of the actual ergot alkaloid content of the specific cereals. Visual screening followed by a chemical analysis of selected samples is a cost effective strategy for monitoring ergot alkaloids in unground products without causing additional risk.

Contents

1 Introduction

Ergot toxins are produced by members of the fungal family of Clavicipitaceae represented among others by *Claviceps purpurea, C. paspali*, and *C. fusiformis*, occurring predominantly on rye, wheat and barley, but also on rice, maize, sorghum, oats and millet (EFSA, 2005). Intoxications induced by *Claviceps purpurea* have been known in Europe for many centuries. In humans these intoxications are described in the medieval literature as St. Anthony's Fire or Holy Fire, with reference to the intense pain resulting from vasoconstriction and subsequent gangrene, as well as the neurotoxic symptoms associated with the ingestion of ergot alkaloids. Ergot alkaloids exert toxic effects in all animal species. Typical clinical symptoms are vasoconstriction that may progress into vaso-occlusion and gangrenous changes, but also into abortions. The neurotoxic signs comprise feed refusal and dizziness but also convulsions (Woolf, 2000; Keller et al, 2005, EFSA, 2005).

The term ergot alkaloids refers to a diverse group of up to forty toxins, comprising clavines, lysergic acids, lysergic acid amides and ergopeptines (Betina, 1994, Schardl et al, 2006). The fungal structures develop instead of kernels on grain ears or seeds on grass heads, being visible as large dark or purple sclerotia. These sclerotia contain different classes of alkaloids, the most prominent being the lysergic acid derivative ergometrine, and the ergopetines ergotamine, ergosine, ergocristine, ergocryptine and ergocornine. Of interest are also their related C-8 epimers - the so called - inines.

In the European Union, a maximum limit of 1000 mg/kg of rye ergot *(Claviceps purpurea)* sclerotia has been established for feed containing unground cereals (European Union, 2002). The EFSA has stated that the physical determination of the contamination rate is often inaccurate as size, weight and composition of the sclerotia may vary considerably (EFSA, 2005). In addition, sorting is impossible in processed feed materials. Moreover, sclerotia show significant differences in their total alkaloid content that varies between 0.01% and 0.21% (Lorenz, 1979; Wolff and Richter, 1989) and show differences in the pattern of produced alkaloids that is determined by the individual fungal strain in a geographical region and the host plant. Hence, it has been suggested by EFSA to replace the physical methods by chemical analysis (EFSA, 2005).

Available data indicate that adverse effects may occur in agricultural animals particularly in pigs after intake of feed contaminated with ergot at levels close to the current EU limit (EFSA, 2005). At present, the data on the toxicological properties of individual ergot alkaloids are too limited to select individual marker toxins for monitoring the extent of contamination (EFSA, 2005). For that reason EFSA has stated that more data are needed to define the variability of ergot alkaloid patterns in European feed materials for future establishment of maximum limits of individual ergot alkaloids.

Recently, the European Commission has recommended to the member states to perform monitoring on the presence of ergot alkaloids, among others in cereals and cereal products intended for animal feeding and in compound feed (European Union, 2012). The European Commission has also requested to relate the presence of ergot alkaloids to the amount of sclerotia present and to focus the monitoring on the six predominantly present ergot alkaloids i.e. ergometrine, ergotamine, ergosine, ergocristine, ergocryptine and ergocornine and their -inine epimers (European Union, 2012).

Based upon available data, a provisional relation between the amount of sclerotia and the level of these individual ergot alkaloids has been proposed by Verstraete (2010). The following levels of individual ergot alkaloids would correspond to a content of 1 g or rye ergot sclerotia: ergocristine: 600 µg; ergotamine: 300 µg; ergocryptine: 100 µg; ergometrine: 100 µg; ergosine: 100 µg; ergocornine: 100 µg. However, it was stressed that this relationship includes a high level of uncertainty/variability and that it is absolutely necessary that more reliable data are generated to establish this relationship with more certainty (Verstraete, 2010).
Furthermore, it is recognised that epimerisation at the C-8 position of the lysergic acid moiety may readily occur in the sclerotia and is difficult to control during the analytical procedure (EFSA, 2009). Although these iso-lysergic acid isomers are generally considered being less biologically active than the corresponding lysergic acid derivatives, it has been advised to incorporate them in the analytical method (Müller et al, 2009).

The aim of the work described in this report was to study the relationship between the presence of sclerotia and the content of ergot alkaloids and to study the differences in the pattern of the individual ergot alkaloids. The study was confined to cereals, intended for use as feed materials and to compound feeds. The samples were taken by the Netherlands Food and Consumer Product Safety Authority (NVWA) in the framework of the Dutch National Plan for Feed. The data presented in this report have been submitted to the EFSA to be included in their comprehensive database.

2 Material en methods

2.1 Origin and sampling

The samples have been collected on site by the Netherlands Food and Consumer Product Safety Organisation (NVWA) in the framework of the Dutch National Monitoring Plan on animal feeding stuffs in the years 2007 till 2010. In 2007 part of the samples was obtained by selective sampling of 'suspect' lots. In the other years random sampling was performed.

2.2 Visual inspection

The method for physical determination was followed as described by the International Association of Feedingstuff Analysis (IAG) section Feedingstuff Microscopy (IAG, s.n.). No treatment prior to visual inspection was applied to the samples. Each entire grain sample was weighted. Grain samples were spread on a flat surface in a large bin. All grains were inspected visually and grains showing black ergot infection (sclerotia) in whole or partly were selected. The selected grains were weighted and the weight of the infected portion was recorded in mg and as fraction of the complete sample (mg/kg).

It is extremely difficult to collect reliable information of the amount of infestation of ground material, whether ground cereals or compound feeds. These types of samples were therefore not investigated visually.

2.3 Chemical method

Analytical standards of the ergot alkaloids were obtained from several commercial sources (Sigma-Aldrich, Zwijndrecht, The Netherlands; Phytolab, Vestenbergsgreuth, Germany; Alfarma, Prague, Czech republic). See Annex I for the chemical structures of the ergot alkaloids included in the method. Stock solutions were prepared in acetonitrile and these were stored at -20^{o}C to minimize epimerisation.

2.3.1 Sample preparation of cereals and feed

Cereal and feed samples were ground to 0.5 mm and homogenized. Ergot alkaloids were extracted from cereals or feed (4 g) with 40 ml methanol/water/formic acid (600/400/4) by rotary tumbling for 30 min. After centrifugation (15 min, 3500 g), an aliquot (2 ml) of the extract was filtered through a 30kD ultrafilter at 3500 g. The eluate was analysed for ergot alkaloids by LC-MS/MS.

Quantification was performed by means of standard addition. For the analysis of the cereal and feed samples a two-step procedure was followed. First a screening was carried out. Two subsamples (4 g) were taken, of which one subsample was spiked with a mixture of the ergot alkaloids at 100 µg/kg. Concentrations were calculated on the basis of the spiked sample. Samples containing no ergot alkaloids or only ergot alkaloids in an individual concentration of less than 50 µg/kg were not processed further. When in the sample one or more ergot alkaloids were detected at a concentration of more than 50 µg/kg, the sample was reanalysed by multi-level standard addition (MLSA).

2.3.2 Chemical analysis of sclerotia

Ergot alkaloids in ergot were determined by multi-level standard addition to an extract of the sclerotia. Sclerotia were crushed in a mortar. The crushed bodies were accurately weighted into a 10 ml tube. Methanol/water/formic acid (600/400/4) was added to the ergot in the tube in a volume to weight ratio of 20 to 1. Extraction was performed by rotary tumbling for 30 min After centrifugation (15 min, 3500 g), 10 µl aliquots were transferred to seven different 2-ml glass vials. To five vials a mixture of ergot alkaloid standards was added, in a concentration range of 50 to 1000 µg/g ergot.

2.3.3 LC-MS/MS analysis

Analysis of the samples was performed on a Shimadzu UFLC system equipped with a LC-20AD binary pump, a SIL-HTC autosampler and a CTO-20A column oven coupled to a Waters-Micromass Quattro Ultima tandem mass spectrometer. Ergot alkaloids were separated on a Waters XBridge C18 column (150 x 3.1 mm, 5 µm) using an acetonitrile/water/ammonia (6 mm) gradient. Total runtime was 20 min. Ergot alkaloids were recorded with positive electrospray in MRM mode using two precursor to product ion transitions for each compound. The mass spectrometric settings were optimised for each alkaloid individually. See Annex II for the instrumental details. A total of 28 transitions was monitored in a single run. All compounds could be unequivocally characterized on the basis of retention time and fragmentation transitions.

The limit of detection (LOD) for individual compounds in cereals and animal feeds is 2 µg/kg; the limit of quantification (LOQ): 10 µg/kg. Limit of detection for individual compounds in sclerotia: 1 µg/g; the limit of quantification: 5 µg/g.

2.3.4 Validation

The method has been in-house validated for ergocornine, ergocristine, α-ergocryptine, ergometrine and ergotamine in cereals (rye, wheat, triticale) and compound feeds at contamination levels of 50 µg/kg and higher. The method has been additionally in-house validated for the following ergot alkaloids: ergocorninine, ergocristinine, α-ergocryptinine, β-ergocryptine, ergometrinine, ergosine, ergosinine, ergotaminine, agroclavine, chanoclavine-1, elymoclavine, ergine, erginine, festuclavine, lysergol, methylergometrine and methysergide. For β-ergocryptinine and ergovaline no standards were available.

2.3.5 Calculation of ergot alkaloid content

Samples subjected to LC-MS/MS analysis were either complete or split into a sclerotia fraction and a cereal fraction. The ergot alkaloid content of the cereal or animal feed sample was calculated for each individual component (expressed in µg/kg). The alkaloid content of sclerotia was calculated for each individual component (expressed in µg/g). The amounts found in the sclerotia were also expressed for the complete sample (µg/kg). The reported content is the sum of cereal and sclerotia fractions (expressed in µg/kg). Results have not been corrected for moisture content of the sample. β-ergocryptinine co-eluted with α-ergocryptinine and these compounds were therefore reported as the sum of α- and β-ergocryptinine.

3 Results

3.1 Origin of samples

In Table 1 an overview of the samples collected in the framework of the Dutch National Monitoring Program on Animal Feeds from 2007 to 2010 is presented. The majority of the samples could be classified into one of the following four major categories - rye, triticale, wheat and compound feed. Two samples of barley and two samples containing a mixture of cereals were treated separately. The majority of the samples was of Dutch origin. Only 23 samples originated from Germany and of 20 samples the origin was not known or they were single entries of a European country. The number of samples collected per category and per year was variable. A relatively large number of rye samples was collected each year, but triticale was mainly collected during 2008, 2009 and 2010, while most of the wheat samples were collected in 2007. Animal feeds were collected during 2008, 2009 and 2010. The samples collected were randomly selected, except for some 'suspect' lots collected in 2007.

Table 1. Overview of the collected samples.

Commodity	Country of origin	Year of Sampling				Total
		2007	2008	2009	2010	
Rye	The Netherlands	9	11	15	16	51
	Germany	3	3	1	3	10
	Unknown/others	1	5	0	2	8
	Total rye	**13**	**19**	**16**	**21**	**69**
Triticale	The Netherlands	2	14	9	6	31
	Germany	1	3	0	5	9
	Unknown/others	0	2	1	2	5
	Total triticale	**3**	**19**	**10**	**13**	**45**
Wheat	The Netherlands	10	1	0	0	11
	Germany	0	1	0	0	1
	Unknown/others	6	0	0	0	6
	Total wheat	**16**	**2**	**0**	**0**	**18**
Other cereals	The Netherlands	2	0	0	0	2
	Germany	0	1	0	0	1
	Unknown/others	0	0	0	1	1
	Total other cereals	**2**	**1**	**0**	**1**	**4**
Compound feed	The Netherlands	0	10	19	17	46
	Germany	0	0	1	1	2
	Unknown/others	0	0	0	0	0
	Total compound feed	**0**	**10**	**20**	**18**	**48**
Total		**34**	**51**	**46**	**53**	**184**

In Table 2 an overview is given of the samples subjected to visual inspection (physical determination). The set of 120 samples is a subset of all the samples shown in Table 1.

Compound feeds have not been inspected by visual screening. Some of the cereal samples were milled and no visual screening could be performed.

Table 2. Overview of samples evaluated by visual inspection. The samples are a subset from the samples collected in Table 1.

Commodity	Country of origin	Year of Sampling				Total
		2007	2008	2009	2010	
Rye	The Netherlands	7	9	14	16	46
	Germany	3	2	1	3	9
	Unknown/others	0	4	0	1	5
	Total rye	**10**	**15**	**15**	**20**	**60**
Triticale	The Netherlands	2	11	9	6	28
	Germany	0	3	0	5	8
	Unknown/others	0	2	0	2	4
	Total triticale	**2**	**16**	**9**	**13**	**40**
Wheat	The Netherlands	10	1	0	0	11
	Germany	0	1	0	0	1
	Unknown/others	4	0	0	0	4
	Total wheat	**14**	**2**	**0**	**0**	**16**
Other cereals	The Netherlands	2	0	0	0	2
	Germany	0	1	0	0	1
	Unknown/others	0	0	0	1	1
	Total other cereals	**2**	**1**	**0**	**1**	**4**
Total		**28**	**34**	**24**	**34**	**120**

3.2 Visual inspection

Of a total of 184 samples investigated in the present study, 64 ground samples were not visually inspected. Of the remaining 120 samples, 67 samples (56%) showed grains infested with ergot. The results of the visual inspection are presented in Table 3. The average sclerotia content for the complete set of samples was 317 mg/kg.

Rye was the most susceptible species of the cereals investigated. Of the 60 rye samples investigated, 45 (75%) contained ergot sclerotia. Two examples of sclerotia found in samples of rye are illustrated in Figure 1 and 2. The average sclerotia content (including blanks) in rye samples was 449 mg/kg, with a maximum of 3429 mg/kg. There was no significant difference in occurrence and concentrations between the samples originating from The Netherlands and those from Germany or those of unknown/other origin. For wheat and triticale (Figure 3) the share of infested samples was significantly lower than for rye. In approximately two thirds of the triticale and wheat samples no sclerotia were detected. A small number of samples in these groups (one triticale and one wheat sample) contained a high amount of sclerotia, which may bias the overall results. One triticale sample contained as much as 6554 mg/kg ergot, the next highest sample contained only 185 mg/kg. Similarly, the highest wheat sample contained 2940 mg/kg, the next highest one 160 mg/kg.

In 2007 a part of the samples was taken from lots that were under 'suspicion'. In the other years (2008-2010) sampling has been random. When the 2007 samples (28 in total) are removed from the data set, for the remaining 92 samples (including blanks) the average sclerotia content is 197 mg/kg. The average content for rye (271 mg/kg) is significantly higher than for triticale (29 mg/kg). For wheat too few samples remain for a realistic average content. Alternatively, when only the highest wheat sample (dating from 2008) is removed, the remaining 15 samples contain as little as 16 mg/kg.

Table 3. Distribution of the total sclerotia weight in the samples analysed.

Com-modity	Country of origin	No of samples	Distribution according to total sclerotia weight					Content (mg/kg)	
			Blank	1-10 mg/kg	10-100 mg/kg	100-1000 mg/kg	1000-10000 mg/kg	Avg.	Max.
Rye	The Netherlands	46	12	0	13	17	4	360	2760
	Germany	9	2	0	2	2	3	889	3429
	Unknown/ others	5	1	0	2	1	1	468	1886
	Total rye	**60**	**15**	**0**	**17**	**20**	**8**	**449**	**3429**
	(% of total)		(25.0)	(0.0)	(28.3)	(33.3)	(13.3)		
Triticale	The Netherlands	28	17	3	6	1	1	250	6554
	Germany	8	5	0	1	2	0	41	165
	Unknown/ others	4	2	0	0	2	0	80	163
	Total triticale	**40**	**24**	**3**	**7**	**5**	**1**	**191**	**6554**
	(% of total)		(60.0)	(7.5)	(17.5)	(12.5)	(2.5)		
Wheat	The Netherlands	11	5	0	3	0	1	274	2940
	Germany	1	1	0	0	0	0	0	0
	Unknown/ others	4	3	0	0	1	0	40	160
	Total wheat	**16**	**11**	**0**	**3**	**1**	**1**	**198**	**2940**
	(% of total)		(68.8)	(0.0)	(18.8)	(6.2)	(6.2)		
Other cereals	The Netherlands	2	2	0	0	0	0	0	0
	Germany	1	1	0	0	0	0	0	0
	Unknown/others	1	0	0	0	1	0	514	514
	Total other cereals	**4**	**3**	**0**	**0**	**1**	**0**	**129**	**514**
	(% of total)		(75.0)	(0.0)	(0.0)	(25.0)	(0.0)		
Total		**120**	**53**	**3**	**27**	**27**	**10**	**317**	**6554**
	(% of total)		**(44.2)**	**(2.5)**	**(22.5)**	**(22.5)**	**(8.3)**		

In some samples grains were present which were moulded instead of infected by ergot (Figure 4), which were easily distinguishable.

Figures 1 and 2. Rye ergot sclerotia, isolated from contaminated rye grain. In Figure 2 a set of normal rye grains is included for comparison.

Figures 3 and 4. Ergot sclerotium of triticale (left), and moulded grains of triticale, not infected by ergot (right).

3.3 LC-MS/MS analysis

3.3.1 Analysis of cereals and animal feeds

All 184 samples as described in Table 1 were analysed for ergot alkaloid content. The samples were analysed for 24 different ergot alkaloids (Annex I, II), 16 of which were detected above the limit of detection of 2 µg/kg (Annex VIII). In Table 4 the distribution of the total ergot alkaloid concentration is shown. The average ergot alkaloid concentration for all samples was 89 µg/kg. When only the 2008-2010 samples (150) are considered, the average content is 94 µg/kg.

Of the 69 rye samples analysed, 34 (49%) contained a total ergot alkaloid content of less than 10 µg/kg. The other 35 rye samples contained alkaloid concentrations between 10 and a maximum of 1231 µg/kg. An average concentration of 134 µg/kg was obtained for the rye samples (including blanks). For the 56 rye samples collected during 2008-2010 an average of 136 µg/kg was found. A somewhat higher average concentration (185 µg/kg) was obtained for the samples originating

from Germany compared to those originating from The Netherlands. However, the number of German rye samples investigated was very small (only 10 over the period 2007-2010). Regarding the triticale samples, 30 out of 45 samples (67%) did contain (almost) no ergot alkaloids.
The average ergot alkaloid concentration was only 33 µg/kg, significantly lower compared to rye. The maximum amount found was 297 µg/kg, which is also lower compared to rye.

Table 4. Distribution of the total ergot alkaloid concentration in the samples analysed.

Commodity	Country of origin	No of samples	Distribution according to total EA concentration			Concentration (µg/kg)	
			<10 µg/kg*	10-100 µg/kg	>100 µg/kg	Average	Maximum
Rye	The Netherlands	51	27	11	13	105	1231
	Germany	10	4	3	3	185	826
	Other/unknown	8	3	1	4	251	915
	Total rye	**69**	**34**	**15**	**20**	**134**	**1231**
	(% of total)		(49.3)	(21.7)	(29.0)		
Triticale	The Netherlands	31	20	6	5	35	297
	Germany	9	8	1	0	6	50
	Other/unknown	5	2	2	1	68	199
	Total triticale	**45**	**30**	**9**	**6**	**33**	**297**
	(% of total)		(66.7)	(20.0)	(13.3)		
Wheat	The Netherlands	11	6	2	3	79	529
	Germany	1	1	0	0	0	0
	Other/unknown	6	4	2	0	18	56
	Total wheat	**18**	**11**	**4**	**3**	**54**	**529**
	(% of total)		(61.1)	(22.2)	(16.7)		
Other cereals	The Netherlands	2	2	0	0	0	0
	Germany	1	1	0	0	0	0
	Other/unknown	1	0	0	1	961	961
	Total other cereals	**4**	**3**	**0**	**1**	**240**	**961**
	(% of total)		(75.0)	(0.0)	(25.0)		
Compound feed	The Netherlands	46	8	31	7	73	583
	Germany	2	0	1	1	165	317
	Other/unknown	0	--	--	--	--	--
	Total compound feed	**48**	**8**	**32**	**8**	**77**	**583**
	(% of total)		(16.7)	(66.7)	(16.7)		
Total		**184**	**86**	**60**	**38**	**89**	**1231**
	(% of total)		**(46.7)**	**(32.6)**	**(20.7)**		

* *Note: some samples (5) contained traces of one or two ergot alkaloids just above the LOD.*

Only 18 wheat samples were investigated in this study. Eleven samples (61%) did contain no or only traces of ergot alkaloids. The maximum amount found was 529 µg/kg and the average for all wheat samples was 54 µg/kg. In the 'other' cereal category one barley sample contained a relatively high (961 µg/kg) amount of ergot alkaloids. The majority of the compound feed samples

analysed contained ergot alkaloids; only in eight samples (17%) the content did not exceed 10 µg/kg. In eight samples the concentration exceeded 100 µg/kg, with a maximum of 583 µg/kg. The average ergot alkaloid content in compound feed (48 samples) was 77 µg/kg. Interestingly, this is only slightly lower than the average concentration of 93 µg/kg detected in all cereal samples combined (136 samples). It should be noted that the compound feeds can be composed of many ingredients, and that the cereal content can be relatively small. Unfortunately, of most feeds investigated the cereal content was not known. Summarising, of the 184 investigated samples, 86 samples were (almost) blank, containing less than 10 µg/kg. The other 98 samples contained ergot alkaloids in a concentration ranging between 10 and 1231 µg/kg. The average for all samples was 89 µg/kg. The total ergot alkaloid concentration exceeded 100 µg/kg in 38 samples, while 11 samples exceeded 500 µg/kg. As the large majority of samples was from Dutch origin no conclusions can be drawn regarding the correlation between the geographical origin and the ergot alkaloid content.

In Figure 5 the average concentration is shown of the individual ergot alkaloids found in the cereal and feed samples. For each alkaloid the concentrations of the two epimeric forms have been combined for convenience. Interestingly, in the rye samples the overall concentration of two alkaloids -ergosine and ergotamine- is around 30 µg/kg, of two other alkaloids -ergocristine and ergocryptine- the concentration is around 20 µg/kg and for the two remaining alkaloids -ergocornine and ergometrine-, it is around 10 µg/kg. Other ergot alkaloids (mainly agroclavine and chanoclavine-1) are hardly found at all. For the other major feed commodities (triticale, wheat, compound feed) the average individual alkaloid concentrations are lower than in rye. However, the major ergot alkaloids are the same as in rye. Only in wheat the relative concentration of ergotamine and ergosine is somewhat low, but this could be due to the relatively small number of samples analysed. The number of samples originating from Germany is too small to make a meaningful comparison of the ergot alkaloid composition.

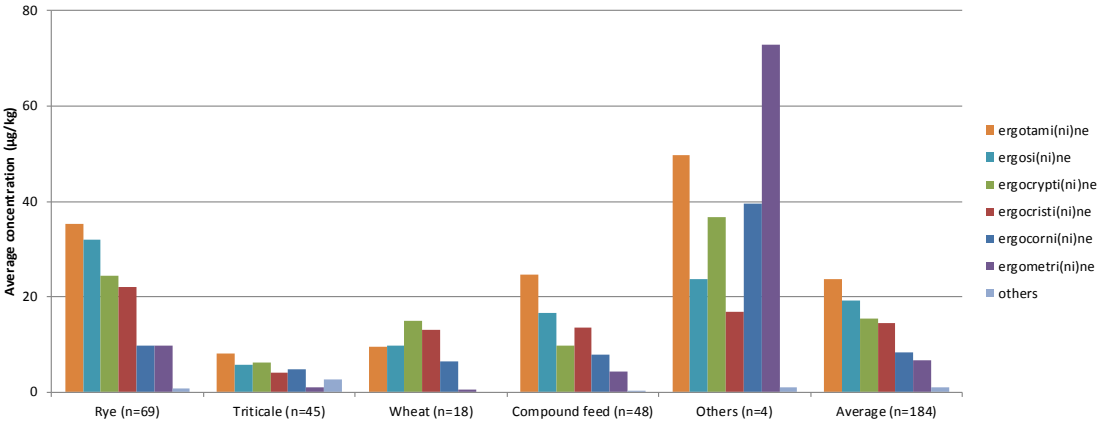

Figure 5. Average concentration of the major ergot alkaloids specified for the different feed commodities.

The relative distribution of the six major alkaloids has been determined on the basis of each individual positive sample as well (Figure 6). From Figure 6 it can be seen that when all samples are considered ergotamine, ergosine, ergocryptine and ergocristine each contribute on average approximately 20% to the total ergot alkaloid content. When the different commodities are considered, for rye a similar distribution is found, but for the compound feeds ergotamine and ergosine make a larger contribution at the expense of ergocryptine. In triticale the contribution of

ergocristine is relatively small and that of ergocornine relatively large. In all commodities and on an individual sample basis, the relative distribution of the ergot alkaloids is highly variable as can be seen from the large standard deviations.

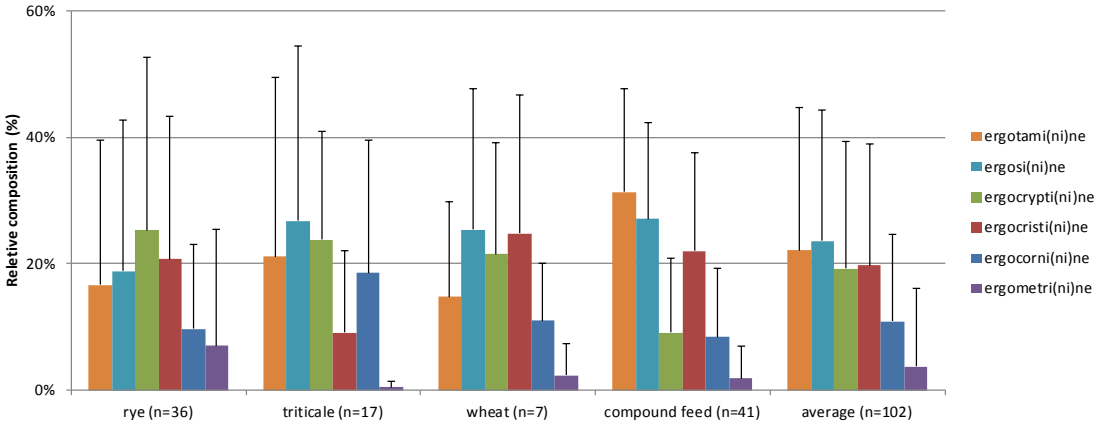

Figure 6. Relative distribution of the major ergot alkaloids in positive cereals and animal feeds. Standard deviation is indicated as an error bar.

The high variability in ergot alkaloid composition becomes also evident from Table 5. In Table 5 the correlation coefficients have been calculated between the six major ergot alkaloids in the cereals and animal feeds. A (strongly) positive correlation between two alkaloids indicates that both alkaloids often co-occur in the samples, or that both are absent. A (strongly) negative correlation indicates that in most cases only one of the two alkaloids is present in substantial amounts. When all samples are considered the strongest correlations found are negative, between ergocryptine and ergotamine (-0.510), between ergocornine and ergotamine (-0.474) and between ergocornine and ergocristine (-0.453). A relatively strong positive correlation (0.406) is observed between ergocornine and ergocryptine. An important result is that in the profiles of rye only one correlation is stronger than 0.4: for ergocryptine and ergotamine (-0.479). In animal feed the correlation between ergocornine and ergocryptine is particularly strong (0.716). A larger number of significantly positive or negative correlations is found in triticale and in wheat. However, the number of investigated triticale and wheat samples is relatively small and the correlations apparently become weaker if more samples are included. Many correlations in the different commodities are weakly positive or negative indicating that the distribution is more or less random within the group. It should be noted that the correlations found in animal feed generally follow the trends found in the combined cereal samples.

Table 5. Coefficients of correlation between the major ergot alkaloids, based on their relative distribution in positive cereal and animal feed samples. Correlations are shown on the basis of the all samples category and in the order of diminishing significance. Correlations stronger than 0.4 or -0.4 are indicated in bold.

Correlation	Rye (30)	Triticale (14)	Wheat (7)	Cereals (51)	Animal Feed (37)	All (88)
Ergocryptine-ergotamine	**-0.479**	**-0.596**	0.194	**-0.451**	**-0.555**	**-0.510**
Ergocornine-ergotamine	-0.370	**-0.602**	**-0.427**	**-0.447**	**-0.502**	**-0.474**
Ergocornine-ergocristine	-0.257	**-0.489**	**-0.787**	-0.393	**-0.510**	**-0.453**
Ergocornine-ergocryptine	0.291	**0.550**	0.242	0.321	**0.716**	**0.406**
Ergocristine-ergocryptine	-0.346	-0.187	**-0.583**	-0.298	**-0.443**	-0.354
Ergosine-ergotamine	-0.269	**-0.517**	**-0.886**	-0.379	0.023	-0.319
Ergocryptine-ergosine	-0.391	0.259	**-0.410**	-0.271	0.037	-0.243
Ergocyptine-ergometrine	-0.222	-0.382	-0.181	-0.225	-0.281	-0.195
Ergocornine-ergometrine	-0.205	-0.302	**0.637**	-0.169	-0.127	-0.148
Ergometrine-ergosine	-0.144	-0.304	**0.542**	-0.139	-0.092	-0.145
Ergocristine-ergometrine	-0.201	0.306	**-0.506**	-0.113	0.018	-0.100
Ergocristine-ergosine	0.005	-0.209	-0.332	-0.071	-0.134	-0.098
Ergocristine-ergotamine	-0.042	-0.185	0.360	-0.095	-0.003	-0.068
Ergometrine-ergotamine	-0.087	0.020	**-0.564**	-0.071	0.158	-0.066
Ergocornine-ergosine	-0.150	**0.542**	0.322	0.067	-0.066	0.019

The average distribution between the two epimeric forms of the ergot alkaloids has been calculated on basis of the data presented in Annex VIII and is shown in Figure 7. As can be seen from Figure 7 the average contribution of the major, lysergic acid, forms is quite constant for the various commodities. Epimerisation to the iso-lysergic acid form is lowest for ergometrine (around 5%), followed by ergotamine (15%). For the other ergot alkaloids it varies between 15 and 25%.

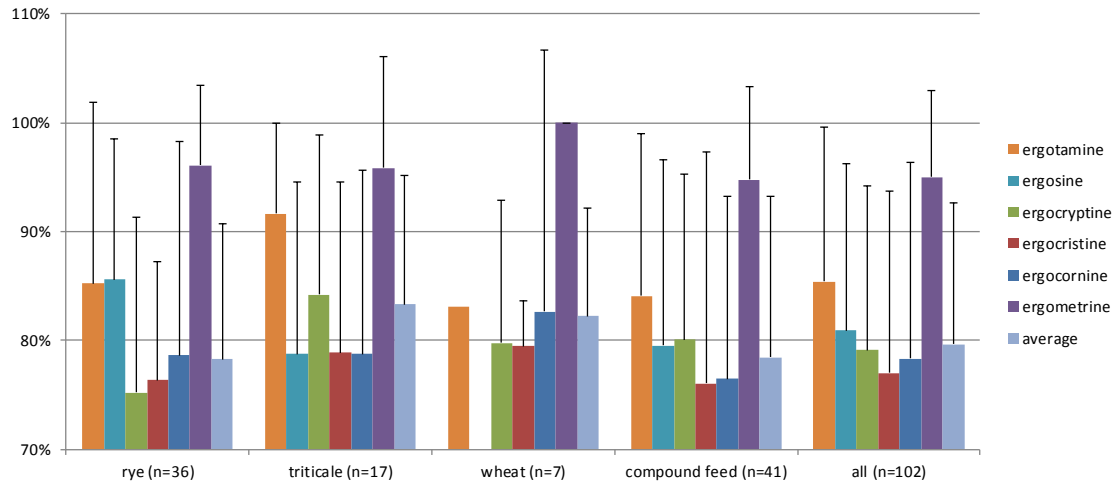

Figure 7. Significance of epimerisation in cereals and compound feed. The contribution of each of the major ergot alkaloids is shown. The remainder is the corresponding epimer. Standard deviation is indicated as an error bar.

3.3.2 Analysis of sclerotia

Of the samples that were visually inspected, 67 contained sclerotia (see section 3.2). Of these samples, 48 sclerotia samples were analysed for ergot alkaloid content (Table 6; see Annex IX for the individual results). The samples contained between 1 and 17 sclerotia (for some samples the number of sclerotia was not determined), that were homogenised and analysed together. The ergot alkaloid concentration in the sclerotia was highly variable. Fourteen out of 48 samples (29%) did not contain measurable amounts of ergot alkaloids. The majority of samples contained alkaloid concentrations between 100 and 1000 µg/g, while nine samples contained high amounts, up to 6000 µg/g in a sclerotia sample from triticale. The average content in sclerotia from rye was 521 µg/g and in sclerotia from triticale it was 959 µg/g. For all samples the average amount was 659 µg/g. From Figure 8 it can be seen that sclerotia in triticale contain relatively high amounts of ergosine, ergocryptine and ergocornine. The number of samples analysed was relatively small, however (only 10 positive samples contribute). With respect to the sclerotia in rye the concentration differences between the alkaloids were smaller.

Table 6. Ergot alkaloid content of sclerotia samples.

Commodity	No of samples	Distribution according to total EA concentration					Concentration (µg/g)	
		Blank	1-10 µg/g	10-100 µg/g	100-1000 µg/g	1000-10000 µg/g	Average	Maximum
Rye	32	10	1	1	15	5	521	3258
Triticale	14	4	0	1	5	4	959	6003
Wheat	1	0	0	0	1	0	144	144
Barley	1	0	0	0	0	1	1412	1412
Total	**48**	**14**	**1**	**2**	**21**	**10**	**659**	**6003**
	(%)	**(29.2)**	**(2.1)**	**(4.2)**	**(43.8)**	**(20.8)**		

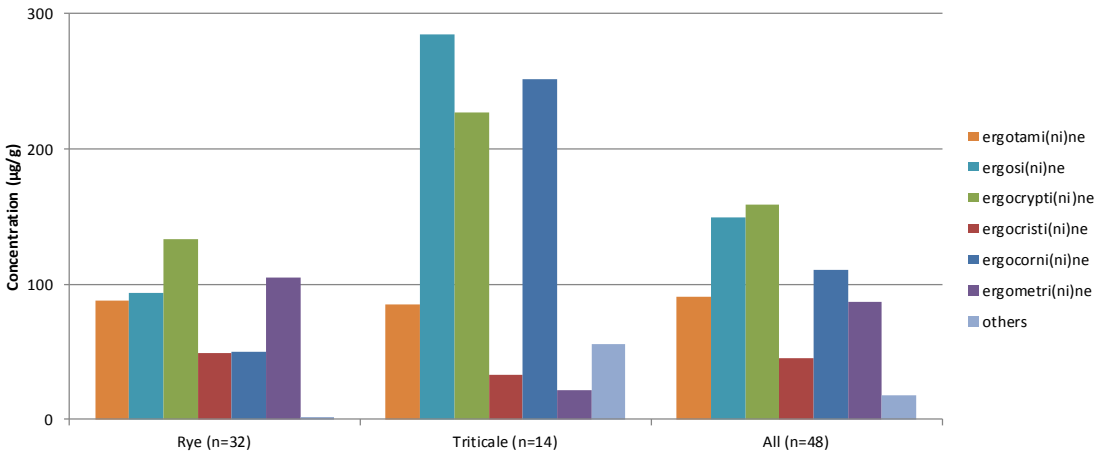

Figure 8. Average ergot alkaloid concentration in sclerotia specified for the major cereal commodities.

3.3.3 Analysis of the residual ergot alkaloid content in visually screened cereal samples

It was investigated how efficient visual screening of samples in combination with removal by hand picking of sclerotia is in removal or reduction of the ergot alkaloid content. In total 120 samples have been analysed by visual means. Of 19 samples that contained sclerotia, the sclerotia fraction was combined with the rest (cleaned) fraction and ground before chemical analysis was performed. These samples were excluded. The qualitative results for the remaining 101 samples are shown in Table 7. In total 53 samples did not contain sclerotia according to visual inspection. For these samples the 'rest' fraction is identical to the complete sample. In 42 out of the 53 samples that did not contain sclerotia, no ergot alkaloids could be detected in the 'rest' fraction. These samples were truly blank. However, in 11 samples (traces of) alkaloids were detected. This was relatively often the case for triticale (25% of the samples) and for wheat (36% of the samples). Only in one rye sample (7%) screened negative for sclerotia, ergot alkaloids could be detected.

Table 7. Qualitative assessment of the presence of ergot alkaloids in the cleaned fraction of cereal samples. Samples were screened for sclerotia content, which subsequently was physically removed by hand. (-): no ergot alkaloids present; (+): ergot alkaloids present as indicated in the headings.

Commodity	Cleaned fraction No of samples	Original sample: sclerotia absent			Original sample: sclerotia present		
		Total	Alkaloids (-)	Alkaloids (+)	Total	Alkaloids (-)[a]	Alkaloids (+)
Rye	47	15	14	1	32	21	11
Triticale	38	24	18	6	14	13	1
Wheat	12	11	7	4	1	0	1
Others	4	3	3	0	1	0	1
Total	**101**	**53**	**42**	**11**	**48**	**34**	**14**
(%) of all samples		**(52.5)**	**(41.6)**	**(10.9)**	**(47.5)**	**(33.6)**	**(13.9)**

[a] *In these samples sclerotia were present, that however did not always contain ergot alkaloids. This was the case for 10 rye and 4 triticale samples (13.9% of the total number of samples).*

The other 48 samples did contain sclerotia and of these samples the rest fraction was chemically analysed after removal of the sclerotia (section 3.3.2). Of these 48 samples measurable amounts of ergot alkaloids were detected in 14 cleaned fractions, indicating that visual screening and handpicking of the sclerotia, did not fully remove the ergot alkaloids. The majority of these samples were rye. In the remaining 34 samples no residual ergot alkaloid content was detected. In 14 of these cleaned samples the sclerotia themselves did not contain measurable amounts of alkaloids (section 3.3.2). For the other 20 samples the sclerotia however did contain alkaloids, indicating that the handpicking of sclerotia effectively removed the ergot alkaloid content. No samples were encountered in which the sclerotia were free of ergot alkaloids but the cleaned fractions did contain ergot alkaloids.

In Figure 9 the quantitative results are shown. The relatively high average amount of ergotamine found in the rye samples is mainly due to one sample (acc.nr. 244312) that contained 991 µg/kg in the cleaned fraction. For this particular sample the sclerotia fraction contributed only 39 µg/kg to the ergotamine content of the sample. When this sample is removed from the set, the average

ergotamine content of the rye rest fractions is only 1.4 µg/kg, which is in line with the other ergot alkaloids found. From Figure 9 it can be seen that for wheat the residual ergot alkaloid content after sclerotia picking is still relatively high. The relatively high amounts found in the small 'Others" category is due to a barley sample that contained a high amount of ergot alkaloids, both in the sclerotia fraction as well as in the rest fraction. Finally, comparison of the ergot alkaloid content of the 'cleaned' fractions to the ergot alkaloid content of the 'complete' samples, reveals that on average 29.3% of the ergot alkaloid content remains in the 'cleaned' cereal fraction, while the sclerotia contain 70.7% of the ergot alkaloid content. Residual alkaloid content on average is highest in cleaned triticale (52.3%) and wheat (45.8%), while it is lowest in rye (23.7%).

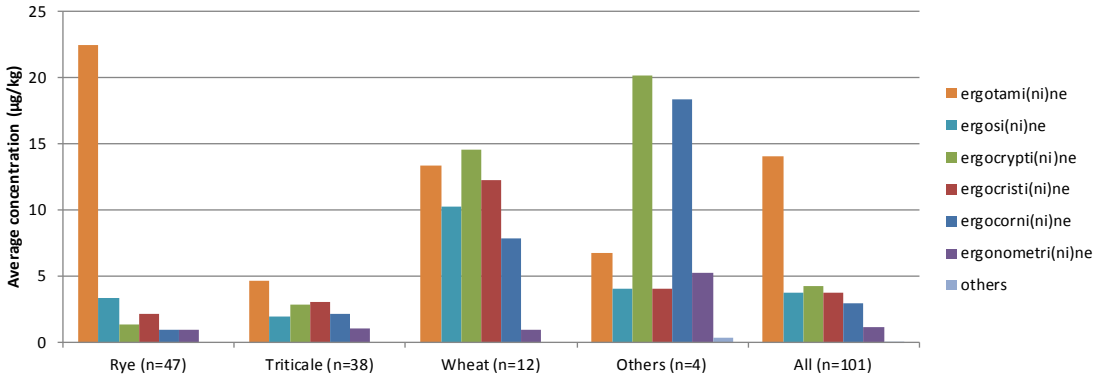

Figure 9. Average concentration of the major ergot alkaloids in the cleaned fraction of the visually screened samples (sclerotia removed).

3.4 Correlation between visual inspection results and ergot alkaloid levels

Results of both the visual inspection as well as the chemical research are available for a total of 120 samples (Table 8).

Table 8. The number of positive and negative samples for the visual inspection method and for the chemical (LC-MS/MS) method. The share of the total number of cereal samples is also indicated.

Combined results (n=120)[a]		n	%
Visually positive / chemically positive	A	43	35.8%
Visually positive / chemically negative	B	24	20.0%
Visually negative / chemically positive	C	11	9.2%
Visually negative / chemically negative	D	42	35.0%

In 85 samples (70.8%, the total of samples indicated A and D in Table 8) the result of the visual inspection, either the presence or absence of sclerotia, matches with the result of the chemical analysis. In 24 samples (indicated B) a positive visual result was followed by a negative chemical result, the absence of ergot alkaloids ("false positives"; see Annex III). In these 24 occasions the average amount of sclerotia was 367 mg/kg (minimum 3 mg/kg, three samples above 1000 mg/kg, maximum 2760 mg/kg). In 11 cases (indicated C) no sclerotia were found although ergot alkaloids appeared to be present ("false negatives"). The average amount of ergot alkaloids found was 75 µg/kg and the median amount was 56 µg/kg. In two samples only trace amounts of ergot

alkaloids (less than 10 µg/kg) were found, seven samples contained relatively low levels (between 10 and 100 µg/kg), one sample contained 124 µg/kg and one contained 297 µg/kg. The latter sample was a triticale (acc.nr. 223562 in Annex IV). The "false negative" results were reported in the year 2007 (4), 2008 (2) and 2010 (5); in 2009 no "false negatives" were established.

In order to get a reliable view on the correlation between a visually positive result and a chemically positive result it is necessary to distinguish between the "false positive" and "false negative" results. Moreover, the use of a series of data pairs with zero's (samples D in Table 8) might seriously influence the resulting correlation coefficient. A breakdown of the results obtained for sample types B and C of Table 8 is presented in Table 9. For rye a significant number of "false positives" were obtained, while "false negative" results for rye were rare. For triticale and wheat the number of "false positives" and "false negatives" appears to be more in balance.

The correlation between the amount of sclerotia, if any, and the amount of ergot alkaloids, if present, is 0.316 when all results are taken into account (Table 9). The correlation is slightly lower (0.251) when the double negative samples (D) are removed from the set. Similar results are obtained when the rye samples and the triticale samples are considered separately. Most of the correlation coefficients between the sclerotia content and the ergot alkaloid level are between 0.25 and 0.35 (Table 9). In contrast, the correlation for the wheat samples is very high since the lowest scores and the highest score (acc.nr. 218770) are matching very well (see Annex V).

Table 9. "False positives" and "false negatives" and correlation coefficients for the total set of cereal samples, and the break down for the type of cereal. The samples indicated D in Table 8 are excluded for the values in the column indicated as "excluding zero values".

	False positives (B)	False negatives (C)	Including double negatives (D)	Excluding double negatives (D)
Correlation between sclerotia and alkaloid content, all samples	24	11	0.316 (120)	0.251 (78)
Correlation for rye	17	1	0.330 (60)	0.264 (46)
Correlation for wheat	2	4	0.929 (16)	0.937 (9)
Correlation for triticale	5	6	0.317 (40)	0.227 (22)

4 Discussion en conclusions

The current study focuses on different topics; the (visual) inspection of sclerotia, the (chemical) detection of ergot alkaloids, either in complete samples, in sclerotia, or in the remaining material of the cleaned samples and the correlation between visual and chemical analysis. These different topics will be discussed in the view of literature data.

4.1 Sclerotia content in cereals for animal consumption

In this study 120 samples of cereals have been analysed for sclerotia content. To put the results in a broader perspective, data from other investigations should be taken into account. However, accounts of sclerotia determinations in grains and feeds are quite scarce in the scientific literature. Ruhland et al (2010) recently presented the results obtained by the Bavarian Health and Food Safety Authority. From 2006 to 2010 366 grain samples (ground and unground, no further classification given) were analysed by visual screening. In 25.6% of samples sclerotia were detected, with an average content of 194 mg/kg and a maximum of 2130 mg/kg. Four samples (1.1%) exceeded the official limit. Compared to the German study, in our samples the rate of positives (67 out of 120, 56%) is approximately twice as high, the number of violations eight times higher (10 out of 120, 8.3%) and also the average content is higher (317 mg/kg, Table 3). When the 2007 samples (28) are removed from our data set, the average ergot content in the remaining 92 samples, collected in 2008-2010, is 197 mg/kg, which is comparable to the report of Ruhland et al (2010), and our set contains four samples (4.3%) exceeding the official limit.

Unfortunately the composition of the German grain samples is not known, so no further comparison of the data can be made.

4.2 Ergot alkaloid levels in cereals and compound feeds

There are only a few studies available in the literature that report on ergot alkaloid content of compound feeds and cereals applied as feed materials. In Table 10 an overview of the results is presented. Ruhland and Tischler (2008) have reported on ergot alkaloid content in 64 feed grains and 60 mixed (compound) feeds, collected in Bavaria (Germany) during 2005-2007 and determined by LC-fluorescence. Ruhland and Tischler (2008) did not report average concentrations, but only median concentrations of the positive samples (LOD: 5 µg/kg). Recently, Ruhland et al (2010) presented an update of their work in the form of a poster presentation on an annual meeting of the IAG in Belgium: in total 169 feed samples (consisting of cereal and complete feeds, unfortunately only the overall results are given) have been analysed over the period 2006-2010 (samples partly overlap with the earlier report).

Diana Di Mavungu et al (2011) recently reported on the presence of ergot alkaloids in 148 rye and 137 wheat feed and 27 triticale wheat samples, collected from feed mills during 2009 and 2010 in the framework of an EU survey on ergot alkaloids in cereals intended for human consumption and animal feeding. An LC-MS/MS method was applied with a very low LOQ (1 µg/kg). The samples originated from 10 European countries: Germany, Poland, Finland, The Netherlands, Belgium, Czech Republic, Denmark, Switzerland, Sweden and France. Diana Di Mavungu et al (2011) reported average and median concentrations for the cereals (all samples). From the individual

data reported median concentrations for the positive samples (≥ 5 µg/kg) was calculated for comparison with the Ruhland and Tischler (2008) data.

The results obtained in this study are presented in Table 10 for comparison.

Table 10. Ergot alkaloid content of animal feeds (µg/g). Summary of available data.

Studies published	Year of sampling	Method of analysis	LOD/LOQ (µg/kg)	Feed commodity	No of samples	Percentage of positives	Mean conc (µg/kg)	Median* conc (µg/kg)	Median** conc (µg/kg)	Max. conc (µg/kg)	Samples > 1000 µg/kg
Germany, 2008[a]	2005-2007	HPLC-FLD	5	Rye	15	100	not reported	not reported	96	1067	1
				Triticale	14	93	not reported	not reported	25	1103	1
				Wheat	21	86	not reported	not reported	29	1236	1
				Other grains	14	93	not reported	not reported	44	140	0
				Compound feed	60	90	not reported	not reported	70	4883	3
				All	124	92	not reported	not reported	70	4883	6
Germany, 2010[b]	2006-2010	HPLC-FLD	5	All	169	90	255	not reported	83	5044	9
Europe, 2011[c]	2009-2010	LC-MS/MS	1	Rye	148	52	319	1	85	12340	11
				Triticale	27	48	62	<1	104	1103	1
				Wheat	137	34	18	<1	16	702	0
				All	312	44	165	<1	61	12340	12
The Netherlands, 2012[d]	2007-2010	LC-MS/MS	2	Rye	69	54	134	10	121	1231	1
				Triticale	45	45	33	<2	63	297	0
				Wheat	18	39	54	<2	56	529	0
				Other grains	4	25	240	<2	961	961	0
				Compound feed	48	85	77	29	31	583	0
				All	184	57	89	14	56	1231	1

[a] *Ruhland and Tischler, 2008.* [b] *Ruhland et al, 2010.* [c] *Diana Di Mavungu et al, 2011. Samples collected in 10 different European countries.* [d] *This study. * All samples considered. ** Only positive samples considered with an ergot alkaloid content of 5 µg/kg or more. Note: the German studies partly overlap.*

A high incidence of positive samples was reported by Ruhland and Tischler (2008): in 92% of the cereals and 90% of the mixed feeds ergot alkaloids were detected above the LOD of 5 µg/kg). In the study of Diana Di Mavungu et al (2011) and in our study a considerable lower percentage of positive findings is reported for the cereals, notwithstanding the lower LOD/LOQ applied. In both studies an incidence of positive samples of approximately 50% for rye and triticale is reported and a somewhat lower incidence for wheat (34-39%). The different results could be related to the analytical techniques applied: Ruhland and Tischler (2008) used HPLC-FLD and in the other studies LC-MS/MS was applied. LC-MS/MS is a more specific detection technique allowing for a better discrimination between the analytes and matrix interferences, in particular at low residue levels. Interestingly, in our study a high incidence of positive compound feeds (85%) was observed, which is in line with the results presented by Ruhland and Tischler (2008).

When the median concentrations of the positive samples are considered, a good correlation is found between the studies presented. Ruhland and colleagues reported a median level of 70 µg/kg in their 2008 study, and a median of 83 µg/kg for their 2010 study. The EU survey reported 61 µg/kg as median value for the positive samples and in our study it is 56 µg/kg. In general somewhat higher median levels are reported for the rye samples and lower levels for the wheat samples. The median levels for triticale are fluctuating.

Regarding the maximum levels found in cereal and compound feeds, the EU survey has reported the highest concentration of ergot alkaloids for rye (12340 µg/kg) (Diana Di Mavungu et al, 2011). This was determined in a sample originating from Switzerland. In that study very high levels were reportedly found in most of the other Swizz samples as well. The maximum levels found in our study for rye from The Netherlands (1231 µg/kg) are in line with the maximum reported in the study of Ruhland and Tischler (2008) for the German rye samples. Diana Di

Mavungu et al (2011) reported a maximum of 1257 µg/kg for the German rye samples (n = 9) and a maximum of 417 µg/kg for the rye samples collected in The Netherlands (n = 32). It was noted in their study that most of the samples from The Netherlands were free of ergot alkaloids or contained relatively low amounts. As can be seen in Table 10, in none of the studies the amounts of ergot alkaloids exceeded 1250 µg/kg in the other cereal samples (wheat, triticale, others).

Regarding the compound feeds, a much higher maximum concentration (4883 µg/kg) has been reported in the study of Ruhland and Tischler (2008) than in our study (583 µg/kg). Of the 60 compound feeds analysed by Ruhland and Tischler (2008), three samples exceeded 1000 µg/kg. Compared to our results the feed samples analysed in Bavaria appear to be contaminated with significantly higher levels of ergot alkaloids. In a personal communication Dr. Ruhland indicated that the reported data on the Bavarian feed samples included several samples that were selected from conspicuous lots, which may explain the relatively large number of highly contaminated samples in their reports.

Nevertheless, it is interesting to note that -without drawing pertinent conclusions due to the limitations of the available data- in this study, the average ergot alkaloid content in compound feeds is approx. half of the average content in rye and higher than the average content in triticale and wheat. From the results of the study of Ruhland and Tischler (2008) the same observations can be made for the median concentrations of compound feeds versus cereals. Compound feeds can be composed of many ingredients, and the cereal content can be relatively small. Unfortunately, of most feeds investigated no information on the cereal content was available.

4.3 Ergot alkaloid levels in isolated sclerotia

Although the alkaloid content is known to be highly variable with respect to composition and concentration, it is generally assumed that for sclerotia from Central Europe the average total content is around 2000 µg/g for the 12 major compounds (Müller et al, 2009; BfR, 2004, Wolff et al, 1988). The six bioactive -ine isomers account for approximately 1300 µg/g and the corresponding epimeric -inines for 600 µg/g (the rest consists of minor or unknown alkaloids). The provisional relation for ergot content as proposed by Verstraete (2010) is based on this assumption. Only the six bioactive compounds are mentioned in this paper.

In the literature a number of studies can be found that provide detailed information on the concentrations of individual -ines and -inines and the total ergot alkaloid content of single or pooled samples of rye ergot sclerotia. A summary is presented in Table 11 and a more comprehensive overview can be found in Annex X. In the last decade most studies on ergot alkaloid content of sclerotia have been conducted in Germany. The data originate from monitoring studies of ergot alkaloid content of specific harvest years (Appelt and Ellner, 2009; Mainka et al, 2007a; this study), analysis of single sclerotia (Franzmann et al, 2010) and analytical data for batches of rye ergot used for toxicological studies (Schumann et al, 2009; Mainka et al, 2007b, Mainka et al, 2005). The number of samples analysed varied widely in these studies, which makes it somewhat difficult to compare and generalise the results. Results can be weighted on the basis of production year, on the basis of a specific entry or study or on an individual sample basis.

It is evident from the available experimental data that in general the ergot alkaloid content in sclerotia is significantly less than the proposed levels. This is clearly the case not only for the data reported in this study, but also for the available data on German rye ergot. The average obtained

for the Dutch samples (642 µg/g) is only slightly lower than the average obtained for the German samples (845 µg/g). When all the reported samples are considered, an average value of 800 µg/g is derived, in which the six major alkaloids contribute for 600 µg/g and the six -inine epimers for 200 µg/g. The highest yearly average reported (Annex X) is 1234 µg/g for 48 samples of rye sclerotia dating from 2004, while the lowest average is 239 µg/g for the harvest of 2003 (23 samples). It was noted, however, that the harvest of 2003 was exceptional, due to the extremely warm and dry summer season (Lauber et al, 2005). Climatic conditions vary from year to year and can have a significant impact on the ergot alkaloid content.

Table 11. Ergot alkaloid content of sclerotia (µg/g). Summary of available data.

	Year of harvest	Years/ Samples	Ergo-cornine	Ergo-corninine	Ergo-cristine	Ergo-cristinine	α/β-Ergo-cryptine	α/β-Ergo-cryptinine	Ergo-metrine	Ergo-metrinine	Ergo-sine	Ergo-sinine	Ergo-tamine	Ergo-taminine	Sum total	Sum ines	Sum inines	% ines	%inines
Proposed average																			
Verstraete, 2010			100		600		100		100		100		300		1300	1300			
Muller et al, 2009			80	40	620	260	100	60	100	40	80	40	340	160	1920	1320	600	69%	31%
Year weighted average																			
GER[a]	2002-2009	8	37	16	142	39	32	19	44	9	115	41	114	51	660	484	176	73%	27%
NL[b]	2008-2010	3	79	32	35	11	123	40	66	5	117	35	74	15	634	495	139	78%	22%
Total	2002-2010	9	46	20	119	33	52	23	55	9	122	42	105	43	669	500	169	75%	25%
Sample weighted average																			
GER[a]	2002-2009	174	42	18	156	46	36	20	56	13	195	67	137	59	845	622	223	74%	26%
NL[b]	2008-2010	48	80	31	34	11	122	37	82	6	115	34	76	15	642	508	134	79%	21%
Total	2002-2010	222	50	21	129	38	55	24	62	11	178	60	124	49	801	597	204	75%	25%
Relative composition (% of total alkaloids)																			
Proposed Verstraete, 2010			7.7%		46.2%		7.7%		7.7%		7.7%		23.1%		100.0%	100.0%			
Proposed Muller et al, 2009			4.2%	2.1%	32.3%	13.5%	5.2%	3.1%	5.2%	2.1%	4.2%	2.1%	17.7%	8.3%	100.0%	68.8%	31.3%		
GER[a]	2002-2009	174	5.0%	2.2%	18.4%	5.5%	4.3%	2.4%	6.6%	1.5%	23.1%	7.9%	16.2%	7.0%	100.0%	73.6%	26.4%		
NL[b]	2008-2010	48	12.4%	4.8%	5.4%	1.7%	18.9%	5.8%	12.7%	0.9%	18.0%	5.3%	11.8%	2.4%	100.0%	79.2%	20.8%		
Total	2002-2010	222	6.3%	2.6%	16.2%	4.8%	6.8%	3.0%	7.7%	1.4%	22.2%	7.5%	15.5%	6.2%	100.0%	74.6%	25.4%		

[a] *Compilation of available literature data.* [b] *This study.*

The relative composition of the sclerotia is also different from the proposed composition in the literature (Müller et al, 2009). In this study, ergocryptine and ergosine are the most abundant alkaloids (18-19%), followed by ergometrine, ergocornine and ergotamine (around 12%) (Table 11). The contribution of ergocristine and the -inine epimers is 5% or less. In the German data, ergosine, ergocristine and ergotamine (16-23%) are the major alkaloids, the others are accounting for less than 8%, on an individual basis. Based on the proposed composition of Müller et al (2009), ergocristine was expected to be the most abundant ergot alkaloid. This is not reflected in the data (Table 11): it is true that ergocristine is an important ergot alkaloid in the German ergot, but not the dominant one and it is a minor component in this study. Based on the combined Dutch and German dataset, the order of importance is roughly: ergosine, ergocristine, ergotamine > ergocryptine > ergocornine, ergometrine ≥ -inine epimers. The contribution of the six -ine alkaloids to the total is approximately 75%, that of the -inine epimers 25%; thus the relative ratio between the -ine forms and the -inine forms is approx. 3:1.

It is important to realise that the assumed values for ergot alkaloid content (Müller et al, 2009; BfR, 2004; and others) are primarily based on the work that was carried out 30 years ago by Young (1981a,b, 1982), who investigated the ergot alkaloid content of sclerotia in Canadian rye, triticale, wheat and barley. He used a colorimetric method, based on derivatisation with dimethylaminobenzaldehyde for determination of total alkaloid content of sclerotia extracts. For identification and semi-quantification of individual ergot alkaloids HPLC-UV, HPLC-FLD and direct-MS analysis were applied. The studies were conducted under the assumption that the total ergot alkaloid content is more or less equal to the overall alkaloid content in the sclerotia. No quantitative assessment using ergot alkaloid standard calibration curves was made. However, the

colorimetric method used is known to be sensitive to other alkaloids present in the sample as well (Lorenz, 1979), which may result in an overestimation. All German studies have used HPLC-FLD and in the current study LC-MS/MS has been used for determination of the ergot alkaloid content and quantification has been based on calibration curves of the individual standards. As described above significantly lower average total ergot alkaloid concentrations were obtained in the sclerotia samples from Germany and The Netherlands.

Only one recent study on rye ergot content could be found from outside Europe (Blaney, 2009), who analysed Australian ergot samples from various host plants using HPLC-UV (Annex X). Interestingly, he found a high average total content of 2700 µg/g. It remains to be seen if this investigation indicates that Australian ergot on average contains more ergot alkaloids than European ergot or that the results are (in part) biased due to the analytical technique used.

4.4 The effectiveness of manual removal of ergot sclerotia

The manual removal of the sclerotia from the bulk of the sample reduced the ergot alkaloid content on average with 70%. This implies that on average 30% of the ergot alkaloids still remained in the cleaned sample. Chemical analysis showed that the effectiveness can in practice vary between 100% (no alkaloids detected above the limit of detection in the cleaned sample) and 0% (for samples that contained ergot alkaloids, but no sclerotia detected). It is not known if the residual ergot alkaloid content is due to very small sclerotia parts or dust that escape detection, or that cereal grains have been (partly) infected/contaminated with *Claviceps* fungi. The latter explanation is perhaps more likely for those samples in which removal of sclerotia was not very effective in reduction of the ergot alkaloid content. An intriguing case is rye sample 244312. A total amount of 1231 µg/kg ergot alkaloids was found in the complete sample (Annex III). However, the sclerotia (284 mg/kg) only contributed 180 µg/kg, while the cleaned rye fraction contained 1051 µg/kg or 85.4%. The rye fraction was analysed in total three times and similar results were obtained on each occasion. Furthermore, there was no relation found between the ergot alkaloid composition of the sclerotia and that of the rye fraction. The sclerotia contained a mixture of ergocristine, ergosine and ergotamine and their epimers (Annex IX), while the rye fraction contained ergotamine and its epimer in large excess (>90%). Chemical contamination is highly unlikely because the samples were ground at a different location than where the samples were analysed.

No detailed studies are known in which the residual ergot alkaloid content in samples that were manually cleaned from sclerotia was determined. Only from the poster presentation of Ruhland et al (2010) some information can be deducted. Ruhland et al (2010) analysed 56 grain samples (26 ground and 30 unground) both for sclerotia and ergot alkaloid content. They reported 17 "false negative" visual screening results, seven of which concerned unground cereal samples. For these samples only low levels of ergot alkaloids (up to 19 µg/kg) were detected. The other 10 were ground cereal samples, containing levels up to a maximum of 381 µg/kg. The fact that these samples were ground to a small particle size (>0.5 mm) may well have hampered an adequate detection of ergot particulates.

4.5 Correlation between visual screening of sclerotia and chemical analysis of ergot alkaloids

The presented results indicate that in more than 70% of the total of 120 samples a good prediction was achieved of the presence of ergot alkaloids in cereals (Table 8). The correlation between the amount of sclerotia (in mg/kg) and the level of ergot alkaloids (in µg/kg) is generally between 0.2 and 0.35 (Table 9). This relatively low level indicates that in general the amount of sclerotia is a poor predictor of the expected amount of ergot alkaloids. On the other hand, none of the coefficients is negative which indicates that a fully random distribution (no correlation between the two types of results) does not apply.

In a rather high proportion of the samples (20%) ergot was detected, but no ergot alkaloids. Most of these "false positives" were rye samples. Not only samples containing low levels of sclerotia tested negative for ergot alkaloids, in fact three samples exceeded the EU limit for sclerotia content. Apparently these samples were taken from consignments infested with non-toxin producing strains of *Claviceps purpurea*, as has been observed in other studies as well (Battilani et al, 2009).

In 11 samples (9%) no sclerotia were found but ergot alkaloids were detected ("false negatives"). The average amount of ergot alkaloids in these samples was 75 µg/kg (median: 56 µg/kg) with a maximum of 297 µg/kg. As discussed in section 4.3 the average total ergot alkaloid content of sclerotia in North-west and Central Europe is approximately 800 µg/g. Consequently, the average EA concentration that can be expected from a sample containing 1000 mg/kg sclerotia is approximately 800 µg/kg. The "false negative" sample containing 297 µg/kg is still well below this level. A "false negative" rate of only 9% is the most important parameter indicating the reliability of the visual screening. Most of the "false negatives" contain only low amounts of ergot alkaloids. The risk of erroneously accepting a cereal lot based on the visual screening therefore appears to be reasonable low.

As described above, Ruhland et al (2010) analysed 56 feed grain samples (26 ground and 30 unground) both for sclerotia and ergot alkaloid content. They reported two "false positive" (3.6%) and 17 "false negative" (30.4%) results. The two "false positive" samples contained 293 and 429 mg/kg sclerotia. Of the 17 "false negative" samples, seven were unground cereal samples with only low levels of ergot alkaloids (up to 19 µg/kg).

5 Conclusions and recommendations

5.1 Conclusions

Of a total of 120 samples of cereals that were visually inspected; 67 samples (56%) contained sclerotia, with an average content (including blanks) of 317 mg/kg and a maximum of 6554 mg/kg. Ten samples exceeded the official limit of 1000 mg/kg: eight rye samples, one triticale and one wheat sample.

Of the 184 samples of cereals and compound feeds that were chemically analysed; 98 (53%) contained ergot alkaloids in a concentration exceeding 10 µg/kg, with an average content (including blanks) of 89 µg/kg and a maximum of 1231 µg/kg. Compound feeds were most often contaminated (83%) and the average content was 77 µg/kg with a maximum of 583 µg/kg. The average contamination for the cereals was 93 µg/kg and 43% of the samples was contaminated with ergot alkaloids. Rye contained the highest amounts of ergot alkaloids, with respect to average concentration (134 µg/kg) and maximum (1231 µg/kg). Triticale and wheat, respectively, showed lower averages (33 and 54 µg/kg) and maximum amounts (297 and 529 µg/kg). Major ergot alkaloids detected were ergosine, ergotamine, ergocristine and ergocryptine.

Only a proportion of the compound feeds is composed of cereal grains like rye, wheat, triticale and barley. It is therefore surprising that the average contamination with ergot alkaloids is almost the same for unground cereals as for compound feeds. Unfortunately, information on the composition of the compound feeds analysed in this study is rather limited, and no further conclusions can be drawn.

In total 48 samples of sclerotia were chemically analysed. The composition of the sclerotia was highly variable with respect to ergot alkaloid concentrations as well as composition. In total 34 samples contained ergot alkaloids in a concentration exceeding 1 µg/g, with an average concentration for all samples of 659 µg/g and a maximum of 6003 µg/g. The six major ergot alkaloids (ergocornine, ergocristine, ergocryptine, ergometrine, ergosine and ergotamine) accounted for 508 µg/g, the corresponding epimeric -inines for 134 µg/g. Major ergot alkaloids detected were ergocryptine, ergocornine, ergosine and ergotamine. None of the individual ergot alkaloids contributed more than 20% to the total. Of the minor ergot alkaloids only agroclavine and chanoclavine-1 were found and they contributed 17 µg/kg to the total.

The proposed level (2000 µg/g) for average ergot alkaloid content of sclerotia (Müller et al, 2009; Verstraete, 2010) may be too high. The average levels that have been found in this study as well as in other (German) studies indicate that the actual average level of ergot alkaloids in sclerotia originating from North-west and Central Europe is in the order of 800 µg/g, which is only 40% of the proposed level. The available data indicate that in sclerotia the average ratio between the sum of -ines and the sum of- inines is approximately 3:1.

Manual removal of sclerotia resulted in a reduction of the ergot alkaloid content by on average 70%. Remaining alkaloid content on average was highest in cleaned triticale (52.3%) and wheat (45.8%), while it was lowest in rye (23.7%). The remaining alkaloid content could be due to very small sclerotia particles ('ergot dust') or perhaps to non-recognised infestation of cereal grains.

Visual inspection of unground grains is an effective way for identification of samples that pose a potential health risk for human and animal upon consumption. In 9.2% of the samples that contained no sclerotia upon visual inspection (traces of) ergot alkaloids were detected ("false negatives"), with a maximum of 297 µg/kg. The content of the latter sample is still well below the average concentration (800 µg/kg) that can be expected for a sample containing 1000 mg/kg sclerotia. Moreover, only one sample that was compliant according to the current maximum limit for sclerotia, contained a total ergot alkaloid amount exceeding 1000 µg/kg, two other samples contained between 750 and 1000 µg/kg. The application of visual screening followed by a chemical analysis of selected samples is a cost effective strategy for monitoring ergot alkaloids in unground products without causing additional risk.

A weak correlation was found between the sclerotia content and the ergot alkaloid content of samples. This is in accordance with information available from the literature. In a relatively high number of cases (20%) the ergot alkaloid content of cereals contaminated with (sometimes substantial) amounts of sclerotia, was very low or non-detectable. The amount of sclerotia in cereals therefore is not a very reliable indicator of the actual ergot alkaloid content of the specific cereal samples.

5.2 Recommendations

It is recommended to continue the official control of cereals both with the visual and chemical methods, primarily because in a number of cereal samples the maximum limit for ergot sclerotia is exceeded but also because significant percentages of the samples (43% of all inspected cereal samples) are contaminated with ergot alkaloids.

The compound feeds require special attention because the majority (83%) is contaminated with ergot alkaloids. Moreover, the average concentration of ergot alkaloids in compound feeds is in the same order of magnitude as the average concentration in cereals, although cereals compose only part of the ingredients used in compound feeds.

It is advised to investigate the ergot alkaloid content of sclerotia, using a combination of colorimetric, HPLC-FLD and LC-MS/MS methods. This should help to correlate the older data on ergot alkaloid content of sclerotia, that was determined with colorimetric or HPLC-UV analytical techniques, with the more recent data obtained by HPLC-FLD and LC-MS/MS.

The proposed average levels (2000 µg/kg) for ergot alkaloids in sclerotia should be reconsidered on the basis of the currently available data.

Research should be initiated to find out the reason(s) for the relatively high residual ergot alkaloid contents in cereals after manual removal of the sclerotia.

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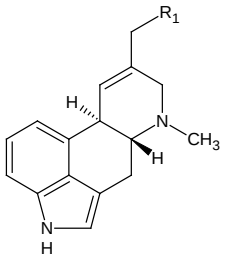
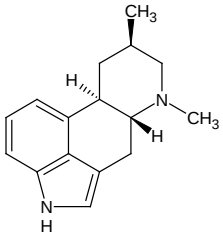
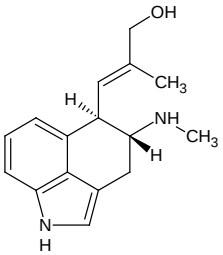
Annex I
Ergot alkaloids analysed by LC-MS/MS



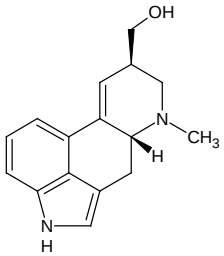
	R_1		MW
Agroclavine	H	$C_{16}H_{18}N_2$	238.1469 Da
Elymoclavine	OH	$C_{16}H_{18}N_2O$	254.1419 Da



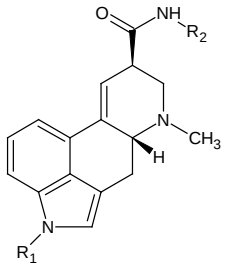
Festuclavine
$C_{16}H_{20}N_2$
MW 240.1626 Da



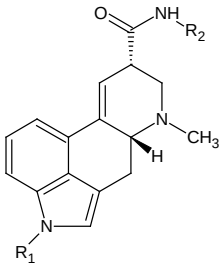
Chanoclavine I
$C_{16}H_{20}N_2O$
MW 256.1576 Da



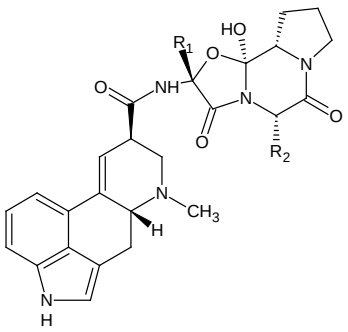
Lysergol
$C_{16}H_{18}N_2O$
MW 254.1419 Da



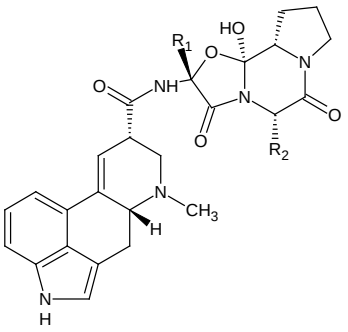
	R_1	R_2	MW	
Ergine	H	H	$C_{16}H_{17}N_3O$	267.1372 Da
Ergometrine	H	$CH(Me)CH_2OH$	$C_{19}H_{23}N_3O_2$	325.1790 Da
Methylergometrine	Me	$CH(Me)CH_2OH$	$C_{20}H_{25}N_3O_2$	339.1947 Da
Methysergide	Me	$CH(Et)CH_2OH$	$C_{21}H_{27}N_3O_2$	353.2103 Da



	R_1	R_2		MW
Erginine	H	H	$C_{16}H_{17}N_3O$	267.1372 Da
Ergometrinine	H	$CH(Me)CH_2OH$	$C_{19}H_{23}N_3O_2$	325.1790 Da



	R_1	R_2		MW
Ergovaline	Me	$CHMe_2$	$C_{29}H_{35}N_5O_5$	533.2638 Da
Ergosine	Me	$CHCH_2Me_2$	$C_{30}H_{37}N_5O_5$	547.2795 Da
Ergocornine	$CHMe_2$	$CHMe_2$	$C_{31}H_{39}N_5O_5$	561.2951 Da
α-Ergocryptine	$CHMe_2$	$CHCH_2Me_2$	$C_{32}H_{41}N_5O_5$	575.3108 Da
β-Ergocryptine	$CHMe_2$	CH(Me)Et	$C_{32}H_{41}N_5O_5$	575.3108 Da
Ergotamine	Me	CH_2Ph	$C_{33}H_{35}N_5O_5$	581.2638 Da
Ergocristine	$CHMe_2$	CH_2Ph	$C_{35}H_{39}N_5O_5$	609.2951 Da



	R_1	R_2		MW
Ergosinine	Me	$CHCH_2Me_2$	$C_{30}H_{37}N_5O_5$	547.2795 Da
Ergocorninine	$CHMe_2$	$CHMe_2$	$C_{31}H_{39}N_5O_5$	561.2951 Da
α-Ergocryptinine	$CHMe_2$	$CHCH_2Me_2$	$C_{32}H_{41}N_5O_5$	575.3108 Da
β-Ergocryptinine	$CHMe_2$	CH(Me)Et	$C_{32}H_{41}N_5O_5$	575.3108 Da
Ergotaminine	Me	CH_2Ph	$C_{33}H_{35}N_5O_5$	581.2638 Da
Ergocristinine	$CHMe_2$	CH_2Ph	$C_{35}H_{39}N_5O_5$	609.2951 Da

Annex II
MS/MS conditions used for the analysis of ergot alkaloids

Alkaloid	Precursor ion (m/z)	Product ion 1 (m/z)	Collision energy (eV)	Product ion 2 (m/z)	Collision energy (eV)
Agroclavine	239.2	183.1	20	208.1	20
Festuclavine	241.2	154.1	35	210.1	20
Elymoclavine	255.2	181.1	30	224.1	15
Lysergol	255.2	197.1	20	240.1	20
Chanoclavine-1	257.2	168.1	25	226.1	10
Ergine	268.2	208.1	25	223.1	20
Erginine	268.2	208.1	25	223.1	20
Ergometrine	326.2	208.1	30	223.1	25
Ergometrinine	326.2	208.1	30	223.1	25
Methylergometrine	340.2	208.1	30	223.1	25
Methysergide	354.2	222.1	30	237.1	25
Ergovaline	534.3	223.1	40	268.1	25
Ergosine	548.3	208.1	40	223.1	30
Ergosinine	548.3	208.1	40	223.1	30
Ergocornine	562.3	223.1	35	305.1	25
Ergocorninine	562.3	223.1	35	305.1	25
α-Ergocryptine	576.3	223.1	35	305.1	30
α-Ergocryptinine	576.3	223.1	35	305.1	30
β-Ergocryptine	576.3	223.1	35	305.1	30
β-Ergocryptinine	576.3	223.1	35	305.1	30
Ergotamine	582.3	208.1	45	223.1	35
Ergotaminine	582.3	208.1	45	223.1	35
Ergocristine	610.3	223.1	35	305.1	30
Ergocristinine	610.3	223.1	35	305.1	30

Annex III
Rye and rye products: Determination of sclerotia and ergot alkaloid content

Product	Code EU classification	RIKILT No.	Country of origin	Sampling date	Sclerotia (mg/kg)	Ergot alkaloids (µg/kg)
Rye	1.7.1	199231	NL	29-8-2007	2200	n.d.
	1.7.1	199232	NL	29-8-2007	2100	11
	1.7.1	202077	GER	24-9-2007	738	826
	1.7.1	202078	NL	3-10-2007	2760	n.d.
	1.7.1	202081	NL	9-10-2007	50	n.d.
Rye midlings	1.7.2	202218	NL	10-10-2007	n.a.	n.d.
Rye	1.7.1	205225	GER	14-11-2007	1060	n.d.
	1.7.1	205226	NL	12-11-2007	550	n.d.
	1.7.1	205869	NL	20-11-2007	n.a.	16
	1.7.1	206303	GER	29-11-2007	3429	63
	1.7.1	206943	Unknown	03-12-2007	n.a.	5
	1.7.1	206965	NL	3-12-2007	62	n.d.
	1.7.1	207055	NL	3-12-2007	408	677
	1.7.1	210966	Unknown	28-2-2008	n.a.	717
	1.7.1	213101	NL	7-4-2008	800	287
	1.7.1	213677	NL	17-4-2008	95	14
Rye feed	1.7.3	214807	NL	8-5-2008	n.a.	91
Rye midlings	1.7.2	215363	NL	20-5-2008	n.a.	115
Rye	1.7.1	217777	NL	7-7-2008	753	373
	1.7.1	218722	Unknown	30-7-2008	368	126
	1.7.1	218723	NL	30-7-2008	n.d.	n.d.
	1.7.1	218867	Unknown	4-8-2008	18	n.d.
	1.7.1	219064	NL	11-8-2008	n.d.	n.d.
	1.7.1	219435	NL	21-8-2008	39	9
	1.7.1	220522	NL	11-9-2008	1767	861
	1.7.1	220548	GER	12-9-2008	25	n.d.
	1.7.1	220724	Unknown	16-9-2008	n.d.	n.d.
	1.7.1	222606	Unknown	3-10-2008	1886	915
	1.7.1	223303	NL	23-10-2008	79	n.d.
	1.7.1	223304	NL	21-10-2008	297	141
	1.7.1	223556	GER	29-10-2008	n.a.	18
	1.7.1	224155	GER	29-10-2008	n.d.	n.d.
	1.7.1	233248	NL	10-6-2009	96	237
	1.7.1	233621	NL	16-6-2009	660	n.d.
	1.7.1	234215	NL	2-7-2009	n.d.	n.d.
	1.7.1	235081	NL	23-7-2009	429	187

Product	Code EU classification	RIKILT No.	Country of origin	Sampling date	Sclerotia (mg/kg)	Ergot alkaloids (µg/kg)
	1.7.1	236080	NL	18-8-2009	192	74
	1.7.1	239289	NL	20-10-2009	461	4
	1.7.1	239290	NL	20-10-2009	n.d.	n.d.
	1.7.1	239291	NL	20-10-2009	314	224
	1.7.1	239292	NL	20-10-2009	281	29
	1.7.1	239293	NL	20-10-2009	n.d.	n.d.
	1.7.1	239465	NL	23-10-2009	584	177
	1.7.1	239696	NL	26-10-2009	32	47
	1.7.1	239805	GER	28-10-2009	n.d.	n.d.
	1.7.1	239806	NL	28-10-2009	n.d.	n.d.
	1.7.1	241079	NL	25-11-2009	n.a.	37
	1.7.1	241932	NL	9-12-2009	267	n.d.
	1.7.1	243263	GER	18-1-2010	2459	819
	1.7.1	244312	NL	15-2-2010	284	1231
	1.7.1	244478	NL	19-2-2010	n.d.	n.d.
	1.7.1	245271	NL	18-2-2010	n.d.	56
	1.7.1	245500	GER	1-3-2010	197	20
	1.7.1	245626	NL	11-3-2010	n.d.	n.d.
	1.7.1	245852	NL	17-3-2010	298	262
	1.7.1	245885	POL	19-3-2010	66	212
	1.7.1	247859	GER	3-5-2010	94	106
	1.7.1	250445	NL	18-5-2010	225	n.d.
	1.7.1	253210	NL	17-8-2010	58	n.d.
	1.7.1	254143	NL	6-9-2010	n.d.	n.d.
	1.7.1	254144	NL	6-9-2010	62	n.d.
	1.7.1	254720	NL	15-9-2010	n.d.	n.d.
	1.7.1	254962	NL	21-9-2010	57	197
	1.7.1	254963	NL	21-9-2010	12	14
Rye midlings	1.7.2	255834	EU	20-9-2010	n.a.	31
Rye	1.7.1	255983	NL	27-9-2010	24	n.d.
	1.7.1	256419	NL	12-10-2010	27	n.d.
	1.7.1	257275	NL	14-10-2010	254	n.d.
	1.7.1	257413	NL	25-10-2010	n.d.	n.d.

n.a.: not analysed, n.d.: not detected; NL: Netherlands, GER: Germany, POL: Poland.

Annex IV
Triticale: Determination of sclerotia and ergot alkaloid content

Product	Code EU classification	RIKILT No.	Country of origin	Sampling date	Sclerotia (mg/kg)	Ergot alkaloids (µg/kg)
Triticale	1.10.1	202079	NL	3-10-2007	6554	140
	1.10.1	202541	GER	16-10-2007	n.a.	n.d.
	1.10.1	205228	NL	15-11-2007	n.d.	n.d.
	1.10.1	209658	NL	5-2-2008	n.d.	n.d.
	1.10.1	210822	NL	27-2-2008	n.a.	n.d.
	1.10.1	215362	NL	20-5-2008	n.a.	59
	1.10.1	218771	NL	29-7-2008	n.d.	5
	1.10.1	218772	NL	30-7-2008	n.d.	n.d.
	1.10.1	218866	Unknown	4-8-2008	n.d.	n.d.
	1.10.1	219065	NL	11-8-2008	n.d.	n.d.
	1.10.1	219423	NL	19-8-2008	8	4
	1.10.1	219424	NL	19-8-2008	185	120
	1.10.1	220521	GER	4-9-2008	n.d.	n.d.
	1.10.1	222505	NL	7-10-2008	n.a.	n.d.
	1.10.1	223557	GER	29-10-2008	n.d.	n.d.
	1.10.1	223562	NL	30-10-2008	n.d.	297
	1.10.1	223797	NL	3-11-2008	13	13
	1.10.1	223928	NL	3-11-2008	n.d.	n.d.
	1.10.1	224157	GER	29-10-2008	n.d.	n.d.
	1.10.1	224727	NL(GER?)	19-11-2008	n.d.	n.d.
	1.10.1	224966	NL	25-11-2008	68	17
	1.10.1	225945	Unknown	9-12-2008	163	72
	1.10.1	226818	NL	21-1-2009	n.d.	n.d.
	1.10.1	234821	NL	9-7-2009	3	n.d.
	1.10.1	234822	Unknown	14-7-2009	n.a.	n.d.
	1.10.1	234873	NL	14-7-2009	9	n.d.
	1.10.1	236588	NL	28-8-2009	n.d.	n.d.
	1.10.1	238307	NL	24-9-2009	n.d.	n.d.
	1.10.1	239370	NL	21-10-2009	23	138
	1.10.1	239464	NL	23-10-2009	55	102
	1.10.1	241080	NL	25-11-2009	n.d.	n.d.
	1.10.1	241933	NL	9-12-2009	n.d.	n.d.
	1.10.1	243398	NL	21-1-2010	62	n.d.
	1.10.1	243966	GER	9-2-2010	n.d.	n.d.
	1.10.1	243976	NL	10-2-2010	14	32
	1.10.1	244313	NL	15-2-2010	n.d.	56

Product	Code EU classification	RIKILT No.	Country of origin	Sampling date	Sclerotia (mg/kg)	Ergot alkaloids (µg/kg)
	1.10.1	245499	SLO	1-3-2010	n.d.	67
	1.10.1	246414	GER	29-3-2010	n.d.	n.d.
	1.10.1	247860	LUX	4-5-2010	155	199
	1.10.1	251422	GER	14-7-2010	165	50
	1.10.1	254719	GER	14-9-2010	105	n.d.
	1.10.1	254721	NL	15-9-2010	n.d.	n.d.
	1.10.1	255835	GER	29-9-2010	60	n.d.
	1.10.1	255982	NL	27-9-2010	n.d.	6
	1.10.1	258797	NL	22-11-2010	n.d.	n.d.

n.a.: not analysed, n.d.: not detected; NL: Netherlands, GER: Germany, SLO: Slovakia; LUX: Luxembourg.

Annex V
Wheat: Determination of sclerotia and ergot alkaloid content

Product	Code EU classification	RIKILT No.	Country of origin	Sampling date	Sclerotia (mg/kg)	Ergot alkaloids (µg/kg)
Wheat	1.11.1	202080	Unknown	8-10-2007	160	n.d.
	1.11.1	202219	Unknown	11-10-2007	n.a.	n.d.
	1.11.1	202542	Unknown	17-10-2007	n.a.	n.d.
	1.11.1	203701	NL	25-10-2007	n.d.	n.d.
	1.11.1	203728	Unknown	25-10-2007	n.d.	n.d.
	1.11.1	204393	NL	7-11-2007	n.d.	n.d.
	1.11.1	205227	Unknown	15-11-2007	n.d.	56
	1.11.1	205991	NL	23-11-2007	n.d.	n.d.
	1.11.1	205993	NL	23-11-2007	n.d.	n.d.
	1.11.1	205994	NL	23-11-2007	n.d.	23
	1.11.1	206030	NL	26-11-2007	n.d.	124
	1.11.1	206319	Unknown	28-11-2007	n.d.	49
	1.11.1	206492	NL	29-11-2007	29	n.d.
	1.11.1	206964	NL	3-12-2007	n.d.	n.d.
	1.11.1	207056	NL	6-12-2007	10	159
	1.11.1	207152	FR	4-12-2007	34	28
	1.11.1	218770	NL	30-7-2008	2940	529
	1.11.1	224156	GER	29-10-2008	n.d.	n.d.

n.a.: not analysed, n.d.: not detected; NL: Netherlands, GER: Germany, FR: France.

Annex VI
Various grains: Determination of sclerotia and ergot alkaloid content

Product	Code EU classification	RIKILT No.	Country of origin	Sampling date	Sclerotia (mg/kg)	Ergot alkaloids (µg/kg)
Mixed grain	1.0.0	205992	NL	22-11-2007	n.d.	n.d.
Mixed grains and maize	1.0.0	207057	NL	6-12-2007	n.d.	n.d.
Barley	1.1.1	224154	GER	29-10-2008	n.d.	n.d.
Barley	1.1.1	258015	FR	5-11-2010	514	961

n.d.: not detected; NL: Netherlands, GER: Germany, FR: France.

Annex VII
Compound feeds: Determination of ergot alkaloid content

Product	RIKILT No.	Country of origin	Sampling date	Ergot alkaloids (µg/kg)	Cereal content (%)
CF for ruminants	216067	NL	5-6-2008	n.d.	triticale (10%)
CF for porcines	218158	NL	14-7-2008	n.d.	wheat (9%), rye flour (2.5%), tapioca (35%)
CF for ruminants	218446	NL	24-7-2008	62	semolina, rye, maize, soya
CF for porcines	219196	NL	11-8-2008	40	triticale
CF for ruminants	219302	NL	18-8-2008	16	triticale (5%)
CF for porcines	219303	NL	14-8-2008	59	triticale (7%)
CF for porcines	220523	NL	11-9-2008	22	wheat (25%), barley (15%), maize (14%), triticale (8%), wheat gluten (6%), soya (3.9%), biscuits (3.5%)
CF for ruminants	220524	NL	11-9-2008	96	triticale (8%), maize gluten (4.4%)
CF for ruminants	224834	NL	19-11-2008	n.d.	
CF for ruminants	252200	NL	26-11-2008	5	
CF for porcines	226819	NL	21-1-2009	n.d.	
CF for porcines	228001	NL	18-2-2009	20	wheat, rye, maize, barley, semolina, soya
CF for porcines	234635	NL	8-7-2009	10	rye
CF for ruminants	235855	NL	11-8-2009	30	
CF for ruminants	235856	NL	11-8-2009	58	triticale (4.2%)
CF for ruminants	235857	NL	11-8-2009	31	triticale (8%), semolina (3.7%), wheat (3%)
CF for ruminants	238160	NL	22-9-2009	30	rye
CF for porcines	238161	NL	22-9-2009	33	barley, rye, wheat, triticale, semolina
CF for ruminants	238672	NL	9-10-2009	n.d.	
CF for porcines	238673	NL	9-10-2009	156	
CF for poultry	238674	NL	9-10-2009	n.d.	
CF for porcines	239463	NL	23-10-2009	15	
CF for porcines	240652	NL	16-11-2009	69	
CF for rabbits	240653	NL	16-11-2009	36	
CF for ruminants	240654	NL	16-11-2009	n.d.	
CF for porcines	241078	NL	25-11-2009	17	
CF for ruminants	241764	NL	7-12-2009	20	
CF for porcines	241934	NL	9-12-2009	26	
CF for porcines	241935	NL	9-12-2009	25	
CF for ruminants	242721	GER	31-12-2009	317	
CF for porcines	243724	NL	1-2-2010	28	barley, triticale, wheat, soya,
CF for ruminants	244350	NL	10-2-2010	28	maize, soya, triticale, wheat

Product	RIKILT No.	Country of origin	Sampling date	Ergot alkaloids (µg/kg)	Cereal content (%)
CF for porcines	247395	NL	7-4-2010	10	barley, wheat, soya, maize, triticale
CF for porcines	248856	NL	26-5-2010	210	triticale, barley, wheat, maize, soya
CF for porcines	250642	NL	21-6-2010	31	barley (18%), wheat (17%), semolina (10%), rye (10%), maize (9%), wheat gluten (5%), maize flour (2.8%), biscuits (2.5%)
CF for ruminants	250643	NL	21-6-2010	32	maize gluten (20%), wheat gluten (10%)
CF for equines	253209	NL	12-8-2010	12	triticale, barley, wheat
CF for porcines	254964	NL	20-9-2010	128	wheat, barley, soya, rye, maize
CF for porcines	255392	NL	17-9-2010	47	barley, triticale, wheat flour, wheat, semolina
CF for ruminants	257017	NL	20-10-2010	21	maize, soya, maize gluten, wheat
CF for ruminants	257023	NL	20-10-2010	19	
CF for porcines	257024	NL	20-10-2010	91	wheat, triticale, barley, soya, maize, maize gluten
CF for ruminants	257276	NL	14-10-2010	50	maize gluten, triticale
CF for porcines	257277	NL	14-10-2010	18	wheat, barley, biscuits, semolina, maize, wheat gluten, triticale, soya
CF for porcines	257955	NL	1-11-2010	420	barley, semolina, wheat flour, rye, triticale, maize
CF for porcines	259099	NL	18-11-2010	530	wheat (29%), barley (25%), soya (16%), maize (12%)
CF for porcines	259100	NL	18-11-2010	248	wheat (41%), rye (19%), barley (15%)
CF for porcines	259101	NL	18-11-2010	583	wheat (41%), rye (19%), barley (15%)

n.d.: not detected; NL: Netherlands, GER: Germany. Note: Visual determination of sclerotia content is not possible for compound feeds.

Annex VIII
Ergot alkaloid pattern for all positive samples (see Annex I)

		Ergot alkaloid content (µg/kg)														
RIKILT number	Product	Agro-clavine	Chano-clavine	Ergo-cornine	Ergo-corninine	Ergo-cristine	Ergo-cristinine	α-Ergo-cryptine	α/β-Ergo-cryptinine[1]	β-Ergo-cryptine	Ergo-metrine	Ergo-metrinine	Ergo-sine	Ergo-sinine	Ergo-tamine	Ergo-taminine	Total
199232	Rye					6	5							*		**	11
202077	Rye			6		150	47	33	15		10		428	*	137	**	826
205869	Rye					8	3	5						*		**	16
206303	Rye			7	4			11	11	13			17	*		**	63
206943	Rye							5						*		**	5
207055	Rye			38	29			136	199	220	20		35	*		**	677
210966	Rye			136	19	203	37	8	38	22	17		100	*	104	33	717
213101	Rye			37	22	48	10	41	43	52			22	*	12		287
213677	Rye	6									8			*			14
214807	Rye			8	4	19	9	5	4	5	4		7	*	23	3	91
215363	Rye midlings							3			10		3	*	92	7	115
217777	Rye			38	9	84	13	17	9	17	6		127	*	47	6	373
218722	Rye (Organic)			4		56	15	7	3				37	*	4		126
219435	Rye				4	4							2	*			10
220522	Rye			28	13	155	51	32	19	37	158	19	100	*	215	33	860
222606	Rye	36		32	14	170	34	57	23	36	64	19	202	*	197	32	916
223304	Rye			9	10	10	5	38	19	30			6	*	4	11	142
223556	Rye												18	*			18
233248	Rye			3		5	2	7	5	16	18	2	71	16	80	12	237
235081	Rye			4		31	9	16	10	3	11		13		68	23	188
236080	Rye			2		7	2		3	2			5	2	40	11	74
239289	Rye										2		2				4
239291	Rye			19	9			20	17	45	6		43	19	39	7	224
239292	Rye			12	5			5	2	4			2				30
239465	Rye			5		61	26	3	2		14	2	13	5	35	13	179
239696	Rye			2				8	18	18			2				48
241079	Rye (Organic)			2	4	5	2	3	4	3	2		7	2	3		37
243263	Rye		13	26	11	13		7	7	18	45	7	518	120	28	6	819
244312	Rye		3			74	21				23	4	59	17	855	175	1231
245271	Rye (Organic)					13	4						33	6			56
245500	Rye					4	2	2	4	6					2		20
245852	Rye			13	3	58	32	81	13	7	3		23	5	20	4	262
245885	Rye			2				4	3	10	182	5	6				212
247859	Rye			13	2			9		10	9	2	45	11	5		106
254962	Rye			39	21			32	16	24			26	12	22	5	197
254963	Rye			3		3		2		2			2		2		14
255834	Rye midlings					7	3						7	2	10	2	31

[1] *sum of α- and β-ergocryptinine determined, reported as α-ergocryptinine. *: sum of ergosine and ergosinine determined, reported as ergosine. **: sum of ergotamine and ergotaminine determined, given as ergotamine.*

Annex VIII
Ergot alkaloid pattern for all positive samples - continued

RIKILT number	Product	Ergot alkaloid content (µg/kg)															
		Agro-clavine	Chano-clavine	Ergo-cornine	Ergo-corninine	Ergo-cristine	Ergo-cristinine	α-Ergo-cryptine	α/β-Ergo-cryptinine[1]	β-Ergo-cryptine	Ergo-metrine	Ergo-metrinine	Ergo-sine	Ergo-sinine	Ergo-tamine	Ergo-taminine	Total
202079	Tritciale					34	7	10	8	23	4		6	*	48	**	140
205228	Tritciale													*		**	0
215362	Tritciale			20	6			7	5	7			14	*			59
218771	Tritciale												5	*			5
219423	Tritciale			3				2						*			5
219424	Tritciale			5		9		6					40	*	57	3	120
223562	Tritciale			41	20	24	14	23	20	20	2		31	*	89	13	297
223797	Tritciale					3	2	3	2				2	*	2		14
224966	Tritciale												2	*	15		17
225945	Tritciale			4	3	8	2	3	5	8			28	*	11		72
239370	Tritciale			33	18			13	7	10	2		37	19			139
239464	Tritciale			18	8			27	12	16			14	6			101
243976	Tritciale			7				4		3			12		6		32
244313	Tritciale							2			9				38	7	56
245499	Tritciale					32	8						7	2	16	2	67
247860	Tritciale		116	22	8			16	3	8	4		17	5			199
251422	Tritciale							2							38	10	50
255982	Tritciale					2							4				6
258797	Tritciale					24	13				21	7	6	4	9	2	86
205227	Wheat			10				19	5				6	*	16	**	56
205994	Wheat			5				3			3		12	*		**	23
206030	Wheat					25	8	22	10	18			21	*	20	**	124
206319	Wheat			3		14	3						29	*		**	49
207056	Wheat			16	15	31	7	16	15	10	6		43	*		**	159
207152	Wheat					15	3							*	10	**	28
218770	Wheat			40	25	95	33	49	36	66			63	*	103	21	531
258015	Barley		4	121	37	51	16	83	21	43	261	30	69	26	163	36	961

[1] *sum of α- and β-ergocryptinine determined, reported as α-ergocryptinine.*

**: sum of ergosine and ergosinine determined, reported as ergosine.*

***: sum of ergotamine and ergotaminine determined, reported as ergotamine.*

Annex VIII
Ergot alkaloid pattern for all positive samples - continued

RIKILT number	Product	Ergot alkaloid content (µg/kg)														
		Agro-clavine	Chano-clavine	Ergo-cornine	Ergo-corninine	Ergo-cristine	Ergo-cristinine	α-Ergo-cryptine	α/β-Ergo-cryptinine[1]	β-Ergo-cryptine	Ergo-metrine	Ergo-metrinine	Ergo-sine	Ergo-sinine	Ergo-tamine	Ergo-taminine	Total
218446	CF for ovines			4		19	8	3	4	3			8	*	13		62
219196	CF for porcines					5					3		20	*	12		40
219302	CF for bovines			3		3							4	*	6		16
219303	CF for porcines			17	5			8	6				11	*	12		59
220523	CF for porcines					7							5	*	10		22
220524	CF for bovines			4	2	11	4	6	3	2			26	*	34	4	96
225200	CF for bovines												5	*			5
228001	CF for porcines					3		2	2				4		7	2	20
234635	CF for porcines			2				2					3		3		10
235855	CF for bovines												3	3	12	12	30
235856	CF for bovines					24	12				2		6	3	8	3	58
235857	CF for bovines					5	7				2		4	3	6	4	31
238160	CF for bovines					2	6						5	3	9	5	30
238161	CF for porcines				3	5							7		14	4	33
238673	CF for porcines					22	17	4	2	6	5		28	10	50	12	156
239463	CF for porcines			4							3		8				15
240652	CF for porcines			10	4	8		7	9	11			5	2	9	4	69
240653	CF for rabbits					6	4	3					7	2	10	4	36
241078	CF for porcines					9	3						2		3		17
241764	CF for bovines				2	3	2		2				2	2	4	3	20
241934	CF for porcines				2	4	2		2	3			3	2	5	3	26
241935	CF for porcines			4	2	2	3	2	2	2			4	2	2		25
242721	CF for bovines			17	9	60	14	17	9	9	7		65	10	87	10	314
243724	CF for porcines			4	2	3		4	2	3			5		5		28
244350	CF for bovines					4	2	2			2		4		11	3	28
247395	CF for porcines					2							2		4	2	10
248856	CF for porcines			12	7	30	12	12	9	18	5		31	14	48	12	210
250642	CF for porcines			5	3	2		2	3	6			4	2	4		31
250643	CF for bovines			2	2	4	4	2	2	2			4	3	5	2	32
253209	CF for equines			3	2			2		2			3				12
254964	CF for porcines			3	2	24	10	5	2	2	20	3	10	4	35	8	128
255392	CF for porcines					9	9				2		11	5	8	3	47
257017	CF for bovines			5				3		2			7		4		21
257023	CF for bovines			4		2		3			2		2		6		19
257024	CF for porcines			10		10					10		22		39		91
257276	CF for bovines			9	3	3	2	5	2	4			10	3	7	2	50
257277	CF for porcines					2		2		4			6	2	2		18
257955	CF for porcines		4	18	9	43	13	26	9	17	26	3	80	23	126	23	420
259099	CF for porcines			28	13	56	14	22	7	21	47	9	63	22	202	26	530
259100	CF for porcines			28	13	25	8	19	6	15	17	6	31	12	58	10	248
259101	CF for porcines			70	29	54	25	46	15	31	27	6	99	36	123	22	583

[1] *sum of α- and β-ergocryptinine determined, reported as α-ergocryptinine.*

**:* *sum of ergosine and ergosinine determined, reported as ergosine. Ergot alkaloid content in sclerotia.*

Annex IX
Ergot alkaloid content in sclerotia

RIKILT number	Product	Number of sclerotia	Sclerotia weight (mg)	Ergot alkaloid content (µg/g)															Relative alkaloid content[2]
				Agro-clavine	Chano-clavine	Ergo-cornine	Ergo-corninine	Ergo-cristine	Ergo-cristinine	α-Ergo-cryptine	α/β-Ergo-cryptinine[1]	β-Ergo cryptine	Ergo-metrine	Ergo-metrinine	Ergo-sine	Ergo-sinine	Ergo-tamine	Ergo-taminine	Total	
217777	rye	?	408.3			51	13	112	17	22	12	23	8	1	168	*	62	8	497	0.343
218867	rye	?	870.6			11	3			11	4	10	1		10	*			51	0.033
219435	rye	?	22.0			6		91	8	5			8		39	*	13		169	0.124
220522	rye	?	1540			16	8	85	27	18	11	21	89	11	55	*	118	18	476	0.309
220548	rye	?	19.0													*			0	0.000
222606	rye	?	1210	19		16	7	87	17	30	12	19	32	9	103	*	102	17	471	0.299
223303	rye	?	47.0													*			0	0.000
223304	rye	?	186.0			30	32	34	17	127	64	101			19	*	14	37	475	0.250
233248	rye	2	39.7		8	28	7	2		78	56	166	187	18	719	165	756	127	2317	1.489
233621	rye	2	407.8																0	0.000
235081	rye	9	263.3					72	21	27	18	6	19	3	12	3	121	49	351	0.198
236080	rye	9	123.4			9	6	38	10	6	14	13			28	9	207	60	398	0.231
239289	rye	6	197.4										4		3	2			9	0.006
239291	rye	4	130.0			60	28			62	53	143	18		138	61	75	24	661	0.381
239292	rye	10	120.4			42	16			18	9	13			6	4			107	0.060
239465	rye	17	383.2			8	2	104	44	5	4	2	24	3	22	9	60	22	308	0.172
239696	rye	1	19.1			53	45			243	561	568			54	29			1553	0.706
241932	rye	6	129.7																0	0.000
243263	rye	14*	617.0		5	11	4	5		3	3	7	18	3	211	49	11	3	333	0.205
244312	rye	5	131.1		5			232	74				8		135	37	114	24	627	0.375
245500	rye	4	96.4			7	4	22	9	10	19	33	4		6	3	8	2	126	0.069
245852	rye	11	137.7			24	12	80	35	261	37		10		67	17	50	7	599	0.377
245885	rye	1	26.3		3	31	7			66	42	155	2765	80	92	18			3258	2.391
247859	rye	2	43.9			143	21			100	16	105	16						400	0.280
250445	rye	4	142.7																0	0.000
253210	rye	2	24.9																0	0.000
254144	rye	1	29.5																0	0.000
254962	rye	2	32.4		3	377	143			204	90	191	6		301	128	381	92	1915	1.122
254963	rye	1	5.4			230	87	273	68	143	81	152	15		194	89	173	45	1548	0.907
255983	rye	1	9.9																0	0.000
256419	rye	1	12.5																0	0.000
257275	rye	1	167.3																0	0.000

[1] *sum of α- and β-ergocryptinine determined, reported as α-ergocryptinine. *: sum of ergosine and ergosinine determined, reported as ergosine.*
Note: samples 217777 and 233248 contain traces (2 µg/g or less) of ergine and erginine).

Annex IX
Ergot alkaloid content in sclerotia - continued

RIKILT number	Product	Number of sclerotia	Sclerotia weight (mg)	Ergot alkaloid content (µg/g)														
				Agro-clavine	Chano-clavine	Ergo-cornine	Ergo-corninine	Ergo-cristine	Ergo-cristinine	α-Ergo-cryptine	α/β-Ergo-cryptinine[1]	β-Ergo cryptine	Ergo-metrine	Ergo-metrinine	Ergo-sine	Ergo-sinine	Ergo-tamine	Ergo-taminine	Total
219423	triticale	1	5.0			347	35			200	22	126	8		142	*			880
223797	triticale	1	7.8					248	121	234	162		5	64	125	*	118	20	1097
224966	triticale	2	47.5			7	3			15	5	5	5		30	*	216	20	305
225945	triticale	5	127.8			23	19	51	12	16	31	52	4		170	*	65	5	448
234821	triticale	2	15.5																0
234873	triticale	1	59.8																0
239370	triticale	1	10.6			1415	794			560	289	454	76	11	1594	810			6003
239464	triticale	4	48.5			44	35	19	10	58	78	92	5		38	20	2		400
243398	triticale	2	31.3																0
243976	triticale	1	8.8		25	530	54	1		288	42	241	85	6	825	95	429	27	2648
247860	triticale	2	74.1		750	142	49			104	19	49	27	5	107	32			1283
																			(533)[3]
251422	triticale	8	61.4			7	8			10	6	9					230	60	330
254719	triticale	1	40.7			4	3			9	5	2							24
255835	triticale	2	33.4																0
218770	wheat	?	1995			11	7	22	7	14	10	16			19	*	31	7	144
258015	barley	11	350.0	1	6	137	27	74	26	70	15	43	473	52	112	40	270	63	1411

[1] *sum of α- and β-ergocryptinine determined, reported as α-ergocryptinine.* [3] *excluding chanoclavine.* *: *sum of ergosine and ergosinine determined, given as ergosine.*

Note: samples 243976 and 258015 contain traces (2 µg/g or less) of ergine and erginine).

Annex X
Ergot alkaloid content in sclerotia (µg/g): Available data

Entry	Country	Year of harvest	Technique	No of Samples	Cereal	Ergo-cornine	Ergo-corninine	Ergo-cristine	Ergo-cristinine	α/β-Ergo-cryptine	α/β-Ergo-cryptinine	Ergo-metrine	Ergo-metrinine	Ergo-sine	Ergo-sinine	Ergo-tamine	Ergo-taminine	Sum total	Sum ines	Sum ininines	% ines	%ininines	Reference
1	Proposed, Verstraete, 2010					100		600		100		100		100		300		1300	1300		100%		Verstraete, 2010
2	Proposed, Müller, 2009	(1978,1979)	Color+LC-UV/FLD		Various	80	40	620	260	100	60	100	40	80	40	340	160	1920	1320	600	69%	31%	Müller et al, 2009 (based on Young, 1982)
3	Australia	2008	LC-UV	26	Various	117	36	21	16	405	173	6		77	16	1532	306	2705	2158	547	80%	20%	Blaney, 2009
4	Germany	1988	LC-FLD	1	Rye	69	29	353	101	46	31	82	12	110	30	284	67	1212	943	269	78%	22%	Wolff et al, 1988
5	Germany	2002	LC-FLD	1	Rye	32	20	104	28	40	64	24	4	60	20	56	20	472	316	156	67%	33%	Mainka et al, 2005
6	Germany	2002	LC-FLD	14	Rye	8	4	469	90	17	8	74	13	31	5	58	19	795	656	139	83%	17%	Mainka et al, 2007a
7	Germany	2003	LC-FLD	21	Rye	10	4	35	5	21	8	7	2	23	4	22	6	146	118	28	81%	19%	Mainka et al, 2007a
9	Germany	2003	LC-FLD	2	Rye	74	25	290	57	61	16	111	40	269	43	202	34	1219	1006	213	83%	17%	Mainka et al, 2007b
8	Germany	2004	LC-FLD	48	Rye	27	11	97	28	30	20	88	20	529	172	153	61	1234	922	312	75%	25%	Mainka et al, 2007a
14	Germany	2005	LC-FLD	1	Rye	30	16	31	16	26	20	10	1	35	20	113	71	389	245	144	63%	37%	Franzmann et al, 2010
15	Germany	2006	LC-FLD	3	Rye	36	16	61	22	24	21	33	8	51	21	101	53	446	306	140	69%	31%	Franzmann et al, 2010
10	Germany	2007	LC-FLD	17	Rye+Triticale	85	38	214	73	58	24	60	14	108	46	174	74	968	699	268	72%	28%	Appelt & Ellner, 2007
16	Germany	2007	LC-FLD	37	Rye	43	21	140	56	38	28	30	7	56	27	157	79	682	464	218	68%	32%	Franzmann et al, 2010
12	Germany	2007	LC-FLD	1	Rye	30	19	416	163	31	18	113	29	70	42	434	217	1582	1093	489	69%	31%	Appelt & Ellner, 2007
11	Germany	2008	LC-FLD	6	Rye	30	31	118	86	23	28	65	14	41	37	125	110	708	402	306	57%	43%	Appelt & Ellner, 2007
13	Germany	2008	LC-FLD	1	Rye	32	17	103	46	30	27	39	8	64	31	154	85	635	421	214	66%	34%	Schumann et al, 2009
17	Germany	2008	LC-FLD	15	Rye	126	43	247	64	79	26	77	13	151	58	253	82	1219	933	286	77%	23%	Franzmann et al, 2010
18	Germany	2009	LC-FLD	7	Rye	27	13	69	18	27	16	19	5	41	21	73	30	359	256	103	71%	29%	Franzmann et al, 2010
19	Netherlands	2008	LC-MS/MS	13	Rye+Triticale	40	10	56	17	82	26	12	7	68	0	57	10	384	315	69	82%	18%	This study, 2012
20	Netherlands	2009	LC-MS/MS	14	Rye+Triticale	118	67	17	6	180	77	24	3	187	79	87	20	864	612	252	71%	29%	This study, 2012
21	Netherlands	2010	LC-MS/MS	21	Rye+Triticale	78	20	33	10	107	18	163	7	98	24	79	15	653	558	94	86%	14%	This study, 2012
	Combined results per year																						
	Germany	2002		15	Rye	10	5	445	86	18	12	71	13	33	6	58	19	774	633	140	82%	18%	
	Germany	2003		23	Rye	16	5	57	9	25	9	16	6	44	7	37	8	239	195	44	82%	18%	
	Germany	2004		48	Rye	27	11	97	28	30	20	88	20	529	172	153	61	1234	922	312	75%	25%	
	Germany	2005		1	Rye	30	16	31	16	26	20	10	1	35	20	113	71	389	245	144	63%	37%	
	Germany	2006		3	Rye	36	16	61	22	24	21	33	8	51	21	101	53	446	306	140	69%	31%	
	Germany	2007		55	Rye+Triticale	56	26	168	63	44	26	41	10	72	33	167	80	787	548	239	70%	30%	
	Germany+Netherlands	2008		35	Rye+Triticale	75	28	150	50	69	26	50	11	98	32	155	60	805	598	207	74%	26%	
	Germany+Netherlands	2009		21	Rye+Triticale	88	49	34	10	129	57	22	3	138	60	82	23	696	494	202	71%	29%	
	Netherlands	2010		21	Rye+Triticale	78	20	33	10	107	18	163	7	98	24	79	15	653	558	94	86%	14%	
	Year weigthed average																						
	Germany	2002-2009		8		37	16	142	39	32	19	44	9	115	41	114	51	660	484	176	73%	27%	
	Netherlands	2008-2010		3		79	32	35	11	123	40	66	5	117	35	74	15	634	495	139	78%	22%	
	Germany+Netherlands	2002-2010		9		46	20	119	33	52	23	55	9	122	42	105	43	669	500	169	75%	25%	
	Entry weighted average																						
	Germany	2002-2009		14		42	20	171	54	36	23	54	13	109	39	148	67	775	560	215	72%	28%	
	Netherlands	2008-2010		3		79	32	35	11	123	40	66	5	117	35	74	15	634	495	139	78%	22%	
	Germany+Netherlands	2002-2009		17		49	22	147	46	51	26	56	11	110	38	135	58	750	548	202	73%	27%	
	Sample weighted average																						
	Germany	2002-2009		174		42	18	156	46	36	20	56	13	195	67	137	59	845	622	223	74%	26%	
	Netherlands	2008-2010		48		80	31	34	11	122	37	82	6	115	34	76	15	642	508	134	79%	21%	
	Germany+Netherlands	2002-2010		222		50	21	129	38	55	24	62	11	178	60	124	49	801	597	204	75%	25%	
	Relative composition (% of total alkaloids)																						
	Proposed average, Verstraete, 2010					7.7%		46.2%		7.7%		7.7%		7.7%		23.1%		100.0%			100.0%		
	Proposed average, Müller et al, 2009					4.2%	2.1%	32.3%	13.5%	5.2%	3.1%	5.2%	2.1%	4.2%	2.1%	17.7%	8.3%	100.0%			68.8%	31.3%	
	Germany	2002-2009		174		5.0%	2.2%	18.4%	5.5%	4.3%	2.4%	6.6%	1.5%	23.1%	7.9%	16.2%	7.0%	100.0%			73.6%	26.4%	
	Netherlands	2008-2010		48		12.4%	4.8%	5.4%	1.7%	18.9%	5.8%	12.7%	0.9%	18.0%	5.3%	11.8%	2.4%	100.0%			79.2%	20.8%	
	Germany+Netherlands	2002-2010		222		6.3%	2.6%	16.2%	4.8%	6.8%	3.0%	7.7%	1.4%	22.2%	7.5%	15.5%	6.2%	100.0%			74.6%	25.4%	

RIKILT - Institute of Food Safety is part of the international knowledge organisation Wageningen UR (University & Research centre). RIKILT conducts independent research into the safety and quality of food. The institute is specialised in detecting and identifying substances in food and animal feed and determining the functionality and effect of those substances.

RIKILT advises national and international governments on establishing standards and methods of analysis. RIKILT is available 24 hours a day and seven days a week in cases of incidents and food crises.

The research institute in Wageningen is the National Reference Laboratory (NRL) for milk, genetically modified organisms, and nearly all chemical substances, and is also the European Union Reference Laboratory (EU-RL) for substances with hormonal effects.

RIKILT is a member of various national and international expertise centres and networks. Most of our work is commissioned by the Dutch Ministry of Economic Affairs, Agriculture and Innovation and the new Dutch Food and Consumer Product Safety Authority. Other parties commissioning our work include the European Union, the European Food Safety Authority (EFSA), foreign governments, social organisations, and businesses.

More information: www.rikilt.wur.nl