Special Issue Mycotoxins
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Mycotoxins

Science & Solutions

Know Your Mycotoxin Risk

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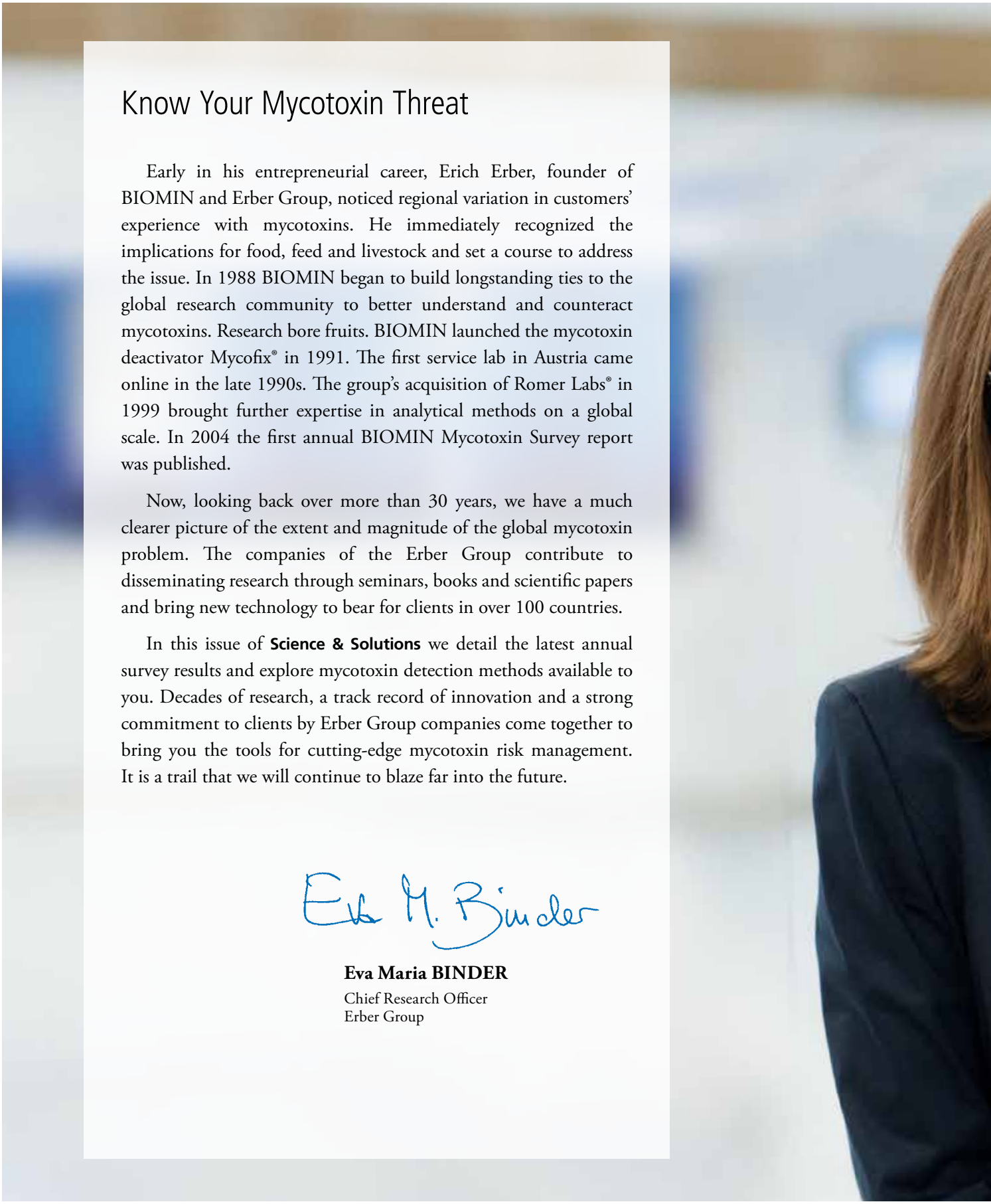
Latest BIOMIN Mycotoxin Survey Results

The Right Detection Tools

Editorial



Know Your Mycotoxin Threat

Early in his entrepreneurial career, Erich Erber, founder of BIOMIN and Erber Group, noticed regional variation in customers' experience with mycotoxins. He immediately recognized the implications for food, feed and livestock and set a course to address the issue. In 1988 BIOMIN began to build longstanding ties to the global research community to better understand and counteract mycotoxins. Research bore fruits. BIOMIN launched the mycotoxin deactivator Mycofix® in 1991. The first service lab in Austria came online in the late 1990s. The group's acquisition of Romer Labs® in 1999 brought further expertise in analytical methods on a global scale. In 2004 the first annual BIOMIN Mycotoxin Survey report was published.

Now, looking back over more than 30 years, we have a much clearer picture of the extent and magnitude of the global mycotoxin problem. The companies of the Erber Group contribute to disseminating research through seminars, books and scientific papers and bring new technology to bear for clients in over 100 countries.

In this issue of **Science & Solutions** we detail the latest annual survey results and explore mycotoxin detection methods available to you. Decades of research, a track record of innovation and a strong commitment to clients by Erber Group companies come together to bring you the tools for cutting-edge mycotoxin risk management. It is a trail that we will continue to blaze far into the future.

Eva Maria BINDER
Chief Research Officer
Erber Group

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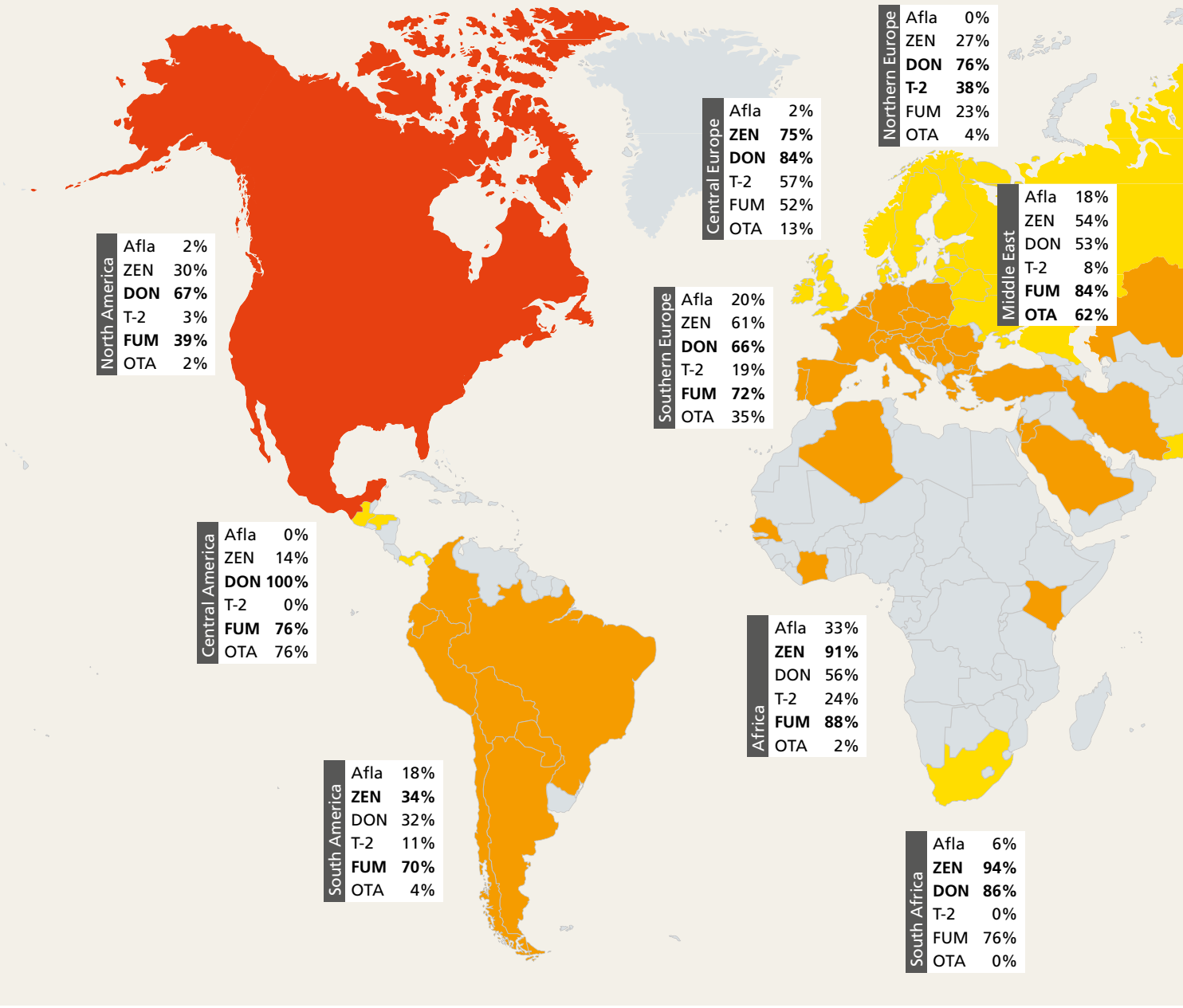


2015 BIOMIN Mycot

By **Michele Muccio** and **Sabine Masching**, Mycotoxin Risk Management Product Managers

The latest edition of the annual survey, covering 8271 agricultural commodity samples from 75 countries with over 31000 analyses, highlights the main dangers from the most important mycotoxins in primary feedstuffs and their potential risk to livestock animal production.

The survey results provide an insight on the incidence of aflatoxins (Afla), zearalenone (ZEN), deoxynivalenol (DON), T-2 toxin (T-2), fumonisins (FUM) and ochratoxin A (OTA) in the primary components used for feed which include corn (maize), wheat, barley, rice, soybean meal, corn gluten meal, dried distillers grains (DDGS) and silage, among others.

Risk levels

Because of the powerful sensitivity of state-of-the-art detection tools (e.g. using LC-MS/MS based

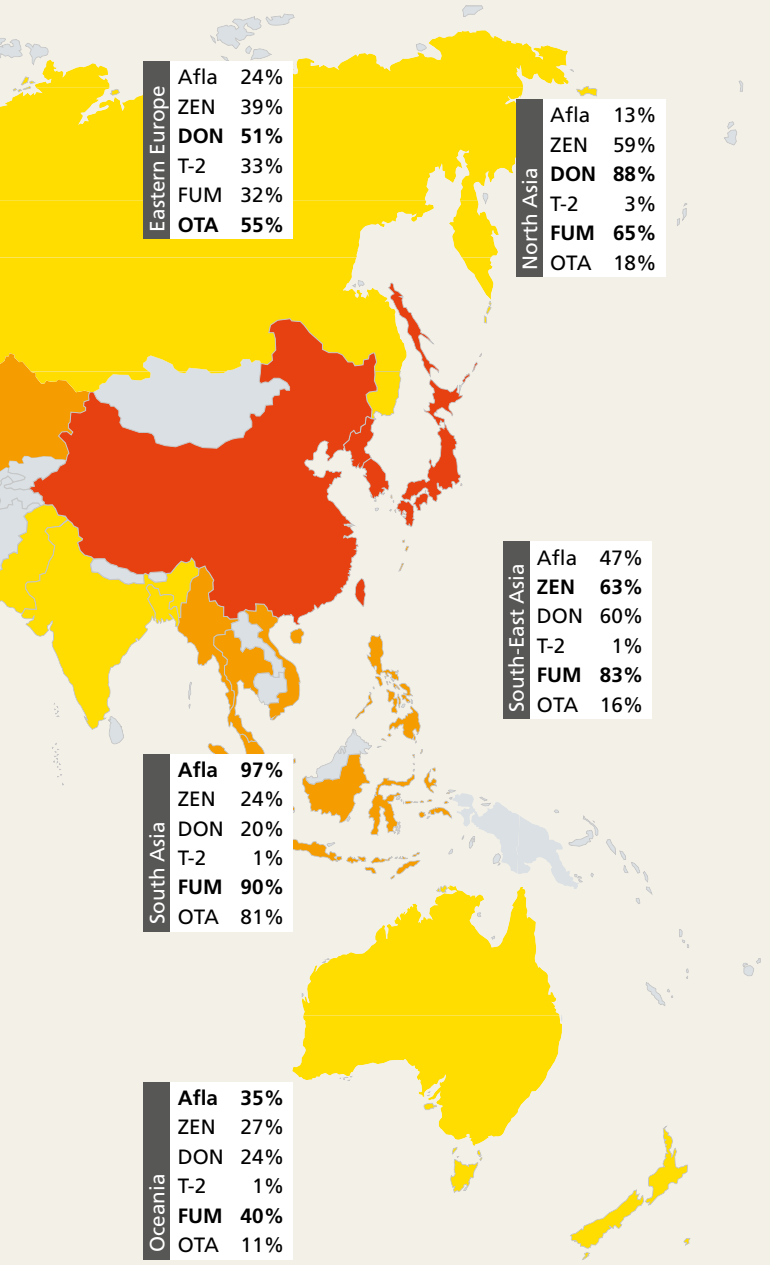


Figure 1. Global map of mycotoxin occurrence and risk in different regions.

Squares indicate the percentage of analyzed samples contaminated by mycotoxins per region. Risk was calculated per region on the number of different average values of mycotoxin contamination above threshold. Colors indicate different risk levels according to the legend below.

Legend

- Moderate risk = 1 or 2 mycotoxins above recommended thresholds
- High risk = 3 or 4 mycotoxins above recommended thresholds
- Severe risk = 5 or 6 mycotoxins above recommended thresholds
- No samples tested

oxin Survey Results

multi-mycotoxin analysis Spectrum 380®, it is no longer sufficient to talk about the mere presence of mycotoxins; concentration levels must be considered. Consequently, the latest results feature a mycotoxin risk map based upon both the presence of mycotoxins and their potential harm to livestock depending on concentration levels associated with known health risks.

Figure 1 shows mycotoxin occurrence data for each region as a percentage of all samples tested. The overall risk level for a particular region (indicated by color according to legend) is determined by the number of single mycotoxins with average contamination levels measured in parts per billion (ppb) which exceed the maximum risk threshold levels for livestock.

Recommended risk threshold of major mycotoxins in ppb

Afla	ZEN	DON	T-2	FUM	OTA
2	50	150	50	500	10

The risk thresholds are based on worldwide practical experience in the field and in scientific trials that were conducted to reflect as closely as possible field situations and take into account the most sensitive species for each mycotoxin.

The average risk levels used as a basis do not preclude specific, severe instances of mycotoxin contamination in farm or fields locally, nor do they account for the negative impacts of multiple mycotoxin presence.

For the second year, the survey includes results of multiple mycotoxin analysis of more than 380 mycotoxins and metabolites, Spectrum 380®, using state-of-the-art liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) in a single analysis step.

It is no longer sufficient to talk about the mere presence of mycotoxins; concentration levels must be considered.

Low risk indicates that average levels of single mycotoxin presence for a given zone do not exceed minimum recommended thresholds for livestock. Moderate risk indicates the presence of one to two major mycotoxins at levels known to cause harm in animals. High risk indicates the presence of three to four major mycotoxins at levels known to cause harm in animals. Severe risk indicates the presence of five or more major mycotoxins at levels known to cause harm in animals.

The mycotoxin risk map relies upon single mycotoxin occurrence which may understate the threat posed by mycotoxins to animals given their known synergistic effects (the presence of multiple mycotoxins compounds the potential harm) and subclinical effects (even low levels of mycotoxin contamination can impair animal health and performance).

Regional insights

North America and North Asia faces the most severe threat of mycotoxin-related risks to livestock. Both regions registered at least five major mycotoxins at average concentration levels above risk threshold levels.

Table 1 provides an overview on the number of samples tested, occurrence, average contamination levels and maximum contamination values. Fumonisins and deoxynivalenol are the top threats in all regions except for Africa where zearalenone constitutes the major threat to livestock.

Europe

Europe ranked as a high risk region, with 4 mycotoxins at average concentrations above risk threshold levels. Samples from Europe showed the highest incidence of DON at 77% and a high average of 1288 ppb, the latter figure being the highest found worldwide. The highest European level of DON was detected in an Austrian corn silage sample at 34861 ppb. Second most occurring mycotoxin was ZEN, present in 64% of the samples. The highest ZEN level in Europe was observed in a German corn sample at 8888 ppb. Samples from Europe showed again the highest incidence of T-2 toxin, close to double compared to past years, at 42%. The highest level of T-2 toxin, 685 ppb, was detected in a corn silage sample from France. FUM occurred in 54% of the samples, with the highest single level from Europe observed in an Italian corn sample (15383 ppb).

Asia

Asia is at a high risk for mycotoxin-related risks to livestock with 4 mycotoxins present at average concentrations above risk threshold levels. DON prevalence and average concentration in Asia were 74% and 857 ppb, respectively. The highest singly occurring DON concentration worldwide was detected in a Chinese DDGS sample (84860 ppb).The second highest occurring mycotoxin was FUM, detected in 67% of the samples at an average concentration of 1032 ppb. The highest occurrence of FUM in Asia was detected in a Thai corn sample (16258 ppb). ZEN was the third highest occurring mycotoxin in Asian samples, detected in 55% of tested samples at an average concentration of 368 ppb.

In Asia, the highest ZEN value was detected in a Chinese finished feed sample (9432 ppb). Afla was found in 25% of the samples at the highest average concentration worldwide (59 ppb). The highest worldwide value for aflatoxins was detected in a Chinese cotton seed sample (9404 ppb).

North America

North America faces again a severe risk for mycotoxin-related threats to livestock with 5 mycotoxins present at average concentrations above risk threshold levels.

Michele Muccio
Sabine Masching
Mycotoxin Risk Management Product Managers

Table 1. Detailed results of mycotoxin occurrence by region.

		Afla	ZEN	DON	T-2	FUM	OTA
Europe	Number of samples tested	1,163	2,894	3,684	2,051	1,543	1,188
	% of contaminated samples	11%	64%	77%	42%	54%	26%
	Average of positives (ppb)	6	213	1,288	25	898	7
	Maximum (ppb)	153	8,888	34,861	685	15,383	150
Asia	Number of samples tested	2,360	2,357	2,420	1,077	1,824	1,454
	% of contaminated samples	25%	55%	74%	2%	67%	20%
	Average of positives (ppb)	59	368	857	39	1,032	7
	Maximum (ppb)	9,404	9,432	84,860	171	16,258	259
N. America	Number of samples tested	484	495	359	354	481	423
	% of contaminated samples	2%	30%	67%	3%	39%	2%
	Average of positives (ppb)	16	244	1,132	44	974	32
	Maximum (ppb)	108	12,900	26,294	223	16,300	200
S. America	Number of samples tested	995	668	333	411	444	202
	% of contaminated samples	18%	34%	32%	11%	70%	4%
	Average of positives (ppb)	6	131	545	28	2,235	2
	Maximum (ppb)	138	2,593	4,195	65	36,489	12
Middle East	Number of samples tested	94	115	117	40	80	26
	% of contaminated samples	18%	54%	53%	8%	84%	62%
	Average of positives (ppb)	1	62	446	20	513	3
	Maximum (ppb)	8	367	1,983	45	2,534	9
Africa	Number of samples tested	182	183	182	182	183	182
	% of contaminated samples	13%	93%	79%	5%	79%	1%
	Average of positives (ppb)	43	41	486	8	599	0
	Maximum (ppb)	258	858	4,974	47	4,368	0

Source: 2015 BIOMIN Mycotoxin Survey

The most frequently occurring mycotoxin is DON, detected in 67% of the samples at an average concentration of 1132 ppb. The highest DON level in North America was detected in a US oat sample (26294 ppb). FUM, ZEN, Afla and OTA were detected in 39%, 30%, 2% and 2% of samples respectively, at average levels of 974, 244, 16 and 32 ppb respectively.

South America

South America faces high mycotoxin-related risks to livestock, having 4 mycotoxins present at average concentrations above risk threshold levels. Fumonisins were present in 70% of the samples at an average concentration of 2235 ppb. The highest FUM value worldwide was detected in a Brazilian corn sample (36489 ppb). Prevalence of DON doubled in comparison to last year, with 32% of samples testing positive.

Middle East

The Middle East registered high mycotoxin-related risks to livestock with 3 mycotoxins present at average concentrations above risk threshold levels. Samples from the Middle East showed high occurrence of FUM, OTA, ZEN and DON, detected in 84%, 62%, 54% and 53% of samples respectively. With the exception of OTA, the average concentrations of these mycotoxins were all above the risk threshold.

Africa

African samples showed the highest prevalence of zearalenone at 93%.The second highest average values of Afla were detected in this region as well. Both deoxynivalenol and fumonisins were detected in 79% of the samples analyzed.

Conclusion

The analysis of the 8271 samples in this survey indicates that constant monitoring of mycotoxins is important. An effective mycotoxin risk management program is essential in order to protect animals from the negative impacts of mycotoxins on animals' health and performance.


AgraStrip® DON
AgraStrip® DON
AgraStrip® DON

Find the Right Mycotoxin Testing Tool

By **Philipp Gruber**, Product Manager at Romer Labs

Feed millers and livestock producers have more choice than in the past when it comes to testing for the presence of mycotoxins in commodity raw materials and finished feed. Here's how to select the appropriate method for your situation.

For decades, taking samples and sending them to an analytical service provider has been the main method for determining the presence of mycotoxins. In recent years on-site rapid test methods have become widely available, offering simplicity and ease-of-use to quickly detect mycotoxins on site. With more options to choose from, finding the right tool has gained importance.

On-site testing vs. analytical service

The first step in finding the right testing solution is to decide whether to conduct the test yourself on-site (e.g. in the field or at the production facility), or send the samples to an analytical service laboratory. That decision depends on three main considerations:

1 Required testing throughput

For high volume or frequent testing (high throughput), it might be worth conducting on-site tests, since costs are generally low.
If you only perform occasional testing or have low throughput, sending your samples to an analytical service lab could be more convenient.

2 Acceptable time to results

On-site rapid tests will deliver results within an hour. This makes rapid tests a useful tool when decision time is short, e.g. when deciding whether to accept a truck delivery. From start to finish, analytical service results take on average one week.

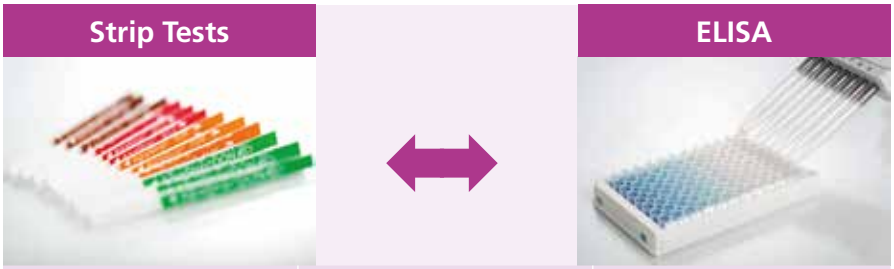
3 Quality of results

On-site testing can be categorized as a screening tool in that it provides a quick indication as to the presence of one analyte per test. Reference methods available at an analytical service laboratory are much more robust, offering greater reliability on a larger number of analytes.

Rapid tests

The two most popular on-site methods are strip tests and ELISA tests. The key differences are illustrated in *Figure 1*. Strip tests are designed to give results as soon as possible, though they can only process two samples at a time. They are therefore widely used at reception points of the supply chain of agricultural raw commodities. ELISA (enzyme-linked immunosorbent assay) test kits can test up to 44 samples simultaneously. In general, ELISA is the better option when you have 6 or more samples:

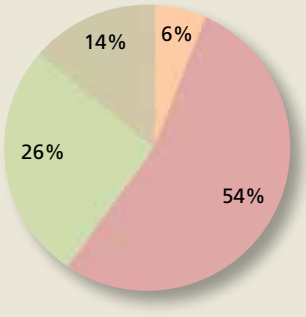
Figure 1. On-site testing methods.



Max.2	**Samples at a time**	Max.44
10 min	**Time to result**	30 min
Low	**Equipment costs**	Medium - low
Low	**Training requirement**	Medium - low

Source: Romer Labs

How do you test for mycotoxins?



Poll results of 407 feed and livestock professionals in 86 countries on 3 March 2016.

Source: BIOMIN, Romer Labs

Philipp Gruber
Product Manager at Romer Labs

Figure 2. Analytical service testing methods.

	ELISA	HPLC	LC-MS/MS
			
Price	Low	Medium	High
No. analytes per run	1 target	Multiple target	< 18
Accuracy	Sreening	Reference method, highly sensitive and precise	Reference method (accredited results), highly sensitive and precise

Source: Romer Labs

the price difference is quickly recuperated due to the need to buy fewer kits and it saves time.

Analytical service testing

When sending samples to an analytical service lab you have to decide which technology should be used. In addition to classic ELISA, reference methods like HPLC (high performance liquid chromatography) and LC-MS/MS (liquid chromatography-tandem mass spectrometry) can be chosen. The key differences are illustrated in *Figure 2*. Reference methods analyze your sample for multiple toxins in one go. For example, the LC-MS/MS multi-mycotoxin method offered by Romer Labs is capable of analyzing up to 18 toxins at a time.

Raw materials vs. finished feed

We recommend to constantly monitor the input and output of a finished feed production line. This means applying rapid tests to screen incoming raw material used in feed production. Most commodities have protocols for rapid test methods. Catching mycotoxin contaminated materials before they enter the supply chain can help prevent more costly problems later on.

Finished feed, being made up of various different materials, demonstrates greater complexity in terms of testing. Depending on the amount of feed that requires monitoring you can apply rapid tests or send samples to analytical service labs. If you have only a small amount of feed to test or your feed composition changes frequently, you will have more convenient, reliable results using an analytical service. For large amounts of feed with an unchanged formula it might be worth to create a customized protocol for rapid tests. Bear in mind that the feed composition often varies with market price, season and use. To reliably apply rapid tests to finished feed, it is recommended to have a validation (customized protocol) tailored to your specific feed formulation.

Conclusion

The growing popularity of rapid tests for mycotoxins creates more choice for millers and farmers. There are a number of factors to consider when choosing the right mycotoxin detection tool. On-site testing methods offer a number of advantages, namely speed, cost and ease of use. The reference methods available from an analytical service laboratory will provide greater precision for a larger number of analytes, delivering a fuller picture of the contamination situation. Rapid tests are a good option for raw commodity screening. For finished feed, an analytical service or validated rapid test may be used. For an effective mycotoxin detection program, it may be worth considering a combination of tactics that best fit your requirements.

Photo: Grassetto

Tips for sampling

Sampling error accounts for 76% of total error when testing for mycotoxins. Taking good, representative samples can help improve the accuracy of the final result. There are several ways to improve sampling:

1. Increase sample number and sample size. According to current European Union recommendations, for up to 50 tons of cereals, take 10 to 100 incremental samples of 100g each.
2. Use a finer grind to improve the results.
3. Consult the mycotoxin sampling tool on the FAO website which provides analysis of a sampling plan.
4. Follow the sampling guide available on www.romerlabs.com.

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