Special Issue Mycotoxins 02
A magazine of Biomin

Mycotoxins

Science & Solutions

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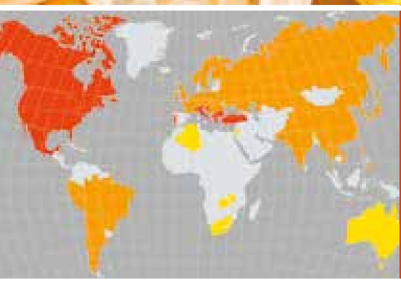
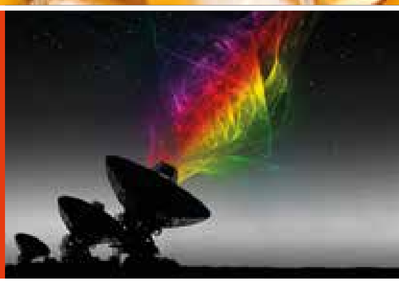
Mycotoxins

What to Expect



Latest BIOMIN Mycotoxin Survey Results



Why Multiple Mycotoxin Detection Matters

Editorial

Charting New Territory

One could expect that, after three decades of research, most of the understanding and insights would have already been gleaned from the subject matter. And yet, the study of mycotoxins advances relentlessly, showing no signs of exhaustion.

Enormous technological gains have been made in mycotoxin detection with sensitivity of tools such as liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) improving 200 fold sensitivity over the past decade. The ability to detect over 380 mycotoxin metabolites provides a much more detailed view than ever before, improving our understanding of the situation in the field and informing the future research agenda. Recent results show that mycotoxin co-occurrence is more common than previously considered and consequently requires a mycotoxin risk management solution that encompasses multiple modes of action.

Nor have the dangers posed by mycotoxins abated. Mycotoxin-related threats to livestock production are severe or high in over 80% of regions worldwide according to the latest BIOMIN Mycotoxin Survey, the longest-running and most comprehensive one of its kind. Ten out of twelve regions registered three or more major mycotoxins at concentration levels known to cause harm in animals. Mycotoxin contamination will be a greater concern for producers in most countries compared to last year since the concentration levels of mycotoxins present in agricultural commodity samples nearly doubled for three of the six major mycotoxins analyzed.

We hope that you enjoy this special issue of **Science & Solutions** dedicated to the topic of mycotoxins.

Ursula HOFSTETTER
Director Competence Center Mycotoxins

Contents

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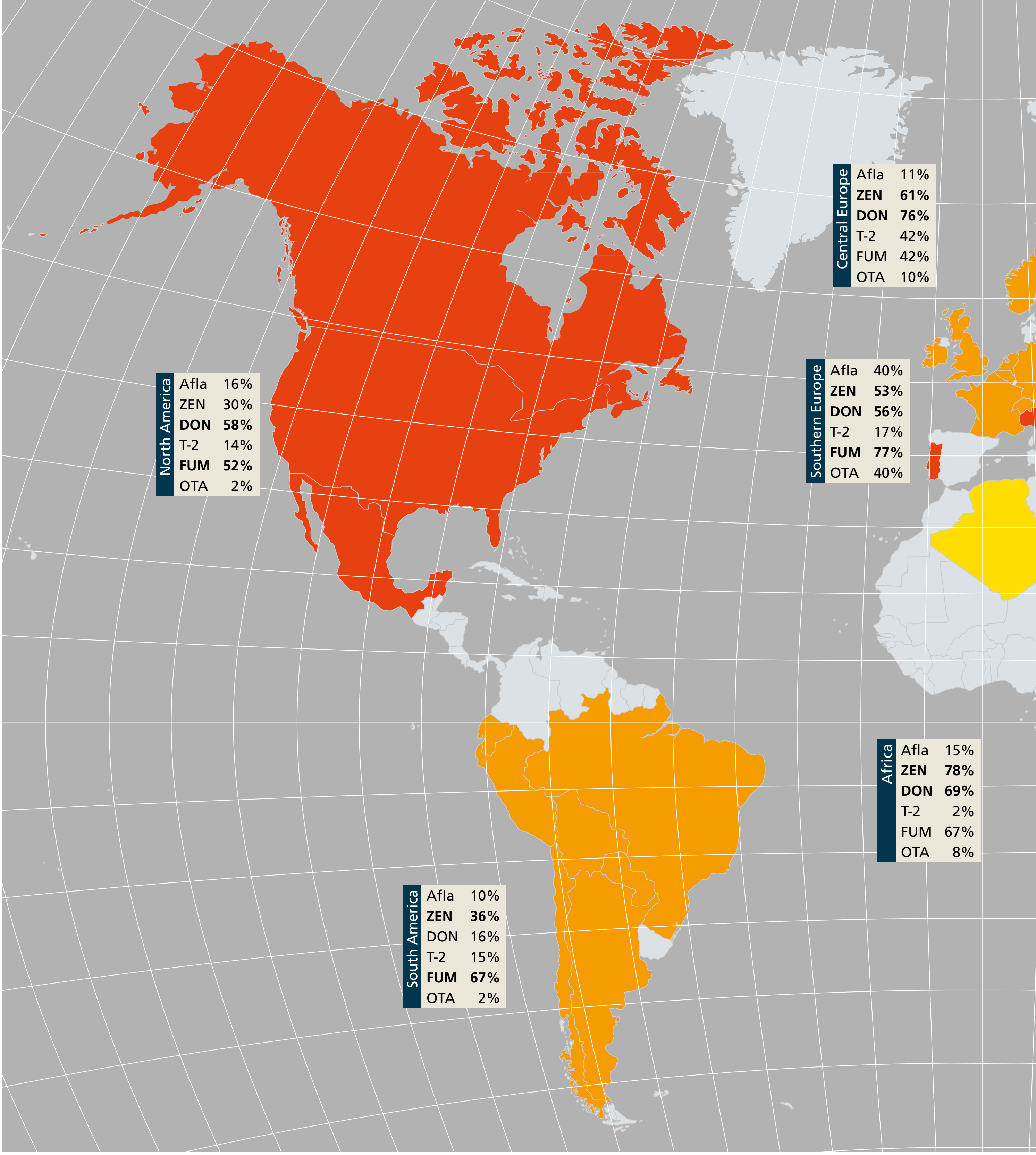
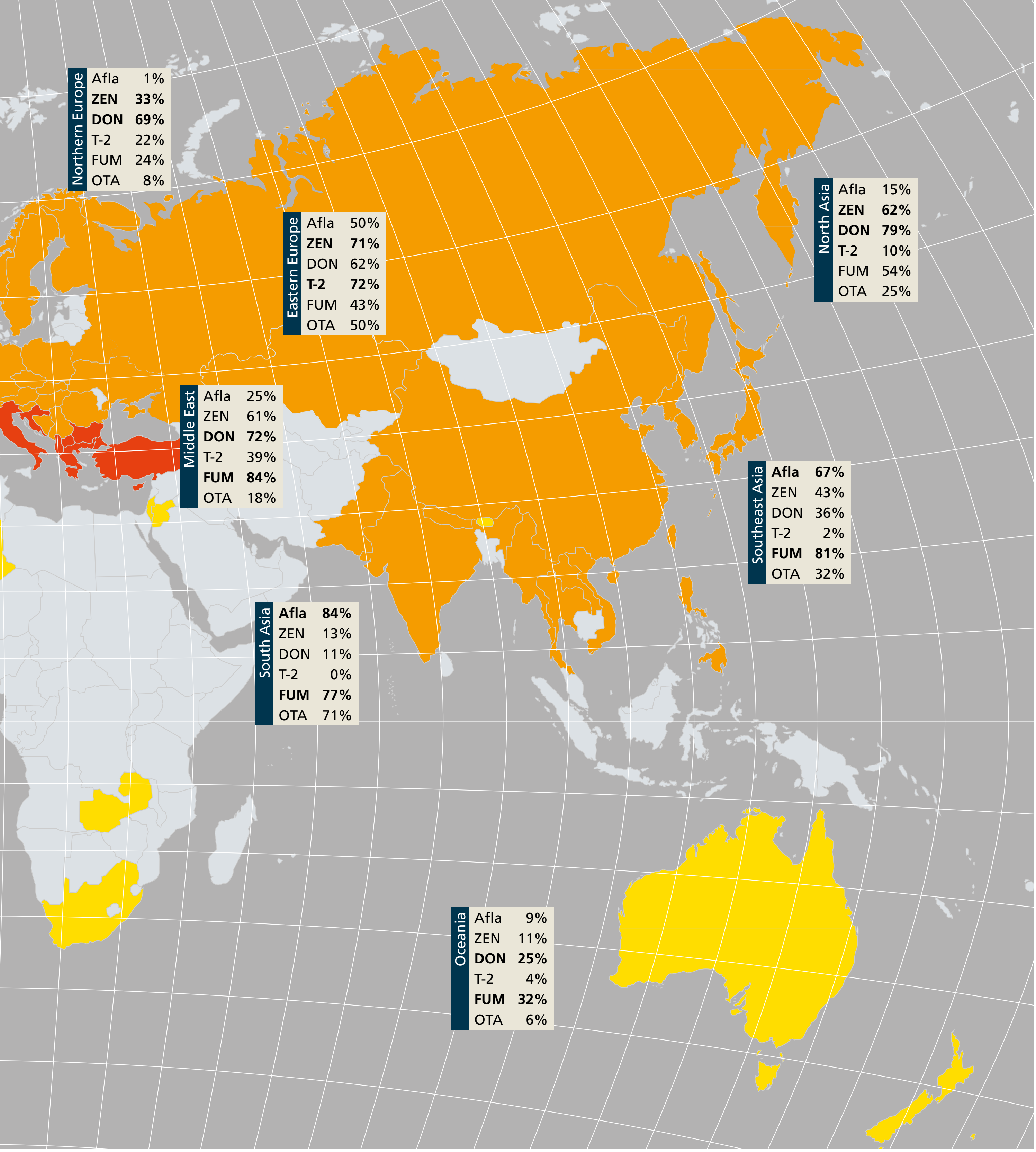


2014 BIOMIN Myco

By **Paula Kovalsky** and **Karin Nährer,** Product Managers Mycotoxin Risk Management

The latest edition of the annual survey covers 6,844 agricultural commodity samples from 64 countries with more than 26,200 analyses and highlights the main dangers from the most important mycotoxins in primary feedstuffs and their potential risk to livestock animal production.



toxin Survey Results

Recommended risk threshold of major mycotoxins in ppb

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

Legend

- Low risk = no mycotoxins above recommended thresholds
- 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

Photo: iStockphoto/Spiritartist

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, 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 to the most sensitive animal species.

Figure 1 shows mycotoxin occurrence data for each region as a percentage of all samples tested. The overall risk level for a particular region is determined by the number of single mycotoxins with average contamination levels (measured in parts per billion, ppb) which lie above the maximum risk threshold levels for livestock. The risk thresholds, listed on page three, 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.

North America and Europe face the most severe threat of mycotoxin-related risks to livestock.

Low risk indicates that average levels of single mycotoxin presence for a given zone do not exceed minimum recommended thresholds for livestock. The average risk level does not preclude specific, severe instances of mycotoxin contamination in farm or fields locally, nor does it account for the negative impacts of multiple mycotoxin presence.

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).

Most common toxins

As in 2013, deoxynivalenol and fumonisins are once again the main threat and are found in over half of all samples tested worldwide (*Figure 2*). Deoxynivalenol poses the most frequent threat to livestock with a prevalence of 66% and average contamination level of 1,394 parts per billion. A full 82% of those samples (marked by a dot in *Figure 2*) exceed risk thresholds for livestock. Levels of fumonisins (56% of samples, 1,594 ppb on average) and zearalenone (53% of samples, 221 ppb on average) also present a cause for concern, with 48% and 54% of positive samples respectively registering concentrations above recommended risk threshold levels.

Regional insights

Overall, North America and Europe face the most severe threat of mycotoxin-related risks to livestock, both severe risk areas, each registering average concentrations for at least five major mycotoxins above risk threshold levels.

Table 1 provides an overview on the number of samples tested, occurrence, average contamination levels

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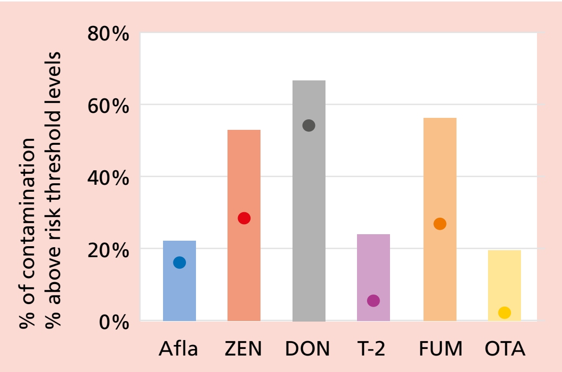
Table 1. Detailed results of mycotoxin occurrence by region

		Afla	ZEN	DON	T-2	FUM	OTA
Europe	Number of samples tested	1,036	2,620	3,373	1,322	1,199	1,021
	Average of positives (ppb)	8	272	1,864	66	1,019	3
	Maximum (ppb)	481	16,495	29,600	1,300	56,948	76
Asia	Number of samples tested	1,751	1,767	1,762	436	1,651	1,686
	Average of positives (ppb)	104	167	512	18	1,399	7
	Maximum (ppb)	5,155	6,215	8,901	61	130,246	854
North America	Number of samples tested	603	566	623	510	556	460
	Average of positives (ppb)	29	282	1,231	60	3,353	6
	Maximum (ppb)	980	13,700	24,792	285	154,000	22
South America	Number of samples tested	483	407	342	314	470	251
	Average of positives (ppb)	40	107	866	9	1,932	3
	Maximum (ppb)	1,352	3,779	7,590	361	52,438	11
Middle East	Number of samples tested	48	54	54	44	43	38
	Average of positives (ppb)	2	19	194	12	541	13
	Maximum (ppb)	8	148	641	37	2,927	67
Africa	Number of samples tested	88	88	88	88	88	88
	Average of positives (ppb)	7	39	599	2	249	13
	Maximum (ppb)	23	452	9,176	2	2,781	57

Source: 2014 BIOMIN Mycotoxin Survey

Figure 2. Worldwide prevalence of major mycotoxins. Bars represent the % of contaminated samples. Dots display the occurrence of mycotoxins above risk threshold levels.



Scource: 2014 BIOMIN Mycotoxin Survey

and maximum contamination values. Deoxynivalenol is the number one threat in all regions except for South America, where fumonisins constitute the most frequent health risk to livestock.

Europe

Southern Europe, with five mycotoxins present at average concentrations above risk threshold levels, ranked as a severe risk region while the rest of Europe ranked as high risk, having four average concentrations above risk threshold levels. Samples from Europe showed the highest incidence of T-2 toxin at 22% and a high average of 91 ppb. The highest level of T-2 toxin was detected in a Russian wheat sample at 1,300 ppb. Nearly three out of four European samples contained deoxynivalenol at the highest average concentration worldwide (1,864 ppb). The highest singly occurring deoxynivalenol level was observed in an Austrian corn sample at 29,600 ppb and the highest singly occurring ZEN level was observed in an Austrian corn sample at 16,495 ppb.

Asia

Asia is at a high risk for mycotoxin-related risks to livestock with four mycotoxins present at average concentrations above risk threshold levels, while Oceania registered moderate risk with three mycotoxins at high ppb levels. The highest prevalence and average aflatoxin concentration worldwide was observed in Asian samples at 22% and 104 ppb. The highest singly occurring aflatoxin level was observed in a Chinese cotton seed sample at 5,155 ppb and the highest ochratoxin A prevalence was observed in Asia at 27% and highest singly occurring ochratoxin A concentration was detected in a Chinese finished feed sample at 854 ppb.

Table 2. Mycotoxins by commodity.

		Afla	ZEN	DON	T-2	FUM	OTA
Finished Feed	Number of samples tested	1,592	1,872	1,983	931	1,676	1,459
	Average of positives (ppb)	32	87	484	57	926	6
	Maximum (ppb)	484	6,215	17,920	1,300	25,041	854
Maize	Number of samples tested	1,169	1,793	2,123	830	1,071	1,012
	Average of positives (ppb)	45	411	2,443	83	2,914	4
	Maximum (ppb)	1,352	16,495	29,600	852	154,000	52
Wheat	Number of samples tested	227	408	592	164	208	228
	Average of positives (ppb)	10	83	860	86	433	3
	Maximum (ppb)	87	2,115	28,864	1,300	4,333	18
Soy	Number of samples tested	158	174	178	142	160	164
	Average of positives (ppb)	3	19	204	18	123	19
	Maximum (ppb)	11	288	1,166	108	977	141
DDGS	Number of samples tested	69	71	72	42	68	64
	Average of positives (ppb)	13	353	997	28	1,894	6
	Maximum (ppb)	196	3,600	8,514	80	27,665	27
Silage	Number of samples tested	139	225	277	112	145	127
	Average of positives (ppb)	6	290	2,521	43	319	2
	Maximum (ppb)	15	3,055	13,920	174	3,939	5

Source: 2014 BIOMIN Mycotoxin Survey

North America

North America faces severe threat for mycotoxin-related risks to livestock. The highest average values of ZEN and FUM were detected in North American samples at 282 ppb and 3,352 ppb, respectively. The average level of deoxynivalenol was second highest in North American samples at 1,231 ppb and the highest singly occurring fumonisin level was observed in a US corn sample at 154,000 ppb.

South America

South America ranked as high risk for mycotoxin-related risks to livestock, having four mycotoxins present at average concentrations above risk threshold levels. Fumonisins were present in almost two-thirds of all South American samples at 1,932 ppb, the second highest average level worldwide. Despite the low prevalence of T-2, the average concentration in South American samples was highest worldwide at 125 ppb. Although only 10% of South American samples were contaminated with Afla, the average concentration was second highest worldwide at 40 ppb.

Middle East

The Middle East registered as facing moderate risk for mycotoxin-related risks to livestock with three mycotoxins present at average concentrations above risk threshold levels. Samples from the Middle East showed the highest average ochratoxin A concentration globally. Deoxynivalenol and fumonisins were present in 72% and 84% of these samples, respectively.

Africa

Africa registered two mycotoxins present at average concentrations above risk threshold levels (aflatoxins, deoxynivalenol). African samples showed the highest prevalence of zearalenone at 78%. Deoxynivalenol and fumonisins were present in more than two-thirds of all African samples.

Feedstuffs

Finished feed and corn (maize) are the commodities most affected by mycotoxins. Deoxynivalenol constitutes the most frequent threat to finished feed, corn, wheat and silage. *Table 2* provides the detailed results of mycotoxin occurrence by commodity.

Conclusion

The analysis of the 6,844 samples in this survey clearly indicated 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.

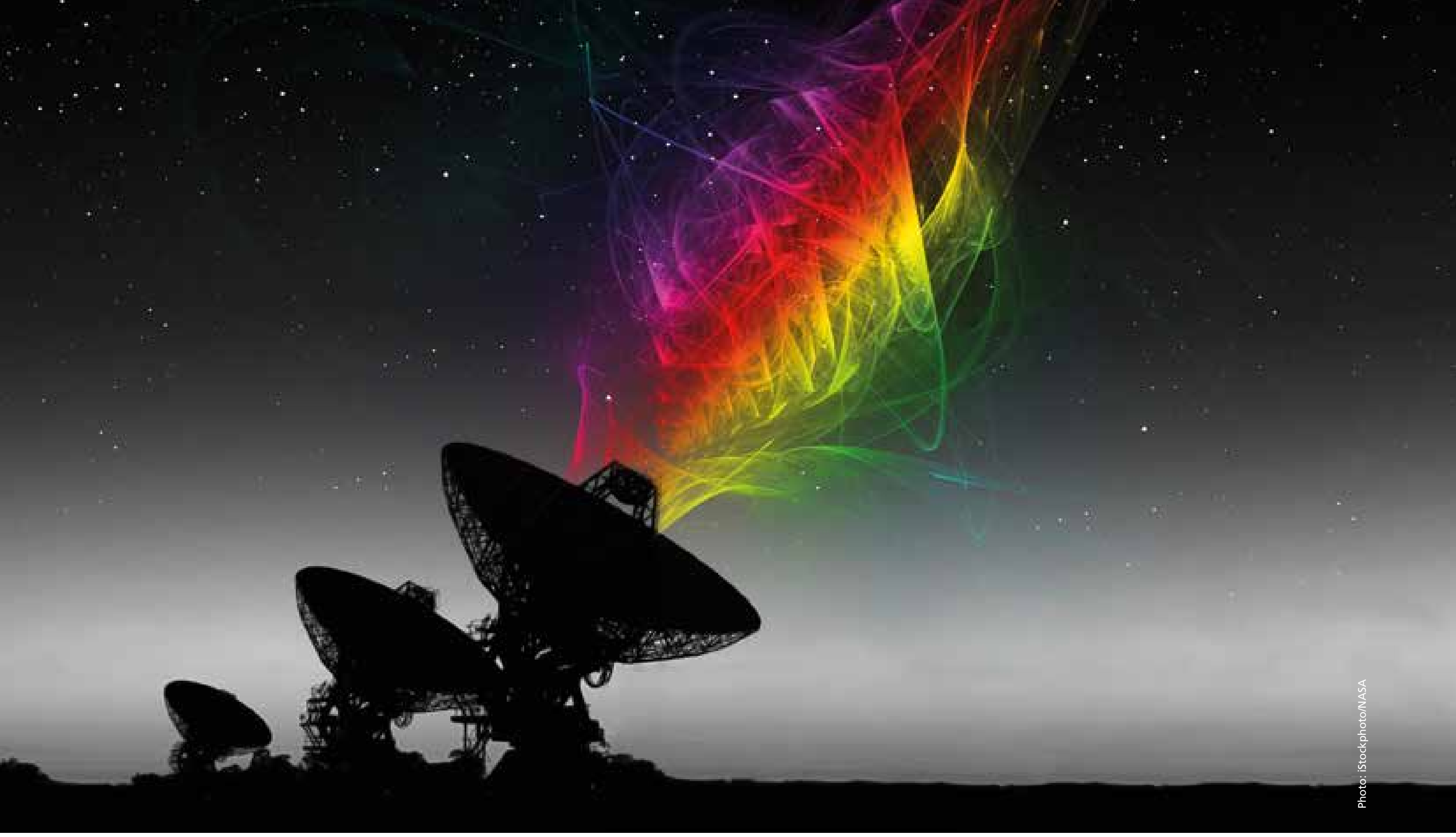


Photo: iStockphoto/NASA

Why Advanced Multiple Mycotoxin Detection Matters

By **Christina Schwab** and **Paula Kovalsky,** Product Managers, Mycotoxin Risk Management

A new technology promises to revolutionize the simultaneous detection of mycotoxins and greatly expand our scientific knowledge. Several cases in the field along with the 2014 BIOMIN Mycotoxin Survey results reveal the value of multiple mycotoxin detection in terms of understanding threats to livestock along with the direction of future research.

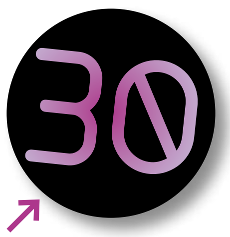
The monitoring of fungal toxins has become indispensable in the feed industry and animal production. Until recently, most of the available analytical methods only covered single classes of mycotoxins such as aflatoxins, type B trichothecenes or fumonisins. Over the past decade, the sensitivity of liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) has increased by 200 fold and it is quickly becoming the reference for multiple mycotoxin detection. The tool's power and accuracy allow for more refined detection of a greater number of mycotoxins and metabolites than ever before. Available commercially for the first time in 2014, next-generation mass spectrometry provides a more detailed picture of the contamination of different feed materials using Spectrum 380® to measure more than 380 mycotoxins and other secondary metabolites in one go.

Better detection in the field

By offering more powerful and accurate mycotoxin detection, Spectrum 380® can help farmers to understand situations that they encounter in the field that are not readily revealed by traditional techniques. Two case studies illustrate the benefits of better detection.

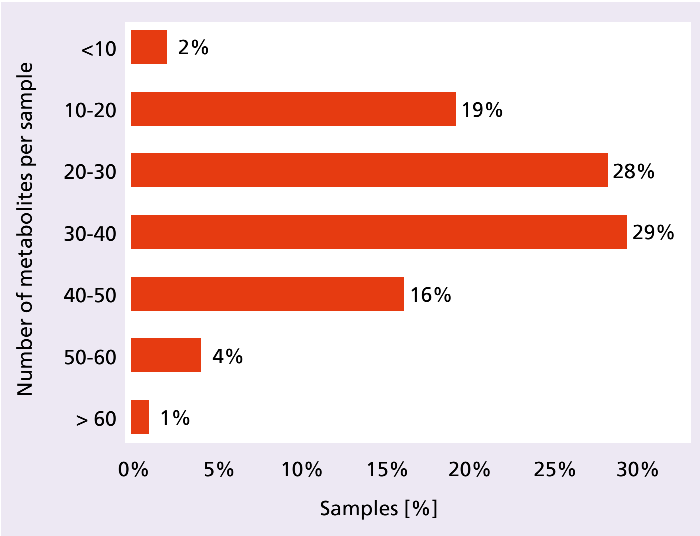
Co-exposure is the norm

A South African cattle farmer noticed that the animals were experiencing tremor



On average 30 different metabolites per sample were detected using Spectrum 380®.

Figure 1. Distribution of 814 samples according to number of mycotoxins per sample.



Source: 2014 BIOMIN Mycotoxin Survey

problems in their hind legs. Black spots on the barley in the feed raised suspicion of an *Aspergillus* contamination. The farmer conducted regular routine analysis that revealed only low levels of fumonisins, which did not explain the tremor problem. Multiple mycotoxin analysis using Spectrum 380® showed quite a different picture: the presence of other mycotoxins at high concentrations including 7 parts per million of cytochalasin E and 500 parts per billion of patulin, a mycotoxin with neurotoxic effects.

While this is just one example, we know from decades of research on mycotoxins that they tend to occur in groups. This phenomenon, known as co-exposure, is quite common as demonstrated by recent data.

For the first time the results of Spectrum 380® analysis of 814 raw materials and finished feed samples collected worldwide are included in the 2014 BIOMIN Mycotoxin Survey. On average, 30 different metabolites per sample were detected (*Figure 1*) based on data for all regions except Asia. The number of mycotoxins per sample ranged from 4 to 75. In 98% of samples more than 10 metabolites were found. (Using older mycotoxin detection methods, quantifying 380 metabolites in 814 samples would have required 309,320 separate analyses, versus 814 analyses with Spectrum 380®).

Compound (synergistic) effects

A Brazilian pig farmer observed ear necrosis, a well-known symptom of deoxynivalenol contamination, in his pigs. Mycotoxin analysis using enzyme-linked immunosorbent assay (ELISA) showed only very low levels deoxynivalenol in the three feed samples analyzed—too low to account for the ear necrosis. Multiple mycotoxin analysis using Spectrum 380® showed that in reality his animals faced a total concentration of mycotoxins ten times higher than ELISA indicated. In addition to deoxynivalenol, the feed samples contained other type B trichothecenes such as nivalenol and the glucosylated form of deoxynivalenol (DON-3-glucoside), and members of the culmorin family, known to intensify the effects caused by deoxynivalenol.

This case reflects the knowledge that some mycotoxins display synergistic effects: the adverse consequences of each mycotoxin (separately, often in a lab) are aggravated by multiple mycotoxin occurrence in the field and thus result in greater overall harm to the animal.

Informing the research agenda

Spectrum 380® analysis reveals that the major mycotoxins, present in over 40% of cases, account for just 6 of the 32 most common mycotoxins in the 814 samples tested (*Figure 2*). The others, sometimes referred to as emerging mycotoxins or exotic metabolites, are less well-understood in part because not enough data is available for toxicological studies to be conducted.

Figure 2 also shows that 95% of the 814 feed samples tested positive for the structurally-related group of beauvericin and enniatins. In December 2014, the European Food Safety Authority (EFSA) published a new scientific opinion on the health risks of these toxins in food and

↗ Further toxicity studies are required to gain sufficient data on the impact of exotic and emerging mycotoxins in animal health and performance.

Christina Schwab
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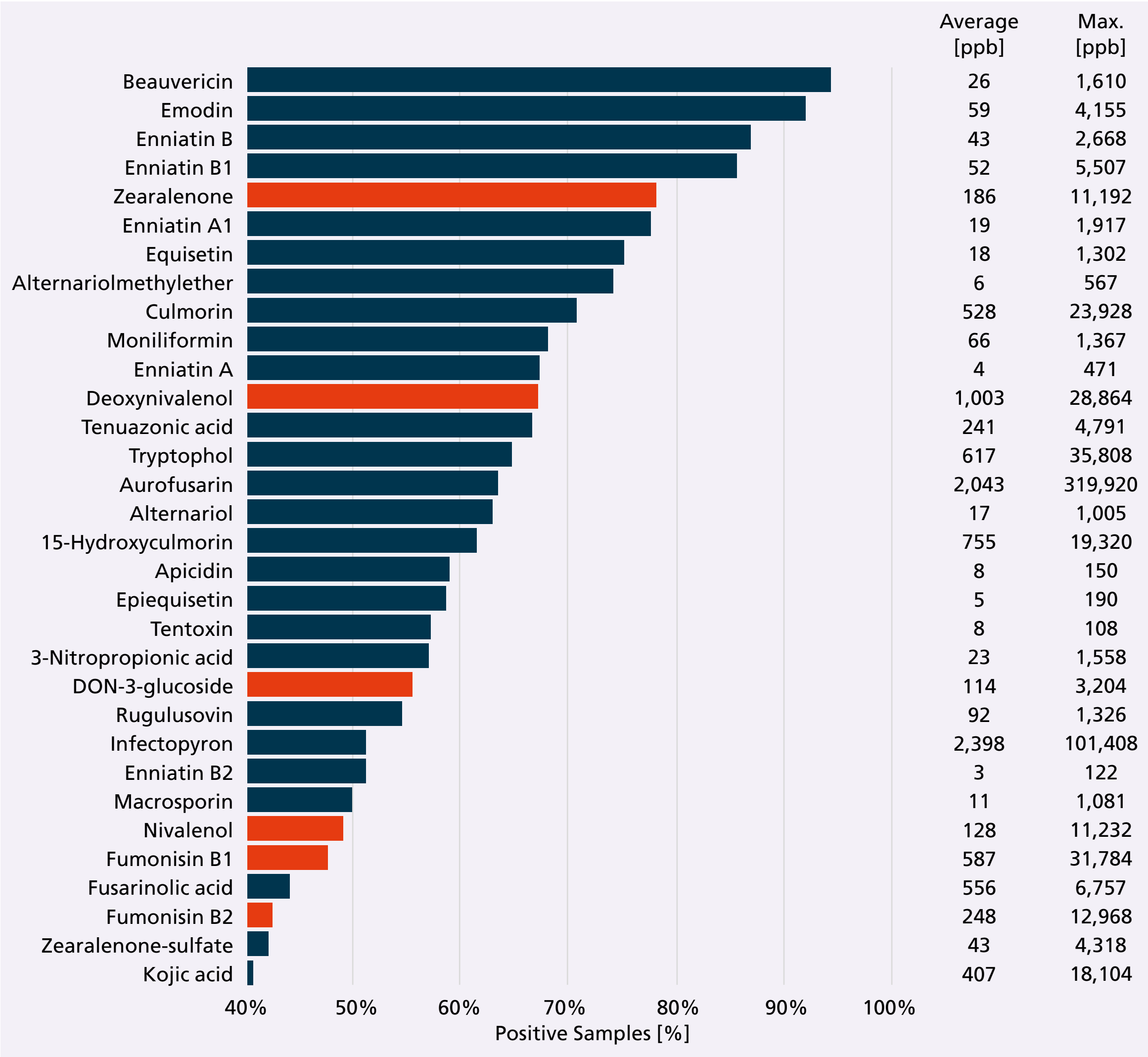
Figure 2. The 32 most common metabolites found in 814 worldwide feed and raw material samples tested (present in more than 40% of samples). Red-colored bars indicate well known mycotoxins. For the purpose of data analysis, limit of quantification (LOQ) level for each mycotoxin was adopted to determine positive samples.



Source: 2014 BIOMIN Mycotoxin Survey

feed highlighted the need for more data, especially in relation to their co-occurrence and possible combined effects with other *Fusarium* toxins.

Conclusion

The availability of LC–MS/MS and multiple mycotoxin detection will shed considerable light on mycotoxin analysis and the mycotoxin situation on the field. Using multiple mycotoxin detection technologies makes sense because of several things we know about mycotoxins. First, co-exposure is quite common. Second, there are negative synergistic effects among certain mycotoxins. Further toxicity studies are required to gain sufficient data on the impact of exotic and emerging mycotoxins in animal health and performance. Given the diversity of mycotoxins coming to light, effective mycotoxin risk management must rely upon the most up-to-date scientific knowledge and multiple modes of action to protect against agriculturally relevant mycotoxins.

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