Occurrence of mycotoxins and pesticides in straw and hay used as animal feed

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Contents

Summary

A total of 149 samples of straw (barley, wheat, rapeseed) and 43 samples of hay, aimed as feed materials and taken in the Netherlands in 2012-2013, were analysed to gain insight in the occurrence of 40 mycotoxins and 219 pesticides.

Mycotoxins (up to 13 in a single sample) were detected in most of the straw samples, in decreasing order of abundance: enniatins, DON, alternaria toxins, zearalenon and T-2/HT-2. Furthermore there were incidental detects for moliniformin, citrinin, sterigmatocystin, mycophenolic acid and ergot alkaloids.
In hay, sterigmatocystin and enniatins were the most frequently detected mycotoxins, followed by alternaria toxins. Other mycotoxins detected at lower frequencies were zearalenon, mycophenolic acid, nitropropionic acid, alpha/beta-zearalenol, ochratoxin A and citrinin.
DON and zearalenon exceeded the EU guidance levels for feed materials in a limited number of samples (4 and 1, respectively). T-2/HT-2 and ochratoxin A were below the EU guidance levels. For the other mycotoxins, no compliance verification with legal or guidance levels could be made because no values are yet available.

Pesticides (up to 10 in a single sample) were detected in most of the straw samples. Besides boscalid, the triazoles epoxiconazole and tebuconazole were detected in more than 50% of the straw samples. Other pesticides detected were mostly other fungicides, pyrethroid- and organophosphorus insecticides, and synthetic auxin herbicides. In hay the abundance of pesticides was much lower, although less so for grass seed hay. No compliance verification with legal or guidance levels could be made because for straw and hay such values have not been established in the EU.

From the survey it is clear that straw and hay contribute to the exposure of livestock to multiple mycotoxins and pesticides (especially triazoles). The significance of the contribution depends on the intake of straw and hay which in turn depends on the animal, region and farming practises. More detailed information on this is required to determine the actual exposure.

1 Introduction

Straw and hay are two types of roughages which can be part of the animal diet. Straw is a low cost feed material with low nutritional value. It is used as source of dietary fibre for ruminants. Animals fed restrictively, e.g. dairy cows in their dry period and gestating sows, are given straw to reach satiety. Furthermore, animals may consume straw when used as bedding material. In such cases intake can be significant and for pigs estimated intakes up to 14% of the diet have been reported [Rohweder 2013]. Hay has a higher nutritional value than straw and is fed to ruminants and horses.

The actual intake of straw and hay for individual animal is highly variable. It not only varies between species but may also vary from country to country, and depend on the farmer's practise which in turn may be influenced by regional, seasonal and feed-cost variations. Table 1 provides some data on intake of straw and hay for the Netherlands [Bikker 2013] and more general for the EU [EU 1996]. Especially regarding the latter, it should be emphasised that diets vary enormously and the figures are only a rough indication of maximum likely intake levels.

Table 1

Indication of intake of straw and hay for cattle and pigs.

Matrix	Netherlands [Bikker 2013]	EU [EU 1996]	
	Straw kg DM/day	Straw kg DM/day	Hay kg DM/day
Cattle			
dairy (lactating period)	0.5	4**	20**
dairy (dry period)	2 - 6		
veal (calves)	0.2		
beef		7.5	15
Fattening pigs	0.2*		0.45

* Mainly from bedding, 0-2 kg/day, average 0.2 kg/day. **dairy cattle

DM = dry matter.

Cereals and grasses may be infested by fungi in the field or post-harvest during storage which may lead to presence of mycotoxins, not only in the kernels/seeds but also in the vegetative part of the plant and hence in the straw and hay. Mycotoxins can adversely affect animal health or productivity [Fink 2008, Bryden 2012] and in some cases may end up in the animal products thereby posing a potential risk for human health.

To combat fungi, and other pests such as insects and weeds, and to facilitate harvest, pesticides (fungicides, insecticides, herbicides, desiccants, respectively) are commonly applied in agricultural production (and storage) of cereals and grasses. As a consequence, besides mycotoxins, straw and hay may also contain residues of pesticides.

In enforcement, monitoring, and control of mycotoxin contamination and pesticide residues in cereals the focus is typically on the grains and, to a lesser extent, on silage. Although straw and hay intake is lower than cereal grains or silage, it cannot be neglected as an (additional) route of exposure of animals to mycotoxins and pesticides, and therefore insight in their presence and levels is relevant.

The aim of this survey was to provide data on the occurrence of mycotoxins and pesticide residues in straw and hay used as animal feed in The Netherlands.

2 Sample analysis

2.1 Samples

Samples were taken according to regulation 152/2009 by the Dutch Food and Consumer Product Safety Authority (NVWA) between September 2012 and September 2013. In total 149 samples of straw were received, mainly from wheat and barley, for as far the crop was specified. Since rapeseed straw is also used as feed ingredient, this type of straw was included in the survey. All straw samples were analysed for mycotoxins, 93 of them also for pesticide residues. Between February and September 2013, 43 samples of hay (of which 5 grass seed hay) were taken and analysed for both mycotoxins and pesticides. The majority of the samples were originating from the Netherlands. Approximately 28% of the samples were from other countries, mostly France and Germany. In Figure 1 the numbers of samples specified by type and origin are graphically shown.

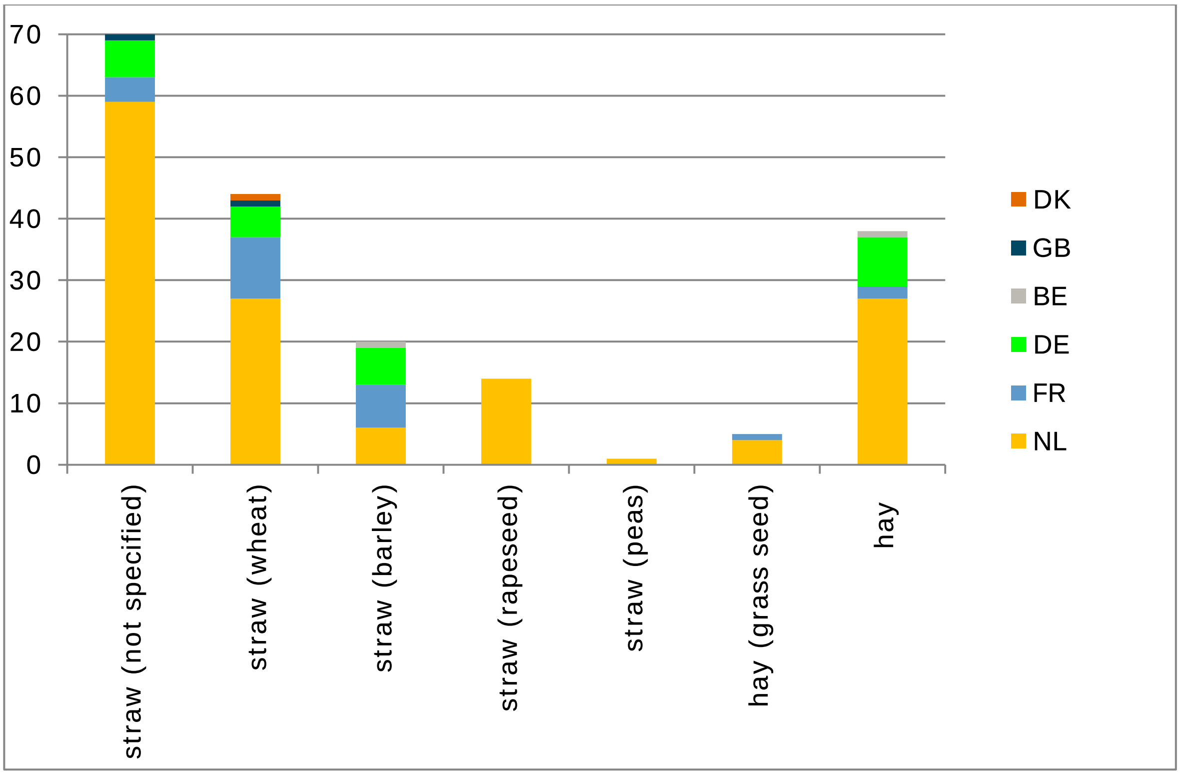

Figure 1 *Numbers of samples included in the survey, specified by type and origin.*
DK = Denmark, GB = Great Britain, BE = Belgium, DE = Germany, FR = France, NL = Netherlands.

2.2 Methods

2.2.1 Moisture

The moisture content of the straw and hay samples was determined gravimetrically by weighing before and after drying in an oven under controlled conditions.

2.2.2 Mycotoxin analysis

Mycotoxins were determined by an in-house validated multi-method used for routine monitoring and enforcement of compound feeds and feed ingredients. The method covered aflatoxins, trichothecenes, fumonisins, zearalenones, alternaria toxins and various other toxins. Enniatins were screened for in 2012 but not always quantified. Following completion of a re-validation, enniatins and ergot alkaloids were added to the method from January 2013, thereby extending the quantitative scope to 40 mycotoxins. In addition, the reporting limit was lowered for a number of mycotoxins (see Annex 1).

Samples were cut and cryogenically homogenized to < 1mm. A method based on liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) was used. In brief, portions of 2.5 gram were extracted with acetonitrile/water (84/16) containing 1% of formic acid (head-over-head, 30 min). After centrifugation, an aliquot was diluted 1:1 with water. Filtered extracts were then analysed by LC-MS/MS measuring two transitions (except for moniliformin and nitropropionic acid, only one transition available). Quantification was performed using the standard addition approach, either by addition prior to extraction (fumonisins and citrinin) or after extraction (other mycotoxins).

For mycotoxins, in line with 2002/32/EC and 2006/576/EC, the results obtained were expressed on a 12% moisture content basis. For this the actual moisture content for each sample was determined as described in 2.2.1 and the results in mg/kg sample 'as is' were converted into the equivalent of that sample with a 12% moisture content.

2.2.3 Pesticide analysis

Pesticides were determined by in-house validated multi-methods used for routine monitoring and enforcement of compound feeds and feed ingredients. In total, the survey covered 219 pesticides, including the regulated organochlorine insecticides from 2002/32/EC, pyrethroids and organophosphorus insecticides, conazole and strobilurin fungicides, and phenoxy acid herbicides. The list of pesticides is provided in Annex 2. The reporting limits for the majority of the pesticides was 0.01 mg/kg and otherwise not higher than 0.05 mg/kg.

Samples were cut and cryogenically homogenized to < 1mm. Extraction and cleanup was based on the QuEChERS approach [Lehotay 2007]. In brief, to 2.5 gram of sample 7.5 mL of water and 10 mL of acidified acetonitrile were added and the pesticides were extracted for 30 min by head-over-head shaking. Then salts were added to induce phase partitioning. For LC-MS/MS analysis, the acetonitrile extract was diluted 1:1 with water, filtered and analysed. For GC-MS/MS analysis, the acetonitrile extract was cleaned by dispersive solid phase extraction (dSPE) using C18 and PSA SPE materials. Quantification was performed using the standard addition approach, adding the standard to the final extract.

3 Results

3.1 Moisture content

The results obtained for the moisture determination of the straw and hay samples are summarized in Table 2. The moisture content for individual samples ranged from 5 to 24% for straw and 6 to 30% for hay. The average and median moisture content of straws were close to the 12% used as basis for limits set for undesirable substances [EU directive 2002/32/EC]. For hay the median was slightly below that.

Table 2
Moisture content of straw and hay samples.

		Moisture content in % w/w			
Matrix	**N**	**min**	**max**	**average**	**median**
straw (all)	149	5.0	23.6	12.1	12.6
straw (barley)	20	5.0	16.5	11.1	10.7
straw (wheat)	44	5.5	23.6	11.8	12.1
straw (rape seed)	14	8.4	15.5	12.3	13.3
straw (not specified)	70	5.4	18.1	12.7	12.8
hay	42	5.9	30.2	11.3	9.8

3.2 Mycotoxins

The individual results for all straw and hay samples analysed are provided as supplemental information (Excel file). For mycotoxins, all results are expressed in mg/kg on a 12% moisture basis.

3.2.1 Straw samples

Mycotoxins (for scope and reporting limits see Annex 1) were detected in 93% of the samples analysed. In most samples, multiple mycotoxins were detected, with a maximum of 13 different toxins. The distribution of multiple mycotoxin occurrence in the samples analysed is shown in Figure 2.

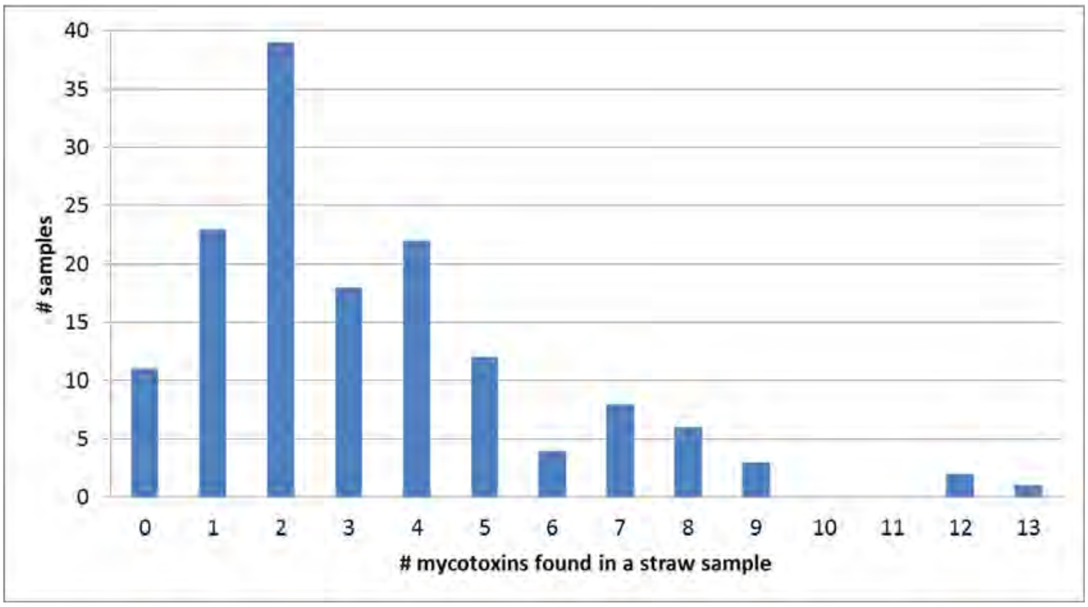

Figure 2 *Frequency of detection of multiple mycotoxins in straw samples.*

The results for straw are summarized in Tables 3 and 4. Fusarium toxins were most frequently found (91% of the samples), followed by alternaria toxins (32%). For the fusarium toxins, the occurrence in decreasing order of abundance was: enniatins (91%), type B trichothecenes (61%, mostly deoxynivalenol (DON)), zearalenone (28%), type A trichothecenes (11%, mostly HT2 toxin). Moniliformin, citrinin, sterigmatocystin, mycophenolic acid, and ergot alkaloids were incidentally detected. Of the toxins for which maximum limits (ML) or guidance limits (GL) have been established, aflatoxins, ochratoxin A, and fumonisins were not detected in straw.

In case detection of DON, other type B trichothecenes co-occurred in approximately 50% of the DON-positive samples. Acetyl-DONs were most frequently co-occurring (39%), followed by DON-3-glucoside (28%) and nivalenol (15%). The concentrations of these toxins relative to DON (min-max/median) varied widely and were 6-500%/31%, 7-44%/15% and 2-60%/11%, respectively.

From Table 4 some differences can be observed in the occurrence of mycotoxins in the different types of straw (as far as specified) although it should be mentioned that the numbers for each type are relatively small. In rapeseed straw, except for enniatins, no fusarium toxins were detected, while alternaria toxins seemed more abundant compared to barley and wheat straw. DON and derivatives were more frequently found and at higher levels in wheat straw compared to barley straw.

3.2.2 Hay samples

Mycotoxins were detected in 77% of the samples analysed (for all samples the 2013 scope and reporting limits were applicable, see Annex 1). In many samples, multiple mycotoxins were detected. The distribution of multiple mycotoxin occurrence in the samples analysed is shown in Figure 3.
A summary of the findings is presented in Table 5. The Aspergillus toxin sterigmatocystin and the Fusarium toxins enniatins were the most frequently detected mycotoxins in hay (in 51% and 49% of the samples, respectively), followed by the alternaria toxins (26%) and ergot alkaloids (19%). Besides a few incidences of nivalenol, no trichothecenes were found in hay. Several other mycotoxins were detected at low frequency, in decreasing order: zearalenon, mycophenolic acid, nitropropionic acid, alpha/beta-zearalenol, ochratoxin A and citrinin.

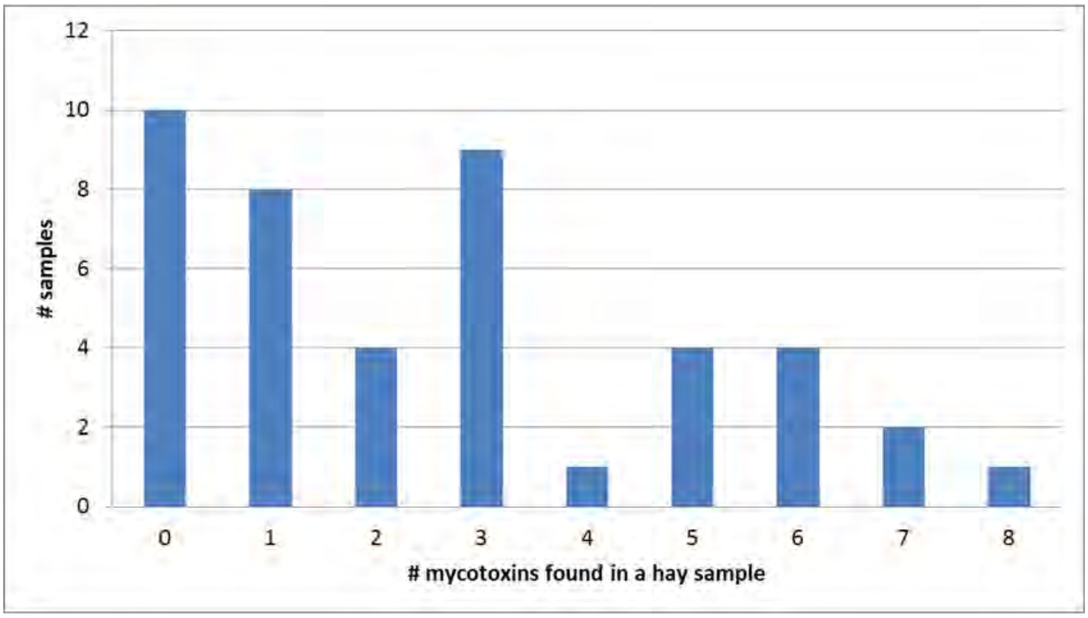

Figure 3 *Frequency of detection of multiple mycotoxins in hay samples.*

Table 3

Summary of mycotoxins found in straw samples (all straw samples combined).

Fungi genus					**Straw (all)**				detection rate				distribution of levels found								
Fusarium	Aspergillus	Penicillium	Alternaria	Claviceps	Mycotoxin class	Mycotoxin	samples analysed	RL mg/kg	number	%	lowest mg/kg	highest mg/kg	mg/kg	No	mg/kg	No	mg/kg	No	mg/kg	No	GL mg/kg
x					trichothecenes (B)	Deoxynivalenol (DON)	149	0.2-0.5	91	61%	0.23	49	>RL, <0.5	9	0.5-4	71	4-8	7	>8	4	8
x					trichothecenes (B)	3/15-acetyl-DON	149	0.4	34	23%	0.40	16	>RL, <0.5	6	0.5-4	26	4-8	0	>8	2	-
x					trichothecenes (B)	DON-3-Glucoside	131	0.25	23	18%	0.20	8.4	>RL, <0.5	11	0.5-4	11	4-8	0	>8	1	-
x					trichothecenes (B)	Nivalenol	126	0.1-0.5	12	10%	0.12	1.6	>RL, <0.5	7	0.5-4	5	4-8	0	>8	0	-
x					trichothecenes (A)	Diacetoxyscirpenol (DAS)	149	0.01-0.1	1	1%	0.029	0.029	>RL, <0.1	1	0.1-0.4	0	0.4-1	0	>1	0	-
x					trichothecenes (A)	T-2 Toxin	149	0.02-0.10	5	3%	0.021	0.20	>RL, <0.05	3	0.05-0.20	2	0.20-0.5	0	>0.5	0	-
x					trichothecenes (A)	HT2 toxin	149	0.02-0.10	17	11%	0.019	0.40	>RL, <0.05	6	0.05-0.25	8	0.25-1	3	>1	0	-
x					zeralenons	Zearalenone	149	0.05	41	28%	0.050	2.10	>RL, <0.1	10	0.1-0.5	24	0.5-2	6	>2	1	2
x					zeralenons	Zearalenol alpha	149	0.01-0.25	1	1%	0.018	0.018	>RL, <0.05	1	0.05-0.20	0	0.20-0.5	0	>0.5	0	-
x					zeralenons	Zearalenol beta	149	0.01-0.25	1	1%	0.034	0.034	>RL, <0.05	1	0.05-0.25	0	0.25-1	0	>1	0	-
x					peptides, enniatins	Beauvericin	149	0.025	22	15%	0.022	0.41	>RL, <0.1	21	0.1-0.4	0	0.4-1	1	>1	0	-
x					peptides, enniatins	Enniatin A	126	0.05-0.10	4	3%	0.050	0.18	>RL, <0.25	4	0.25-1	0	1-2	0	>2	0	-
x					peptides, enniatins	Enniatin A1	126	0.05-0.10	41 (32)*	33%	0.055	0.36	>RL, <0.25	29	0.25-1	3	1-2	0	>2	0	-
x					peptides, enniatins	Enniatin B	126	0.05-0.10	115 (58)*	91%	0.067	3.0	>RL, <0.25	26	0.25-1	22	1-2	8	>2	2	-
x					peptides, enniatins	Enniatin B1	126	0.05-0.10	93 (60)*	74%	0.054	1.4	>RL, <0.25	40	0.25-1	18	1-2	2	>2	0	-
x					other	Moniliformin	149	0.125	8	5%	0.15	0.31	>RL, <0.25	7	0.25-1	1	1-2	0	>2	0	-
	x				aflatoxins & precursors	Sterigmatocystin	149	0.001-0.20	2	1%	0.001	0.038	>RL, <0.005	1	0.005-0.02	0	0.02-0.1	1	>0.1	0	-
	x	x			other	Citrinin	149	0.05-0.15	1	1%	0.072	0.072	>RL, <0.25	1	0.25-1	0	1-2	0	>2	0	-
		x			other	Mycophenolic acid	149	0.05-0.4	4	3%	0.087	0.26	>RL, <0.25	3	0.25-1	1	1-2	0	>2	0	-
			x		alternaria toxins	Alternariol	149	0.02-0.1	48	32%	0.023	8.9	>RL, <0.1	13	0.1-0.4	26	0.4-1	5	>1	4	-
			x		alternaria toxins	Alternariol-methylether	149	0.005-0.025	14	9%	0.008	0.076	>RL, <0.1	14	0.1-0.4	0	0.4-1	0	>1	0	-
				x	ergot alkaloid	Ergocryptine	43	0.005	1	2%	0.005	0.005	>RL, <0.05	1	0.05-0.25	0	0.25-1	0	>1	0	-
				x	ergot alkaloid	Ergotamine	43	0.005	1	2%	0.006	0.006	>RL, <0.05	1	0.05-0.25	0	0.25-1	0	>1	0	-
				x	ergot alkaloid	Ergots total	43	0.005	1	2%	0.012	0.012	>RL, <0.1	1	0.1-0.4	0	0.4-1	0	>1	0	(0.80)**

RL = reporting limit (lower values apply to samples analyzed in 2013, see Annex 1); GL = guidance limit (2006/576/EC). All results are expressed in mg/kg, 12% moisture basis. * between brackets: number of samples in which enniatins were quantified.

** ML for rye ergot (Claviceps purpurea) kernels is 1000 mg/kg, this roughly corresponds to 0.8 mg/kg total ergot alkaloids [EFSA opinion on ergot alkaloids, 2012].

Table 4

Summary of mycotoxins found in different types of straw.

										Wheat straw			Rapeseed straw			Unspecified straw		
Fungi genus							Barley Straw			Wheat straw			Rapeseed straw			Unspecified straw		
Fusarium	Aspergillus	Penicillium	Alternaria	Claviceps	Mycotoxin class	Mycotoxin	analysed No	detected		analysed No	detected		analysed No	detected		analysed No	detected	
x					trichothecenes (B)	Deoxynivalenol (DON)	20	7	35%	44	35	80%	14	0	0%	70	49	70%
x					trichothecenes (B)	3/15-acetyl-DON	20	1	5%	44	12	27%	14	0	0%	70	21	30%
x					trichothecenes (B)	DON-3-Glucoside	18	0	0%	40	10	25%	12	0	0%	60	13	22%
x					trichothecenes (B)	Nivalenol	16	2	13%	39	3	8%	12	0	0%	58	7	12%
x					trichothecenes (A)	Diacetoxyscirpenol (DAS)	20	0	0%	44	1	2%	14	0	0%	70	0	0%
x					trichothecenes (A)	T-2 Toxin	20	2	10%	44	2	5%	14	0	0%	70	1	1%
x					trichothecenes (A)	HT2 toxin	20	5	25%	44	5	11%	14	0	0%	70	7	10%
x					zeralenons	Zearalenone	20	3	15%	44	12	27%	14	0	0%	70	26	37%
x					zeralenons	Zearalenol alpha	20	0	0%	44	0	0%	14	0	0%	70	1	1%
x					zeralenons	Zearalenol beta	20	0	0%	44	0	0%	14	0	0%	70	1	1%
x					peptides, enniatins	Beauvericin	20	3	15%	44	8	18%	14	0	0%	70	11	16%
x					peptides, enniatins	Enniatin A	16	1	6%	39	2	5%	12	0	0%	58	1	2%
x					peptides, enniatins	Enniatin A1	16	3	19%	39	12	31%	12	2	17%	58	15	26%
x					peptides, enniatins	Enniatin B	16	11	69%	39	17	44%	12	8	67%	58	21	36%
x					peptides, enniatins	Enniatin B1	16	10	63%	39	15	38%	12	7	58%	58	28	48%
x					other	Moniliformin	20	1	5%	44	4	9%	14	0	0%	70	3	4%
	x				aflatoxins & precursors	Sterigmatocystin	20	0	0%	44	0	0%	14	1	7%	70	1	1%
	x	x			other	Citrinin	20	0	0%	44	1	2%	14	0	0%	70	0	0%
		x			other	Mycophenolic acid	20	0	0%	44	3	7%	14	0	0%	70	1	1%
			x		alternaria toxins	Alternariol	20	7	35%	44	13	30%	14	7	50%	70	21	30%
			x		alternaria toxins	Alternariol-methylether	20	2	10%	44	4	9%	14	4	29%	70	4	6%
				x	ergot alkaloids	Ergocryptine	9	0	0%	12	0	0%	7	1	14%	14	0	0%
				x	ergot alkaloids	Ergotamine	9	0	0%	12	0	0%	7	1	14%	14	0	0%
				x	ergot alkaloids	Ergots total	9	0	0%	12	0	0%	7	1	14%	14	0	0%

Table 5
Summary of mycotoxins found in hay samples.

Fungi genus					hay (all)				detection rate				distribution of concentrations found								
Fusarium	Aspergillus	Penicillium	Alternaria	Claviceps	Mycotoxin class	Mycotoxin	samples analysed	RL mg/kg	number	%	lowest mg/kg	highest mg/kg	mg/kg	No	mg/kg	No	mg/kg	No	mg/kg	No	GL mg/kg
x					trichothecenes (B)	Nivalenol	43	0.1	2	5%	0.11	0.57	>RL, <0.5	1	0.5-4	1	4-8	0	>8	0	-
x					zeralenons	Zearalenone	43	0.05	6	14%	0.12	0.40	>RL, <0.1	0	0.1-0.5	6	0.5-2	0	>2	0	2
x					zeralenons	Zearalenol alpha	43	0.01	2	5%	0.011	0.019	>RL, <0.05	2	0.05-0.20	0	0.20-0.5	0	>0.5	0	-
x					zeralenons	Zearalenol beta	43	0.01	2	5%	0.017	0.036	>RL, <0.05	2	0.05-0.25	0	0.25-1	0	>1	0	-
x					peptides, enniatins	Beauvericin	43	0.025	2	5%	0.031	0.036	>RL, <0.1	2	0.1-0.4	0	0.4-1	0	>1	0	-
x					peptides, enniatins	Enniatin A1	43	0.05	2	5%	0.08	0.11	>RL, <0.25	2	0.25-1	0	1-2	0	>2	0	-
x					peptides, enniatins	Enniatin B	43	0.05	21	49%	0.051	0.73	>RL, <0.25	12	0.25-1	9	1-2	0	>2	0	-
x					peptides, enniatins	Enniatin B1	43	0.05	9	21%	0.052	0.28	>RL, <0.25	7	0.25-1	2	1-2	0	>2	0	-
	x				aflatoxins & precursors	Sterigmatocystin	43	0.001	22	51%	0.0012	0.17	>RL, <0.005	11	0.005-0.02	6	0.02-0.1	4	>0.1	1	-
	x				other	Nitropropionic acid	43	0.05	2	5%	1.1	2.5	>RL, <0.25	0	0.25-1	0	1-2	1	>2	1	-
	x	x			ochratoxins	Ochratoxin A	43	0.002	1	2%	0.003	0.003	>RL, <0.005	1	0.005-0.02	0	0.02-0.1	0	>0.1	0	0.25
	x	x			other	Citrinin	43	0.05	1	2%	0.076	0.076	>RL, <0.25	1	0.25-1	0	1-2	0	>2	0	
		x			other	Mycophenolic acid	43	0.05	4	9%	0.056	0.33	>RL, <0.25	3	0.25-1	1	1-2	0	>2	0	
			x		alternaria toxins	Alternariol	43	0.02	6	14%	0.039	6.0	>RL, <0.1	4	0.1-0.4	1	0.4-1	0	>1	1	
			x		alternaria toxins	Alternariol-methylether	43	0.005	11	26%	0.006	8.7	>RL, <0.1	10	0.1-0.4	0	0.4-1	0	>1	1	
				x	ergot alkaloid	Agroclavine	43	0.01	1	2%	0.041	0.041	>RL, <0.05	1	0.05-0.25	0	0.25-1	0	>1	0	
				x	ergot alkaloid	Ergocristine	43	0.005	5	12%	0.006	0.12	>RL, <0.05	3	0.05-0.25	2	0.25-1	0	>1	0	
				x	ergot alkaloid	Ergotaminine	43	0.005	1	2%	0.006	0.006	>RL, <0.05	2	0.05-0.25	0	0.25-1	0	>1	0	
				x	ergot alkaloid	Ergosine	43	0.005	4	9%	0.006	0.046	>RL, <0.05	9	0.05-0.25	0	0.25-1	0	>1	0	
				x	ergot alkaloid	Ergots total	43	0.005	8	19%	0.008	0.174	>RL, <0.1	9	0.1-0.4	2	0.4-1	0	>1	0	(0.8)*

RL = reporting limit; GL = guidance limit (2006/576/EC). All results are expressed in mg/kg, 12% moisture basis.

* ML for rye ergot (Claviceps purpurea) kernels is 1000 mg/kg, this roughly corresponds to 0.8 mg/kg total ergot alkaloids [EFSA opinion on ergot alkaloids, 2012].

3.3 Pesticides

The individual results for all straw and hay samples analysed are provided as supplemental information (Excel file). For pesticides, all results are expressed in mg/kg material analysed, i.e. not on 12% moisture basis. However, since the average and median moisture values in straw and hay are not much different from 12% (see Table 1), the impact on interpretation of the results with respect to intake is considered to be minor.

3.3.1 Straw samples

Pesticides (for scope see Annex 2 and 3, reporting limits mostly 0.01 mg/kg and always <0.05 mg/kg) were detected almost in all samples (96%). In the majority of the straw samples, multiple pesticides were detected. The distribution of occurrence of multiple pesticides in the samples analysed is shown in Figure 4.

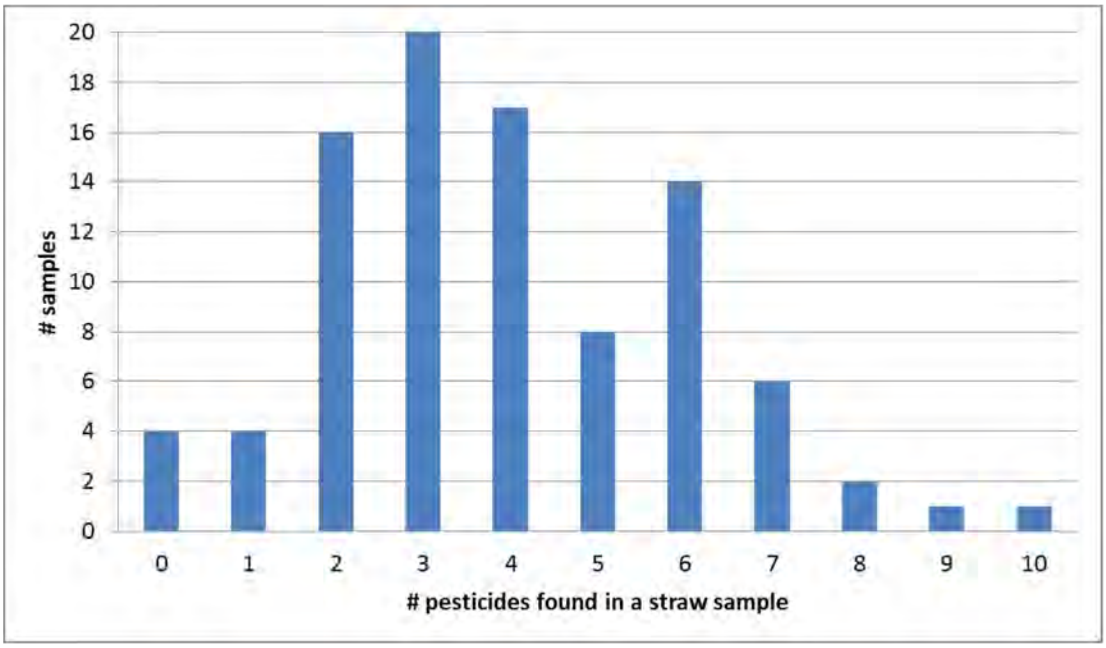

Figure 4 *Frequency of detection of multiple pesticides in straw samples.*

In Table 6 an overview is given of the pesticides detected, and for each pesticide the frequency and concentration ranges. In total, 48 different pesticides were detected. The most frequently detected residues were epoxiconazole, boscalide, and tebuconazole (all in >50% of the samples). Fungicides accounted for the majority (78%) of the residues found in straw samples, insecticides 13% and herbicides 8%. Classified by their chemical class (typically corresponding to a similar mode of action), the conazoles were the dominating class for the fungicides (52% of the fungicide residues), pyrethroids for the insecticides (88% of the insecticide residues), and synthetic auxins (phenoxycarboxylic acids and fluroxypyr) for the herbicides (90%). Especially for the fungicides, multiple active ingredients, sometimes from the same chemical class, were detected.

From Table 7 differences can be observed in the occurrence of pesticide residues in the different types of straw (as far as specified), although it should be mentioned that the numbers for each type are relatively small. The most prominent difference was that in rapeseed straw no herbicides were detected. Also insecticides were not frequently occurring (8% vs 37% for barley and 70% for wheat straw). Fungicides were occurring at high rates in all three types of straw (barley 89%, wheat 100%, rapeseed 92%).

Table 6

Summary of pesticides found in straw samples (all straw samples combined).

PESTICIDES			detection rate		lowest	highest	concentration distribution in mg/kg				MRL**
type	class	active ingredient	number	%	mg/kg	mg/kg	>RL, <0.05	0.05-0.20	0.20-0.50	>0.50	mg/kg
FUNGICIDES	conazole	cyproconazole	16	17%	0.01	0.16	9	7	0	0	0.1-0.4
		difenoconazole	3	3%	0.013	0.31	1	0	2	0	0.05*-0.5
		epoxiconazole	58	62%	0.01	0.87	14	28	10	6	0.05*-1
		fluquinconazole	1	1%	0.036	0.036	1	0	0	0	0.05*-0.1
		flusilazole	1	1%	0.051	0.051	0	1	0	0	0.1-0.2
		flutriafol	4	4%	0.01	0.02	4	0	0	0	0.2-0.5
		metconazole	9	10%	0.029	0.29	2	4	3	0	0.1-0.15
		prochloraz	2	2%	0.018	0.027	2	0	0	0	0.5-1
		propiconazole	6	6%	0.027	0.15	3	3	0	0	0.05*-0.2
		tebuconazole	47	51%	0.016	1.7	8	24	11	4	0.2-2
		triadimenol	1	1%	0.2	0.2	0	1	0	0	0.2
	strobilurin	azoxystrobin	7	8%	0.018	0.17	5	2	0	0	0.3-0.5
		dimoxystrobin	2	2%	0.07	0.14	0	2	0	0	0.05-0.1
		pyraclostrobin	17	18%	0.01	0.28	4	11	2	0	0.05-0.2
		trifloxystrobin	1	1%	0.049	0.049	1	0	0	0	0.05*-0.3
	other	bixafen	11	12%	0.06	0.39	0	6	5	0	0.07-0.5
		boscalid	56	60%	0.01	2.0	20	11	6	19	0.5-3
		carbendazim	1	1%	0.18	0.18	0	1	0	0	0.1*-2
		cyflufenamid	1	1%	0.05	0.05	0	1	0	0	0.02*-0.1
		cyprodinil	2	2%	0.029	0.3	1	0	1	0	0.05*-3
		fenpropidin	12	13%	0.011	0.15	8	4	0	0	0.05*-0.5
		fenpropimorph	9	10%	0.01	0.065	8	1	0	0	0.05*-0.5
		fluopicolide	6	6%	0.01	1.1	3	2	0	1	0.01*
		metrafenone	4	4%	0.03	0.12	2	2	0	0	0.05*-0.5
		pencycuron	1	1%	0.05	0.05	0	1	0	0	0.05*
		spiroxamine	8	9%	0.021	0.11	3	5	0	0	0.05*-0.3
INSECTICIDES	pyrethroid	cyfluthrin	2	2%	0.074	0.13	0	2	0	0	0.02*-0.05
		cyhalothrin (lambda)	17	18%	0.016	0.79	4	10	2	1	0.05-0.2
		cypermethrin	7	8%	0.03	0.32	1	5	1	0	0.2-2
		deltamethrin	7	8%	0.031	0.08	1	6	0	0	0.1-2
		fenvalerate	7	8%	0.078	0.56	0	5	1	1	0.05*-0.2
		fluvalinate (tau)	2	2%	0.1	0.26	0	1	1	0	0.05-0.5
	OPP	diazinon	1	1%	0.02	0.02	1	0	0	0	0.01*-0.02*
		dimethoate	2	2%	0.015	0.023	2	0	0	0	0.02*-0.05
		pirimiphos-methyl	1	1%	0.03	0.03	1	0	0	0	0.05*-5
	NN	clothianidin	1	1%	0.012	0.012	1	0	0	0	0.02*-0.04
		thiacloprid	1	1%	0.067	0.067	0	1	0	0	0.1-1
HERBICIDES	PCA	2,4-D	4	4%	0.1	10	0	1	1	2	0.05*-0.1*
		dichlorprop	1	1%	0.23	0.23	0	0	1	0	0.05*-0.2
		MCPA	8	9%	0.058	0.66	0	6	1	1	0.05*-0.1*
	other	bifenox	1	1%	0.15	0.15	0	1	0	0	0.05*-0.1
		chlorpropham	1	1%	0.06	0.06	0	1	0	0	0.02*-0.1*
		dimethenamid	1	1%	0.14	0.14	0	1	0	0	0.01*-0.02*
		fluroxypyr	15	16%	0.011	0.44	6	6	3	0	0.05*-0.1

OPP = organophosphorus pesticide, NN = neonicotinoid, PCA = phenoxycarboxylic acid.

Concentrations are in mg/kg sample 'as is'.

** maximum residue limit (EU, 396/2005, December 2013) do not apply to straw but to barley/wheat grain or rapeseed. For straw no MRLs have been established in the EU.

* MRL set at the 'limit of determination' as established during registration of the pesticide.

Clarification of MRL values with/without *: 0.05*-0.5 means that in one of the three crops (barley, wheat, rapeseed) no residues should be detected in the grain/rapeseed and the LOD-MRL of, in this example, 0.05 mg/kg applies. The 0.5 mg/kg is the highest tolerance established for any of the three crops.

Table 7

Summary of pesticides found in different types of straw.

			Barley straw (19)			wheat straw (30)			rapeseed straw (12)			unspecified (31)	
PESTICIDES			detection rate			detection rate			detection rate			detection rate	
type	class	active ingredient	No	%	*MRL***	No	%	*MRL***	No	%	*MRL***	No	%
FUNGICIDES	conazole	cyproconazole	2	11%	*0.1*	7	23%	*0.1*	3	25%	*0.4*	4	13%
		difenoconazole	0	0%	*0.05**	1	3%	*0.1*	0	0%	*0.5*	2	6%
		epoxiconazole	7	37%	*1.5*	22	73%	*0.6*	4	33%	*0.05**	25	81%
		fluquinconazole	0	0%	*0.05**	1	3%	*0.1*	0	0%	*0.05**	0	0%
		flusilazole	0	0%	*0.2*	1	3%	*0.1*	0	0%	*0.1*	0	0%
		flutriafol	1	5%	*0.5*	1	3%	*0.5*	1	8%	*0.2*	1	3%
		metconazole	0	0%	*0.1*	6	20%	*0.15*	1	8%	*0.1*	2	6%
		prochloraz	0	0%	*1*	1	3%	*0.5*	0	0%	*0.5*	1	3%
		propiconazole	3	16%	*0.2*	2	7%	*0.05**	0	0%	*0.1**	1	3%
		tebuconazole	5	26%	*2*	20	67%	*0.2*	9	75%	*0.5*	13	42%
		triadimenol	0	0%	*0.2*	1	3%	*0.2*	0	0%	*0.2**	0	0%
	strobilurin	azoxystrobin	1	5%	*0.5*	1	3%	*0.3*	4	33%	*0.5*	1	3%
		dimoxystrobin	0	0%	*0.1*	1	3%	*0.1*	0	0%	*0.05*	1	3%
		pyraclostrobin	4	21%	*0.2*	6	20%	*0.05**	1	8%	*0.05**	6	19%
		trifloxystrobin	0	0%	*0.3*	0	0%	*0.05*	0	0%	*0.05**	1	3%
	other	bixafen	2	11%	*0.5*	5	17%	*0.05*	0	0%	*0.07*	4	13%
		boscalid	11	58%	*3.0*	18	60%	*0.5*	8	67%	*1.0*	19	61%
		carbendazim	0	0%	*2.0*	1	3%	*0.1*	0	0%	*0.1**	0	0%
		cyflufenamid	0	0%	*0.1*	0	0%	*0.05*	0	0%	*0.02**	1	3%
		cyprodinil	2	11%	*3.0*	0	0%	*0.5*	0	0%	*0.05**	0	0%
		fenpropidin	6	32%	*0.5*	2	7%	*0.5*	0	0%	*0.05**	4	13%
		fenpropimorph	2	11%	*0.5*	2	7%	*0.5*	0	0%	*0.05**	5	16%
		fluopicolide	0	0%	*0.01**	2	7%	*0.01**	0	0%	*0.01**	4	13%
		metrafenone	1	5%	*0.5*	2	7%	*0.5*	0	0%	*0.05**	1	3%
		pencycuron	0	0%	*0.05**	0	0%	*0.05**	0	0%	*0.05**	1	3%
		spiroxamine	5	26%	*0.3*	0	0%	*0.05**	0	0%	*0.05**	3	10%
INSECTICIDES	pyrethroid	cyfluthrin	0	0%	*0.02**	2	7%	*0.02**	0	0%	*0.05*	0	0%
		cyhalothrin (lambda)	2	11%	*0.5*	8	27%	*0.05*	0	0%	*0.2*	7	23%
		cypermethrin	1	5%	*2.0*	3	10%	*2.0*	0	0%	*0.2*	3	10%
		deltamethrin	0	0%	*2.0*	5	17%	*2.0*	0	0%	*0.1*	2	6%
		fenvalerate	2	11%	*0.2*	1	3%	*0.05*	0	0%	*0.05**	4	13%
		fluvalinate (tau)	0	0%	*0.5*	1	3%	*0.05*	1	8%	*0.1*	0	0%
	OPP	diazinon	0	0%	*0.01**	0	0%	*0.01**	0	0%	*0.02**	1	3%
		dimethoate	0	0%	*0.02**	2	7%	*0.05*	0	0%	*0.05**	0	0%
		pirimiphos-methyl	0	0%	*5*	0	0%	*5.0*	0	0%	*0.05**	1	3%
	NN	clothianidin	1	5%	*0.04*	0	0%	*0.02**	0	0%	*0.02**	0	0%
		thiacloprid	1	5%	*1*	0	0%	*0.1*	0	0%	*0.3*	0	0%
HERBICIDES	PCA	2,4-D	0	0%	*0.05**	1	3%	*0.05**	0	0%	*0.1**	3	10%
		dichlorprop	0	0%	*0.2*	1	3%	*0.2*	0	0%	*0.05**	0	0%
		MCPA	1	5%	*0.05**	4	13%	*0.05**	0	0%	*0.1**	3	10%
	other	bifenox	0	0%	*0.1*	0	0%	*0.1*	0	0%	*0.05**	1	3%
		chlorpropham	0	0%	*0.02**	0	0%	*0.02**	0	0%	*0.1**	1	3%
		dimethenamid	0	0%	*0.01**	0	0%	*0.01**	0	0%	*0.02**	1	3%
		fluroxypyr	3	16%	*0.1*	4	13%	*0.1*	0	0%	*0.05**	8	26%

OPP = organophosphorus pesticide, NN = neonicotinoid, PCA = phenoxycarboxylic acid.

** maximum residue limit (EU, 396/2005, December 2013)) do not apply to straw but to barley/wheat grain or rapeseed. For straw no MRLs have been established in the EU.

* MRL set at the 'limit of determination' as established during registration of the pesticide.

3.3.2 Hay samples

Pesticides (for scope see Annex 2 and 3, reporting limits mostly 0.01 mg/kg and always <0.05 mg/kg) were detected in 35% of the 43 samples analysed. In a number of the hay samples, multiple pesticides were detected. The distribution of occurrence of multiple pesticides in the samples analysed is shown in Figure 5.

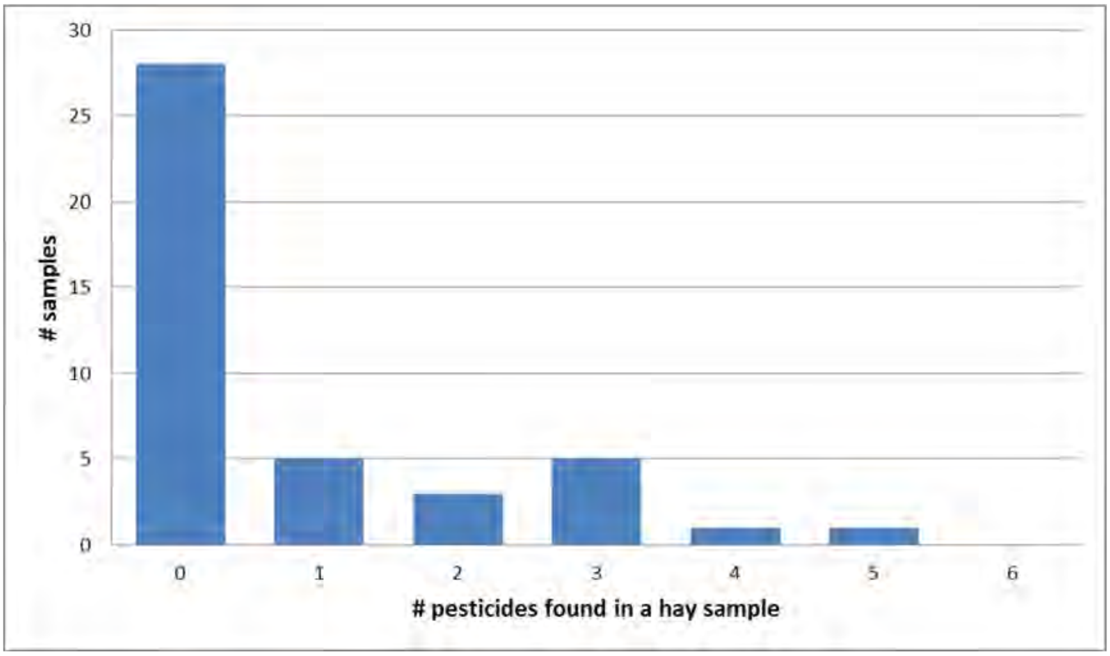

Figure 5 *Frequency of detection of multiple pesticides in hay samples.*

Table 8
Summary of pesticides found in hay samples.

PESTICIDES			detection rate		lowest	highest	concentration distribution in mg/kg			
type	class	active ingredient	number	%	mg/kg	mg/kg	>RL,<0.05	0.05-0.20	0.20-0.50	>0.50
FUNGICIDES	conazole	cyproconazole	2	5%	0.067	0.14	0	2	0	0
		epoxiconazole	2	5%	0.013	0.76	1	0	0	1
		propiconazole	1	2%	0.49	0.49	0	0	1	0
		tebuconazole	3	7%	0.37	0.57	0	0	2	1
		triadimenol	3	7%	0.2	0.95	0	1	0	2
	strobilurin	trifloxystrobin	2	5%	0.015	0.045	2	0	0	0
	other	boscalid	1	2%	0.03	0.0	1	0	0	0
		metalaxyl	1	2%	0.17	0.17	0	1	0	0
		propamocarb	2	5%	0.013	0.031	2	0	0	0
insecticide	pyrethroid	fenvalerate	1	2%	0.055	0.055	0	1	0	0
HERBICIDES	PCA	2,4-D	1	2%	0.096	0.096	0	1	0	0
		MCPA	6	14%	0.019	1.1	2	1	2	1
		mecoprop	3	7%	0.055	0.77	0	2	0	1
	other	fluroxypyr	3	7%	0.19	1.5	0	1	1	1
		linuron	1	2%	0.07	0.07	0	1	0	0

PCA = phenoxycarboxylic acid. Concentrations are in mg/kg sample 'as is'.

In Table 8 an overview is given of the pesticides detected, and for each pesticide the frequency and concentration range. In total, 14 different pesticides were detected. Fungicides and herbicides accounted for the majority (51% and 46% respectively) of the residues found in hay samples. An insecticide was only detected once. Classified by their chemical class (typically corresponding to a similar mode of action), the conazoles were the dominating class for the fungicides and synthetic auxins (phenoxycarboxylic acids and fluroxypyr) accounted for all but one of the herbicides.

Five out of the 43 hay samples analysed were grass seed hay. In these samples the incidence of pesticides was much higher compared to the other hay samples: the grass seed hay samples accounted for 16 out of 35 pesticide detected and also for most of the cases of multiple detections of pesticides.

4 Discussion

From the survey it is clear that a variety of mycotoxins and pesticides are frequently detected in straw and hay, and that livestock is exposed to these contaminants and residues upon intake. Since it was beyond the primary aim of the study, no estimations of actual exposure of mycotoxins and pesticides through straw and hay consumption has been made. It is expected that drawing general conclusion on exposure will be difficult due to the highly variable intake of straw/hay at an individual farm/animal level, as indicated in the introduction. Therefore the discussion below will be brief, qualitative and focus on comparison of the analysis data with legislative and guidance levels.

4.1 Mycotoxins

In the EU, for mycotoxins in feed only aflatoxin B1 and rye ergot have been regulated [directive 2002/32/EC]. For feed materials , a general maximum limit (ML) of 0.020 mg/kg applies for aflatoxin B1. Aflatoxin B1 was not detected in any of the samples and the ML was not exceeded. The ML for rye ergots has been set for rye ergot kernels rather than the toxins themselves, and applies to ‘feed materials containing unground cereals’. Therefore, no direct verification of exceedances of the ML for rye ergots (1000 mg/kg) can be made. However, an indirect verification can be made using the mean total ergot alkaloid content of ergot kernels as indicated in the [EFSA opinion on ergot alkaloids, 2012]. The 1000 mg rye ergots/kg feed material corresponds to approximately 0.8 mg total ergot alkaloids/kg. Targeting at the most abundant alkaloids, the total amount found in the samples did not exceed this value.

Besides the legal maximum limit set in [2002/32/EC], a recommendation has been published [2006/576/EC] that provides guidance levels (GL) for deoxynivalenol (DON), zearalenone, ochratoxin A, and fumonisin B1 and B2, which should not be exceeded in cereals and cereal products. For the sum of T-2 and HT-2 toxin an indicative action level has been established for cereal products for feed [2013/165/EC] which is interpreted here as applicable to barley and wheat straw but not rapeseed straw and hay.

DON, only found in barley and wheat straw, exceeded the guidance limit for feed materials (8 mg/kg) 4 times, corresponding to 2% of the samples analysed.

Zearalenon (ZON), found in barley/wheat straw and hay, exceeded the guidance level for straw/hay as feed material (2 mg/kg) 1 time, corresponding to 0.5% of the samples analysed.

T-2 and HT-2 toxins, found in barley and wheat straw, did not exceed the action level of 0.50 mg/kg for the sum of the two toxins in cereals products applied as feed material.

Ochratoxin A was detected incidentally but did not exceed the guidance level.

Besides the mycotoxins for which legal maximum limits or guidance levels have been established, other mycotoxins were detected in this survey. In straw, enniatins were found in the majority of the samples (91%) and with that were more abundant than DON although in mg total enniatins/kg the levels were somewhat lower. With a 32% detection rate and levels in the mg/kg range, alternaria toxins where the third most occurring toxins in straws after enniatins and DON. In hay, in contrast to straw, sterigmatocystin was frequently detected (51%), mostly in the 0.005-0.10 mg/kg range. Enniatins and alternaria toxins were also frequently detected in hay, but generally at lower levels compared to straw.

The relevance in terms of animal health and productivity is subject to on-going investigations and risk assessment studies. In the past few years, EFSA has published several scientific opinions, including on alternaria toxins [EFSA 2011], sterigmatocystin [EFSA 2013], citrinin [EFSA 2012], and nivalenol [EFSA 2013] and ergot alkaloids [EFSA 2012]. For enniatins/beauvericin an EFSA opinion is currently being prepared. In general, due to lack of toxicity data and occurrence data, no proper risk assessment could be carried out. The analytical data generated in this survey may contribute to fill the gap on occurrence data.

4.2 Pesticides

In the EU, only a limited number of pesticides are regulated for animal feed materials as such [2002/32/EC]. This concerns the classical persistent organochlorine pesticides which have been banned for many years but may still enter the feed chain as environmental contaminant. Pesticides in general are regulated through 396/2005 which provides maximum residue levels (MRLs) for many pesticide/commodity combinations. This regulation applies to both food and feed. For cereal grains for example, the same MRL applies irrespective the use for food or feed. This is because the MRLs are based on good agricultural practise (GAP) and are not necessarily toxicological limits. At this moment, no MRLs have yet been established for commodities that are exclusively used as feeding material (i.e. not for human consumption). This means that for straw and hay, except for a limited number of banned organochlorine pesticides included in 2002/32/EC, no MRLs are available.

Due to the lack of MRLs for straw, a qualitative evaluation based on MRLs for cereal grains was carried out. For this purpose, LOD-MRLs (indicated in Table 6-7 by "*") were distinguished from 'real' tolerances (MRLs without "*"). An LOD-MRL basically means that a residue should not be present either because the pesticide is not registered, or because use according to GAP should not leave residues at the time of harvest in the commodity as specified in the regulation (grain in case of cereals). MRLs without "*" indicate that the pesticide has been registered in the EU (not necessarily in all EU countries) and that treatment of the crop may leave a residue.

As can be seen from Table 6 and 7, for the majority of the pesticides detected in straw, a tolerance for either barley/wheat grain or rapeseed exists. Therefore, the detection of residues can be expected. Since MRLs apply to the cereal grains and not to the straw, no direct quantitative evaluation of the levels found versus the MRLs can be made.

In a limited number of samples, pesticides were detected that had no tolerance in barley/wheat grain or rapeseed (LOD-MRL applies). This was the case for the following pesticides:

MCPA: detected in barley (DE) 0.11 mg/kg; wheat (NL) 0.058, 0.065, 0.10, 0.39 mg/kg; unspecified straw (NL) 0.13, 0.13, 0.66 mg/kg. MCPA is a herbicide with applications in cereals/grasses, registered in the Netherlands for multiple crops including wheat. The detection at levels higher than the LOD-MRL either means that with good agricultural practise (GAP) residues in straw are higher than in cereal grains, or that crop protection products have not been used according to GAP.

2,4-D: wheat straw (FR) 0.82 mg/kg; unspecified straw (FR) 0.10 mg/kg. 2,4-D is a herbicide with applications in cereals/grasses. No registration in the Netherlands for cereals.

Fluopicolide: wheat straw (NL) 0.03, 0.06 mg/kg; straw unspecified (DE) 0.11 mg/kg, (NL) 0.01, 0.04, 1.1 mg/kg. Fluopicolide is a fungicide registered in the Netherlands for other crops (e.g. potatoes) but not for cereals which makes the findings remarkable.

Four other pesticides without a residue tolerance in cereal grains were only incidentally detected in straw: **pencycuron** (straw unspecified (NL) 0.05 mg/kg), **diazinon** (straw unspecified (NL) 0.02 mg/kg); **dimethenamid** (straw unspecified (NL) 0.14 mg/kg); **chlorpropham** (straw unspecified (NL) 0.06 mg/kg).

For hay no qualitative assessment of the residues found using MRLs was made because no MRLs are available. In general, both the number of pesticides and their levels were lower compared to straw, although this seems less true for grass seed hay.

It was beyond the scope of this survey to assess the relevance of the pesticide residues detected in terms of animal health or productivity. For most of the pesticides, toxicity and transfer to animal products for human consumption have been assessed in studies performed for their registration and data can be found in assessment reports available through EFSA. It is however considered relevant to point out here the high abundance of triazole fungicides (all conazole fungicide listed in Tables 6-7 except prochloraz) in straw. Triazole fungicides (especially difenoconazole, epoxiconazole, propiconazole, and tebuconazole) and have been associated with resistance of *Aspergillus fumigatus*, causing *Aspergillus* diseases in humans, against medical triazoles [Snelders 2012]. This has raised concerns and triggered investigations into the abundance of triazoles in agricultural products, household, and the environment [BuRO 2010, Royal HaskoningDHV 2013]. Therefore, for information, the sum of triazole fungicides detected in the straw samples is summarized and graphically shown in Figure 6.

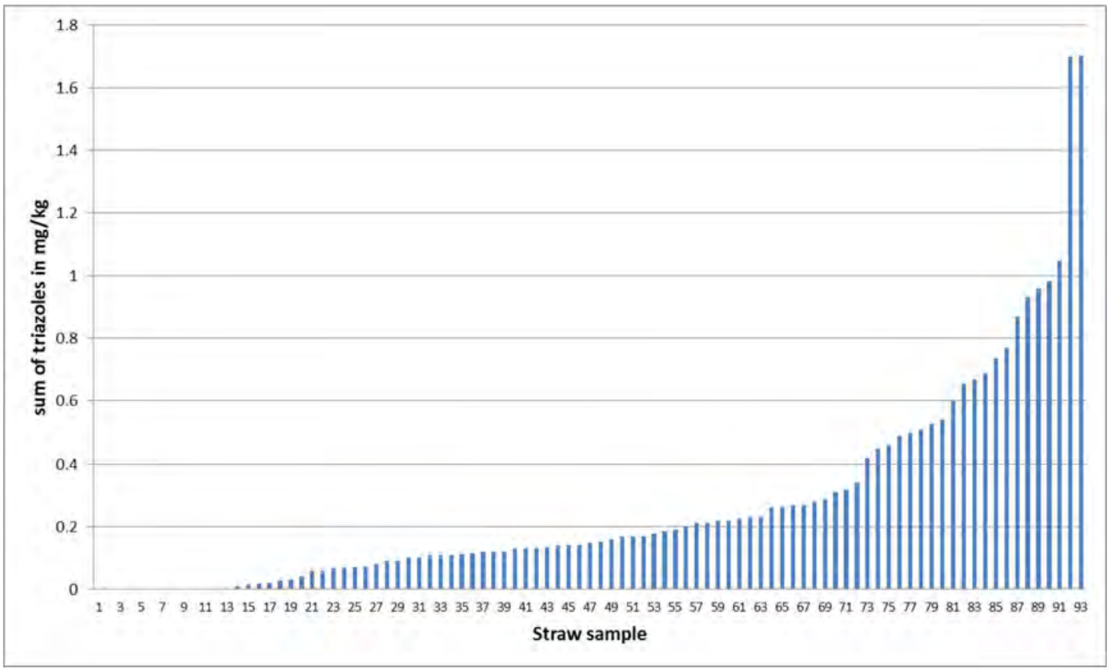

Figure 6 *Distribution of total amount of triazoles found in straw samples.*

5 Conclusions

- Mycotoxins are detected in 93% of the straw samples and 77% of the hay samples.
- Up to 13 mycotoxins were detected in a single straw sample and up to 8 in hay.
- In decreasing order, enniatins, DON, alternaria toxins, zearalenon and T-2/HT-2 were the most frequently detected mycotoxins in straw.
- Wheat straw was more contaminated with mycotoxins than barley straw.
- Rapeseed straw was mainly contaminated with enniatins and alternaria toxins.
- Hay was mainly contaminated with sterigmatocystin and enniatins, followed by alternaria toxins.
- For DON positive samples, acetyl-DON, DON-3-glucoside and nivalenol frequently co-occurred with DON but relative levels varied greatly.
- For the regulated mycotoxins, aflatoxin B1 was not detected. For rye ergots, although ergot alkaloids were detected, there were no indications that the maximum level was exceeded.
- DON and zearalenon exceeded the EU guidance values in a limited number of samples (4 and 1, respectively, corresponding to 2% or less of the samples analysed). T-2/HT-2 and ochratoxin A were below the EU guidance values. For the other mycotoxins, no compliance verification with legal or guidance levels could be made because no values are yet available.

- Pesticide residues were detected in 96% of the straw samples and 35% of the hay samples (especially grass seed hay)
- Overall, 48 different fungicides, insecticides and herbicides were detected.
- Fungicides, mostly triazoles, were detected in all three types of straw and accounted for 78% of the pesticides.
- Rapeseed straw contained no herbicides and only few insecticides
- In hay, especially grass seed hay, (triazole)fungicides and herbicides were the most frequently detected pesticides.

- From the survey it is clear that straw and hay contribute to the exposure of livestock to multiple mycotoxins and pesticides (especially triazoles).
- For determination of actual exposure of animals, more details data on intake of straw and hay (at animal species and farm level) are required.

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Annex 1 Scope mycotoxin method

	2012	2013		Fungi genus producing the mycotoxin				
Mycotoxin	Reportin limit (mg/kg)		mycotoxin class	Fusarium	Aspergillus	Penicillium	Alternaria	Claviceps
15-acetyl-DON	0.4	0.4	trichothecenes (B)	x				
3-acetyl-DON	0.4	0.4	trichothecenes (B)	x				
Aflatoxin B1	0.005	0.0025	aflatoxins & precursors		x			
Aflatoxin B2	0.005	0.0025	aflatoxins & precursors		x			
Aflatoxin G1	0.005	0.0025	aflatoxins & precursors		x			
Aflatoxin G2	0.005	0.0025	aflatoxins & precursors		x			
Agroclavine	0.005	0.025	ergot alkaloid					x
Alternariol	0.1	0.02	alternaria toxins				x	
Alternariol-methylether	0.025	0.005	alternaria toxins				x	
Beauvericin	0.02	0.025	enniatins	x				
Citrinin	0.15	0.05	other		x	x		
Deoxynivalenol (DON)	0.5	0.2	trichothecenes (B)	x				
Diacetoxyscirpenol (DAS)	0.1	0.0125	trichothecenes (A)	x				
DON-3-Glucoside	0.25	0.25	trichothecenes (B)	x				
Enniatin A	0.1	0.05	enniatins	x				
Enniatin A1	0.1	0.05	enniatins	x				
Enniatin B	0.1	0.05	enniatins	x				
Enniatin B1	0.1	0.05	enniatins	x				
Ergocorni(ni)ne	n.a.	0.005	ergot alkaloid					x
Ergocrist(in)ine	n.a.	0.005	ergot alkaloid					x
Ergocrypt(in)ine	n.a.	0.005	ergot alkaloid					x
Ergometr(in)ine	n.a.	0.005	ergot alkaloid					x
Ergos(in)ine	n.a.	0.005	ergot alkaloid					x
Ergotam(in)ine	n.a.	0.005	ergot alkaloid					x
Fumonisin B1	0.1	0.1	fumonisins	x				
Fumonisin B2	0.1	0.04	fumonisins	x				
Fumonisin B3	0.1	0.02	fumonisins	x				
HT2 toxin	0.1	0.02	trichothecenes (A)	x				
Moniliformin	0.125	0.125	other	x				
Mycophenolic acid	0.4	0.05	other			x		
Nitropropionic acid	0.1	0.05	other		x			
Nivalenol	0.5	0.1	trichothecenes (B)	x				
Ochratoxin A	0.025	0.002	ochratoxins		x	x		
Penicillic acid	0.5	0.1	other			x		
Roquefortine C	0.02	0.0025	alkaloid			x		
Sterigmatocystin	0.02	0.001	aflatoxins & precursors		x			
T-2 Toxin	0.1	0.02	trichothecenes (A)	x				
Zearalenol alpha	0.25	0.01	zeralenons	x				
Zearalenol beta	0.25	0.01	zeralenons	x				
Zearalenone	0.05	0.05	zeralenons	x				

Annex 2 Scope Pesticide multi-method (LC-MS/MS)

Scope Pesticide multi-method (LC-MS/MS) 1 from 2.

2,4-D	Dichlorprop (2,4-DP)	Fluazifop
Acephate	Dichlorvos	Fluazifop-butyl
Acetamiprid	Difenoconazole	Fludioxonil
Aldicarb	Diflubenzuron	Flufenoxuron
Aldicarb sulfone	Dimethenamid	Fluquinconazole
Aldicarb sulfoxide	Dimethoate	Fluroxypyr
Atrazine	Dimoxystrobin	Fluroxypyr-methylheptylester
Azoxystrobin	Disulfoton	Flusilazole
Bendiocarb	Disulfoton-sulfone	Flutriafol
Benfuracarb	Disulfoton-sulfoxide	Fuberidazole
Benzoylprop-ethyl	Diuron	Gibberellic acid
Bifenthrin	Dodemorph	Haloxyfop
Bitertanol	Dodine	Hexaconazole
Boscalid	Epoxiconazole	Hexythiazox
Carbaryl	Ethiofencarb	Imazalil
Carbendazim	ethiofencarb sulfone	Imidacloprid
Carbofuran	ethiofencarb sulfoxide	Iprovalicarb
Carbofuran, hydroxy	Etofenprox	Isoprocarb
Chloridazon	Fenazaquin	Isoproturon
Chlorpropham	Fenbuconazole	Kresoxim-Methyl
Chlorpyrifos	Fenhexamid	Linuron
Chlorpyrifos-methyl	Fenobucarb	Lufenuron
Clofentezine	Fenpropidin	Malaoxon
Clothianidin	Fenpropimorph	Malathion
Cypermethrin	fenthion sulfoxide	MCPA
Cyproconazole	fenthion-O	Mecoprop
Cyprodinil	fenthion-O sulfoxide	Mepronil
Deltamethrin	Fenthion-sulfone	Metalaxyl
Demeton-S-methyl-sulfone	Flamprop-isopropyl	Metconazole
Diazinon	Flamprop-methyl	Methacriphos

Scope Pesticide multi-method (LC-MS/MS) 2 from 2.

Methamidophos	Propargite	Triazoxide
Methidathion	Propham	Trichlorfon
Methiocarb	Propiconazole	Tricyclazole
Methiocarb sulfone	Propoxur	Trifloxystrobin
Methiocarb sulfoxide	Pyraclostrobin	Triflumuron
Methomyl	Pyrethrins Pyrethrin I	Trimethacarb 235-
Metolcarb	Pyrethrins Pyrethrin II	Trimethacarb 345-
Metoxuron	Pyridaben	Triticonazole
Metribuzin	Pyrimethanil	Vamidothion
Monocrotophos	Pyriproxyfen	Zoxamide
Omethoate	Quinclorac	
Oxadixyl	Quizalofop	
Oxamyl	Spinosyn A	
Oxamyl - oxime	Spinosyn D	
Oxydemeton-methyl	Spiromesifen	
Paraoxon-methyl	Spiroxamine	
Penconazole	Tebuconazole	
Pencycuron	Tebufenozide	
Pendimethalin	Tebufenpyrad	
Permethrin	Teflubenzuron	
Phosmet-oxon	Terbucarb	
Phoxim	Tetramethrin	
Piperonyl butoxide	Thiabendazole	
Pirimicarb	Thiacloprid	
Pirimicarb, desmethyl-	Thiamethoxam	
Pirimiphos-methyl	Thiodicarb	
Prallethrin	Thiophanate-Methyl	
Prochloraz	Triadimefon	
Promecarb	Triadimenol	
Propamocarb	Triazophos	

Annex 3 Scope Pesticide multi-method (GC-MS/MS)

Aldrin	Difenoconazole	Parathion-methyl
Azoxystrobin	Dimethomorph	Pendimethalin
Bifenthrin	Disulfoton	Permethrin som
Bromopropylate	Edifenphos	Phorate
Buprofezin	Endosulfan alpha-	Piperonyl butoxide
Chlordane cis- (alpha)	Endosulfan beta-	Pirimiphos-methyl
Chlordane trans- (gamma)	Endosulfan sulphate	Prochloraz
Chlorfenvinphos	Endrin	Procymidone
Chlorobenzilate	Endrin ketone	Propargite
Chlorothalonil	Ethion	Propham
Chlorpropham	Fenitrothion	Propiconazole
Chlorpyrifos	Fensulfothion	Propyzamide
Chlorpyrifos-methyl	Fenvalerate	Pyrazophos
Coumaphos	Flucythrinate	Pyridaphenthion
Cyfluthrin	HCB	Pyrimethanil
Cyhalothrin lambda-	HCH alpha-	Pyriproxyfen
Cypermethrin	HCH beta-	Simazine
Cyprodinil	HCH delta-	Tebufenpyrad
DDD o,p'- (TDE)	HCH gamma- (Lindane)	Tetradifon
DDD p,p'- (TDE)	Heptachlor	Tetramethrin
DDE o,p'-	Heptachlor epoxide (iso B)	Triazophos
DDE p,p'-	Isodrin	Trifluralin
DDMU	Malathion	Vinclozolin
DDT o,p'-	Metalaxyl	
DDT p,p'-	Methidathion	
Deltamethrin	Methoxychlor	
Diazinon	Myclobutanil	
Dichlorvos	Oxadixyl	
Dicloran	Oxychlordane	
Dieldrin	Parathion-ethyl	

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