# Newsletter

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#### > SUMMARY

Over the years, scientists involved in the field of mycotoxins have worked on the development of quicker, more reliable and more economical analytical methods which would enable the identification of a wider range of mycotoxins. As a result of that research, a multimycotoxin analytical method is now available that allows one commodity or feed



is now available that allows sample to be tested within 45 minutes. Instead of just a handful of mycotoxins, an accurate and validated analytical *liquid chromatography–mass spectrometry* (LC-MS/MS) is able to identify more than 320 different mycotoxins, including masked or conjugated mycotoxins not detected by routine analytical methods. Although this achievement represents a great step in terms of mycotoxin research, its practical application in the field and on a daily basis is still far from possible as scientists are still working to understand the impact of those previously unknown and invisible fungal metabolites in animals. While further information on these mycotoxins is still unavailable, it is our duty to focus on those mycotoxins for which information is available, which are the well-known aflatoxins, zearalenone, deoxynivalenol, fumonisins and ochratoxin A.

Enjoy reading!

Inês Rodrigues & Karin Nährer

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# Mycofix Broduct line

## **BIOMIN Mycotoxin** Survey Program 2012

From January to December 2012, a total of 4,023 samples collected worldwide were analysed for the presence of mycotoxins. In total, 14,468 analyses were carried out for the most important mycotoxins in terms of agriculture and animal production - aflatoxins (Afla), zearalenone (ZEN), deoxynivalenol (DON), fumonisins (FUM) and ochratoxin A (OTA). In addition to these mycotoxins, European samples were analysed for T-2 toxin (T-2). Due to lab regulations in other parts of the world, the presence of this mycotoxin was not tested for in other regions. As the origins of the samples were varied, analytical procedures had to be carried out in a convenient way from a logistics point of view. Thus, the majority of the analyses were performed at ROMER Labs Diagnostic GmbH (Austria), ROMER Labs Singapore Pte Ltd (Singapore), ROMER Labs Inc (USA) and SAMITEC (Brazil). Eighty-eight percent of the samples were analysed by High Performance Liquid Chromatography (HPLC) and 12 % by Enzyme Linked Immunosorbent Assay (ELISA) (only applied in the European and North American lab). For the purpose of data analysis, non-detection levels were based on the quantification limits of the test method for each mycotoxin. For more details regarding the analytical procedure, please contact the authors.

#### **Overall results**

*Figure 1* shows the percentage of positive results for each region and for each mycotoxin group. As can be seen in *Figure 2*, 25 %, 46 %, 64 %, 56 % and 31 % of all the samples surveyed tested positive for contamination with Afla, ZEN, DON, FUM and OTA, respectively. Compared with data from the previous year 2011 (*Table 1*), an increase in the occurrence of fusariotoxins (ZEN, DON and FUM) was observed together with a slight decrease in Afla.

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Figure 1 - Worldwide mycotoxin contamination

Table 1 – Overview of worldwide survey results (2011 and 2012)

Global results	Afla 2011	Afla 2012	ZEN 2011	ZEN 2012	DON 2011	DON 2012	FUM 2011	FUM 2012	OTA 2011	OTA 2012
Number of tests	2,770	2,636	3,061	3,320	3,509	3,712	2,548	2,570	1,966	2,230
Percent positive (%)	27	25	40	46	59	64	51	56	27	31
Average of positive (µg/kg)	60	34	241	251	1,056	1,088	1,722	1,350	9	5
Maximum (µg/kg)	2,230	6,323	23,278	9,854	49,307	30,200	77,502	42,120	400	170
Commodity tested	Maize	Ground- nut cake	Wheat	Corn Gluten Meal	Wheat	Maize	Finished feed	Maize	Maize	Maize
Source country	Pakistan	Myanmar	Australia	China	Australia	USA	China	Malaysia	India	India



**Figure 2** – Percentage of positive and negative samples worldwide

#### **Results by geographic region**

*Table 2 (from 2a to 2l)* shows the prevalence of mycotoxins in different regions worldwide. The following observations are highlighted to show the developments in the Asia-Pacific in 2012 compared with 2011:

- an increase in average levels of DON in North Asian samples;
- a reduction of DON levels in Oceania (which can be explained by the milder weather conditions for 2012 compared to the previous extremely rainy year);
- an overall increase in FUM levels (which can be correlated to the increase in FUM levels in Brazil and Argentina, important exporters of commodities to Asia).

In comparison with 2011, DON prevalence in North America was reduced (50 % vs. 37 %) but average levels were increased (919 vs. 2827 ppb). On the other hand, there was a sharp increase in FUM prevalence (from 27 % in 2011 to 78 % in 2012) as well as contamination levels (Average of positive: 1295 ppb in 2011 vs. 1673 ppb in 2012). The situation in South America and the Middle East remained quite similar to previous years, with FUM showing dominance in terms of prevalence and average levels. Due to the information gathered from previous years, samples originating from Northern Europe

were mainly analysed for ZEN, DON and T-2. As expected, these were the major contaminants of commodities and feeds sourced from this region. Especially in terms of trichothecenes (DON and T-2) contamination levels were found to be fairly high. In Central Europe, DON remained the most commonly occurring mycotoxin followed by ZEN. In Southern Europe, similar to previous years, FUM was the most prevalent mycotoxin, followed by OTA.

#### Table 2 - Survey results by geographic region

<b>Table 2a</b> - North Asia (China, Taiwan, Korea, Japan)									
North Asia	Afla	ZEN	DON	FUM	ΟΤΑ				
Number of tests	1,145	1,176	1,181	1,089	1,049				
Percent positive (%)	10	58	82	60	25				
Average* (µg/kg)	27	464	1,600	1,501	6				
Maximum (µg/kg)	341	9,854	28,005	13,495	103				

**Table 2b** - South East Asia (Malaysia, Philippines, Thailand, Vietnam, Indonesia)

South East Asia	Afla	ZEN	DON	FUM	ΟΤΑ
Number of tests	333	333	333	333	333
Percent positive (%)	57	37	40	53	30
Average* (µg/kg)	67	182	555	1,120	3
Maximum (µg/kg)	6,323	9,096	8,074	42,120	26

Table 2c - South Asia (India, Pakistan, Bangladesh)

South Asia	Afla	ZEN	DON	FUM	ΟΤΑ
Number of tests	75	75	75	75	75
Percent positive (%)	73	19	19	45	49
Average* (µg/kg)	60	79	290	755	8
Maximum (µg/kg)	1,224	182	1,843	3,209	170

#### Table 2d - Oceania (Australia and New Zealand)

Oceania	Afla	ZEN	DON	FUM	ΟΤΑ
Number of tests	208	208	208	208	208
Percent positive (%)	2	13	45	12	11
Average* (µg/kg)	6	261	312	269	3
Maximum (µg/kg)	18	896	6,715	1,110	18

#### Table 2e - North America (USA and Canada)

North America	Afla	ZEN	DON	FUM	ΟΤΑ
Number of tests	124	117	125	123	25
Percent positive (%)	30	26	37	78	20
Average* (µg/kg)	18	171	2,827	1,673	14
Maximum (µg/kg)	132	834	30,200	24,300	40

#### Table 2f - South America (Brazil and Argentina)

South America	Afla	ZEN	DON	FUM	ΟΤΑ
Number of tests	325	147	145	333	75
Percent positive (%)	18	26	21	64	3
Average* (µg/kg)	11	205	379	1,750	3
Maximum (µg/kg)	87	6,320	4,782	21,355	3

**Table 2g -** Middle East (Israel, Jordan, Egypt, Lebanon, SaudiArabia, Sudan, Syria, United Arabic Emirates and Yemen)

Middle East	Afla	ZEN	DON	FUM	ΟΤΑ
Number of tests	37	40	40	31	40
Percent positive (%)	35	15	43	65	68
Average* (µg/kg)	2	15	121	1,313	4
Maximum (µg/kg)	7	33	331	3,961	60

 Table 2h - Africa (Ivory coast, Algeria and South Africa)

Africa	Afla	ZEN	DON	FUM	ΟΤΑ
Number of tests	20	25	25	20	25
Percent positive (%)	80	36	68	100	28
Average* (µg/kg)	19	11	326	1,760	1
Maximum (µg/kg)	140	64	1,253	4,450	2

\*Average of positive

Table 2i - North Europ	pe (Noi	way, S	weden, Fi	inland a	and De	nmark)
North Europe	Afla	ZEN	DON	FUM	ΟΤΑ	T-2

4	103	103	0	6	103
0	71	88	-	67	29
-	41	1564	-	1	106
0	861	21,540	0	2	273
	4 0 - 0	4 103 0 71 - 41 0 861	4         103         103           0         71         88           -         41         1564           0         861         21,540	4         103         103         0           0         71         88         -           -         41         1564         -           0         861         21,540         0	4         103         103         0         6           0         71         88         -         67           -         41         1564         -         1           0         861         21,540         0         2

**Table 2j -** Central Europe (Austria, Belgium, Czech Republic,

Germany, France, Hungary, Romania, Slovakia, Slovenia and Poland)								
Central Europe	Afla	ZEN	DON	FUM	ΟΤΑ	T-2		
Number of tests	119	829	1158	111	135	232		
Percent positive (%)	19	50	68	23	23	16		
Average* (µg/kg)	7	41	744	113	10	31		
Maximum (µg/kg)	36	675	12,000	493	71	137		

**Table 2k** - South Europe (Italy, Greece, Portugal, Spain, Croatia, Bulgaria and Turkey)

South Europe	Afla	ZEN	DON	FUM	ΟΤΑ	T-2
Number of tests	173	193	215	166	168	192
Percent positive (%)	44	43	51	84	70	8
Average* (µg/kg)	6	35	330	879	4	258
Maximum (µg/kg)	87	604	10,455	13,457	64	3,051

Table	21 -	East	Europe	(Ukraine,	Belarus,	Lithuania,	Estonia,
Latvia	and	Russ	ia)				

East Europe	Afla	ZEN	DON	FUM	ΟΤΑ	T-2
Number of tests	73	74	104	81	91	94
Percent positive (%)	71	42	67	54	79	56
Average* (µg/kg)	5	32	285	485	6	70
Maximum (µg/kg)	10	340	960	1,930	50	200

#### **Results by commodity**

#### Table 3 - Survey results by commodity

Corn	Afla	ZEN	DON	FUM	ΟΤΑ
Number of tests	746	734	772	712	532
Percent positive (%)	25	43	64	86	10
Average* (µg/kg)	38	375	1,039	1,942	8
Maximum (µg/kg)	818	6,320	30,200	42,120	170
Corn gluten meal	Afla	ZEN	DON	FUM	ΟΤΑ
Number of tests	41	42	44	41	31
Percent positive (%)	32	86	95	90	81
Average* (µg/kg)	102	1,278	3,195	2,836	11
Maximum (µg/kg)	1,224	9,854	15,408	13,457	103
Corn DDGS	Afla	ZEN	DON	FUM	ΟΤΑ
Number of tests	64	63	65	57	50
Percent positive (%)	31	76	85	79	46
Average* (µg/kg)	14	505	5,012	1,807	7
Maximum (µg/kg)	105	2,606	28,005	11,594	49
Soybean	Afla	ZEN	DON	FUM	ΟΤΑ
Soybean Number of tests	<b>Afla</b> 114	<b>ZEN</b> 116	<b>DON</b> 134	<b>FUM</b> 108	<b>OTA</b> 97
Soybean Number of tests Percent positive (%)	Afla 114 18	<b>ZEN</b> 116 26	<b>DON</b> 134 20	FUM 108 8	<b>OTA</b> 97 30
Soybean Number of tests Percent positive (%) Average* (µg/kg)	Afia 114 18 1	ZEN 116 26 13	DON 134 20 344	FUM 108 8 218	<b>OTA</b> 97 30 3
Soybean Number of tests Percent positive (%) Average* (µg/kg) Maximum (µg/kg)	Afla 114 18 1 3	ZEN 116 26 13 65	DON 134 20 344 2,513	FUM 108 8 218 866	<b>OTA</b> 97 30 3 12
Soybean Number of tests Percent positive (%) Average* (µg/kg) Maximum (µg/kg) Wheat	Afla 114 18 1 3 Afla	ZEN 116 26 13 65 ZEN	DON 134 20 344 2,513 DON	FUM 108 8 218 866 FUM	OTA 97 30 3 12 0TA
Soybean Number of tests Percent positive (%) Average* (µg/kg) Maximum (µg/kg) Wheat Number of tests	Afla 114 18 1 3 Afla 172	ZEN 116 26 13 65 ZEN 341	DON 134 20 344 2,513 DON 434	FUM 108 8 218 866 FUM 189	OTA 97 30 3 12 OTA 186
Soybean Number of tests Percent positive (%) Average* (μg/kg) Maximum (μg/kg) Wheat Number of tests Percent positive (%)	Afla 114 18 1 3 Afla 172 5	ZEN 116 26 13 65 ZEN 341 31	DON 134 20 344 2,513 DON 434 70	FUM 108 8 218 866 FUM 189 10	OTA 97 30 3 12 0TA 186 13
Soybean Number of tests Percent positive (%) Average* (μg/kg) Maximum (μg/kg) Wheat Number of tests Percent positive (%) Average* (μg/kg)	Afla 114 18 1 3 Afla 172 5 4	ZEN 116 26 13 65 ZEN 341 31 123	DON           134           20           344           2,513           DON           434           70           1,206	FUM 108 8 218 866 FUM 189 10 327	OTA 97 30 3 12 OTA 186 13 3
Soybean Number of tests Percent positive (%) Average* (μg/kg) Maximum (μg/kg) Wheat Number of tests Percent positive (%) Average* (μg/kg) Maximum (μg/kg)	Afla 114 18 1 3 Afla 172 5 4 4	ZEN 116 26 13 65 ZEN 341 31 123 2,991	DON           134           20           344           2,513           DON           434           70           1,206           12,000	FUM 108 8 218 866 FUM 189 10 327 2,273	OTA 97 30 3 12 OTA 186 13 3 28
Soybean Number of tests Percent positive (%) Average* (μg/kg) Maximum (μg/kg) Wheat Number of tests Percent positive (%) Average* (μg/kg) Maximum (μg/kg) Wheat bran	Afla 114 18 1 3 Afla 172 5 4 11 Afla 11	ZEN 116 26 13 65 ZEN 341 31 123 2,991 ZEN	DON           134           20           344           2,513           DON           434           70           1,206           12,000           DON	FUM 108 8 218 866 FUM 189 10 327 2,273 FUM	OTA 97 30 31 2 0TA 186 13 3 3 28 0TA
Soybean Number of tests Percent positive (%) Average* (µg/kg) Maximum (µg/kg) Wheat Number of tests Percent positive (%) Average* (µg/kg) Maximum (µg/kg) Wheat bran Number of tests	Afla 114 18 1 3 Afla 7 4 172 5 4 11 Afla 47	ZEN 116 26 13 65 ZEN 341 31 123 2,991 ZEN 47	DON           134           20           344           2,513           DON           434           70           1,206           12,000           DON           40	FUM 108 8 218 866 FUM 189 10 327 2,273 FUM 47	OTA 97 30 31 2 0TA 186 13 3 28 28 0TA 32
Soybean Number of tests Percent positive (%) Average* (µg/kg) Maximum (µg/kg) Wheat Number of tests Percent positive (%) Average* (µg/kg) Maximum (µg/kg) Wheat bran Number of tests Percent positive (%)	Afla 114 18 1 3 Afla 172 5 4 11 Afla 47 2	ZEN 116 26 13 65 ZEN 341 31 123 2,991 ZEN 47 26	DON           134           20           344           2,513           DON           434           70           1,206           12,000           DON           40           78	FUM 108 8 218 866 FUM 189 10 327 2,273 FUM 47 30	OTA 97 30 31 22 OTA 186 13 3 28 28 OTA 32 28
Soybean Number of tests Percent positive (%) Average* (µg/kg) Maximum (µg/kg) Wheat Number of tests Percent positive (%) Average* (µg/kg) Wheat bran Number of tests Percent positive (%) Average* (µg/kg)	Afla 114 18 1 3 Afla 172 5 4 11 Afla 47 2 3	ZEN 116 26 13 65 ZEN 341 31 123 2,991 ZEN 47 26 295	DON           134           20           344           2,513           DON           434           70           1,206           12,000           DON           40           78           1,710	FUM 108 8 218 866 FUM 189 10 327 2,273 FUM 47 30 404	OTA 97 30 31 20 0TA 186 13 32 28 0TA 32 28 32 28 5

Rice	Afla	ZEN	DON	FUM	ΟΤΑ
Number of tests	19	20	20	18	19
Percent positive (%)	32	15	35	11	11
Average* (µg/kg)	7	128	229	395	2
Maximum (µg/kg)	25	159	655	454	4
Rice bran	Afla	ZEN	DON	FUM	ΟΤΑ
Number of tests	22	22	22	21	22
Percent positive (%)	45	41	18	24	32
Average* (µg/kg)	15	70	242	173	1
Maximum (µg/kg)	61	165	648	220	2
Barley	Afla	ZEN	DON	FUM	ΟΤΑ
Number of tests	39	231	348	39	42
Percent positive (%)	3	40	66	15	10
Average* (µg/kg)	4	22	669	175	7
Maximum (µg/kg)	4	215	4,279	205	18
Lupines and other pulses	Afla	ZEN	DON	FUM	ΟΤΑ
Number of tests	17	17	17	17	17
Percent positive (%)	18	47	29	6	24
Average* (µg/kg)	9	231	95	173	5
Maximum (µg/kg)	18	498	183	173	6
Finished feed	Afla	ZEN	DON	FUM	ΟΤΑ
Number of tests	908	1,109	1,201	894	804
Percent positive (%)	36	57	66	68	50
Average* (µg/kg)	15	209	969	835	5
Maximum (µg/kg)	350	2,968	18,233	7,219	71
Corn silage	Afla	ZEN	DON	FUM	ΟΤΑ
Number of tests	219	281	319	212	198
Percent positive (%)	7	48	68	27	20
Average* (µg/kg)	4	135	600	562	4
Maximum (µg/kg)	22	1,808	8,755	4,042	15
Straw	Afla	ZEN	DON	FUM	ΟΤΑ
Number of tests	19	21	21	19	19
Percent positive (%)	0	24	67	5	32
Average* (µg/kg)	-	458	544	461	2
Maximum (µg/kg)	0	1,260	1.256	461	2

#### From corn to corn by-products

Similar to previous years, corn was the most extensively tested commodity and FUM was the most prevalent mycotoxin present in 86 % of tested corn samples. Average levels found for all mycotoxins were similar or higher than those observed in 2011, except for Afla, for which levels were lower (38 ppb in 2012 vs. 101 ppb in 2011). As expected, levels of ZEN and DON found in corn by-products such as Corn Gluten Meal (CGM) and Dried Distillers Grains with Solubles (DDGS) far exceeded those found in unprocessed corn.

#### Wheat, rice and respective brans

DON was the most prevalent mycotoxin in wheat, present in 70 % of tested samples. As expected, wheat bran samples had higher contamination levels than wheat itself, even if differences were not as great as expected.

In comparison with wheat, rice samples tested showed a lower prevalence of all mycotoxins, except for Afla, which can be easily explained by the weather conditions favorable to Afla occurrence (higher temperature) in countries producing this commodity.

### Soybean and other protein sources, such as lupines and other pulses

As observed in previous years, both the prevalence and concentration of mycotoxins in soybean were lower than that of cereal grains such as corn and wheat. A similar pattern was observed for other protein sources such as lupines and other pulses which originated mainly from Oceania and were sourced in the Asia-Pacific region. This occurrence was not surprising as it is commonly known that other mycotoxins, namely hepatotoxic Phomopsins produced by *Diaporthe toxica*, are of greater concern in the case of these commodities.

#### **Finished feed**

The contamination pattern of finished feed is quite similar to that of corn, as this is the major commodity used globally for livestock diets. As such, FUM, DON and ZEN were the most prevalent mycotoxins in finished feed samples with contamination levels approaching 4-digit figures in the case of DON and FUM and around 200 ppb for ZEN.

#### Silage and straw

Similarly to previous surveys, the main mycotoxins present in silage were DON and ZEN which were present in 68 % and 49 % of tested samples. The most prevalent mycotoxin found in straw was DON, with 67 % of samples testing positive for this trichothecene. Often overlooked, this fact is of particular relevance for dairy cows and swine and poultry for which straw material is used as bedding. As animals frequently eat bedding material, the contamination levels in straw should not be ignored.

#### **Conclusions**

In view of the results shown for the more than 4000 samples analysed in the year 2012, it is clear that the majority of commodities and feed used in animal nutrition is contaminated with at least one mycotoxin. More frequently than not, more than one mycotoxin will be present in the same ingredient or feed. Prevention of the negative effects of these hazardous substances in animal health and performance is crucial.

BIOMIN has developed effective mycotoxin risk management tools to avoid unnecessary costs and losses originating from the presence of these toxins in animal feeds.

#### > DISCLAIMER

BIOMIN GmbH and the authors had no influence on the sampling process of the investigated samples. Therefore, the contamination levels found in the samples do not necessarily reflect the actual contamination level of these regions/commodities. However, the samples provide more insight into the range and levels of mycotoxins which can be found in diverse commodities of various regions.

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