Trend analysis of mycotoxins in animal feed

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Summary

Feed materials were analysed for the mycotoxins deoxynivalenol, ochratoxin A, zearalenone, aflatoxin B1, fumonisin B1 and B2, and HT-2- and T-2-toxins. In this report trends in the average content during the period 2001-2009 are reported for these mycotoxins. Monitoring data from the National Feed monitoring program and from the Commodity Board Animal Feed are used for this study.

In most cereals or cereal products no increase or decrease of the average levels of mycotoxins is observed. Wheat straw (deoxynivalenol) and wheat products (zearalenone), barley (zearalenone, ochratoxin A) and maize silage (deoxynivalenol, zearalenone) show a slight increase. Some individual samples exceed the limit (e.g. deoxynivalenol in maize kernels and maize silage), but in general levels are low. The increases may be related to high moisture and temperature in the growing season, especially in 2007. The amount of samples taken is low (or decreasing) in some cases. In rice and rice products levels above the limit for aflatoxin B1 have been found, especially in 2005. However, the amount of samples taken each year is very low and in 2008 and 2009 there are no samples. It is recommended to continue monitoring this product regularly.

Oil-containing seeds in general do not contain high levels of mycotoxins. Except for aflatoxin B1, there are no limits or guidance values for these products. In sunflower seeds both average and individual aflatoxin B1 levels exceed the limit, especially in 2001 and 2002. The average aflatoxin B1 level in sunflower seed expeller/extracted increases in the time period studied. It was recommended in a previous report to continue monitoring this product regularly, but the amount of samples taken each year continues to be low. Both in coconut and groundnut aflatoxin B1 levels above the limit have been detected. However, the amount of samples taken are quite low , especially in the most recent years. It is recommended to continue monitoring this product regularly.

In general the mycotoxin levels in compound feed for pigs are low. In some individual samples the guidance values for deoxynivalenol or zearalenone have been exceeded. The average deoxynivalenol content in complete and complementary feed for pigs decreases. The amount of samples taken drops from ca. 200 in 2007 to ca. 50 in 2008 and 2009. Considering the decrease of the average deoxynivalenol content this does not seem to be a problem. In liquid feed for pigs the number of years monitored is too low to identify trends. However, in several individual samples (10% of total) deoxynivalenol levels exceed the limit which indicates that more samples of liquid feed for pigs should be taken. The level of fumonisin B1 + B2 in complete feed for pigs increases but is still low.

There appears to be a decrease in the levels of ochratoxin A and aflatoxin B1 in compound feed for cattle. A few individual samples exceed the guideline for zearalenone.

In general the mycotoxin levels in feed for poultry are low. The levels in individual samples remain below the limit or guidance value. The amount of samples taken decreases.

Recommended is to increase the amount of samples for cereals and cereal products, especially in years with high humidity and/or temperature; maize silage and liquid feed for pigs (high deoxynivalenol and zearalenone levels). The other feeds can be sampled at a minimum level.

Gathering more samples, especially from the industry, will give a more representative indication of the trends in Dutch animal feed. The trend analyses can be improved with additional statistical methods (Mann-Kendall test and Sen's slope estimator). New trend analyses are recommended for ergot alkaloids and possibly other products and/ or countries that are considered at risk using the recently developed model for statistical underpinning of risk-based monitoring.

Contents

1 Introduction

In this report historical values are used to give insight into the trends in levels of several mycotoxins in compound feeds and feeding materials for animals in the Netherlands. This analysis was performed on request of the NVWA (Dutch Food and Consumer Product Safety Authority). The results of these analyses will enable the NVWA to develop a more risk-directed sampling strategy in the National Feed Monitoring program.

In 2007 and 2009 reports have been published on the trends in levels of aflatoxin B1 and dioxins en dioxin-like PCB's (Adamse *et al.*, 2007) and heavy metals (Adamse *et al*., 2009) in compound feeds and feed materials. These trend analyses have been carried out in order to use acquired knowledge to reach a more risk-driven sampling in the National Feed Monitoring program. For these reports data have been used from the KAP-database. The Quality Program Agricultural Products (KAP) is a collaboration between agricultural businesses and the Dutch Government. KAP has been designed to focus on continuous monitoring of the level of contaminants and residues in agricultural products such as vegetables, fruit, milk, meat, fish and feed. KAP processes the results of monitoring programmes and has more than 200,000 measurement results per year, from 1989 to date (van Klaveren, *et al*., 1997). The data used for this report have been submitted to the KAP by the National Feed Monitoring program (NVWA, NP)) and by the Product Board Animal Feed (PDV, 2001-2007) and relate only to monitoring research

Mycotoxins are secondary metabolites produced by fungi that are toxic to animals (and humans) consuming the products. Aflatoxin B1 causes growth retardation in animals. *Fusarium* toxins, especially fumonisins , are neurotoxic and possible carcinogens, trichothecenes are immunotoxic and zearalenone is estrogenic. Ochratoxin A is a nephrotoxin. Mixtures of mycotoxins present in feed probably have at least an additive, if not synergistic, effect. These toxins are primary sources of both yield losses and increase of management costs in animal husbandry worldwide .

For the National Feed monitoring program and Product Board Animal Feed feed materials were analysed for deoxynivalenol (DON), ochratoxin A (OTA), zearalenone (ZEN), aflatoxin B1 (AFLA), fumonisin B1 and B2 (FUM), and HT-2- and T-2-toxins. In this report trends in the average content are reported for these mycotoxins.

2 Material en methods

2.1 Material

2.1.1 Data from KAP database

The data used are collected from the databank of the Program for the Quality of Agricultural Products (KAP). The KAP databank has been maintained by the RIKILT and since 2010 by the RIVM; the results of approximately 9.000 feeding material samples reported here have been submitted to the databank by the National Feed Monitoring program (NVWA, NP)) and by the Product Board Animal Feed (PDV, 2001-2007) and relate only to monitoring research (Table 2.1). The data for this analysis are from the period between 2001 and 2009.

For fumonisin B1 + B2 only data from NP have been used since the PDV only reported fumonisin B1. The data from PDV did not contain values above 1 mg/kg, well below the guidance values.

Table 2.1. Number of samples analysed for certain mycotoxins from PDV and NP.

	Number of samples									
Year	Ochratoxin A		Deoxynivalenol		Zearalenone		Fum B1+B2		Afla B1	
	PDV	NP	PDV	NP	PDV	NP	PDV	NP	PDV	NP
2001	2	355	21	355	3	355	-	-	395	355
2002		386	13	386	161	386	-	-	182	386
2003	184	349	618	349	523	349	-	272	514	349
2004	451	484	1038	483	832	483	-	484	648	484
2005	400	520	928	520	664	518	-	518	574	520
2006	258	457	831	457	601	456	-	457	395	457
2007	770	336	1429	351	1318	350	-	349	840	236
2008	-	224	-	322	-	321	-	321	-	224
2009	-	407	-	304	-	300	-	300	-	407
Total	2063	3518	4878	3527	4102	3518	-	2701	3548	3418

2.2 Methods

2.2.1 Selection of products for trend analysis

The products studied are feed materials such as cereals, roughages and forages, oil-containing plants as well as compound feeds. Aflatoxin B1 Regulatory limits for aflatoxin B1 are laid down in Directive 2002/32/EC (+ amendments) DON, OTA and fumonisins are regulated by recommended guidance values in Recommendation 2006/576/EC (Table 2.2). Products analysed in this study are grouped according to the grouping for limits or guidance values. Only products with a reasonable amount of products analysed per year are shown in this report.

Table 2.2. Limits (2002/32/EC) and guidance values (2006/576/EC), for mycotoxins in animal feed mg/kg (12% moisture).*

	AB1	deoxynivalenol*	zearalenone*	ochratoxin A *	fumonisin B1 + B2 B1+B2*
All feed materials with the exception of:	0.02				
cereals and cereal products[1,2]		8	2	0.25	
maize by-products		12	3		
maize and maize products[1]					60
Complete feedingstuffs with the exception of:	0.01	5			
pigs	0.02	0.9		0.05	5
gilts			0.1		
piglets			0.1		
sows			0.25		
pigs for fattening			0.25		
ruminants (> 4 months)					50
bovine	0.02				
calves (< 4 months)		2			20
calves	0.01		0.5		
dairy cattle	0.005		0.5		
sheep	0.02		0.5		
lambs	0.01	2	0.5		20
goats	0.02		0.5		
goat kids		2	0.5		20
poultry	0.02			0.1	20
horses					5
rabbits					5
pets					5
fish					10
mink					50
Complementary feeding-stuffs with the exception of:	0.005	5			
pigs	0.02	0.9		0.05	5
gilts			0.1		
piglets			0.1		
sows			0.25		
fattening pigs			0.25		
ruminants (> 4 months)					50
bovine	0.02				
calves (< 4 months)		2			20
calves			0.5		

dairy cattle			0.5		
sheep	0.02		0.5		
lambs		2	0.5		20
goats	0.02		0.5		
goat kids		2	0.5		20
poultry	0.02			0.1	20
poultry-young					
horses					5
rabbits					5
pets					5
fish					10
mink					50

[1] Including forages and roughages [2] including maize.

2.2.2 Strategy used for trend analysis

The strategy for the trend analysis is to first combine data on a high clustering level, for example all products from one feed group of all countries of origin. These clustering levels are described in more detail in the report from 2007 (Adamse *et al*., 2007). If trends are visible at this level, new analyses are performed on specific countries/country groups, (for example Peru or Denmark) and/or specific products (for example maize kernels instead of all maize products) to gain more insight into the origin of this trend.

2.2.3 Mycotoxin detection methods

2.2.3.1 Mycotoxins - screening

PDV-related companies use the ELISA method as described in osp-19-2-mycotoxinen_screening.pdf ('bundel onderzoekmethoden diervoeder deel II for screening of products for mycotoxins deoxynivalenol, zearalenone en ochratoxine A').

2.2.3.2 Mycotoxins - confirmation

PDV-related companies use HPLC with fluorescence detection (zearalenone and ochratoxin A) c.q. UV-detection (deoxynivalenol) or LC/MS-MS, as described in osp-19-1-mycotoxinen_confirmatie.pdf, ('bundel onderzoekmethoden diervoeder deel II for confirmation of mycotoxin (deoxynivalenol, zearalenone en ochratoxine A) content'). The limit of detection (LOD) for these mycotoxins are: deoxynivalenol: 0.2 mg/kg; zearalenone: 0.02 mg/kg; ochratoxin A : 0.01 mg/kg.

PDV reports several HPLC methods for determining aflatoxin B1 content of feed. The LODs range from 0.5 - 1 µg/kg. The HPLC method Determination of aflatoxin B1, Commission Directive 92/95/EEC of 9 November 1992 amending the Annex to the Seventh Directive 76/372/EEC establishing Community methods of analysis for the official control of feedingstuffs, publication

13-11-1992; Nr L 327/55-62, has an LOD of 1µg/kg (osp-01a-aflatoxine_b1.pdf), 1 September 1995.

The HPLC method for the determination of aflatoxin B1 in Animal Feed ' published in the EC-report EUR 19058 EN (2000) 'Validation of an Analytical Method to Determine the Content of Aflatoxin in Animal Feed Stuff' has an LOD of 0.5µg/kg (osp-01e-aflatoxine_b1.pdf), 1 December 2002.

NEN-ISO 14718:1999-Animal feeding stuffs- Determination of Aflatoxin B1 content of mixed feeding stuffs- method using high-performance liquid chromatography has an LOD of 1µg/kg (osp-01a-aflatoxine_b1.pdf), 1 February 2005.

RIKILT uses LC/MS-MS to detect aflatoxin B1, deoxynivalenol, fumonisin B1, fumonisin B2, ochratoxin A, T-2/HT-2 toxin and zearalenone (and more mycotoxins) in animal feed. The method uses standard addition and is validated and accredited for all mycotoxins mentioned above. The reporting limits are aflatoxin B1: 0.005 mg/kg, deoxynivalenol: 0.50 mg/kg, fumonisin B1 and B2: 0.40 mg/kg, ochratoxin A: 0.025 mg/kg, T-2/HT-2 toxin : 0.10 and zearalenone: 0.05 mg/kg.

RIKILT used until mid-2004 a HPLC method with a limit of quantification (LOQ) of 1 µg/kg. Then, in consultation with the NVWA with the purpose of cost saving, they switched to a multi-LC-MS method which, in addition to aflatoxin B1, also measures a number of other mycotoxins. The LC-MS method has a higher LOQ than the HPLC method, 5 µg/kg and 1 µg/kg, respectively. As of August 1, 2005 the HPLC method is reintroduced due to non-solvable problems with LC-MS measurement equipment. In the period from mid-2004 until August 2005, in a limited number of cases measurements were still carried out with the HPLC method, i.e. for those samples for which the LOQ of 5 µg/kg was not feasible.

The samples with the result < 1 µg/kg (HPLC method) and < 5 µg/kg (LC-MS method) are saved as 0-values in KAP. Due to the transition to the LC-MS method in 2004, there are many more 0-values per year and the median is lower (usually 0). A method to adjust this could be replacing the null values with half the LOQ ('adjusted zero'). In this report 0-values are not adjusted, as explained by (Adamse, 2007). Where necessary we report samples measured with HPLC and with LC-MS methods separately.

2.2.3.3 12% moisture

Many limits are expressed as levels relative to a product moisture content of 12%. Therefore, for the majority of products, the results of the analyses are converted to 12% moisture. However, the PDV-data and the RIKILT-data prior to 2005 are generally reported on product base. As of 2005, the results from RIKILT are converted into standard 12% moisture. For products measured by PDV and by RIKILT before 2005 this report assumes that the average moisture content of most products was 12%. This is probably correct for most products. However, for products with a high moisture content (e.g. liquid feeds) it is important to interpret the results with the above information in mind.

2.2.4 Reading manual

Results are shown as averages, median values and 90-percentile values, with median and 90-percentile absent when less than 5 and 10 samples, respectively, have been analysed. Histograms are used to display the data, with sample year on the X axis and contaminant content on the left Y axis (see example in Figure 2.1). The amount of samples is shown as squares connected by a line, with values on the right Y-axis. Furthermore, the limit of the contaminant analysed is shown by the grey area. When no average values approach the limit the entire plot area will be grey. The limit(s) are presented in the figure caption. When interpreting the figure it is important to keep in mind the order of magnitude of the left Y-axis. In many cases the measured value is much lower than the regulatory limit and could the uncertainty of the analysis be playing a role. Therefore, when the LOQ of the method used for determining the level of a specific mycotoxin and the calculated averages are in the same order of magnitude, the trends in that measuring range are not reliable.

The average (avg) is the mathematical average of all data in the specific year. The median is the amount where 50% of the samples in this year had mycotoxin level higher than this amount and 50% a lower level. The median is the value in the middle of all values or the average of the two values in the middle with an even number of samples. The median can be more useful than the average when the distribution of values is skewed (many zeros), because the average is more sensitive to outliers. The 90-percentile (90Per) indicates that 90% of the values are below this 90-percentile value.

The results of the regression analysis are shown in the right-hand corner of the figure. The equation y= ax+b is the result of a linear regression between the level of the contaminant and the year of sampling. The equation algebraically describes a straight line for a set of data with one independent variable where x is the independent variable, y is the dependent variable, a represents the slope of the line, and b represents the y-intercept (the y-value when x = 0, the year before the first year shown in the graph). R^2 , the coefficient of determination, indicates how much of the variation can be explained, i.e. whether a low x-value (year) coincides with a high or low contaminant level. If R^2 is close to 1 there is a close relation. If R^2 is lower than 0.30 there is (almost) no relation. With a R^2 above 30 the effect is considered to be significant in this report. N indicates the total number of measurements in the entire period. On the right Y-axis the number of samples per year is shown.

Using simple regression analysis, a trend line through the averages is calculated and displayed (dotted line, extrapolated for missing years). This line reflects the evolution of the mycotoxin content in the years analysed. However, no extensive statistical tests have been used to prove the significance of the trends observed. This requires statistical prerequisites that are not available. The way the results are presented in this report will give sufficient information for discussing sampling strategies and suggest additional trend analyses of other elements and products.

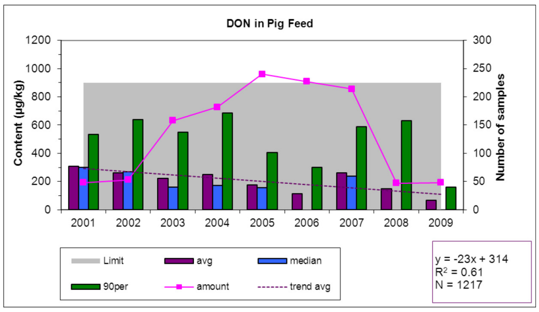


Figure 2.1. Deoxynivalenol content of feed for Pigs, Complete; limit = 900 µg/kg.

Using the reading manual above Figure 2.1 can be interpreted as follows: The amount of samples analysed each year fluctuates between 50 and 250. The average deoxynivalenol content in feed for pigs in the period 2001 through 2009 decreases with 23 µg/kg each year. The R^2 value of 0.61 indicates that this decrease is significant. The mean values (blue bars) remain below the limit (grey area) and also average (purple) and 90percentile (green) values do not exceed the limit. However, most 90 percentile values are higher than 50% of the limit, suggesting that there might be individual values close to or above the limit. Further discussion of these results in 3.6.1.

3 Results

3.1 Trends in general

Of the 11768 samples analysed in total, 5853 contain at least one mycotoxin (50%). Only 2109 are analysed for all 8 mycotoxins. None of them contain all mycotoxins, but one sample (maize flour) contains 6 out of 8 mycotoxins (no ochratoxin A and T-2).

The PDV-dataset (Table 2.1 and Table 3.3) contains a large number of samples from maize products for deoxynivalenol (1449 total/801 positive;554/470 in 2007) and wheat (1065 total/651 positive; 12/8 in 2001; 10/3 in 2002; 150/116 in 2003; 266/180 in 2004; 203/105 in 2005; 181/67 in 2006; 243/172). This is more than sampled from other products and also more than analysed for other mycotoxins. The samples from the NP dataset (Table 3.2) are distributed more equally over products, years and mycotoxins.

Table 3.1. Mycotoxin analysis results.

	Afla B1	DON	OTA	ZEN	FUM B1	FUM B2	HT-2	T-2
Number of samples tested	6966	8405	5600	7620	3076	2903	2528	2978
Positive (#)	1100	3637	283	2623	702	196	19	15
Positive (%)	16	52	4	38	10	3	1	1
Average of positives (µg/kg)	9	676	5	126	1	1	343	164

Table 3.2. Mycotoxin analysis results NP.

	Afla B1	DON	OTA	ZEN	FUM B1	FUM B2	HT-2	T-2
Number of samples tested	3418	3527	3535	3518	2701	2701	2562	2563
Positive (#)	212	656	163	637	404	196	19	1
Positive (%)	3	9	2	9	6	3	1	0
Average of positives (µg/kg)	13	1026	5	164	1	1	343	2457

Table 3.3. Mycotoxin analysis results PDV.

	Afla B1	DON	OTA	ZEN	FUM B1	FUM B2	HT-2	T-2
Number of samples tested	3548	4878	2065	4102	375	0	0	449
Positive (#)	888	2981	120	1986	298	0	0	14
Positive (%)	13	43	2	29	4	0	0	0
Average of positives (µg/kg)	9	599	5	113	0.2	-	-	176

3.2 Trends of HT-2/T-2 toxins in feeding materials of plant origin

3.2.1 Preview

In a Technical report for EFSA about occurrence of T-2 and HT-2 toxins in Europe Van der Fels-Klerx (2010a) reports that especially oats tend to be contaminated with T-2/HT-2-toxin, with an increase in 2005 in Scandinavian countries. The occurrence and concentration in barley has increased across Europe since 2004. In some occasions, maize is contaminated with T-2 toxin and HT-2 toxin, usually at a moderate level. T-2 toxin and HT-2 toxin contamination of wheat occurs very infrequently and at a low concentration level. These trends cannot be shown (significantly) in the data analysed for the current report.

3.2.2 HT-2 and T-2 toxins

In the monitoring data analysed for this report infrequent contamination with HT-2 and T-2 toxin can be detected as well. Some samples of barley (3 of 83), maize silage (3 of 218), maize products (6 of 230), oats grain (5 of 24) and wheat product (1 of 133) contain low levels of HT-2 toxin, ranging from 100 (wheat, 2003) to 1800 µg/kg (maize silage, 2005). All samples are from the NP dataset and analysed with a detection limit of 100 µg/kg.

Table 3.4. HT-2 in feeding materials from plant origin (µg/kg).

	2003	2004	2005	2006	2007	2008	2009
# of samples	136	265	299	268	300	257	242
Average	2	0	6	2	10	0	1
StdDev	15	0	104	22	90	0	9

In some samples of barley (1 of 134), maize products (8 of 218), oats grain (2 of 323) and wheat product (1 of 265) low levels of T-2 toxin are detected ranging from 7 (barley) to 1300 µg/kg (maize). Most positive samples originate from the PDV-dataset. Only one sample (1300 µg/kg) is from the NP dataset. However, the limit of detection of the method used for the NP dataset is 500 µg/kg, so no values below 500 µg/kg are registered for these samples.

Table 3.5. T-2 in feeding materials from plant origin (µg/kg).

	2004	2005	2006	2007	2008	2009
# of samples	303	356	328	482	275	247
Average	0.3	0	1.7	3.5	0	0
StdDev	4.2	0	30.8	59.6	0	0

3.3 Trends in cereals and cereal products

3.3.1 Preview

No significant increase or decrease of the average levels can be observed for deoxynivalenol or zearalenone. In 2007, a year with high moisture and temperature, some individual wheat samples exceed the guidance value, in accordance with reports from others. Levels of deoxynivalenol in wheat are in general higher than zearalenone levels. This is in accordance with Van der Fels-Klerx (unpublished data). Aflatoxin B1, ochratoxin A, fumonisin B1 and B2 in general show no significant increase or decrease and do not exceed the regulatory limit (for aflatoxin) or guidelines. Only in rice and rice product some individual samples exceed the regulatory limit for aflatoxin B1.

3.3.2 Deoxynivalenol

Commission Recommendation 2006/576/EC recommends a guidance value of 8000 µg deoxynivalenol/kg feeding stuff for cereals and cereal products. This includes cereal forages and roughages. Figures 3.1 and 3.2 show no values above or close to this limit. In barley (Figure 3.1A) and rye (Figure 3.1B) no increase or decrease of deoxynivalenol values can be observed in the timeframe 2001-2009. Rye has not been sampled after 2007 and the number of samples of barley and triticale (Figure 3.1C) sharply drops after 2007. For a large part this can be explained by the absence of samples from PDV after 2007. A mycotoxin survey from PDV in 2008 (published on their website: http://www.pdv.nl/nederland/kwaliteit/page3937.php) found two samples of barley (out of nine) with levels above 100 µg/kg; 457 and 500 µg/kg. According to their 2009 report no levels above the guidance value were found in 2009 (GMP+, 2009). In unspecified grains (Figure 3.1D) there appears to be an increase of DON. However, the amount of years and samples is too low for any real significance, even though R^2 = 0.7. The highest level observed (in 2007) is 1378 µg deoxynivalenol/kg 'grainmix'.

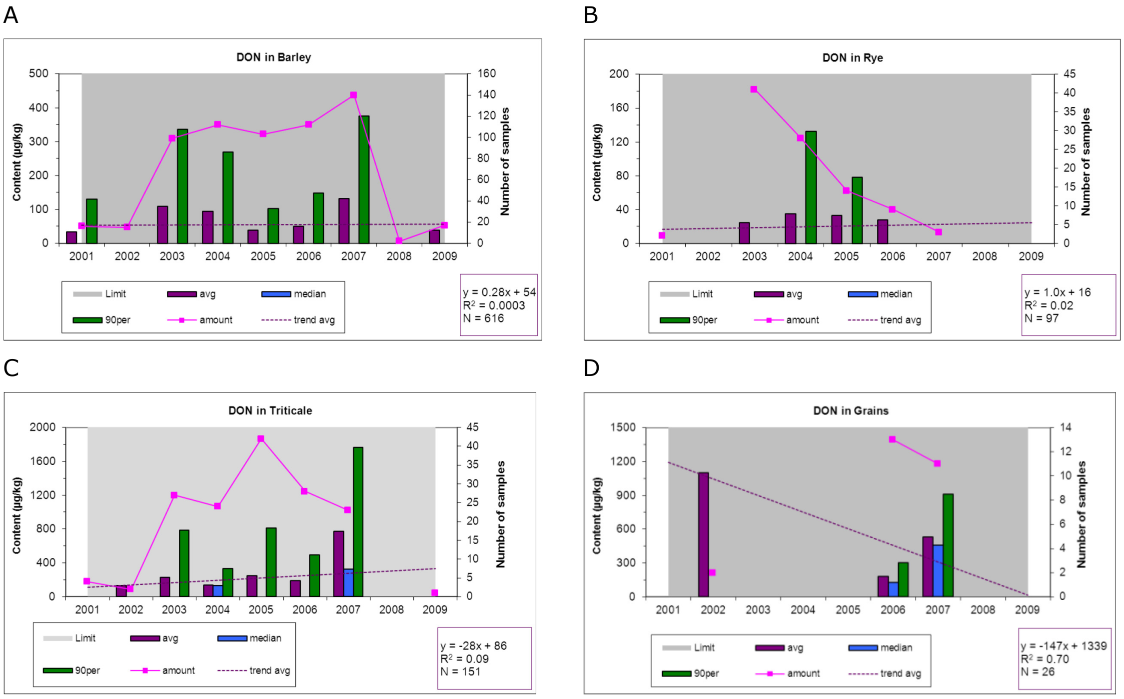


Figure 3.1. DON content of A= Barley ; B= Rye; C= Triticale; D= Grains (unspecified); limit = 8000 µg/kg.

The deoxynivalenol content of wheat (Figure 3.2A) and wheat products (Figure 3.2C) does not increase or decrease significantly in the period 2001-2009. Neither does it approach the limit (guidance value) of 8000 µg deoxynivalenol/kg. However, some individual samples exceeded the limit. The relatively high average in wheat in 2008 is caused by a sample (from an unspecified EU country) with 12900 µg deoxynivalenol/kg. And in 2007 two samples contained 10000 and 8870 µg deoxynivalenol/kg respectively. These samples were registered with the Netherlands as country of origin. The mycotoxin survey from PDV in 2008 (http://www.pdv.nl/nederland/kwaliteit/page3937.php) found no samples (of 19) of wheat exceeding the guidance value of 8000 µg/kg. According to their 2009 report no levels above the guidance value were found in 2009 either (GMP+, 2009).

Several analyses of wheat sampled from Dutch fields showed that the variation in deoxynivalenol levels are highly correlated with variation in temperature and humidity (Waalwijk *et al.*, 2003, Waalwijk *et al.*, 2004, Franz *et al.*, 2009, Van der Fels-Klerx et al, 2010b). The prediction from the models for deoxynivalenol in Dutch winter wheat used by Franz *et al.*, 2009 was confirmed with actual data and showed that with high average postheading temperature, increased precipitation and high relative humidity the deoxynivalenol levels increase. With increased number of hours with postheading temperatures higher than 25°C the deoxynivalenol levels decrease. They also reported that 2007 was characterized by high levels of deoxynivalenol (Figure 3.2B) which coincided with relatively high postheading precipitation, low postheading number of hours with postheading temperatures higher than 25°C and high postheading relative humidity. This corresponds with the two samples from the dataset used for the current report where two samples originating from the Netherlands contained high levels of deoxynivalenol.

Wheat straw is also covered by the guidance limit value of 8000 µg deoxynivalenol/kg. None of the individual samples exceeded this limit (Figure 3.2D). However, there appears to be an increase in average deoxynivalenol content in the 4 years this product was sampled (by PDV).

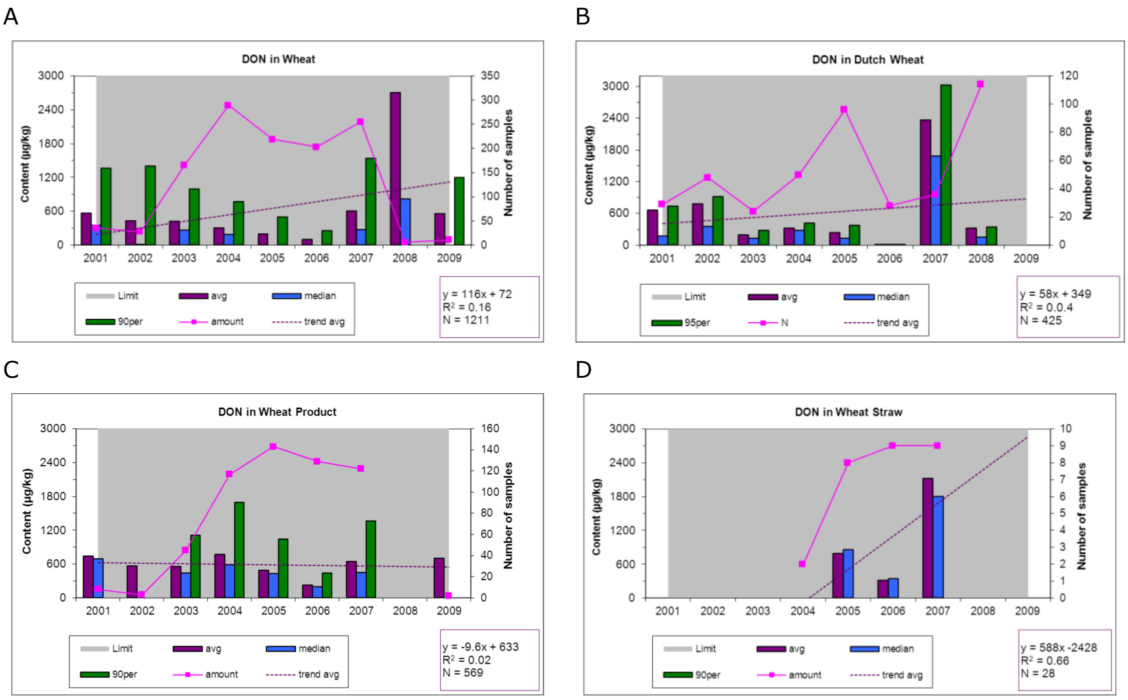


Figure 3.2. Deoxynivalenol content of A = Wheat ; B = Dutch Wheat (data derived from Van der Fels-Klerx et al, 2010b) ; C = Wheat product; D = Wheat straw; limit = 8000 µg/kg.

3.3.3 Zearalenone

The guidance value for zearalenone in cereals and cereal products is 2000 µg/kg. No average values or individual values of the cereal samples analysed are close to this value. The highest values are 750 and 860 µg/kg for two samples from 2005 of wheat middling ('tarwegries in Dutch') and whole wheat, respectively. No increase or decrease in average zearalenone levels are observed.

The amount of samples of whole wheat (Figure 3.3A), triticale (Figure 3.3B), barley (Figure 3.3C) and wheat products (Figure 3.3E) drops significantly in 2008 and 2009. This is caused by the absence of data from PDV in these years. When trends are determined for the period 2001-2007 an increase in average zearalenone content can be observed in barley (Figure 3.3 D) and wheat products (Figure 3.3 F), not in wheat (excluding wheat products, data not shown). However, the levels are well below the guidance value so this trend is probably not relevant. The 2009 report with PDV data (GMP+, 2009) reports that 2 out of 92 samples of (whole) wheat have levels above the guidance value for pigs in 2009 (359 and 373 µg/kg).

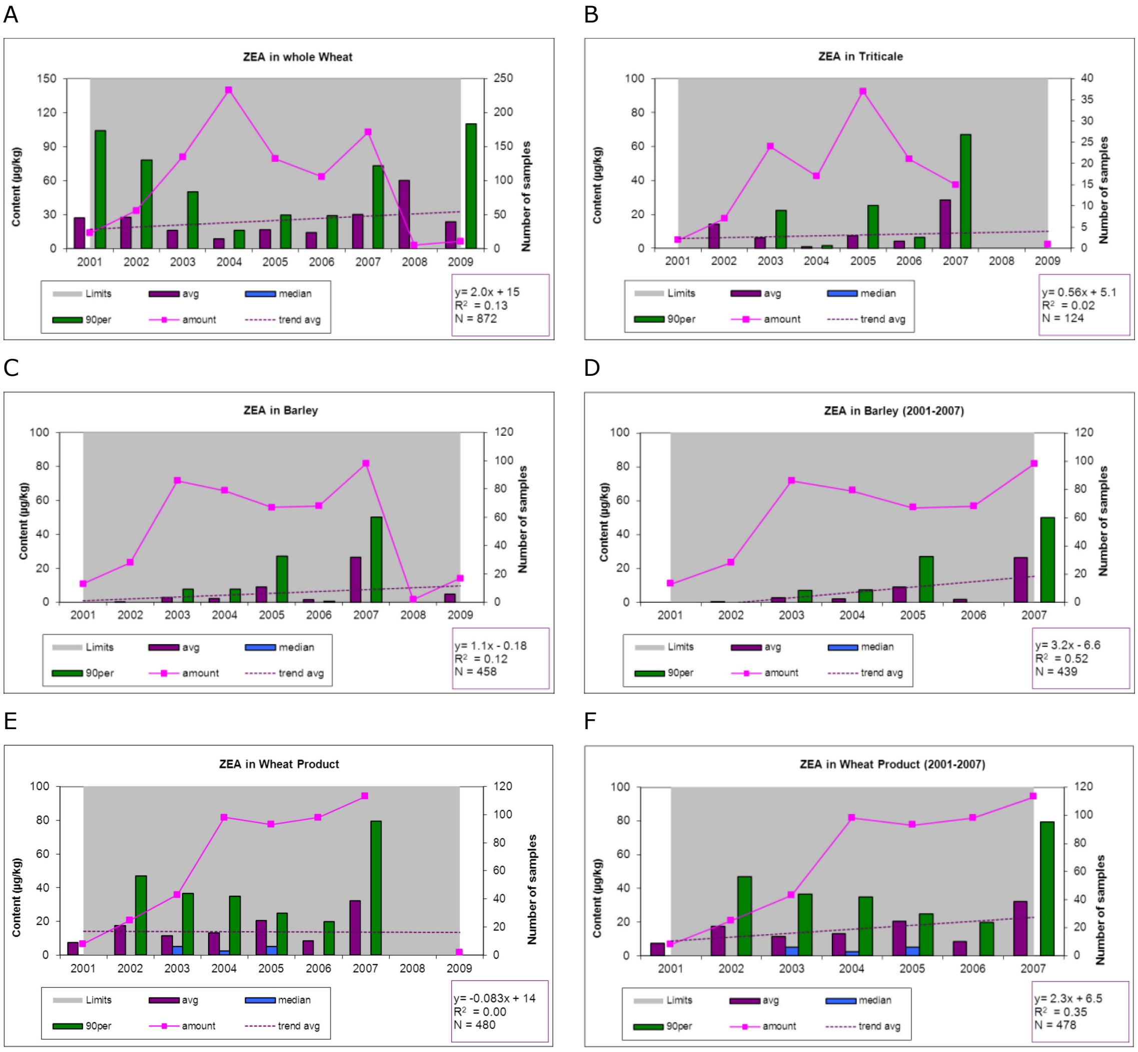
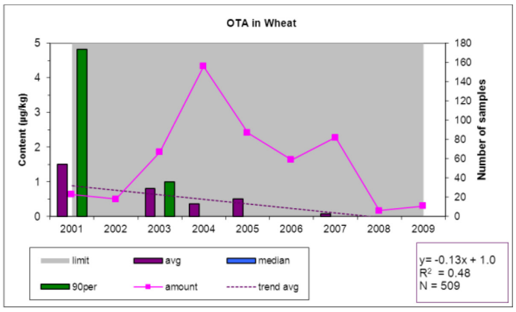


Figure 3.3. Zearalenone content of A = Wheat ; B = Triticale; C = Barley (2001-2009); D = Barley (2001-2007); E = Wheat product (2001-2009); F = Wheat product (2001-2007); guidance value = 2000 µg/kg.

3.3.4 Ochratoxin A

The guidance value for ochratoxin A in cereals and cereal products is 250 µg/kg. The average ochratoxin A levels measured in wheat (Figure 3.4A), wheat products (Figure 3.4B), triticale (Figure 3.4C) and barley (Figure 3.4D) stay well below this limit. No increase or decrease in average ochratoxin A content is observed in wheat product and triticale. Wheat (Figure 3.4A) shows a decrease of the average ochratoxin A content. The amount of samples taken also decreases. In wheat products one individual sample contains ochratoxin A around 50% of the limit: 117 µg/kg. Barley (Figure 3.4D) shows an increase of the average ochratoxin A levels. The highest individual levels are 63.6 (2007) and 100 µg/kg (2009).

A



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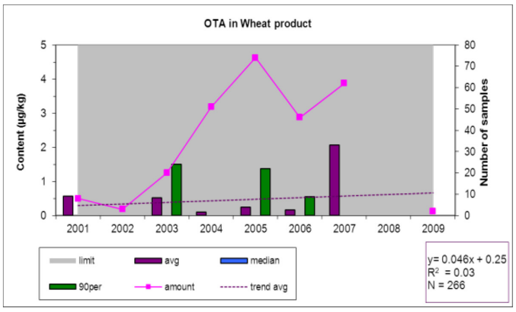

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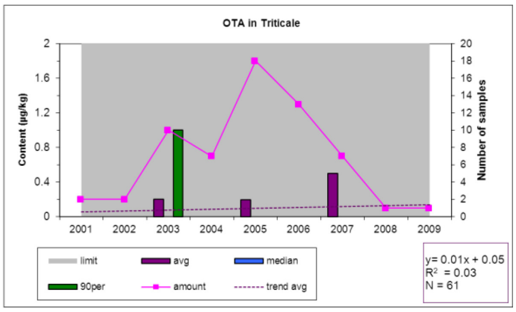

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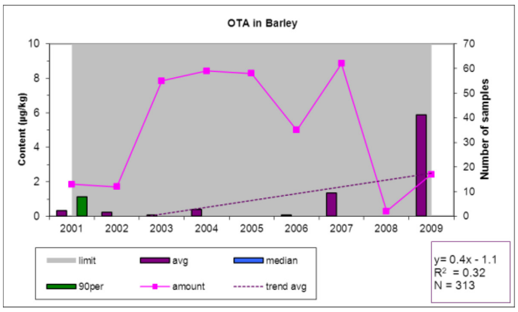

Figure 3.4. Ochratoxin A content of A = Wheat ; B = Wheat product; C = Triticale; D = Barley; guidance value = 250 µg/kg.

3.3.5 Aflatoxin B1

The limit for aflatoxin B1 in cereals and cereal products is 50 µg/kg. None of the products in Figure 3.5 show an increase or decrease of the average aflatoxin B1 content and none of the individual samples of wheat (Figure 3.5A), wheat products (Figure 3.5B), rye (Figure 3.5E) and sorghum (Figure 3.5F) exceed the limit. The average concentrations stay well below this limit. Only in rice (Figure 3.5C) and rice products (Figure 3.5D) individual samples with values close to or exceeding the limit are detected. The four samples of rice from 2005 are relatively high: 9, 11, 14 and 20 µg/kg, but not exceeding the limit. Nineteen samples of rice products (rice bran /rijstevoermeel) are high, ranging from 12 to 74 µg/kg. Four of these samples, all from 2005, (out of 24) exceed the limit with values between 57 and 74 µg/kg. The samples originate (according to the sample registration) from NL, EU or unknown (2). The limit of detection (LOD) varies over the years between 1 or 5 µg/kg. This does not seem relevant for the trends analysed here.

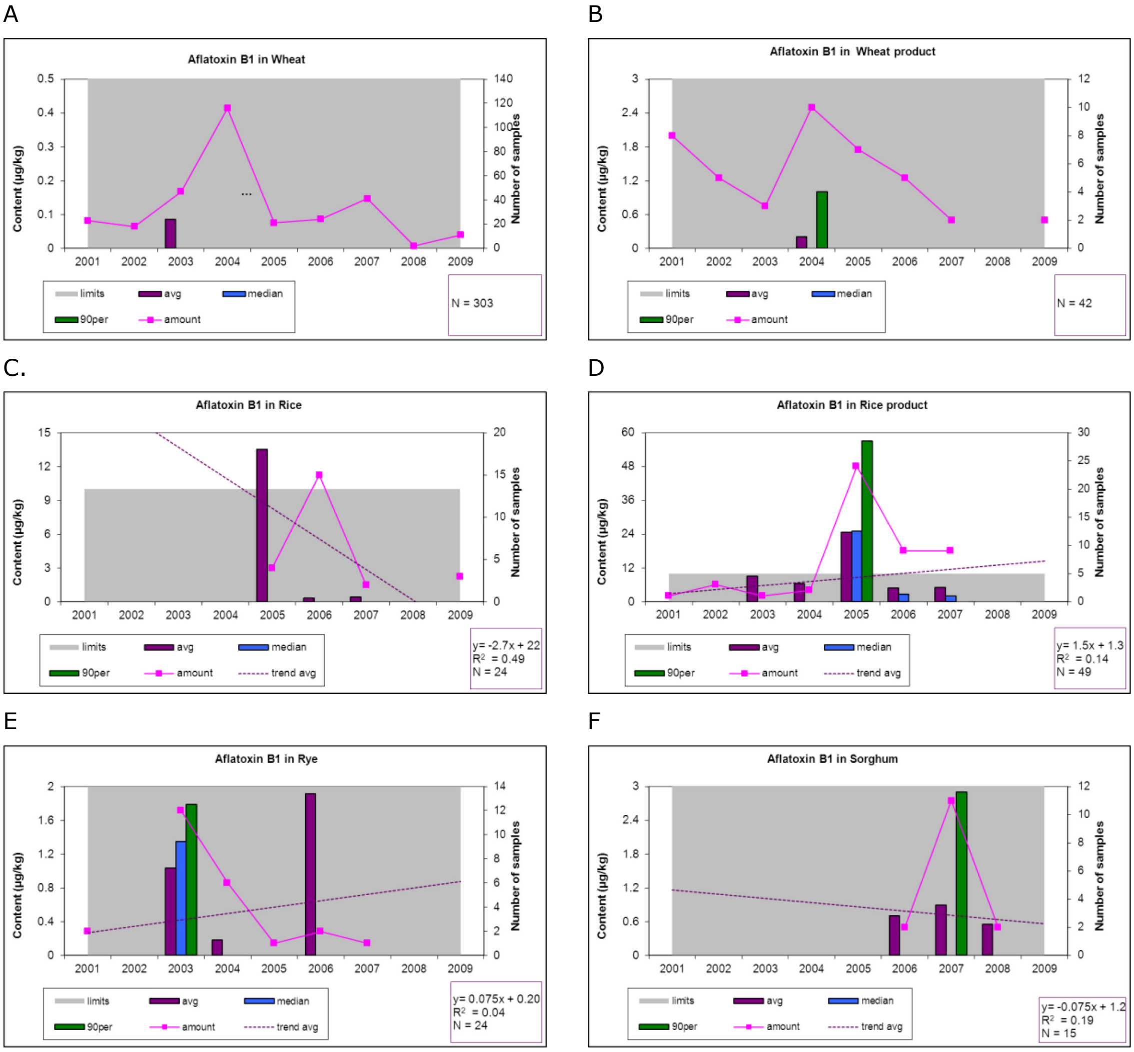

Figure 3.5. Aflatoxin B1 content of A = Wheat ; B = Wheat product; C = Rice; D = Rice product; E = Rye; F = Sorghum; limit = 50 µg/kg.

3.3.6 Fumonisin B1 + B2

There is no guidance value for fumonisin B1 + B2 in cereals and cereal products. Only a few samples of wheat (4 out of 98) and sorghum (2 out of 11) contain fumonisin B1 + B2 above the LOD (0.1 mg/kg). The highest level is 1.4 mg/kg in wheat and 1.9 mg/kg in sorghum. The average fumonisin B1 + B2 content in sorghum (Figure 3.6B) appears to increase but can not be considered relevant with only 11 samples in three years of sampling. Six samples (out of 138) of other cereals and cereal products (data not shown) contain fumonisin B1 + B2 levels between 0.1 and 1.07 mg/kg.

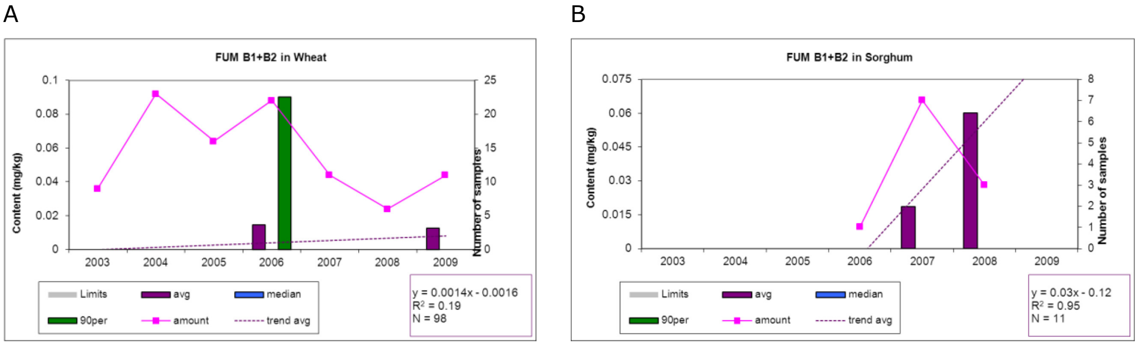

Figure 3.6. Fumonisin B1 + B2 content of A = Wheat ; B = Sorghum; no guidance value.

3.4 Trends in maize and maize products

3.4.1 Preview

Deoxynivalenol levels decrease for maize products, but increase for maize silage. A few individual samples of both maize products and maize silage exceed the guidance value. All samples are from 2007, similar to what was found for wheat. In maize silage the zearalenone levels increase as well. Ochratoxin A levels decrease slightly in maize products whereas aflatoxin B1 increases in maize products. Fumonisin B1 + B2 has only been measured a few times and no relevant increase or decrease can be observed.

3.4.2 Deoxynivalenol

The guidance value (limit) for deoxynivalenol in maize and maize products is the same as for cereals and cereal-products (8000 µg/kg).). There is an additional guidance value for deoxynivalenol in maize by-products (12000 µg/kg), but this product group has not been specified by the EU[1]. Since most individual levels of the different maize (by)products were already below the limit for maize products (8000 µg/kg) and all below the limit for maize by-products (12000 µg/kg) we decided to analyse all maize products together in one group. So in this report the group maize products consists of the products maize gluten feed, maize gluten meal, maize feed flour, maize starch, maize flakes, maize middlings, maize solubles from starch extraction and maize (unspecified). In maize products (Figure 3.7A) the average deoxynivalenol content decreases with no individual samples exceeding the limit. PDV reports one sample (out of 1010) of maize product with 5900 µg/kg in 2009 (GMP$^+$, 2009). The average deoxynivalenol content in maize kernels (fresh kernels and corn cobs) (Figure 3.7B) does not change in the period 2001-2009. However, in 2007 two individual samples of maize kernels (broken and fresh) exceeded the limit for maize and maize products with a content of 8600 and 8200 µg deoxynivalenol/kg respectively. In maize silage (Figure 3.7C) the average deoxynivalenol content increases significantly. In 2007 two individual samples contain 8200 and 9700 µg deoxynivalenol/kg respectively, exceeding the limit for maize and maize products. In 2008 two individual samples contain 6100 and 7300 µg deoxynivalenol/kg respectively, close to the limit.

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[1] For example, COUNCIL DIRECTIVE 96/25/EC on the circulation and use of feed materials defines maize gluten as " Dried by-product of the manufacture of maize starch", whereas the new COMMISSION REGULATION (EU) No 575/2011 defines it as "Product of the manufacture of maize starch".

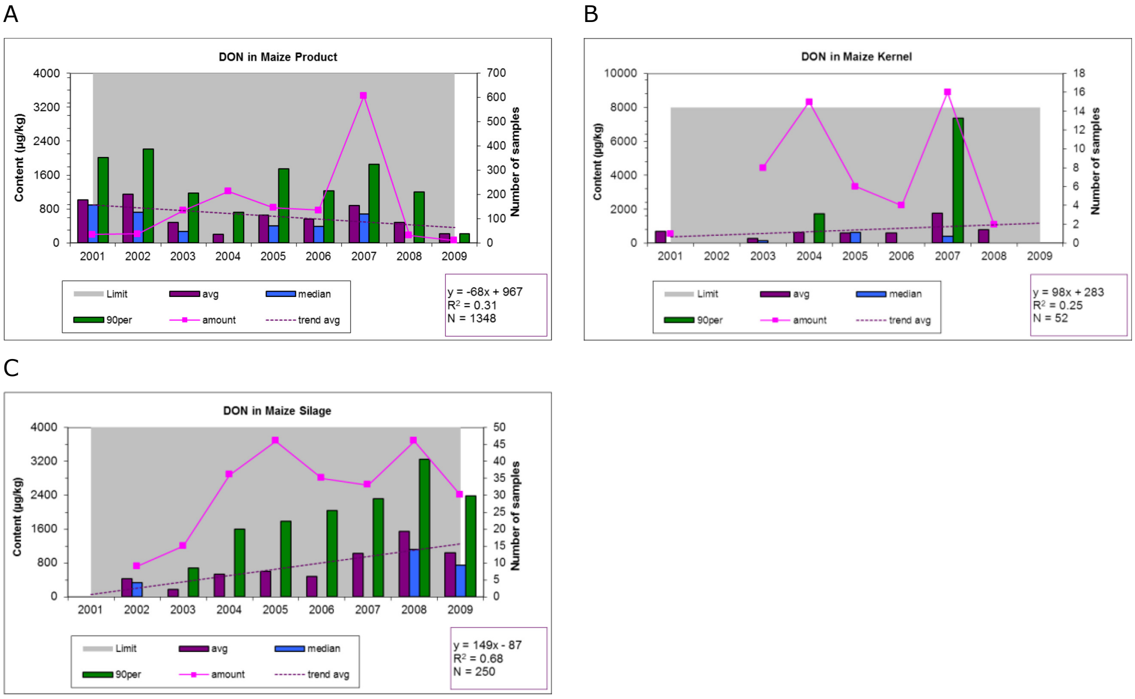


Figure 3.7. Deoxynivalenol content of A = Maize product; B= Maize Kernel; C = Maize Silage; guidance value = 8000 µg/kg (maize and maize products), 12000 µg/kg (maize by-products).

3.4.3 Zearalenone

The guidance value for zearalenone in maize by-products is 3000 µg/kg. This concerns for example by-products obtained from wet milling such as maize gluten feed, maize gluten meal and maize oil. The guidance value for maize and maize products is the same value as for cereals and cereal products: 2000 µg/kg. No average values except one (see below, 2970 µg/kg) exceed this limit. The average zearalenone content in both maize kernels and maize silage increases in the period 2001-2009 (Figure 3.8B and C). In maize products no increase or decrease can be observed (Figure 3.8A). However, in 2007 a few individual maize product samples have high values: seven samples contain 1000 µg zearalenone /kg or more, ranging from 1000 to 2970 µg/kg. In maize kernels one sample from 2007 contains 1400 µg zearalenone /kg and in maize silage five samples (from 2007, 2008 and 2009) contain zearalenone between 1130 and 1300 µg/kg.

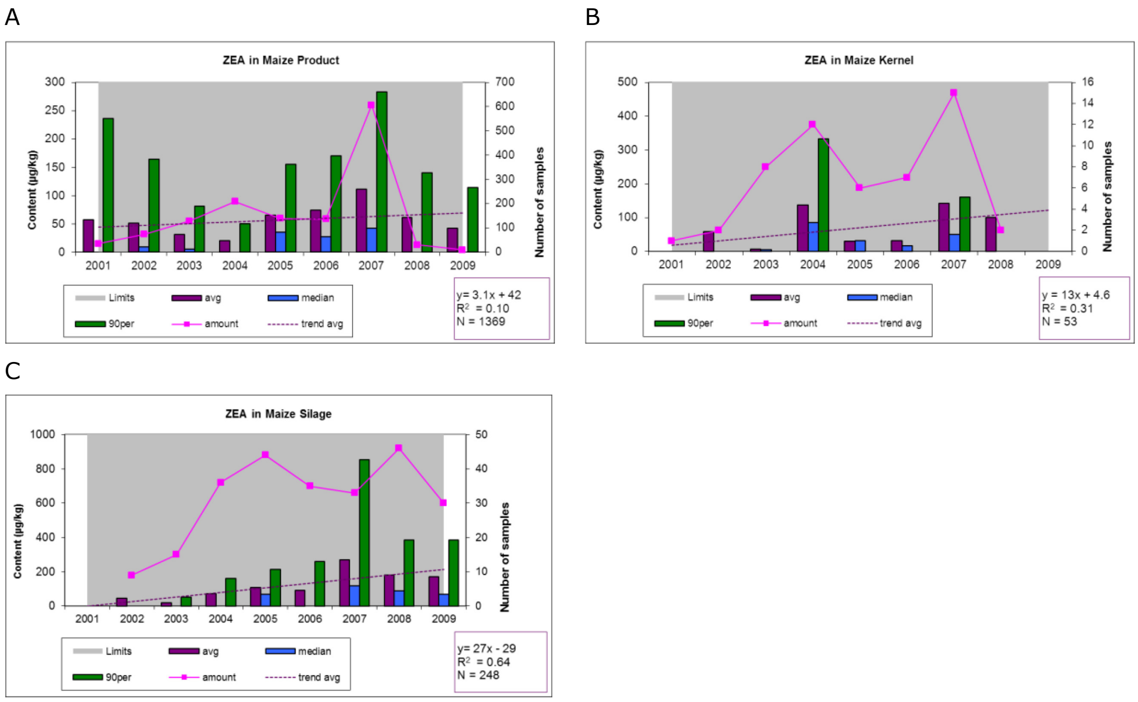


Figure 3.8. Zearalenone content of A = Maize product; B = Maize kernel; C = Maize silage; guidance value = 2000 µg/kg.

3.4.4 Ochratoxin A

The guidance value for ochratoxin in cereals and cereal products (including maize and maize (by-) products) is 250 µg/kg . All average and individual levels remain below this value. Of the 1179 maize-related samples analysed, only 30 contained ochratoxin A levels above the detection limit (LOD). The LOD varies between 1 and 50 µg/kg for samples analysed by RIKILT and between 1 and 20 µg/kg for PDV samples. All of these samples concerned maize products, as shown in Figure 3.9. The individual levels ranged from 1 to 11.4 µg/kg, far below the guidance value of 250 µg/kg. There appears to be a decrease, but because the number of samples in the last two years is very low and the average values are below the LOD this decrease does not seem to be significant. Striking is the large amount of samples taken in 2007. Most of these samples (416 out of 469) originate from PDV.

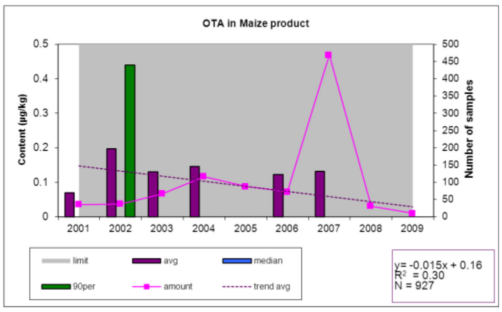


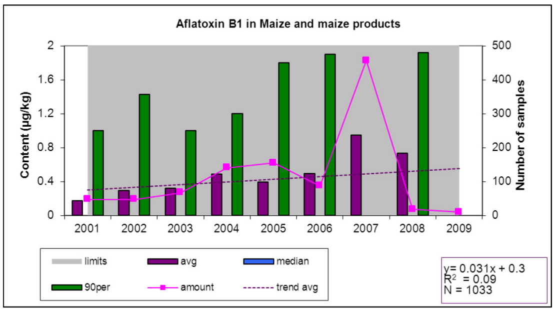
Figure 3.9. Ochratoxin A content of Maize product, complete ; guidance value = 250 µg/kg.

3.4.5 Aflatoxin B1

The limit for aflatoxin B1 in maize and maize products is 20 µg/kg. In maize and maize products (Figure 3.10A) six samples (out of 1033) exceed this limit, all in 2007, with values between 20 and 115 µg/kg. PDV reports for 2009 two samples of maize (from 215) exceeding the limit with 34.7 µg/kg as highest value (GMP+, 2009). The R^2 value of 0.09 indicates that the apparent increase in average aflatoxin B1 content is not significant. When values measured with a LOD of 5 µg/kg or higher are not taken into account (Figure 3.10C), the increase is significant. However, the levels are still very low indicating that this increase might not be very important.

The average aflatoxin B1 content in maize kernels seems to decrease (Figure 3.10B). However, the amount of samples taken is quite low and irregular, which makes it difficult to determine the significance of this observation.

A



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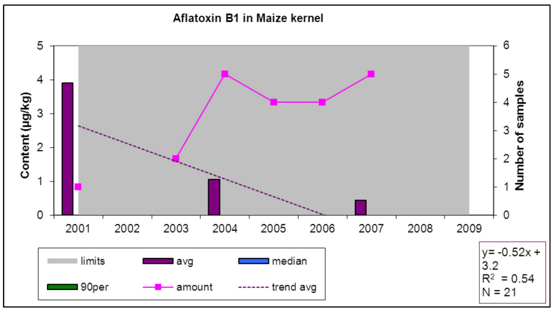

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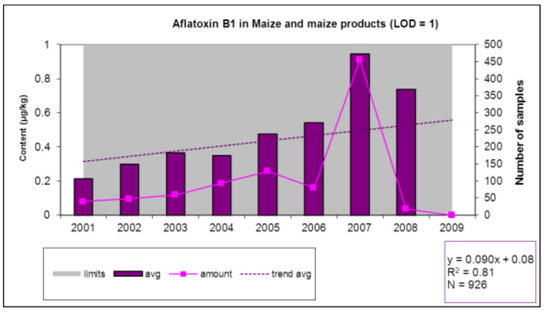

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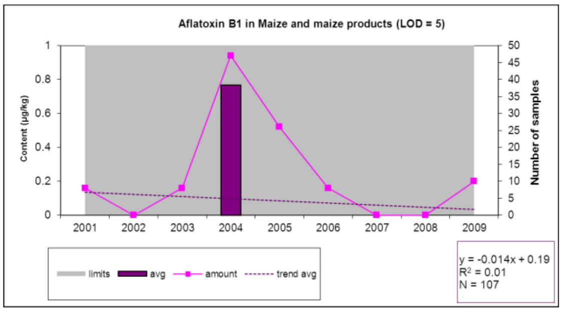


Figure 3.10. Aflatoxin B1 content of A = Maize and maize products; B = Maize kernel ; C = Maize and maize products (LOD 1); D = Maize and maize products (LOD 5); limit = 20 µg/kg.

3.4.6 Fumonisin B1 + B2

The guidance value for fumonisin B1 + B2 in maize and maize products is 60 mg/kg. No increase or decrease of the average fumonisin B1 + B2 content can be observed (Figure 3.11). The average values remain well below the guidance value. Five maize product samples (out of 222) contain fumonisin B1 + B2 levels between 11.7 and 30 mg/kg.

A B

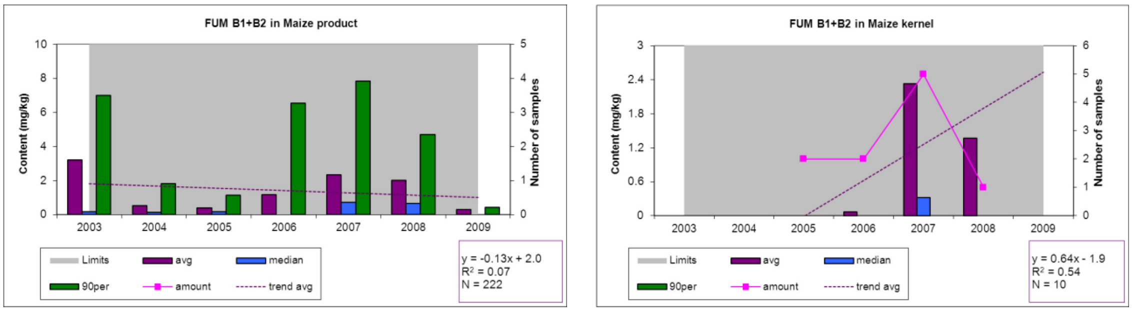


Figure 3.11. Fumonisin B1 + B2 content of A = Maize and maize product; B = Maize kernel; guidance value = 60 mg/kg.

3.5 Trends in oil-containing seeds

3.5.1 Preview

Oil-containing seeds in general do not contain high levels of mycotoxins. Except for aflatoxin B1, there are no limits or guidance values for these products. In sunflower seeds both average and individual aflatoxin B1 levels exceed the limit, especially in 2001 and 2002. A few samples or oil-containing seeds contain levels of mycotoxins, other than aflatoxin B1, that are above the guidance values set for other feeding materials from plants (i.e. cereals), for example zearalenone in soya products.

3.5.2 Deoxynivalenol

There is no guidance value for deoxynivalenol in oil-containing seeds. For comparison, the guidance value for deoxynivalenol in cereal and cereal products is 8000 µg/kg and for maize by-products 12000 µg/kg. Some of the oil-containing seeds have been monitored for deoxynivalenol, with sampling amounts of 5 to 80 per year (Figure 3.12). No increase or decrease of average deoxynivalenol levels can be observed. In 2004 in 5 samples (out of 488) of soya bean expeller/ extracted (Figure 3.12A) the deoxynivalenol content exceeded 1000 µg/kg (from 1018 up to 2714 µg/kg). A few samples of soya bean products (soya bean hulls, 8 out of 255 samples) contain detectable levels of deoxynivalenol, between 50 and 448 µg/kg (Figure 3.12B). Sunflower seed (Figure 3.12C) is monitored during the entire period, but only in 2002 in three samples levels of deoxynivalenol above the detection limit are registered (200, 260 and 530 µg/kg). In 52 samples of sunflower seed expeller/extracted and sunflower oil no deoxynivalenol is detected (results not shown).

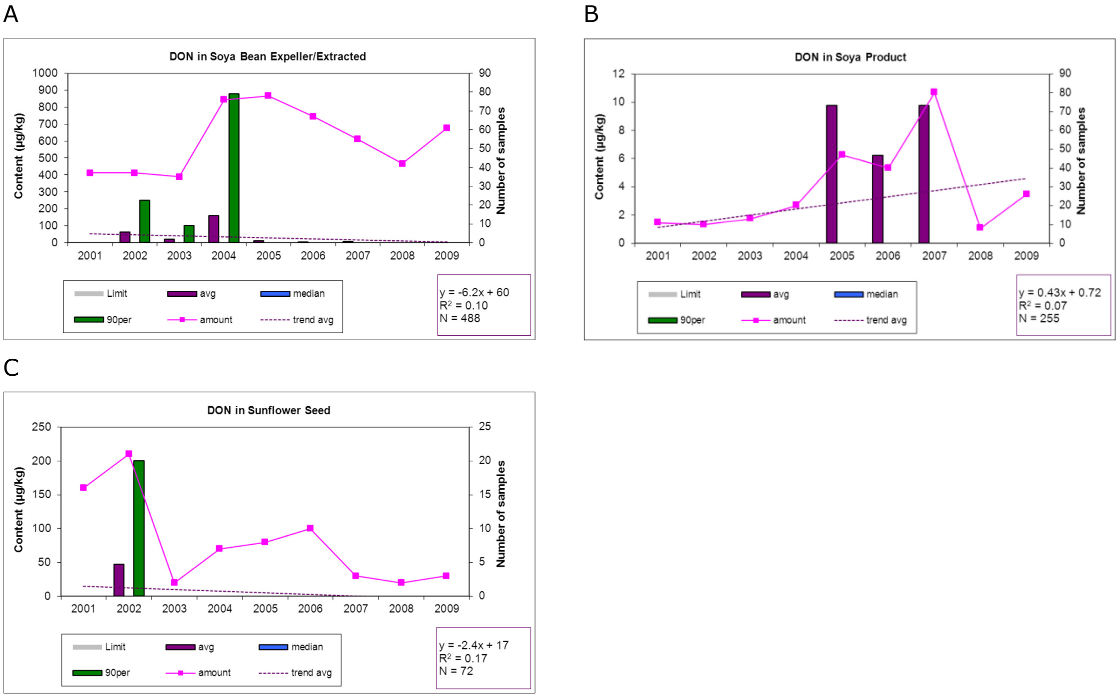
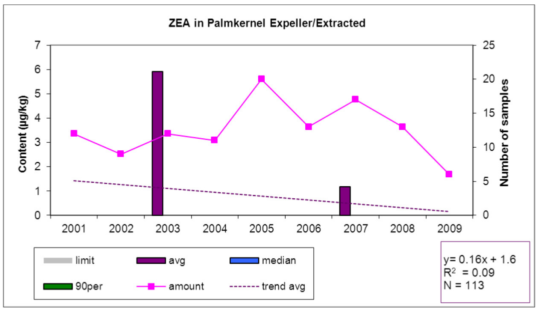


Figure 3.12. Deoxynivalenol content of A = Soya bean expeller/extracted ; B = Soya product; C = Sunflower seed; no guidance value.

3.5.3 Zearalenone

There is no guidance value for zearalenone in oil-containing seeds. For comparison, the guidance value for zearalenone in cereal and cereal products is 2000 µg/kg and for maize by-products 3000 µg/kg. In general the zearalenone levels measured in oil-containing seeds remain below these guidance values. No increase or decrease of the average level can be observed (Figure 3.13). However, in soya products (Figure 3.13C) a sharp increase in the number of samples analysed and in the average zearalenone content can be observed. In 2007 a large number (92) of samples of soya bean hulls (included in product group soya products) are analysed by PDV. In 30 of these samples zearalenone levels ranging from 1000 to 7040 µg/kg are detected. Most of the soya bean hulls samples with high zearalenone content originate from Argentina (Figure 3.13D). For example, 61 of the 109 samples taken in 2007 are from Argentina. Of these 61 samples 25 samples have a zearalenone content of 1000 µg/kg or more. The highest level detected (7040 µg/kg) is from Argentina as well.

A



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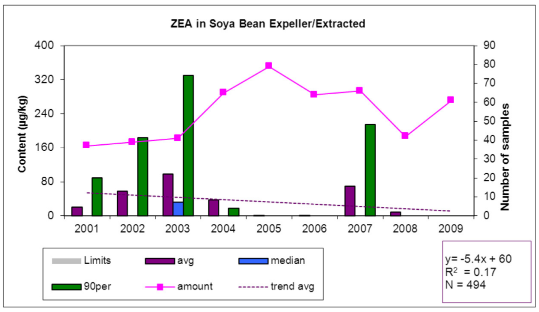

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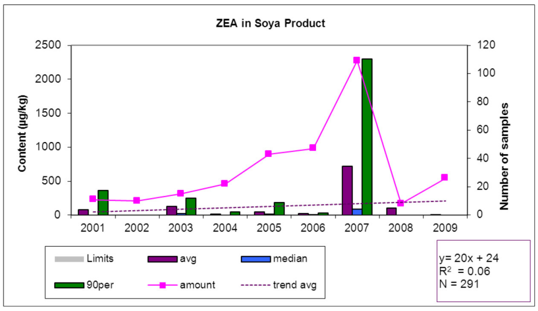

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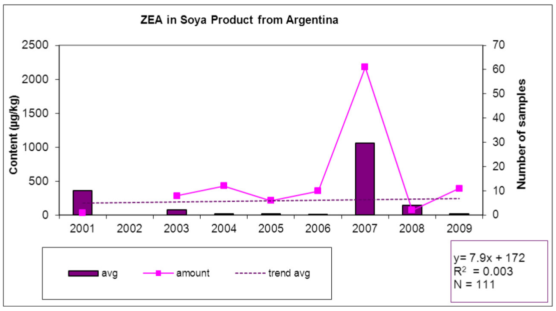


Figure 3.13. Zearalenone content of A = Palm kernel expeller/extracted ; B = Soya bean expeller/extracted; C = Soya product; D: Soya Product from Argentina; no guidance value.

3.5.4 Ochratoxin A

There is no guidance value for oil-containing seeds. Most samples analysed after 2002 or 2003 do not contain ochratoxin A levels above the detection limit (Figure 3.12A-D). In all four product groups this results in a decrease of average ochratoxin A content in the period studied. The individual values are between 1 and 11.7 µg/kg.

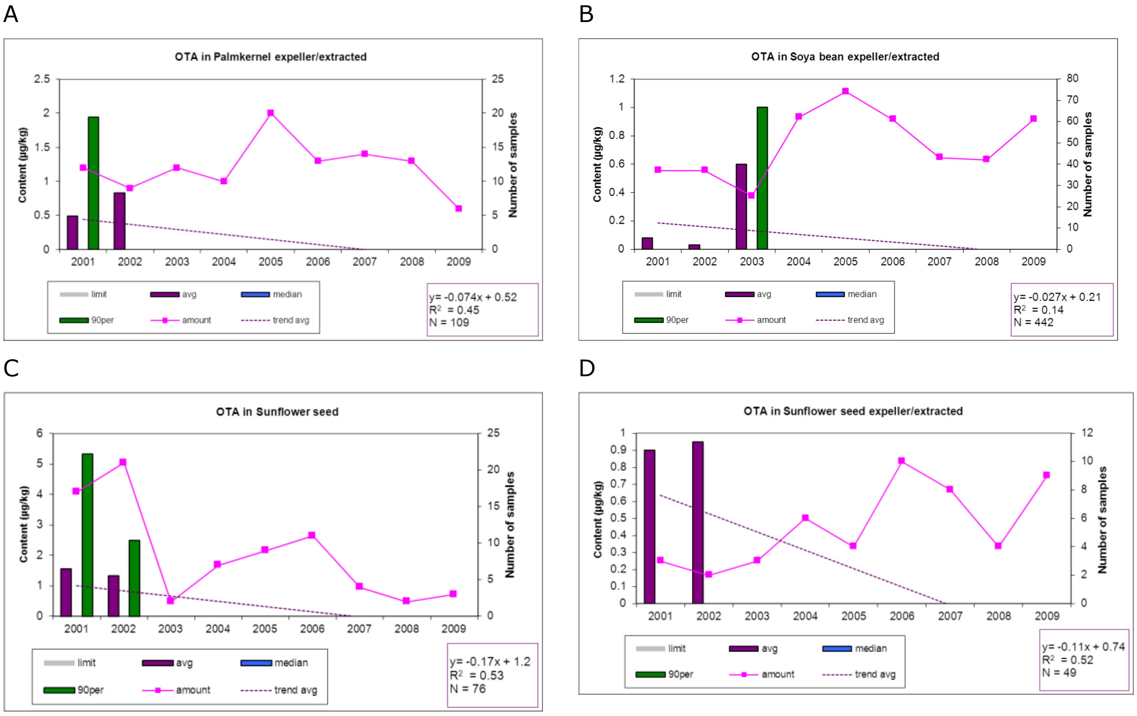


Figure 3.14. Ochratoxin A content of A = Palm kernel expeller/extracted ; B = Soya bean expeller/extracted; C = Sunflower seed; D = Sunflower seed expeller/extracted; no guidance value.

3.5.5 Aflatoxin B1

The regulatory limit for aflatoxin B1 in oil-containing seeds is 20 µg/kg. Neither average or individual aflatoxin B1 levels in palm kernel expeller/extracted (Figure 3.16) exceed this value. No increase or decrease of the average level is observed.

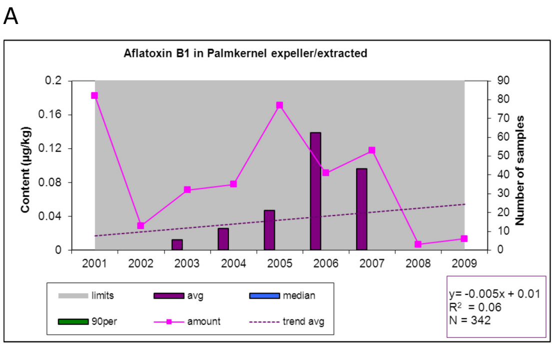
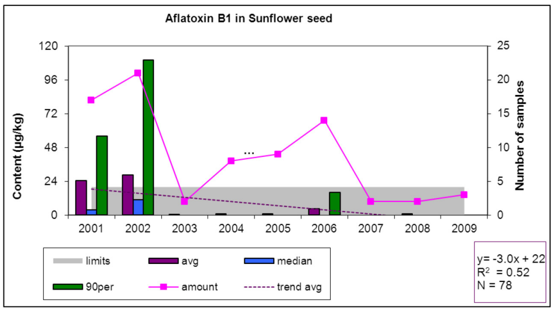


Figure 3.15. Aflatoxin B1 content of A = Palm kernel expeller/extracted ;limit = 20 µg/kg.

In sunflower seeds (Figure 3.16A) both average and individual aflatoxin B1 levels exceed the limit, especially in 2001 and 2002. In 2001 two samples out of 17 exceeded the limit (130 and 240 µg/kg), in 2002 four samples out of 21 (28, 110, 160 and 180 µg/kg) and in 2006 one sample out of 14 (31 µg/kg). Except in 2006 (EU) all samples exceeding the limit originate from Bolivia. The average aflatoxin B1 level appears to decrease, but due the low amount of samples taken after 2002 it is not clear if this trend can be called significant. In a previous report (Adamse, 2007) the aflatoxin B1 content of sunflower seeds appears to be very variable so sunflower seeds with high aflatoxin B1 content could have been missed in the years after 2002 due to the low amount of samples taken.

In sunflower seeds expeller/extracted (Figure 3.16B) no individual samples exceeded the limit. However, there appears to be an increase in the average level.

A



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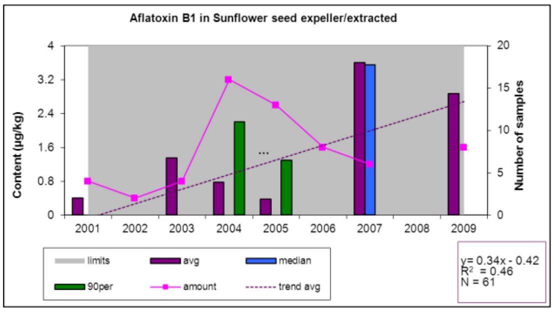
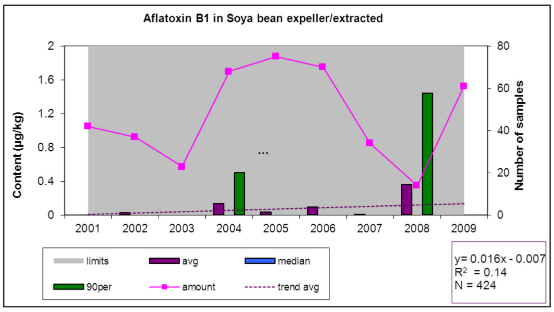


Figure 3.16. Aflatoxin B1 content of A = Sunflower seed; B = Sunflower seed expeller/extracted (all LODs); limit = 20 µg/kg.

In soya bean expeller/extracted no increase or decrease can be observed (Figure 3.17A). There appears to be an increase in the average aflatoxin B1 content when the samples measured wit LOD 1 are analysed (Figure 3.17B). However, no average or individual samples exceed the limit and most values are below 5 µg/kg. This is also the case in other soya products (data not shown). In 2009 none of the 61 samples analysed contained more than 5 µg/kg aflatoxin B1 (=LOD). This suggests that the increase should not be considered relevant.

A



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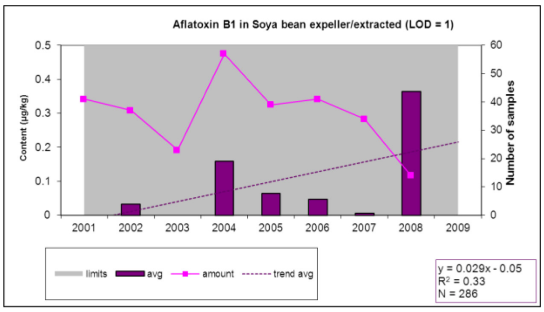

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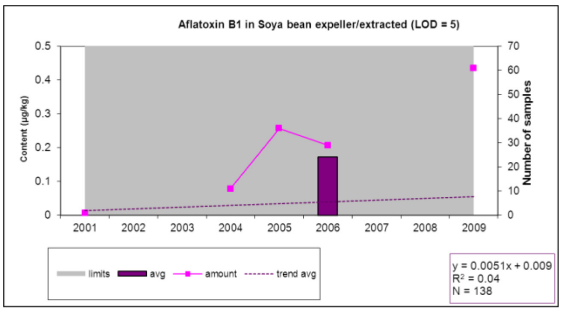


Figure 3.17. Aflatoxin B1 content of A = Soya bean expeller/extracted; B = Soya bean expeller/extracted (LOD 1); C = Soya bean expeller/extracted (LOD 5); limit = 20 µg/kg.

In several other feed materials from plant origin samples are found with aflatoxin levels exceeding the limit. No relevant increase or decrease of the average values can be observed. In cocoa waste (Figure 3.18A) only a few samples are analysed. One of them, cocoa hulls, contains 45 µg/kg. In coconut products (Figure 3.18B) the amount of samples is low as well. After 2006 no samples have been taken. One sample exceeds the limit (54 µg/kg). More samples are analysed of coconut expeller/extracted (Figure 3.18C), but no samples are taken in 2008 and 2009. Of the 401 samples analysed, 72 exceed the limit (18%), ranging from 20.3 to 100 µg/kg. From both groundnut and groundnut expeller/extracted a limited amount of samples is analysed. Of these 153 samples 8 samples exceed the limit, ranging from 22 to 67 and one sample with exceptionally high content of 905 µg/kg in 2008.

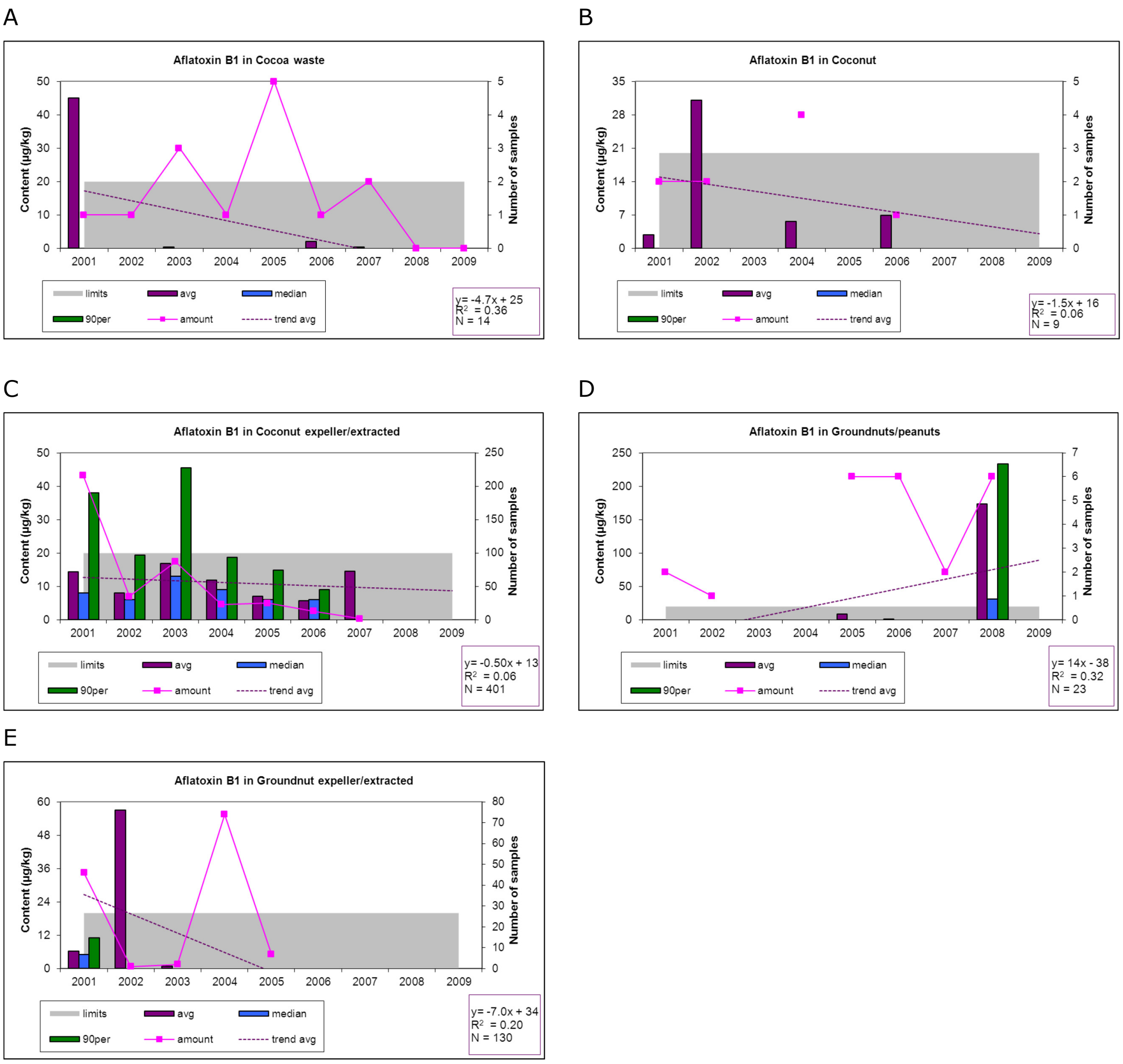


Figure 3.18. Aflatoxin B1 content of A = Cocoa waste; B = Coconut; C = Coconut expeller/extracted; D = Groundnuts/peanuts; E = Groundnut expeller/extracted; limit = 20 µg/kg.

3.5.6 Fumonisin B1 + B2

There is no guidance value for fumonisin B1 + B2 in oil-containing seeds. From 298 samples of soya bean expeller/extracted only four contain fumonisin B1 + B2 levels above the LOD (0.11, 0.11, 0.67 and 0.85 mg/kg respectively). In other oil containing seeds (655 samples, data not shown) only three samples (sunflower seed expeller/extracted, cole-/rapeseed expeller/extracted and linseed expeller/extracted) contain detectable amounts of fumonisin B1 + B2: 0.1, 0.1 and 0.2 mg/kg respectively.

A

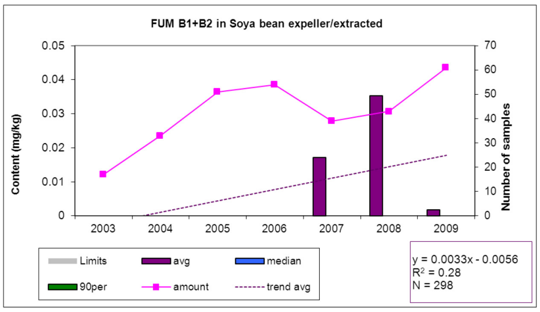


Figure 3.19. Fumonisin B1 + B2 content of A = Soya bean expeller/extracted; no guidance value.

3.6 Trends in compound feed for pigs

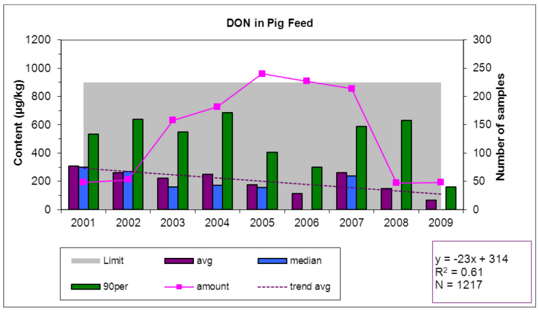
3.6.1 Preview

In general the mycotoxin levels in compound feed for pigs are low. In some individual samples the limits for deoxynivalenol or zearalenone have been exceeded. The average deoxynivalenol content in complete and complementary feed for pigs decreases, but 10% of individual the levels of DON in liquid feed for pigs exceed the limit. The amount of samples taken for this feed is too low to identify trends. The level of fumonisin B1 + B2 in complete feed for pigs increases but is still low.

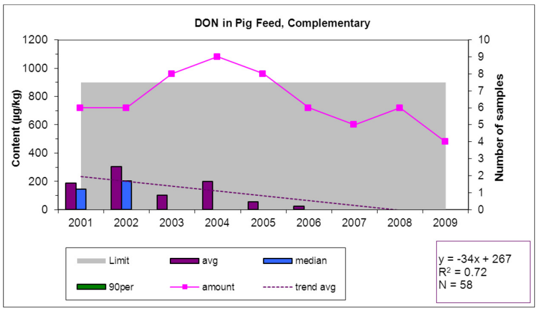
3.6.2 Deoxynivalenol

The guidance value of deoxynivalenol in feed for pigs is lower than the limit for other feeds, i.e. 900 µg/kg. The reason is that pigs are especially sensitive to deoxynivalenol. Both Figure 3.20A and Figure 3.20B show a decrease in the average deoxynivalenol content in complete and complementary feed for pigs. In liquid feed for pigs (Figure 3.20C) the number of years monitored is too low to identify trends. However, individual deoxynivalenol levels exceed the limit in 36 out of 1380 samples - 15 in complete feed (1.2%), 1 in complementary (1.7%) and 10 in liquid feed (10%) - with levels up to 4800 µg/kg in one liquid feed sample.

A



B



C

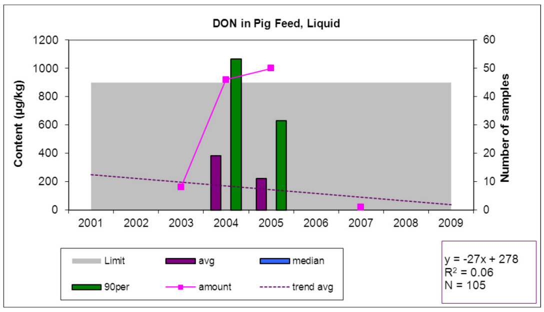


Figure 3.20. Deoxynivalenol content of A = Pig feed, complete ; B = Pig feed, complementary; C = Pig feed, liquid; guidance value = 900 µg/kg.

3.6.3 Zearalenone

The guidance value for zearalenone in compound feed for piglets and gilts (young sows) is 100 µg/kg and for sows and fattening pigs 250 µg/kg. No relevant increase or decrease of the average zearalenone content can be observed (Figure 3.21). The significance of the high R^2 value in Figure D is diminished by the low and variable amount of samples taken. In complete and complementary pig feed (not including feed for piglets, Figure 3.21 A and B) 44 out of 996 samples contain a zearalenone level above 100 µg/kg, the limit for piglets and gilts. Four of these are at or above the guidance value (250, 250, 259 and 290 µg/kg); all of these feeds are intended for sows and fattening pigs. In piglet feed and complementary piglet feed (Figure 3.21 C and D) 13 out of 138 samples contain a zearalenone level above 50 µg/kg (=LOD), two of these samples (from 2003) exceed the guidance value for piglets (120 and 130 µg/kg). One sample (out of 29) of liquid feed for piglets from 2005 (data not shown) contains 190 µg/kg.

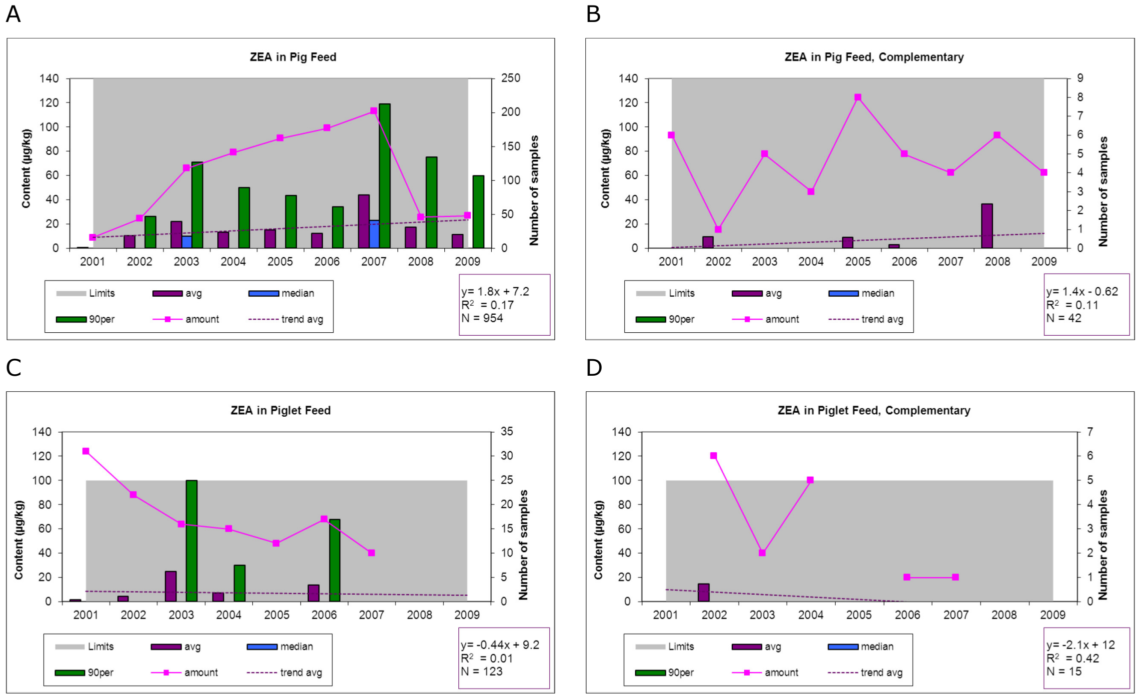


Figure 3.21. Zearalenone content of A = Pig, complete ; B = Pig, complementary; C = Piglet, complete ; D = Piglet, complementary; guidance value = 100 µg/kg (piglets and gilts) or 250 µg/kg (sows and fattening pigs).

3.6.4 Ochratoxin A

The guidance value for ochratoxin A in compound feeds for pigs is 50 µg/kg. The average ochratoxin A content in both complete and complementary feed stays well below this value. No significant increase or decrease of the average ochratoxin A content can be observed. Two individual samples of complete feed contain more than 50% of the guidance value; 28 (in 2006) and 49 (in 2005) µg/kg respectively.

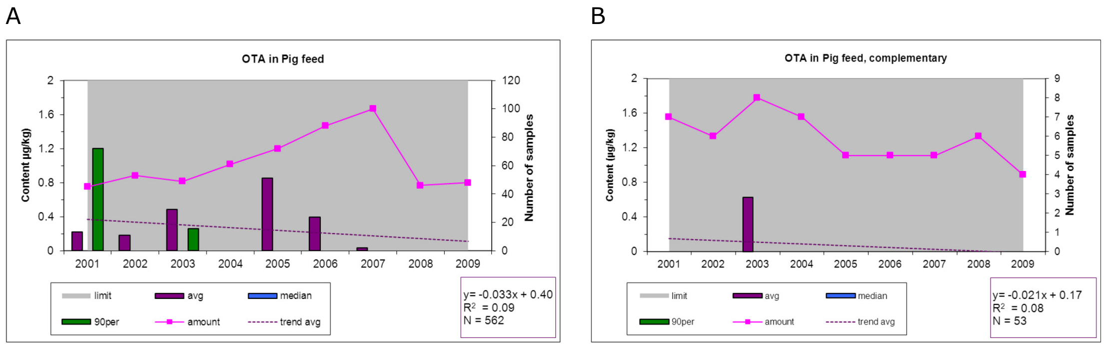


Figure 3.22. Ochratoxin A content of A = Pig, complete ; B = Pig, complementary; guidance value = 50 µg/kg.

3.6.5 Aflatoxin B1

The limit for aflatoxin B1 in compound feed for pigs is 20 µg/kg. Neither the average content or the level in individual samples analysed exceed this limit. Only 10 samples exceed the LOD (1 or 5, depending upon year), but all are 4.2 µg/kg or less. No relevant increase or decrease of the average aflatoxin B1 content can be observed (Figure 3.23).

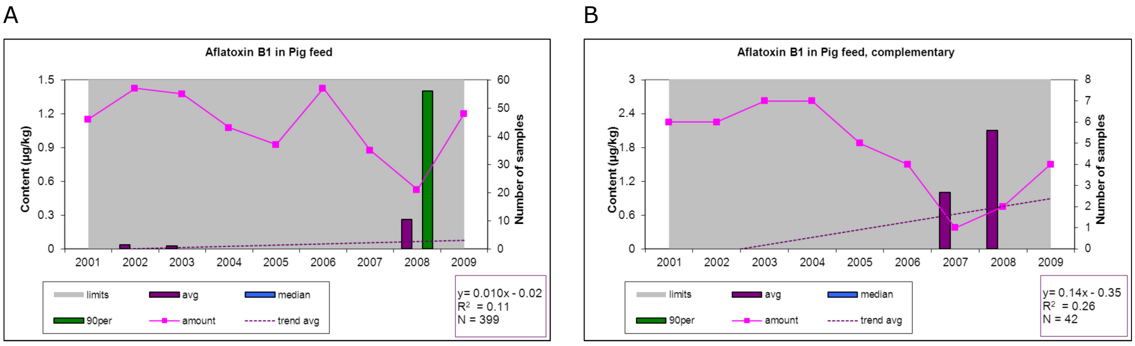


Figure 3.23. Aflatoxin B1 content of A = Pig, complete ; B = Pig, complementary; limit = 20 µg/kg.

3.6.6 Fumonisin B1 + B2

The guidance value for fumonisin B1 + B2 in compound feed for pigs is 5 mg/kg. Out of 430 samples 67 samples contain fumonisin B1 + B2 levels above the LOD. None of them are above de guidance value; the highest value is 2.42 mg/kg (complementary feed, 2008). In complete feed for pigs an increase in the average fumonisin B1 + B2 content can be observed (Figure 3.24A). Of the 46 samples in 2008, 23 were above the LOD, ranging from 0.12 to 1.51 mg/kg. In 2009 the average level decreases again. The amount of samples taken from complementary feed (Figure 3.24B) and liquid feed (Figure 3.24C) is limited and irregular, making it impossible to observe reliable increase or decreases in average fumonisin B1 + B2 content.

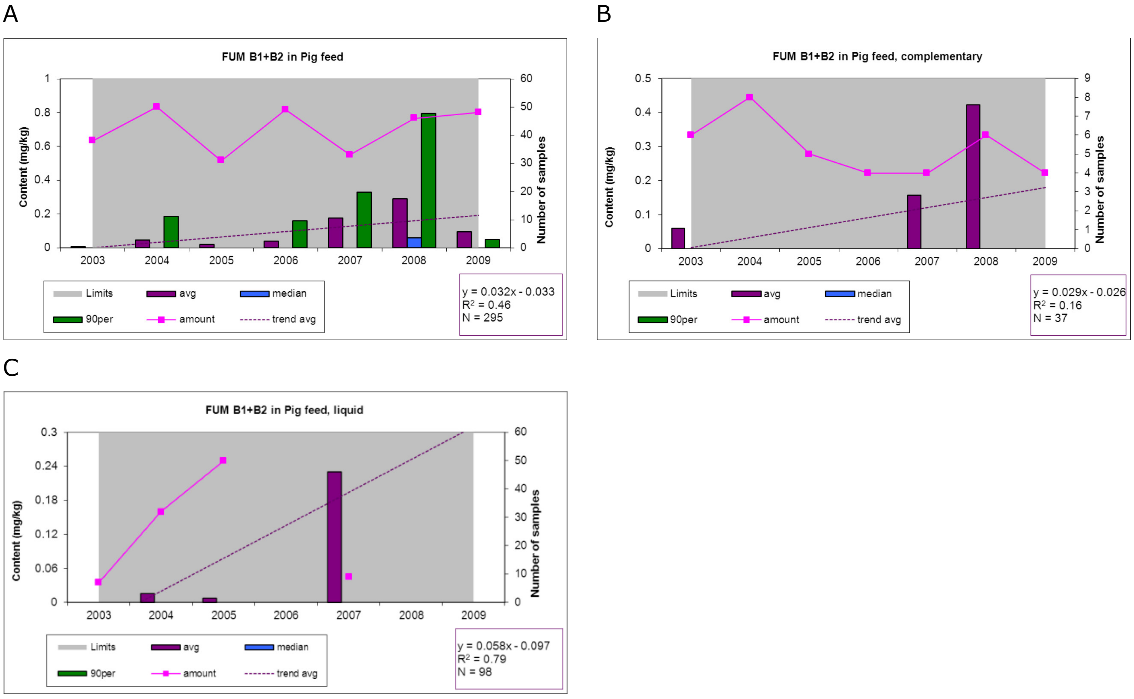


Figure 3.24. Fumonisin B1 + B2 content of A = Pig, complete ; B = Pig, complementary; C = Pig, liquid; guidance value = 5 mg/kg.

3.7 Trends in compound feed for cattle

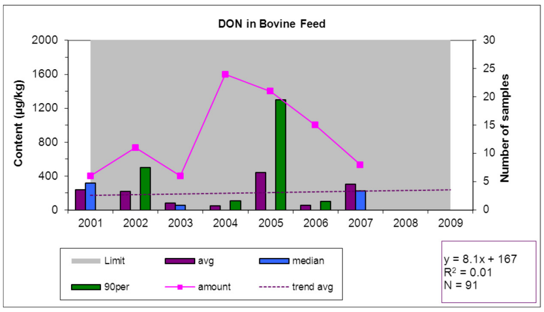
3.7.1 Preview

There appears to be a decrease in the levels of ochratoxin A and aflatoxin B1 in compound feed for cattle. A few individual samples exceed the guideline for zearalenone.

3.7.2 Deoxynivalenol

The guidance value for deoxynivalenol in complementary and complete feed for bovines is 5000 µg/kg, with the exception of calves (< 4 months) where it is 2000 µg/kg. Both in complete (Figure 3.25A) and in complementary (Figure 3.25B) feed the average deoxynivalenol levels do not exceed the lowest limit (set for calves) and do not increase or decrease in the period studied. Looking at individual samples, 11 samples (out of 438) contained more than 1000 µg/kg. One of these samples (in 2005) contained 3000 µg/kg, however, it was identified as general bovine feed, not specifically for calves.

A



B

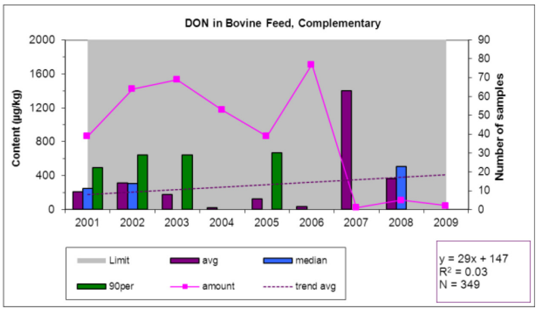


Figure 3.25. Deoxynivalenol content of A = Bovine feed, complete ; B = Bovine feed, complementary; guidance value = 5000 µg/kg (calves 2000 µg/kg).

In complementary feed for ruminants (Figure 3.26A) deoxynivalenol levels are below the limit (5000 µg/kg). No increase or decrease in average levels deoxynivalenol can be observed.

A

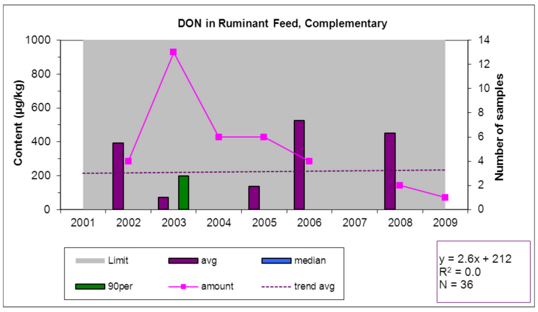
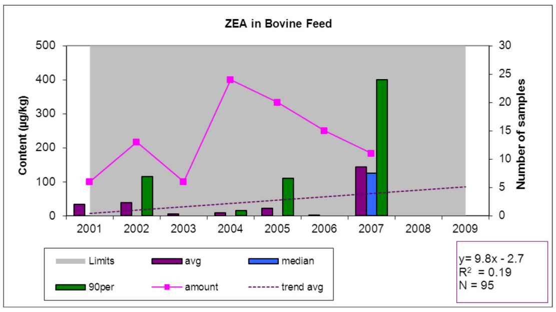


Figure 3.26. Deoxynivalenol content of A = Ruminant feed, complementary ; guidance value = 5000 µg/kg.

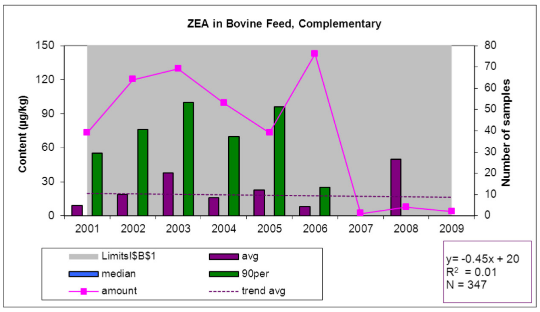
3.7.3 Zearalenone

The guidance value for zearalenone in compound feed for bovines (calves and dairy cattle) is 500 µg/kg. In bovine feed (Figure 3.27A) one individual sample (feed for dairy cattle) exceeds this value; 553 µg/kg, in 2007. No increase or decrease of the average zearalenone content can be observed. In complementary bovine feed (Figure 3.27B) two samples from 2003 contain zearalenone levels close to the limit; 460 and 480 µg/kg, respectively). These samples concern complementary feed for calves. No increase or decrease of the average zearalenone content can be observed. In complementary feed for ruminants (no further specification, potentially for bovines) one sample from 2003 exceeds the guidance value with a zearalenone level of 609 µg/kg. The other values are all below 300 µg/kg. No increase or decrease of the average zearalenone content can be observed.

A



B



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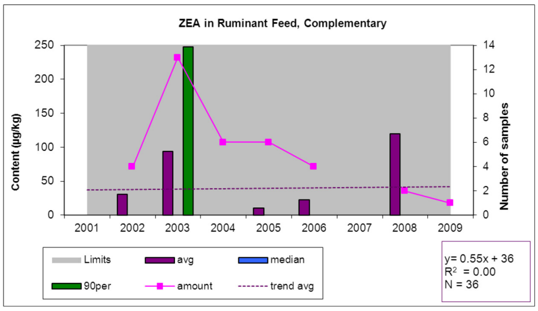
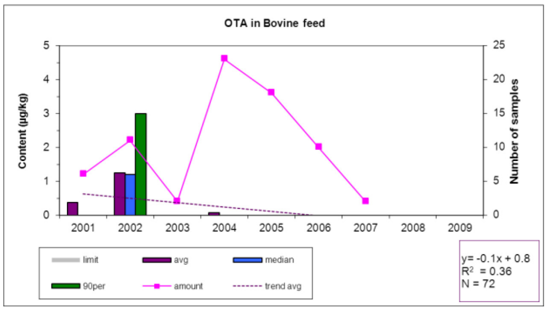


Figure 3.27. Zearalenone content of A = Bovine, complete ; B = Bovine, complementary; C = Ruminant feed, complementary; guidance value = 500 µg/kg (calves and dairy cattle).

3.7.4 Ochratoxin A

There is no official regulatory limit or guidance value for ochratoxin A in compound feeds for bovine animals, only for pigs (50 µg/kg) and poultry (100 µg/kg). The highest level detected in individual samples of compound feed for bovine is 5.4 µg/kg (in 2002). After 2004 no ochratoxin A has been detected. This results in a decrease of the average ochratoxin A content in both complete and complementary feeds. After 2007 no samples of complete feed for bovines have been analysed for ochratoxin A anymore.

A



B

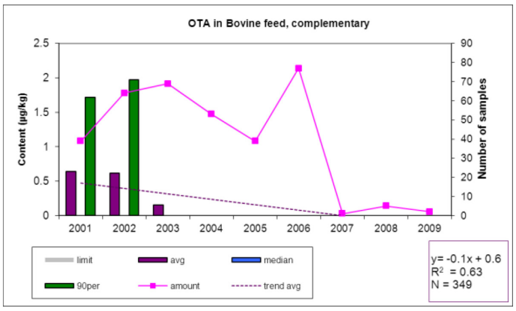
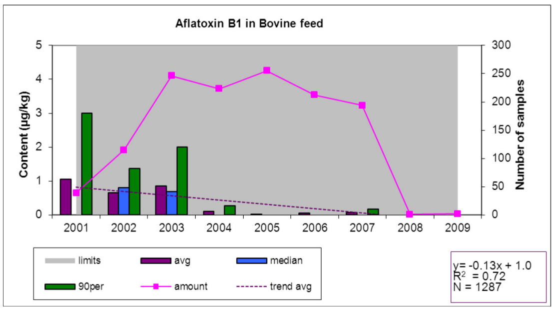


Figure 3.28. Ochratoxin A content of A = Bovine, complete ; B = Bovine, complementary; no guidance value.

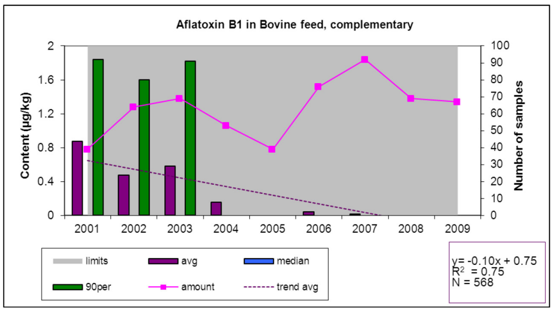
3.7.5 Aflatoxin B1

The limit for aflatoxin B1 in compound feed for bovines ranges from 5 µg/kg (complete feed for dairy cattle), via 10 µg/kg (complete feed for calves), to 20 µg/kg (complete and complementary feed for cattle). No individual or average values exceed 10 µg/kg. Only five samples exceed 5 µg/kg (between 6.8 and 7.5 µg/kg), but this concerns feed for calves, not dairy cattle, so the values do not exceed the limit. In both complete and complementary feed for bovines the average aflatoxin B1 content decreases significantly (Figure 3.29A and B). It should be noted that the amount of samples taken from complete feed for cattle drops significantly after 2007. This is mainly due to the absence of samples from PDV in 2008 and 2009. In the period 2001-2007 1230 of the 1284 samples are analysed by PDV with only a few each year from NP.

A



B



C

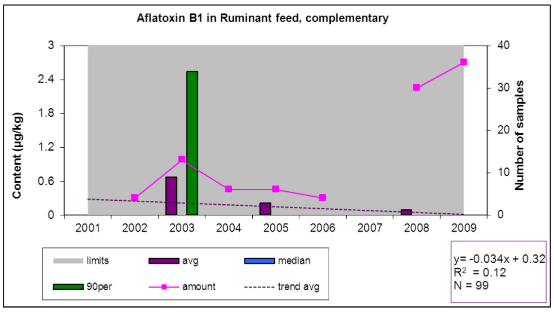
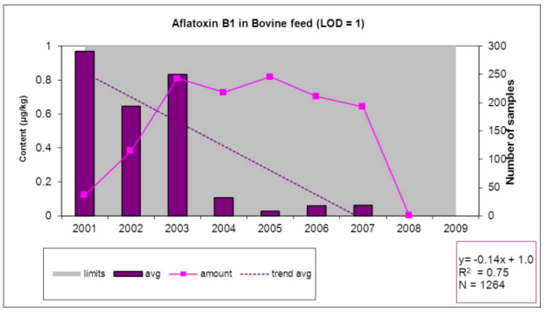


Figure 3.29. Aflatoxin B1 content of A = Bovine, complete ; B = Bovine, complementary (LOD 1); C = Ruminants, complementary (LOD 1); limit = 5 µg/kg (dairy cattle), 10 µg/kg (calves), 50 µg/kg (cattle).

All complementary feed samples are analysed with an LOD of 1 µg/kg (Figure 3.29B and C). If the complete feed samples are divided into groups with equal LOD (Figure 3.30A and B) the decrease found in Figure 3.29A is still relevant for both groups.

A



B

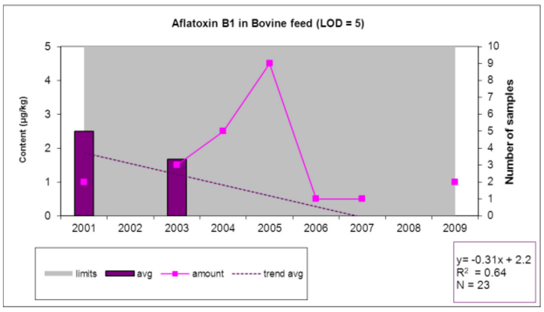


Figure 3.30. Aflatoxin B1 content of A = Bovine, complete (LOD 1); B = Bovine, complete (LOD 5); limit = 5 µg/kg (dairy cattle), 10 µg/kg (calves), 50 µg/kg (cattle).

3.7.6 Fumonisin B1 + B2

The guidance value for fumonisin B1 + B2 in compound feed for bovines (ruminants > 4 months) is 50 mg/kg and for (< 4 months) it is 20 mg/kg. Whereas 121 out of 244 individual samples are above the LOD (0.1 mg/kg) all are below 6 mg/kg. No increase or decrease of the average fumonisin B1 + B2 level can be observed. After 2006 the amount of samples analysed drops sharply, with no samples after 2006 for complete feed (Figure 3.31A) and only nine samples after 2006 for complementary feed (Figure 3.31B). Ruminant feeds are analysed separately because it is not always clear from the sample description if it is intended for bovines or for other ruminant species like sheep. Due to the limited number of samples in Figure 3.31C the decrease cannot be considered relevant. The decrease observed in Figure 3.31D is more reliable and the values are similar to the values from Figure 3.31B.

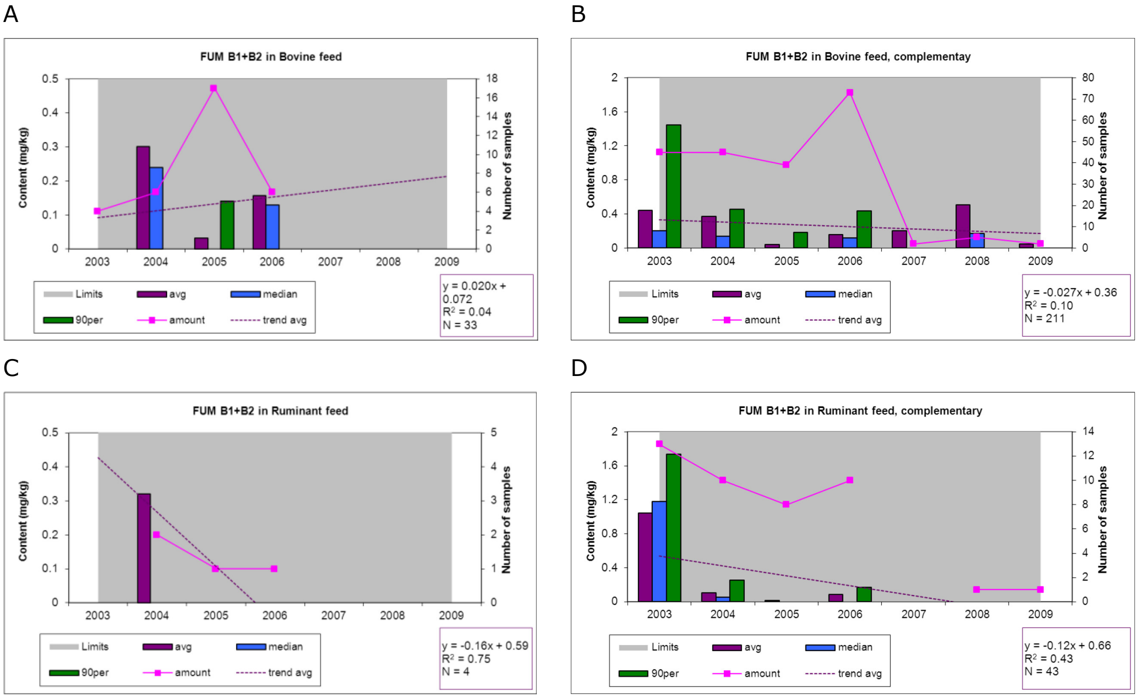


Figure 3.31. Fumonisin B1 + B2 content of A = Bovine, complete ; B = Bovine, complementary; C = Ruminant, complete ; D = Ruminant, complementary; guidance value = 50 mg/kg (> 4 months) or 20 mg/kg (< 4 months).

3.8 Trends in compound feed for poultry

3.8.1 Preview

In general the mycotoxin levels in feed for poultry are low. The levels in individual samples remain below the limit or guidance value. The number of samples taken decreases over the years.

3.8.2 Deoxynivalenol

The guidance value for deoxynivalenol in poultry feed is 5000 µg/kg. No individual samples are found containing more than the guidance value. No increase or decrease of average deoxynivalenol levels is detected in complete poultry feed (Figure 3.32A). The highest deoxynivalenol content measured is 3700 µg/kg in 2007. In complementary poultry feed a decrease can be seen (Figure 3.32B). However, no samples are taken after 2005. The highest deoxynivalenol content measured is 630 µg/kg in 2002, about a factor 10 below the guidance value.

A B

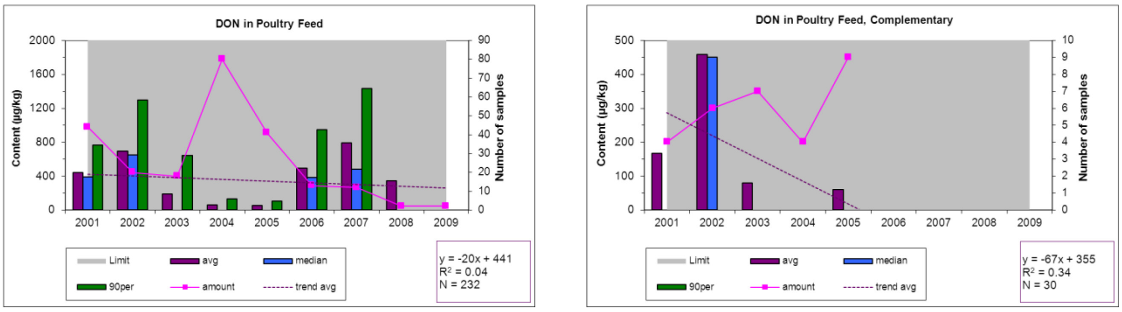


Figure 3.32. Deoxynivalenol content of A = Poultry feed, complete ; B = Poultry feed, complementary; guidance value = 5000 µg/kg.

3.8.3 Zearalenone

There is no guidance value for zearalenone in compound feed for poultry. No relevant increase or decrease of the average zearalenone content can be observed (Figure 3.33). In general the zearalenone levels are below 200 µg/kg with the exception of one sample from 2003 containing 650 µg/kg. The amount of samples analysed drops after 2004, resulting in no samples taken after 2005 from complementary poultry feed (Figure 3.33B) and only a few samples taken each year for complete feed (Figure 3.33A).

A B

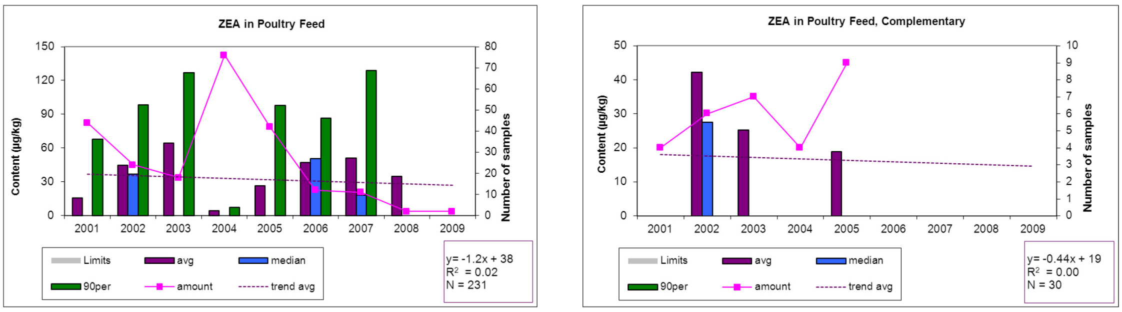


Figure 3.33. Zearalenone content of A = Poultry, complete ; B = Poultry, complementary; no guidance value.

3.8.4 Ochratoxin A

The guidance value for ochratoxin A in compound feeds for poultry is 100 µg/kg. The average ochratoxin A content in both complete and complementary feed stay well below this level. The highest individual level detected is 12 µg/kg (in 2001). After 2003 no ochratoxin A above the detection limit has been detected. No complementary feed has been sampled after 2005 and the amount of complete feed samples drops considerably after 2005.

A B

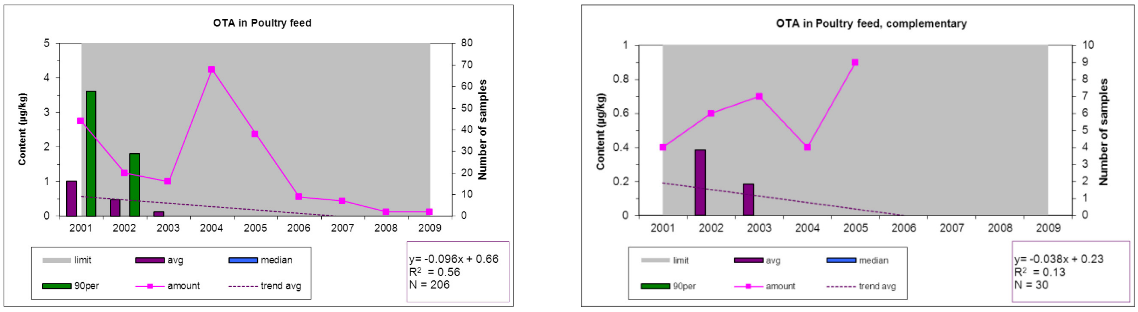


Figure 3.34. Ochratoxin A content of A = Poultry, complete ; B = Poultry, complementary; guidance value = 100 µg/kg.

3.8.5 Aflatoxin B1

The limit for aflatoxin B1 in compound feed for poultry is 20 µg/kg. No average or individual aflatoxin B1 levels exceed this value. No relevant increase or decrease of the average content can be observed in either complete of complementary poultry feed (Figure 3.35A and B).The amount of samples analysed drops sharply after 2005.

A B

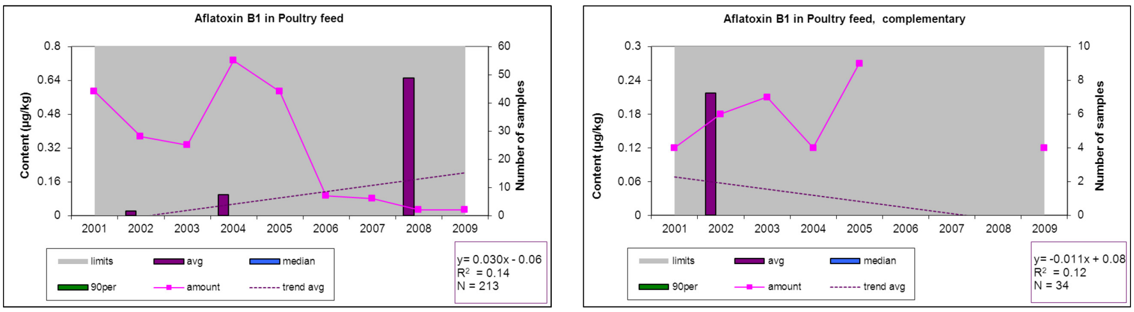


Figure 3.35. Aflatoxin B1 content of A = Poultry, complete ; B = Poultry, complementary; limit = 20 µg/kg.

3.8.6 Fumonisin B1 + B2

The guidance value for fumonisin B1 + B2 in compound feed for poultry is 20 mg/kg. In 33 out of 124 individual samples the fumonisin B1 + B2 content is above the LOD with the highest level (8.1 mg/kg, complete feed, 2005) well below the guidance value. The amount of samples taken is too low and too irregular to determine a significant increase or decrease of the average fumonisin B1 + B2 content (Figure 3.36).

A B

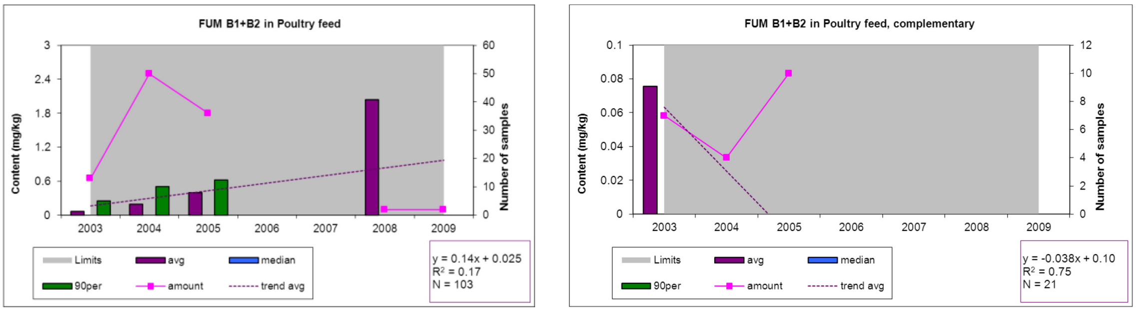


Figure 3.36. Fumonisin B1 + B2 content of A = Poultry, complete ; B = Poultry, complementary; guidance value = 20 mg/kg.

3.9 Trends in compound feed for other animals

3.9.1 Deoxynivalenol

Only a limited amount of samples are available of other animal feeds. In 9 samples of complementary feed for sheep only 3 contained deoxynivalenol concentrations above the limit of detection (LOD). The individual levels are 210, 220 and 370 µg/kg. All of these feeds are intended for lambs. The guidance value for lambs is 900 µg/kg and these individual levels stay well below this limit. One sample of feed for goats (out of 5) contained a measurable deoxynivalenol level of 1300 µg/kg, well below the limit. There is no indication that this feed is intended for young goats (kids, with a limit of 900 µg/kg) implying that the limit for this feed is 5000 µg/kg.

3.9.2 Zearalenone

The guidance value for zearalenone in feed for sheep is 500 µg/kg. None of 17 the samples analysed exceed this value. The highest values measured are 100, 110 and 120 µg/kg in complementary feed (data not shown). There are no samples from feed for other animals analysed.

3.9.3 Ochratoxin A

Only one of the seventeen samples of feed for goats and sheep contains a ochratoxin A level above the LOD (2.3 µg/kg). Four out of 23 samples unspecified compound feed contain levels above the LOD, ranging from 1.3 to 9 µg/kg. The lowest limit for ochratoxin A in feed is 50 µg/kg (pig feed). None of these values are approaching this value.

3.9.4 Aflatoxin B1

The limit for aflatoxin B1 in feed for sheep and goats is 20 µg/kg and 10 µg/kg for lambs and goat kids. None of the 30 samples analysed exceed these values (data not shown). Only three values exceed the LOD (2.1, 2.5 and 2.6 µg/kg). Four samples of feed for horses are analysed, all of them below the LOD. Six samples of compound feed can not be contributed to a specific animal.

Two of these samples contain 24.3 μg/kg. This exceeds the regulatory limit for feed for pigs, bovines, sheep and poultry (20 μg/kg).

3.9.5 Fumonisin B1 + B2

There are guidance values for fish (5 mg/kg), horses, rabbits and pet animal (5), lambs (20) as well, but the amount of samples analysed for these species is too low (ranging from 0 to 4 samples) to warrant a figure. Furthermore, no individual values above the guidance values are present for any of these species.

4 Discussion en conclusions

4.1 Feed materials from plant origin

In the previous report about analysis of aflatoxin B1 in feed (Adamse *et al*., 2007) it was recommended to evaluate the influence of weather and climate on mycotoxins in feed. The current study shows that in 2007 more samples are found with high levels of deoxynivalenol. This is observed by others as well; increased levels of deoxynivalenol in Dutch winter wheat are highly correlated with variation in temperature and humidity (Waalwijk *et al*., 2003, Waalwijk *et al*., 2004, Franz *et al*., 2009, Van der Fels-Klerx et al, 2010b). The predicted climate change with more high extremes in humidity and temperature will probably cause a shift in the dominance of certain *Fusarium* species (i.e. a change in the *Fusarium* profile) which will also lead to a shift in the relative contribution of deoxynivalenol, zearalenone and HT-2/T-2 toxins. Van der Fels-Klerx (unpublished data) observed this in winter wheat from the Netherlands.

In most cereals or cereal products no increase or decrease of the average levels of mycotoxins is observed. Wheat straw (deoxynivalenol) and wheat products (zearalenone), barley (zearalenone, ochratoxin A) and maize silage (deoxynivalenol, zearalenone) show a slight increase. Some individual samples exceed the limit, but in general levels are well below the regulatory limit. In rice and rice products levels above the limit for aflatoxin B1 have been found, especially in 2005. However, the amount of samples taken each year is very low and in 2008 and 2009 there are no samples. It is recommended to continue monitoring this product regularly.

The amount of samples of whole wheat, triticale, barley and wheat products drops significantly in 2008 and 2009. This is caused to a large extent by the absence of data from PDV in these years. When trends are determined for the period 2001-2007 an increase in average zearalenone content can be observed in barley and wheat products, not in wheat (excluding wheat products). However, the levels are well below the guidance value so this trend is probably not relevant.

In the previous trend analysis report (Adamse *et al*., 2007) the trend in average aflatoxin B1 content between 1990 and 2005 has been analysed. A significant decrease in aflatoxin B1 in maize and maize products (combined) is reported. However, this increase was not significant when only the period between 2000 and 2005 was taken in to account. The current report shows an increase of the aflatoxin B1 content of maize products in the period 2001-2009. However, the levels are still very low, indicating that the increase might not be very important.

Oil-containing seeds in general do not contain high levels of mycotoxins. Except for aflatoxin B1, there are no regulatory limits or guidance values for these products. A few samples contain levels above the limit for other feeding materials from plants (cereals), for example deoxynivalenol (soya bean expeller/extracted) and zearalenone (soya products).

In sunflower seeds both average and individual aflatoxin B1 levels exceed the limit, especially in 2001 and 2002. As already reported in the previous trend analysis report (Adamse *et al*., 2007) there is a large variation in aflatoxin levels in sunflower seeds. The average aflatoxin B1 level in sunflower seed expeller/extracted increases in the time period studied. It was recommended to keep monitoring this product regularly, but the amount of samples taken each year continues to be low, also after 2007.

Coconut has been identified in the previous trend analysis report (Adamse *et al*., 2007) as a product with occasionally high levels of aflatoxin B1. In the current report 18% of the samples from coconut expeller/extracted exceed the limit. However, no samples have been analysed after 2007 which makes it impossible to determine whether this fluctuation of levels continues.

Groundnut occasionally high levels of aflatoxin B1 are found, above the regulatory limit. However, sampling of this product has not occurred every year and at low levels. It is recommended to sample more and more regularly when groundnuts continue to be used as animal feed.

In the previous trend analysis report (Adamse *et al*., 2007), analysing the period between 2000 and 2005, soya bean products with high zearalenone levels often originate from Argentina. In the current report this period has been extended to 2009. In this period samples with high zearalenone content, especially in 2007, originate from Argentina as well.

4.2 Compound feeds

Pigs are especially sensitive to mycotoxins. In general the mycotoxin levels in compound feed for pigs are low. In some individual samples the guidance values for deoxynivalenol or zearalenone have been exceeded. The average deoxynivalenol content in complete and complementary feed for pigs decreases. In liquid feed for pigs the number of years monitored is too low to identify trends. However, in several individual samples deoxynivalenol levels exceed the limit which indicates that more samples of liquid feed for pigs should be taken. The level of fumonisin B1 + B2 in complete feed for pigs seems to increase but is still low.

There appears to be a decrease in the levels of ochratoxin A and aflatoxin B1 in compound feed for cattle. A few individual samples exceed the guideline for zearalenone. The amount of samples taken decreases as well. Considering the low amounts of mycotoxins detected in compound feed for cattle this should not be a problem. Especially since the main staple food for cattle are forages and roughages. However, the compound feeds should continue to be monitored with a minimum amount of samples since exceedances of the limits and guidance values do occur occasionally.

In general the mycotoxin levels in feed for poultry are low. The levels in individual samples remain below the regulatory limit or guidance value. The amount of samples taken decreases. Considering the low levels found it should not be a problem when only a minimum amount of samples is taken each year.

4.3 General

Using methods with a relatively high LOD could result in missing a trend at low levels. This can be seen for aflatoxin B1 in maize and maize products and in bovine feed. Analysing all measurements with either an LOD of 5 µg/kg or LOD of 1 µg/kg no significant trend could be detected. However, when measurements with an LOD of 5 µg/kg are excluded from the trend analysis the trend becomes significant. Apparently the trend occurs in the low values. If trends at low levels are considered important, methods with low LODs should be used.

5 Recommendations

5.1 Sampling strategy

Increase amount of samples for:

- - cereals and cereal products, especially in years with high humidity and/or temperature
- - maize silage
- - liquid feed for pigs (high deoxynivalenol and zearalenone levels)

Keep amount of samples at a minimum level:

- - maize and maize products
- - oil-containing seeds
- - compound feed for pigs, cattle and poultry

Since 2008 no samples have been included from PDV. It is recommended to gather more samples, especially from the industry. This will give a more representative indication of the trends in Dutch animal feed.

5.2 Trend analysis

Use additional statistical test to improve the trend analysis. For example with the Mann-Kendall test and/or Sen's slope estimator trends do not need to be adjusted for outliers and it is advised to use these statistical tests in future when enough years are included in the dataset, 4 and 10 years respectively. These tests have been used in the trend analysis report for dioxins and dioxin-like PCBs in feed (Schoss and Adamse, 2011).

Analyse the trends in ergot alkaloids in feed. EFSA carried out a risk assessment on ergot alkaloids as undesirable substance in animal feed in 2005. The Panel on Contaminants in the Food Chain recommended that data on the variability of the ergot alkaloid patterns in feed materials should be collected in Europe and that validated analytical methods for the quantification of the major ergot alkaloids in feed materials should be developed (EFSA 2005).

Use the model recently developed by RIKILT (unpublished) to select products and/or countries of origin that are identified as potentially high risk and analyse trends in these products and countries.

5.3 New ideas

If more up-to-date information about trends is required it is advised to investigate the possibility of presenting updated trend analyses of important compounds and products on a regular basis (more times a year) using an online system.

With specific statistical tests it is possible to get more insight into the minimum amount of samples needed for certain feed groups. These amounts are related to the amount of feed available and the range and distribution of the contaminant concentrations found. It is recommended to perform this test in a future project to improve the effectiveness of the risk-based sampling.

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