Controllo e soluzioni per il problema delle micotossine nei mangimi

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DSM Austria

May 4th, 2023

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Mycotoxin analysis …

… from rapid detection to advanced multi-mycotoxin methods

Mycotoxin analysis

Analysis can be only as good as the sampling

Same average mycotoxin concentration

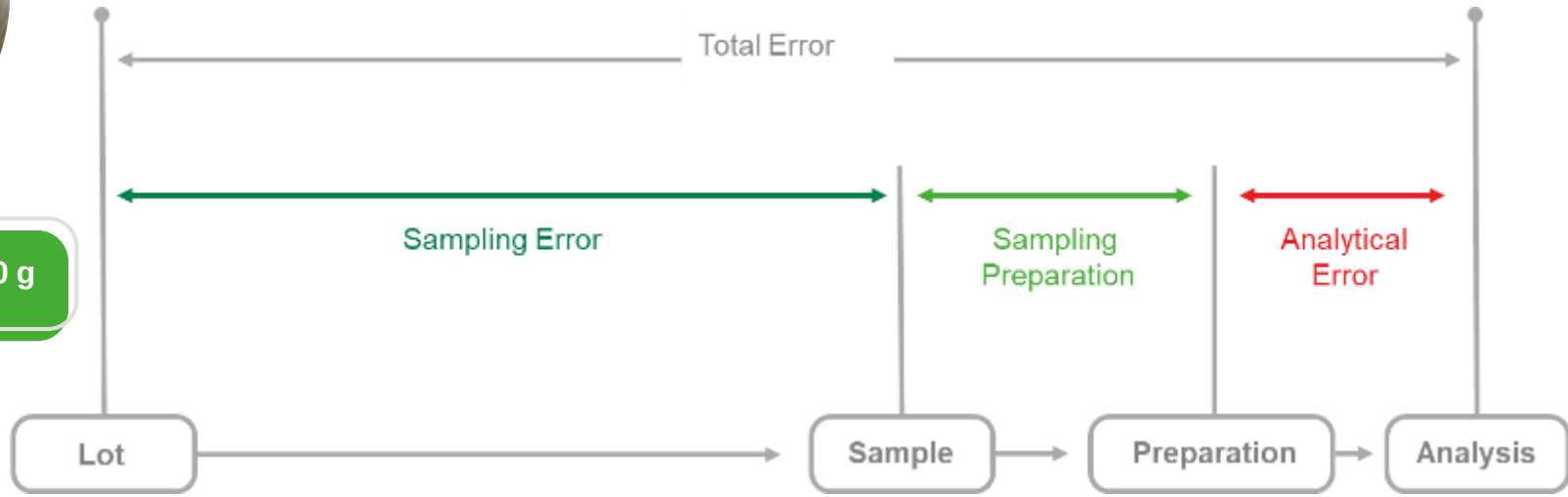
=

Grain Truck: 50 000 kg

Analytical Sample: 10-50 g

"Sampling contributes to up to 98% of the total uncertainty"

Whitaker, 2013

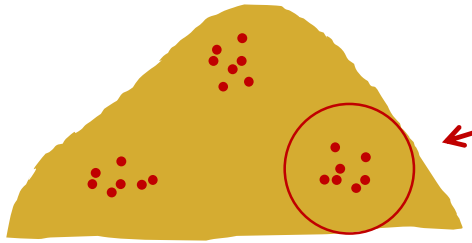


Mycotoxin Analysis: Sampling

Analysis can be only as good as the sampling

1 ppb =
1 gram of sugar
in an Olympic
swimming pool

Uneven distribution of **MYCOTOXINS** in grains



Based on EU Regulation (EC) No 401/2006

1. Collect incremental samples (1 kg) according to lot weight

2. Mix incremental samples to form the aggregate sample: >3 kg

3. Lab sample: Collect scoops from different points in the aggregate (1kg, e.g. 5x200g)

4. Send samples to lab for analysis

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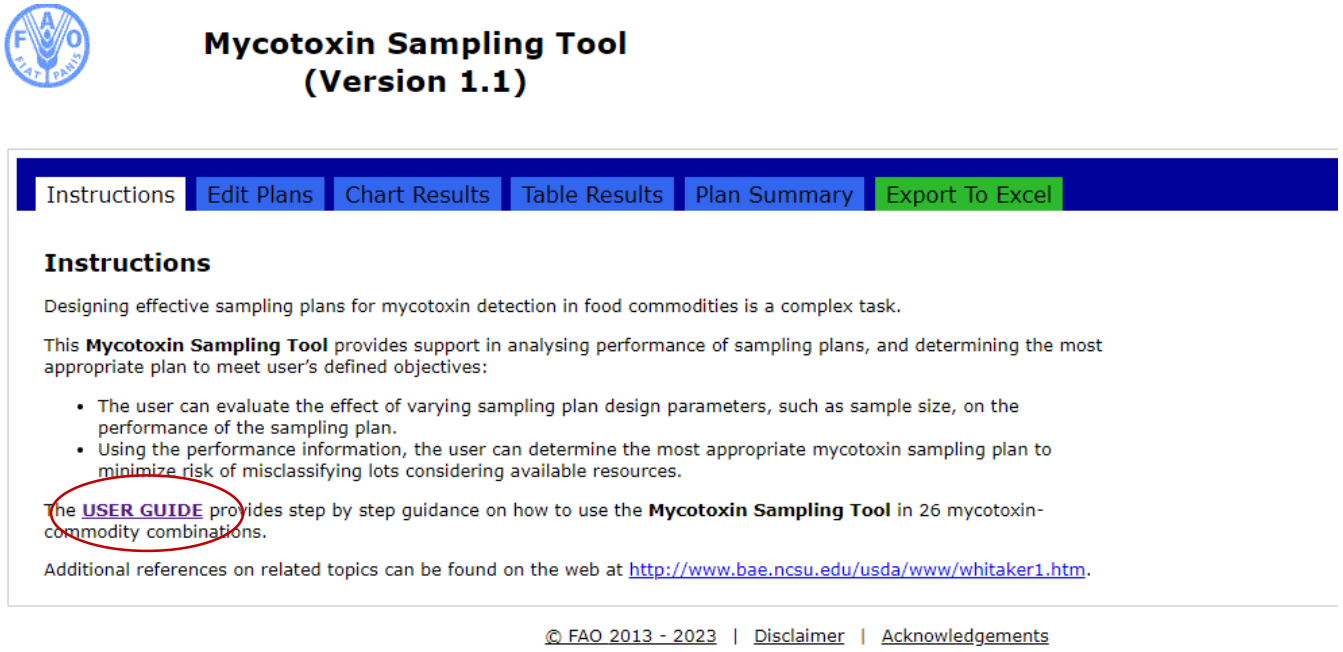
Mycotoxin Sampling Plan

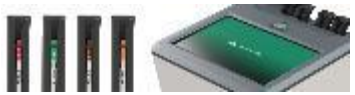
Effective sampling procedure: to obtain a representative sample **by** collecting sufficient (sub)samples to reduce analytical variability **and hence minimize the buyer's and seller's risk.**

- **FAO Mycotoxin Sampling Tool:** www.fstools.org/mycotoxins/



- **Commission Regulation (EC) No 401/2006** of 23 February 2006: "Laying down the Methods of Sampling and Analysis for the Official Control of the Levels of Mycotoxins in **Food**stuffs"
- **EN ISO 6497:2005 No 76/371/EC:** "Animal Feeding Stuffs – Sampling".

Mycotoxin analysis *Which method to choose WHEN?*



ELISA/Lateral Flow Strips

- ✓ Results needed straight away
- ✓ Screening of raw materials
- ✓ Check for single mycotoxins (suspicion)
- ✓ Quantification of main mycotoxins in specific matrices
- ✓ Less expensive than LC-MS/MS
- ✓ On-site detection possible

State-of-the-art

LC-MS/MS: Liquid chromatography coupled to mass spectrometry
➔ **multi-mycotoxin analysis**

Spectrum 380® and Spectrum Top® 50

- ✓ Fulfills the legal requirements
- ✓ Highly sensitive method
- ✓ Check complex matrices (final feed, CSI)
- ✓ More insights and detailed clarification
- ✓ Include common, masked & emerging mycotoxins
- ✓ Supporting differential diagnosis

spectrum TOP 50

Includes the
- ✓ Common mycotoxins and
- ✓ Most important masked & emerging mycotoxins

Spectrum 380®

- ✓ **Full picture** of the total toxic load in a sample (>800 plant and bacterial metabolites)
- ✓ Masked mycotoxins, emerging mycotoxins
- ✓ Phytoestrogens
- ✓ Not a routine analysis

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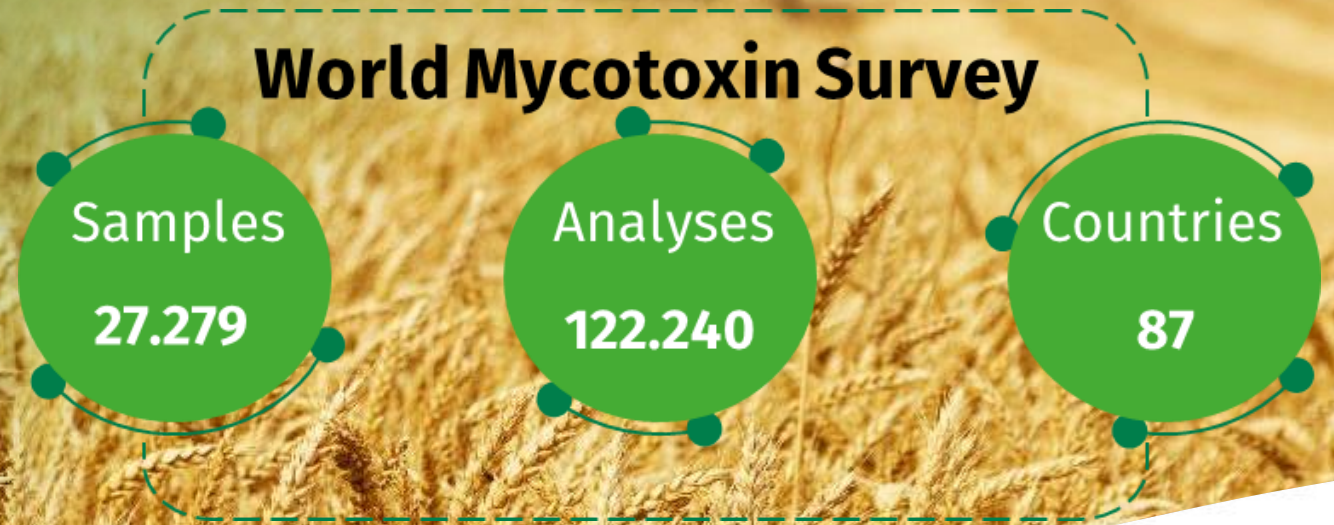
DSM World Mycotoxin Survey

Impact 2023

New insights from the world's largest mycotoxin survey



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- PRECISION SERVICES

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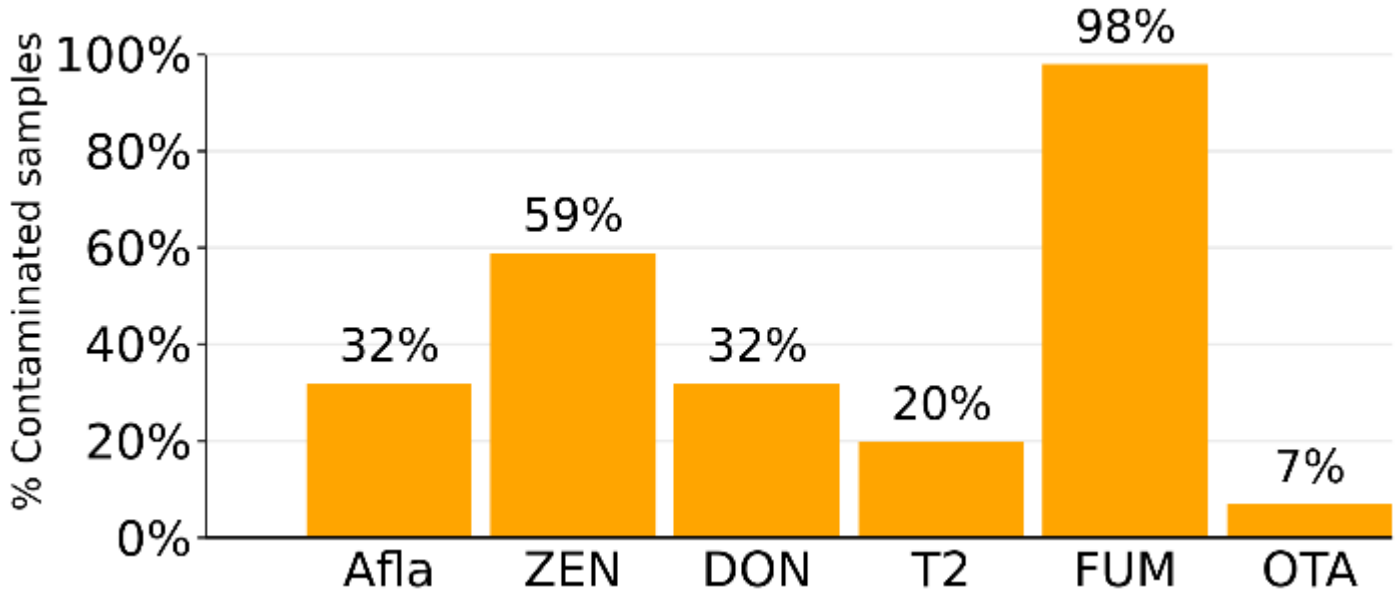
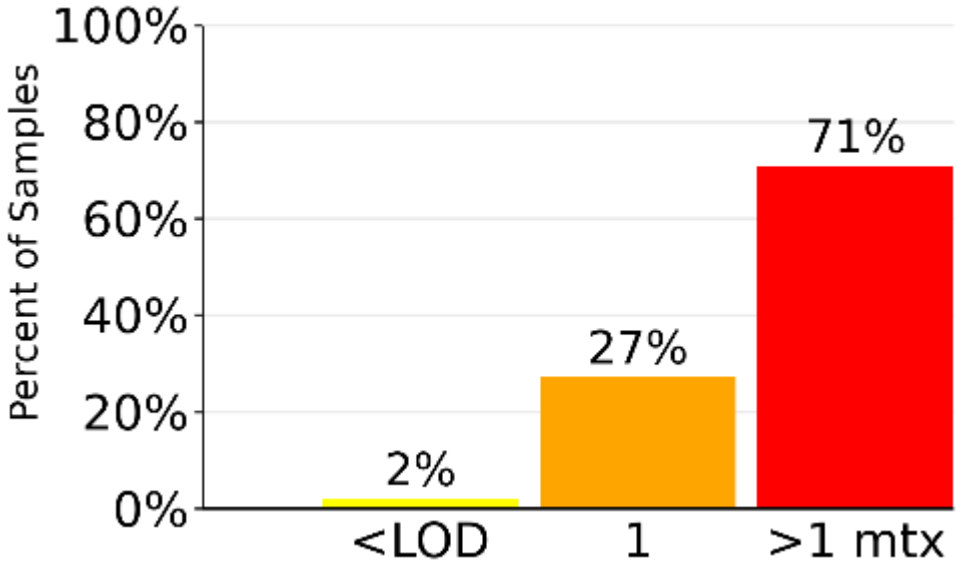
All commodities in Italy (Jan 2023 - Mar 2023)

Parameter	Afla	ZEN	DON	T2	FUM	OTA
Number of samples	103	103	103	103	103	103
% Contaminated samples	32%	59%	32%	20%	98%	7%
% Above risk threshold	21%	5%	15%	2%	55%	2%
Average of positives (ppb)	11	22	224	21	3195	8
Median of positives (ppb)	4	7	133	14	544	6
Maximum (ppb)	101	309	1057	103	37540	16

Prevalence of Mycotoxins Detected



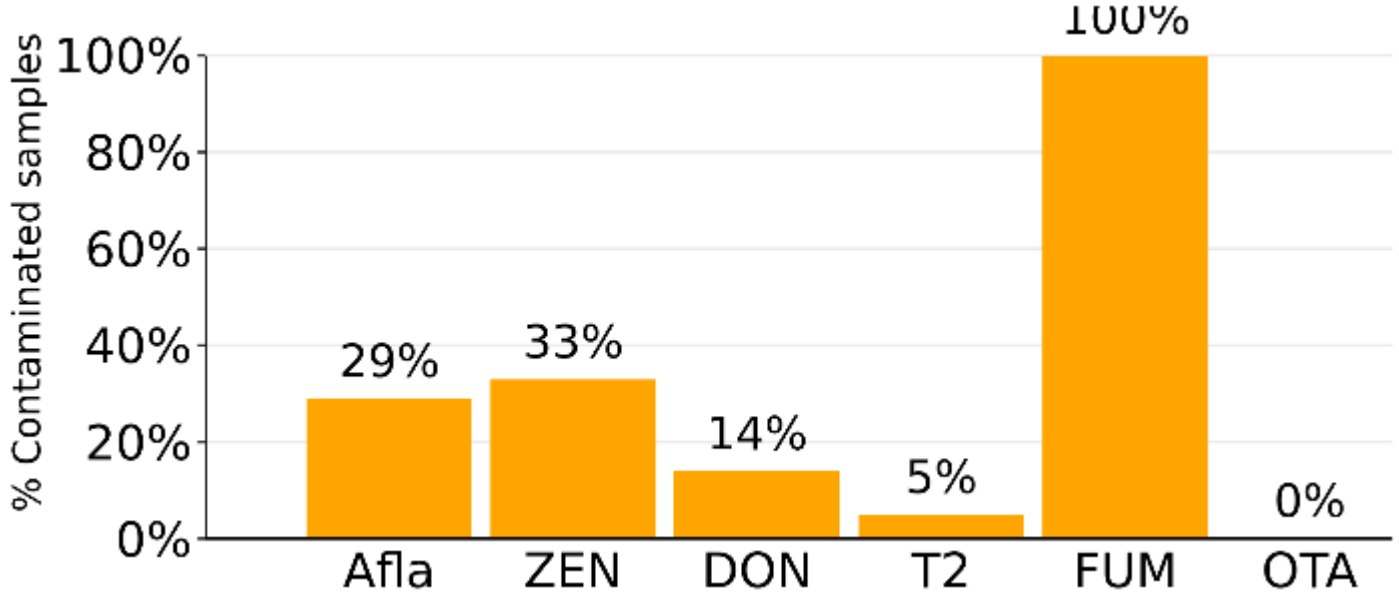
No. of Mycotoxins per Sample



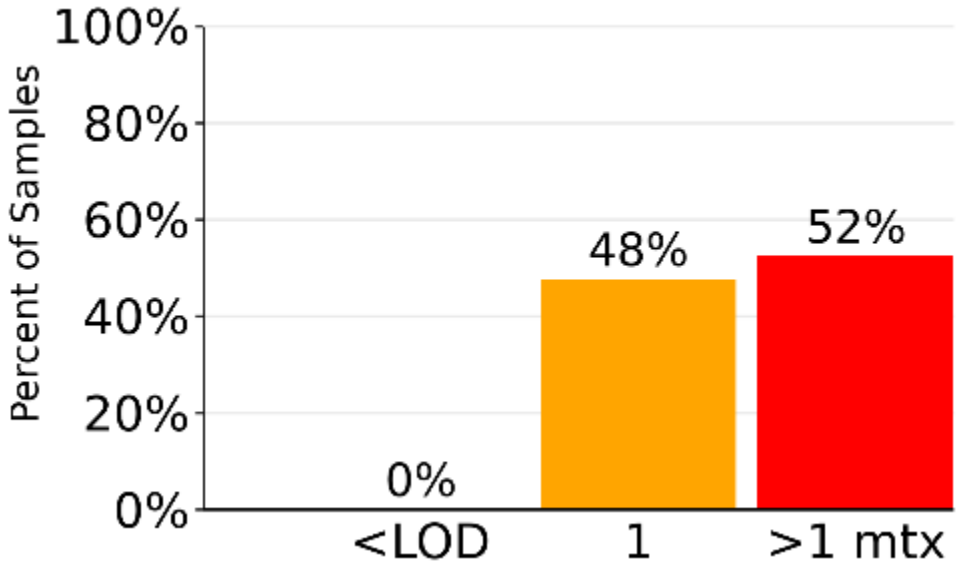
Corn kernels in Italy (Jan 2023 - Mar 2023)

Parameter	Afla	ZEN	DON	T2	FUM	OTA
Number of samples	21	21	21	21	21	21
% Contaminated samples	29%	33%	14%	5%	100%	0%
% Above risk threshold	29%	5%	5%	0%	76%	0%
Average of positives (ppb)	21	19	320	3	8445	
Median of positives (ppb)	6	6	149	3	3976	
Maximum (ppb)	101	84	706	3	37540	0

Prevalence of Mycotoxins Detected



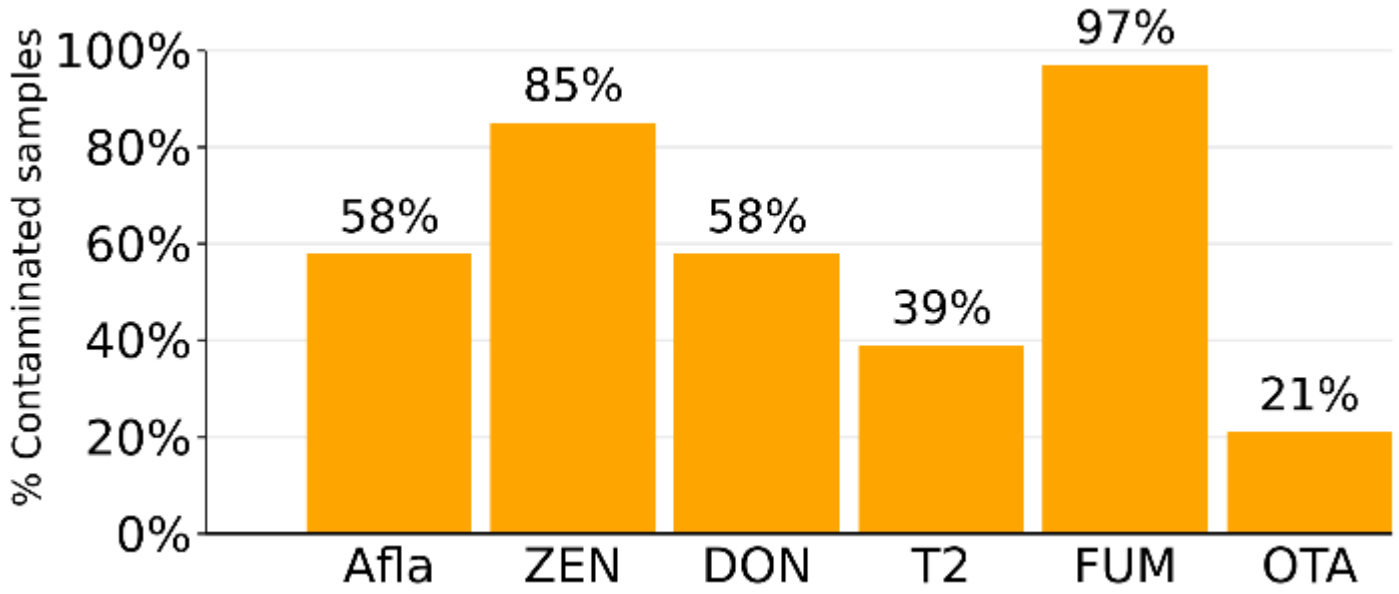
No. of Mycotoxins per Sample



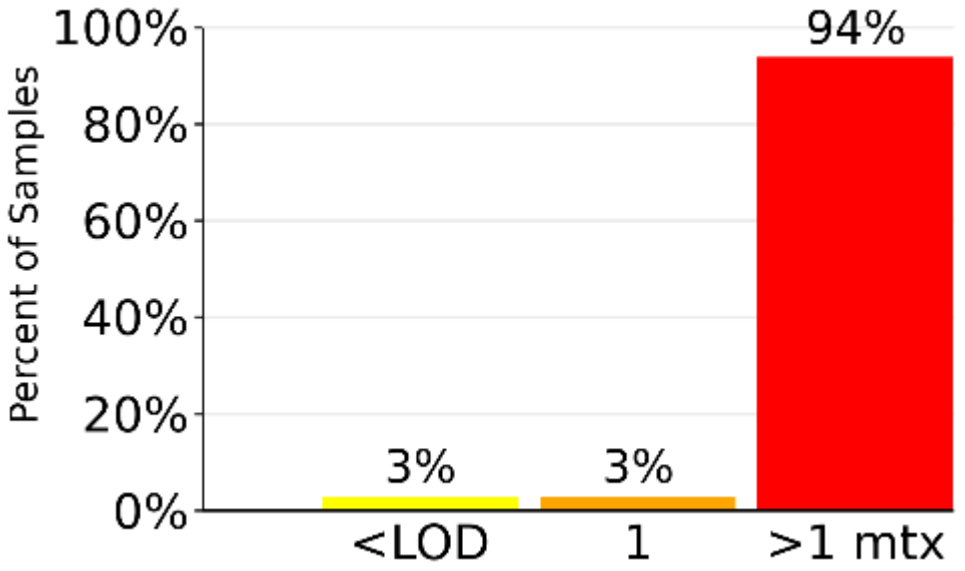
Finished Feed Poultry in Italy (Jan 2023 - Mar 2023)

Parameter	Afla	ZEN	DON	T2	FUM	OTA
Number of samples	33	33	33	33	33	33
% Contaminated samples	58%	85%	58%	39%	97%	21%
% Above risk threshold	36%	3%	27%	6%	52%	6%
Average of positives (ppb)	9	23	158	31	1923	8
Median of positives (ppb)	4	9	133	21	504	6
Maximum (ppb)	63	309	368	103	36719	16

Prevalence of Mycotoxins Detected



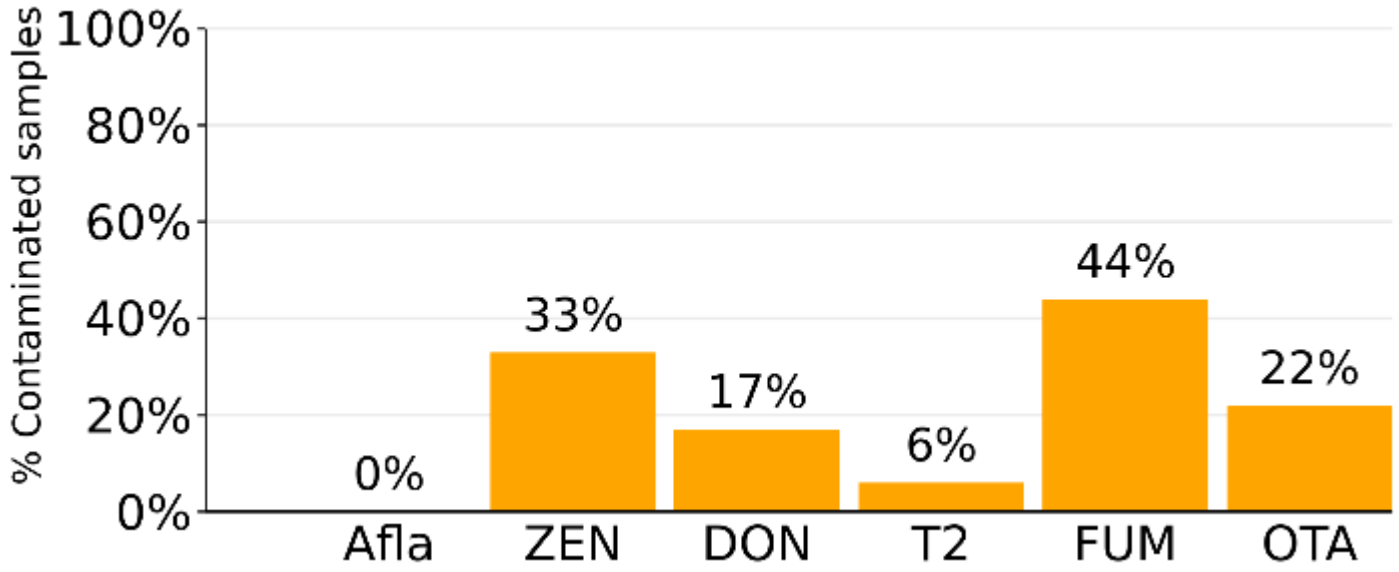
No. of Mycotoxins per Sample



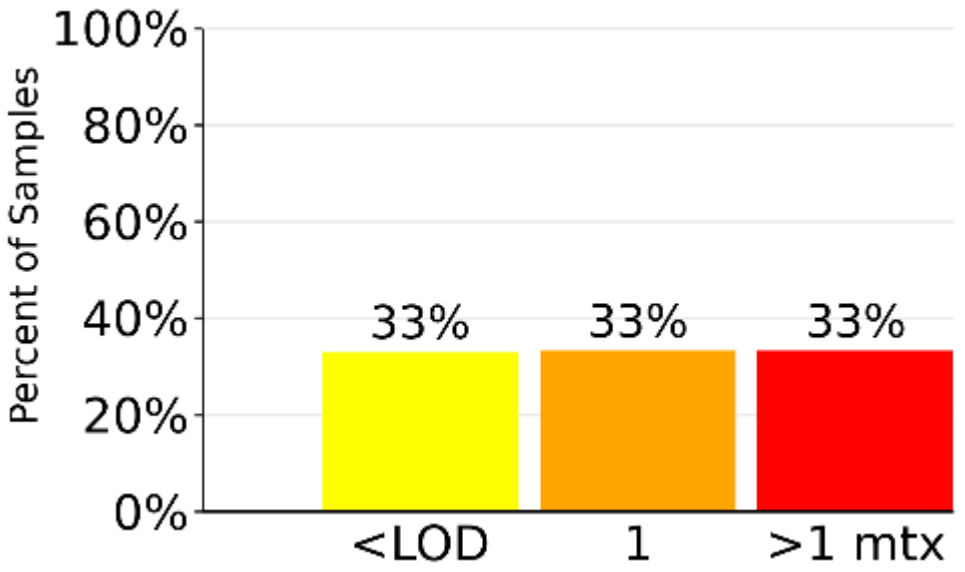
Soybean from USA (Jan 2023 - Mar 2023)

Parameter	Afla	ZEN	DON	T2	FUM	OTA
Number of samples	18	18	18	18	18	18
% Contaminated samples	0%	33%	17%	6%	44%	22%
% Above risk threshold	0%	0%	6%	0%	0%	0%
Average of positives (ppb)		19	149	5	24	3
Median of positives (ppb)		18	102	5	21	3
Maximum (ppb)	0	38	309	5	44	4

Prevalence of Mycotoxins Detected



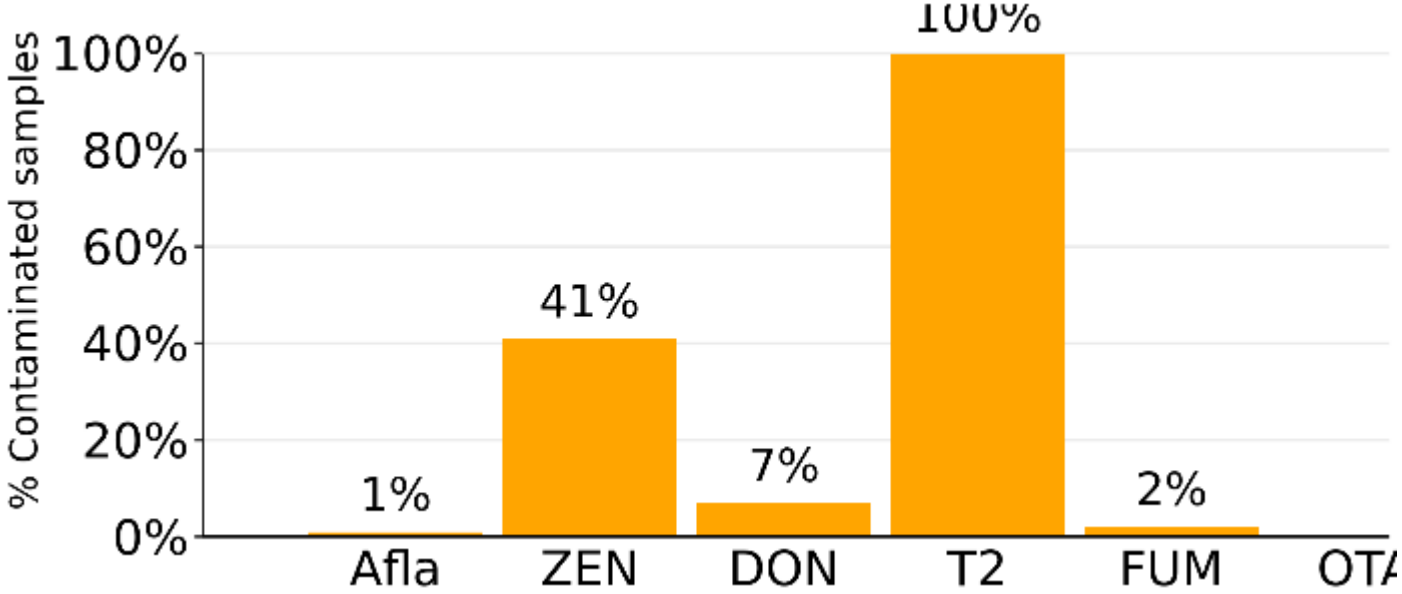
No. of Mycotoxins per Sample



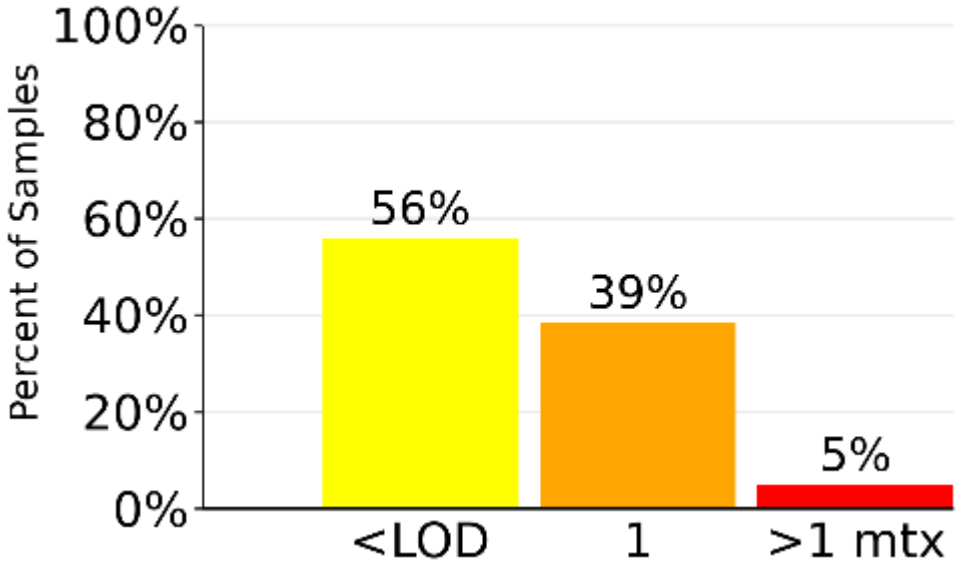
Soybean in Brazil (Jan 2023 - Mar 2023)

Parameter	Afla	ZEN	DON	T2	FUM	OTA
Number of samples	101	101	100	1	101	0
% Contaminated samples	1%	41%	7%	100%	2%	NA%
% Above risk threshold	1%	5%	7%	100%	2%	NA%
Average of positives (ppb)	3	36	724	59	1204	
Median of positives (ppb)	3	32	670	59	1204	
Maximum (ppb)	3	80	1700	59	1710	

Prevalence of Mycotoxins Detected



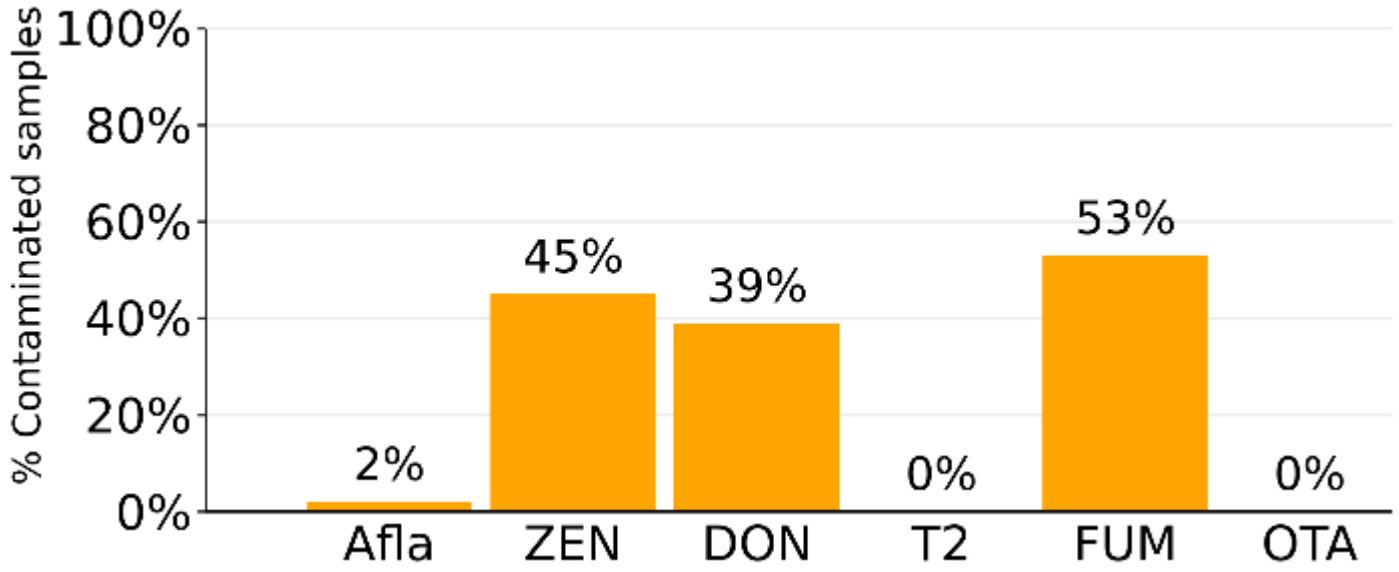
No. of Mycotoxins per Sample



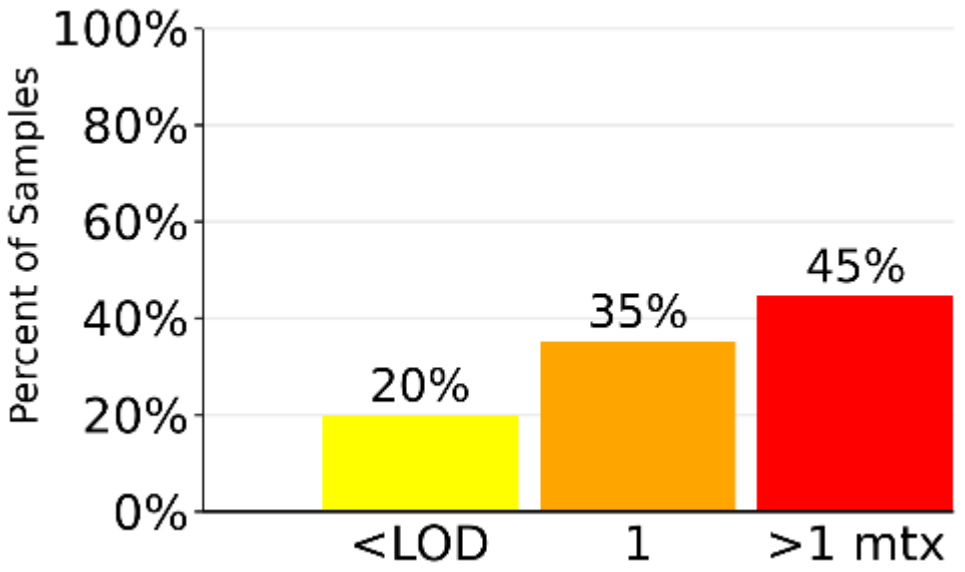
Corn kernels in Brazil (Jan 2023 to Mar 2023)

Parameter	Afla	ZEN	DON	T2	FUM	OTA
Number of samples	286	285	286	7	286	7
% Contaminated samples	2%	45%	39%	0%	53%	0%
% Above risk threshold	2%	32%	37%	0%	40%	0%
Average of positives (ppb)	27	132	613		1011	
Median of positives (ppb)	10	97	520		745	
Maximum (ppb)	119	522	1930	0	5640	0

Prevalence of Mycotoxins Detected



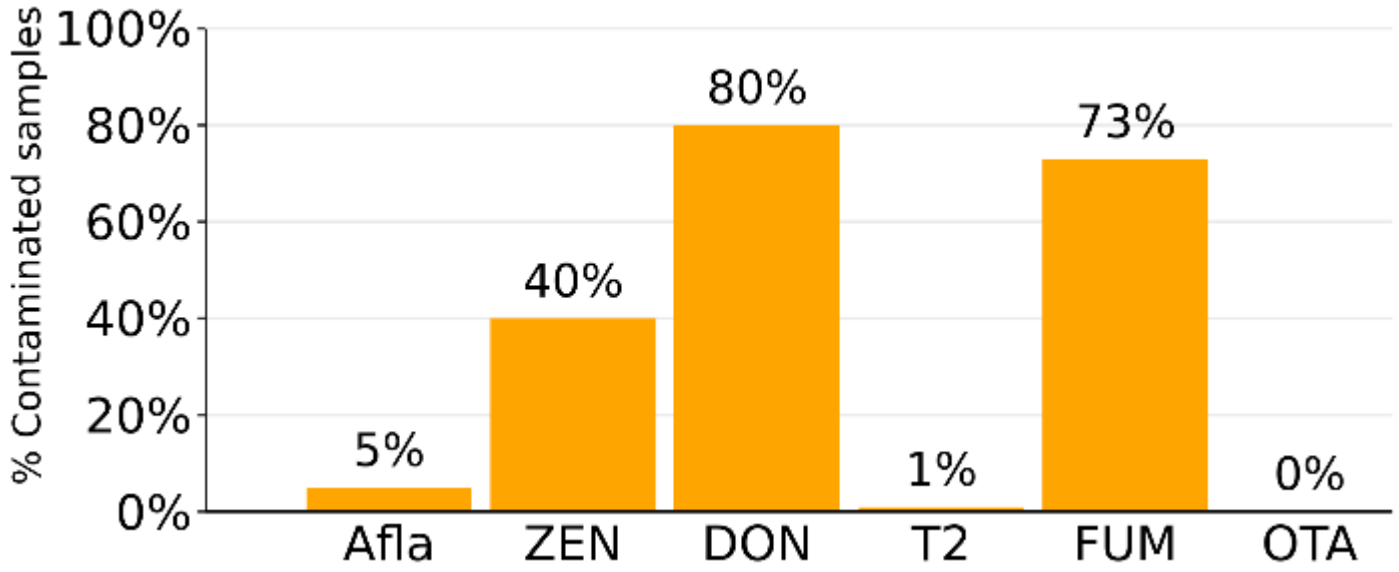
No. of Mycotoxins per Sample



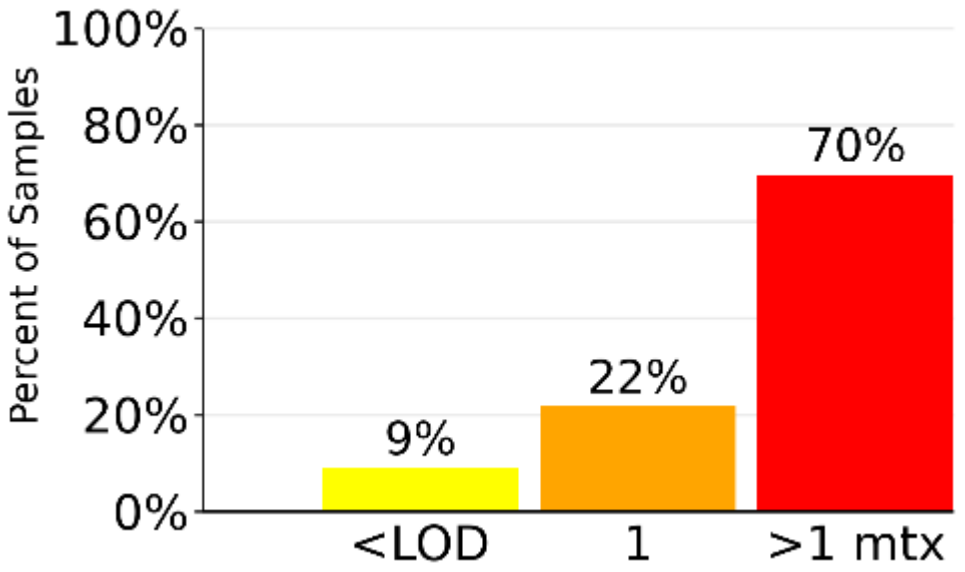
Corn kernels in USA from (Jan 2023 - Mar 2023)

Parameter	Afla	ZEN	DON	T2	FUM	OTA
Number of samples	142	142	142	142	142	142
% Contaminated samples	5%	40%	80%	1%	73%	0%
% Above risk threshold	4%	39%	76%	0%	47%	0%
Average of positives (ppb)	34	349	1267	1	4032	
Median of positives (ppb)	17	134	805	1	1080	
Maximum (ppb)	118	4310	8798	1	83175	0

Prevalence of Mycotoxins Detected



No. of Mycotoxins per Sample



Advanced detection with Multi-mycotoxin analysis methods

Emerging mycotoxins

spectrum TOP 50

ROMER Romer Labs®

Spectrum 380®

FULL 380

BOKU

IFA TULLN

Emerging mycotoxins: toxicity in a nutshell

Fusarium metabolites:

- **Enniatins** and **Beauvericin** show toxicity *in vitro*, but *in vivo* data are limited.
- **Moniliformin** is toxic to poultry (heart and immune system).
- **Fusaric Acid** is neurotoxic (poultry) and teratogenic (zebrafish). Synergism with DON and FUM.
- **Culmorin** shows synergistic effects with DON

Aspergillus metabolites:

- **Sterigmatocystin** structurally related to AfB1. Toxic *in vitro* to several cell lines. *In vivo* it is toxic to ruminants, pigs and chicken embryos.

Alternaria metabolites:

- **Alternariol** is toxic to mammalian cells *in vitro*. It is estrogenic and exhibits synergism with ZEN (inhibition of progesterone synthesis on porcine cell lines).
- **Tenuazonic Acid** is toxic in vivo on dogs, rats, monkeys and chicken. In poultry it causes lesions to several organs and decreases weight gain and feed intake

Alternaria sp.

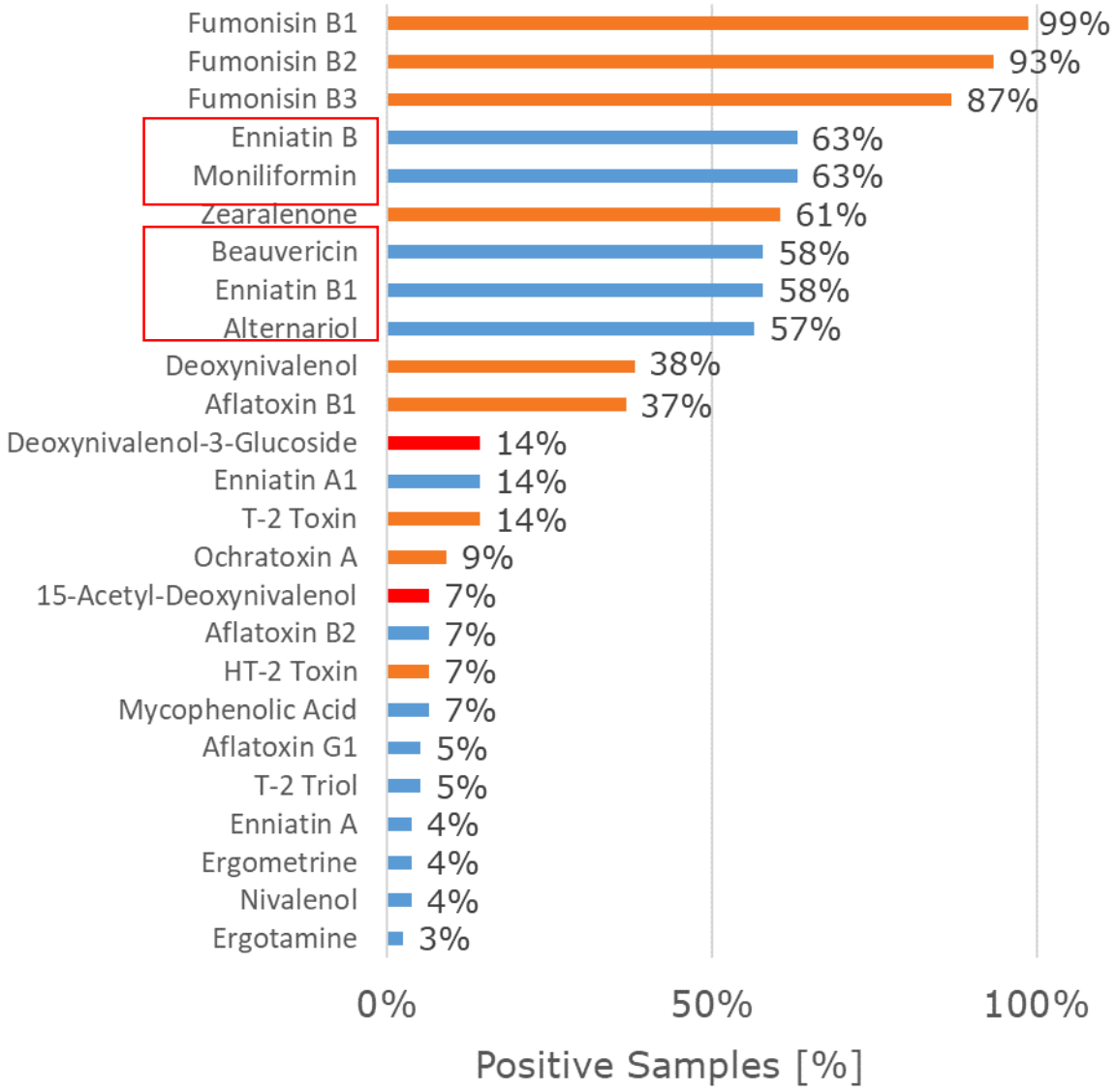
Aspergillus sp.

Fusarium sp.

Gruber-Dorninger et al. 2016

Multi-mycotoxin overview: Spectrum Top® 50; Focus Italy

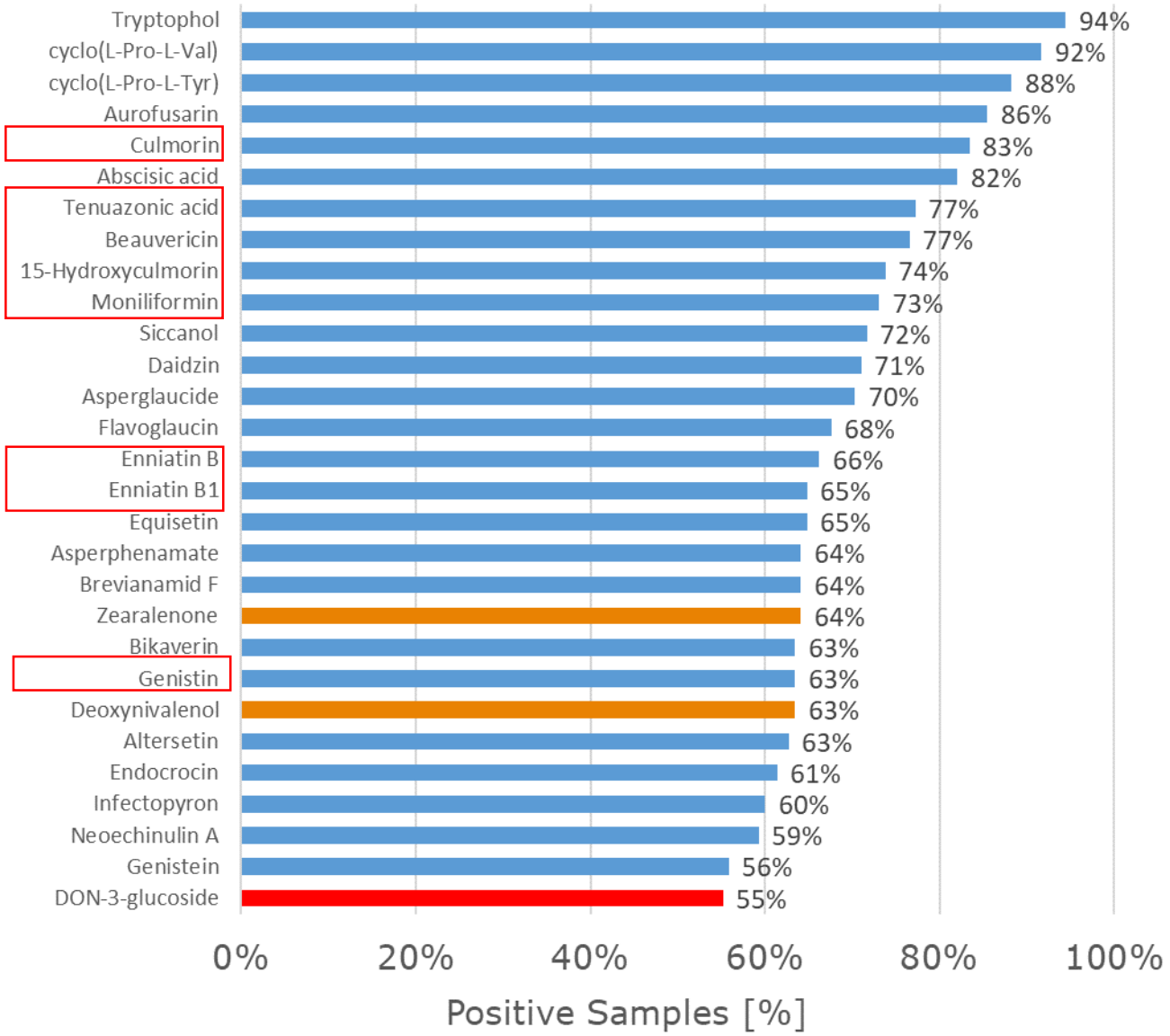
Italy data Jan-March 2023; n=76; Top25 metabolites shown



Metabolite	Average of positives (ppb)	Maximum (ppb)
Fumonisin B1	1513	25375
Fumonisin B2	538	8734
Fumonisin B3	231	2930
Enniatin B	5	26
Moniliformin	319	8951
Zearalenone	24	309
Beauvericin	87	1459
Enniatin B1	3	25
Alternariol	7	42
Deoxynivalenol	217	1057
Aflatoxin B1	8	60
Deoxynivalenol-3-Glucoside	53	118
Enniatin A1	2	4
T-2 Toxin	25	103
Ochratoxin A	8	16
15-Acetyl-Deoxynivalenol	76	182
Aflatoxin B2	2	4
HT-2 Toxin	25	25
Mycophenolic Acid	32	71
Aflatoxin G1	6	8
T-2 Triol	19	33
Enniatin A	2	2
Ergometrine	8	8
Nivalenol	739	943
Ergotamine	13	19

Multi-mycotoxin overview: Spectrum 380®

Global data Jan-March 2023; n=145; all results >=55% prevalence shown



Metabolit

Tryptophol
cyclo(L-Pro-
cyclo(L-Pro-
Aurofusarin
Culmorin
Abscisic aci
Tenuazonic
Beauvericin
15-Hydroxyc
Moniliformi
Siccanol
Daidzin
Asperglauci
Flavoglaucir
Enniatin B
Enniatin B1
Equisetin
Asperphena
Brevianamic
Zearalenone
Bikaverin
Genistin
Deoxynivaler
Altersetin
Endocrocin
Infectopyron
Neoechinulin A
Genistein
DON-3-glucoside

40

Average number of Mycotoxins and Metabolites per sample

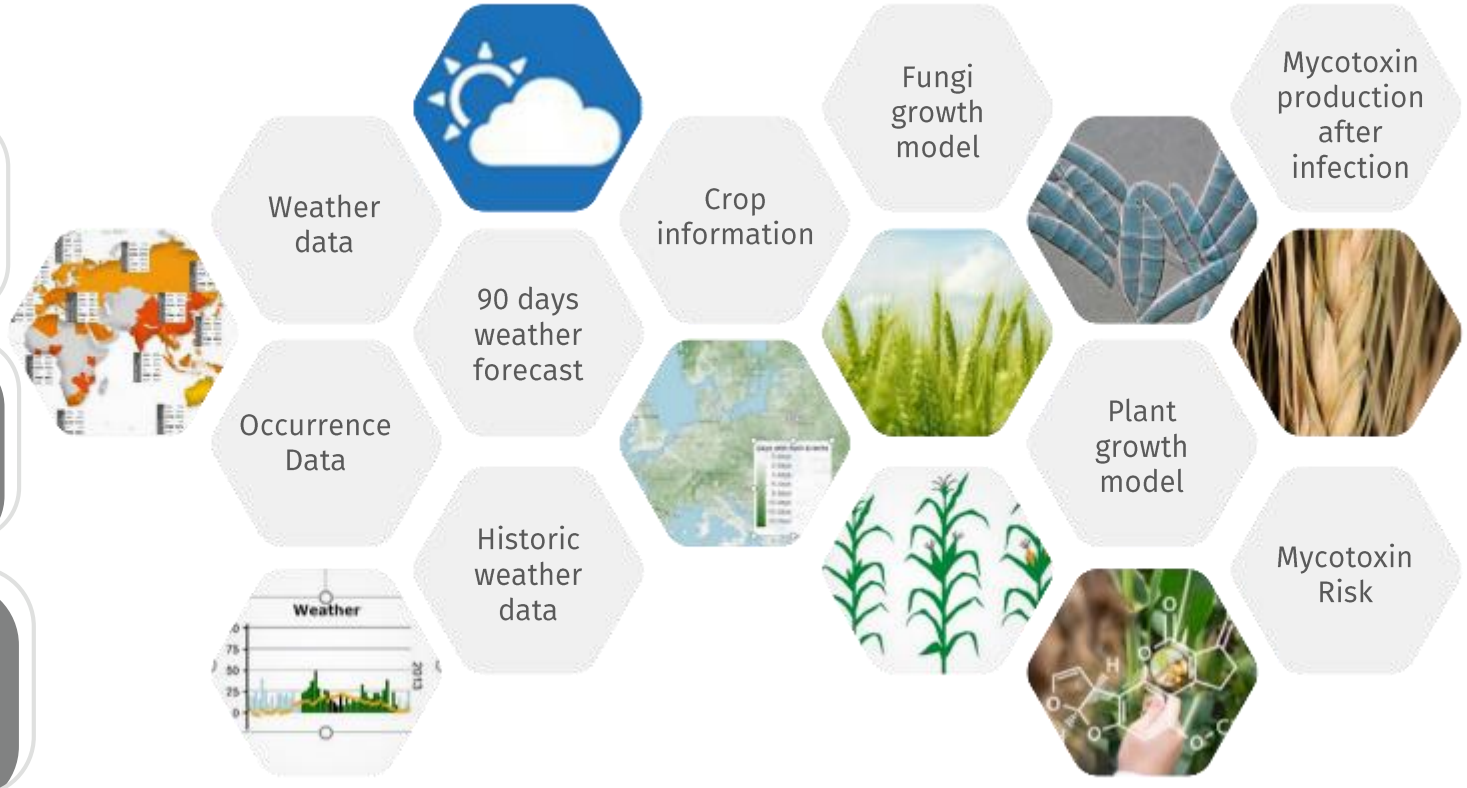
Mycotoxin Prediction Tool – looking into the future…

Mycotoxin Prediction

Corn: risk of Afla, DON, FUM and ZEN

Wheat: risk of DON and ZEN

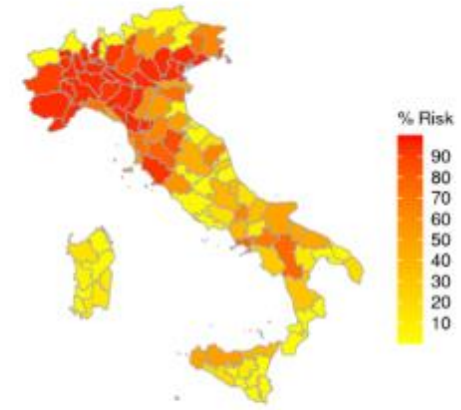
- Based on weather data, cultivar, tillage, fungicide, …
- Incorporating mechanistic models of Battilani et al
- Models are adapted with Mycotoxin Survey results and linked to global weather data and crop data



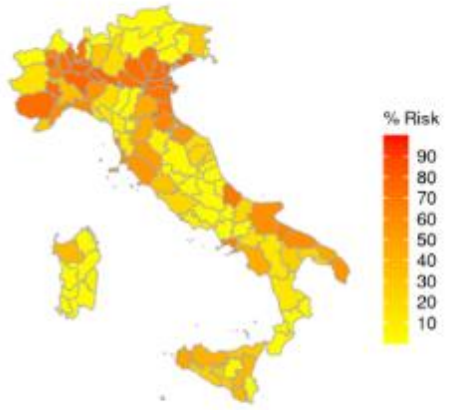
Battilani, P., Leggieri, M.C., Rossi, V. and Giorni, P., 2013. AFLA-maize, a mechanistic model for Aspergillus flavus infection and aflatoxin B1 contamination in maize. Computers and electronics in agriculture, 94, pp.38-46.
Battilani, P., Rossi, V. and Pietri, A., 2003. Modelling Fusarium verticillioides infection and fumonisin synthesis in maize ears. Aspects of applied biology, 2003(1), pp.91-100.

Reaching the clients - Country reports example

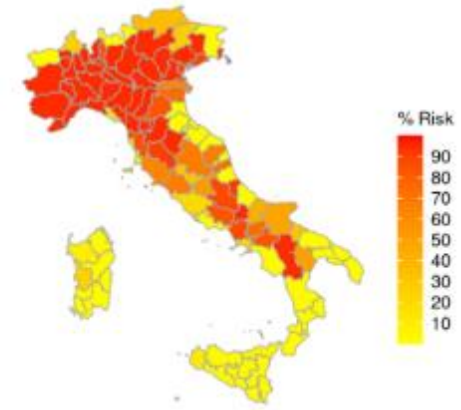
Italy risk of FUM in Corn = 31%



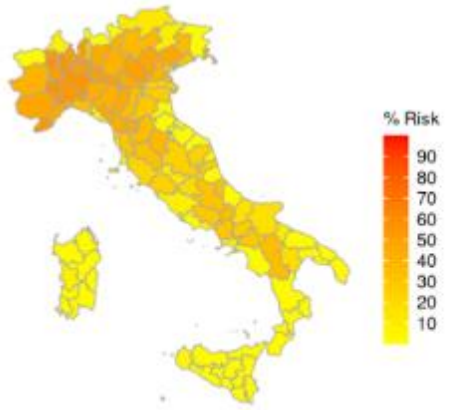
Italy risk of AFLA in Corn = 28%



Italy risk of DON in Corn = 33%

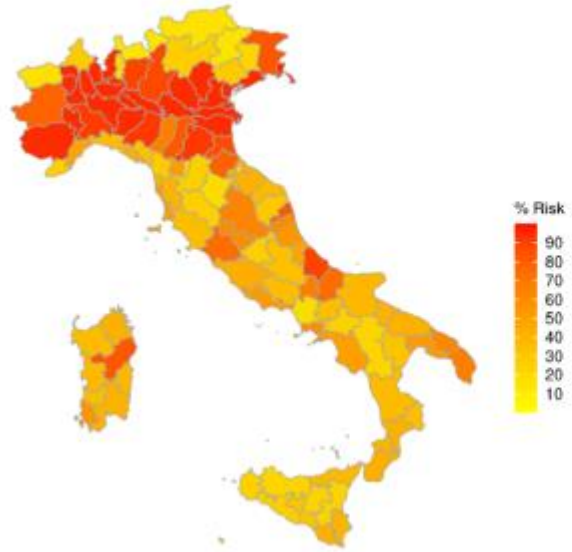


Italy risk of ZEN in Corn = 13%



- List of over 20 countries around the world
- Automatically created bi-weekly
- Disclaimer: *As mycotoxin predictions are based on statistical methods, DSM neither gives any warranties with regard to the content, nor accepts any liabilities in connection with the predictions or its consequences, if any.*

Italy risk of DON in Wheat = 41%



Italy risk of ZEN in Wheat = 6%

Accessed April 28th, 2023

Mycotoxin counteracting strategies –

why do we need them and what solutions there are …

Mycotoxin occurrence leads to ….

Significant annual losses for livestock industry and agriculture

Due to:

- Loss of crop production
- Disposal of contaminated feed
- Reduced livestock production
- Increased animal health care costs
- Analytical and regulatory costs

Estimations of economic losses associated with mycotoxin contamination:

annual losses in US only due to ***fumonisins*** in animal feed

US$ 1-20M

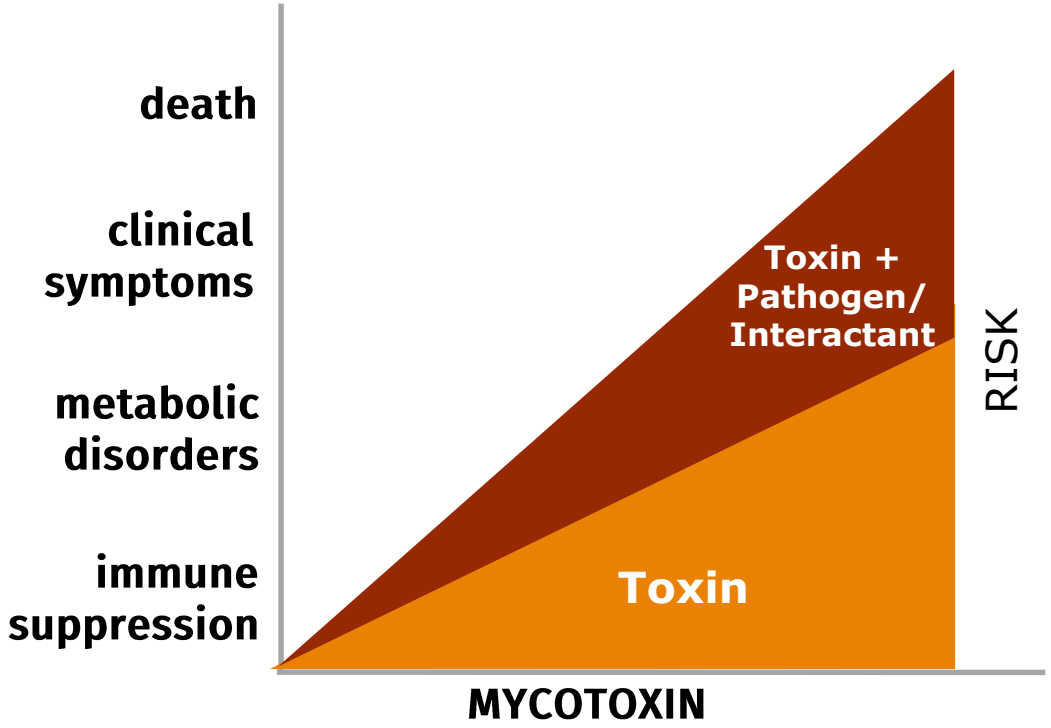
annual losses of corn industry in US due to ***aflatoxins***

US$ 52.1M to US$ 1.68B

Bryden W.L., 2012; Mitchell *et al.*, 2016; Pinotti *et al.*, 2016; Wu F., 2015; Wu F., 2007

Adverse effects already at low mycotoxin contamination levels

There are no safe levels: major effects can be observed in the immune system at low mycotoxin contamination levels

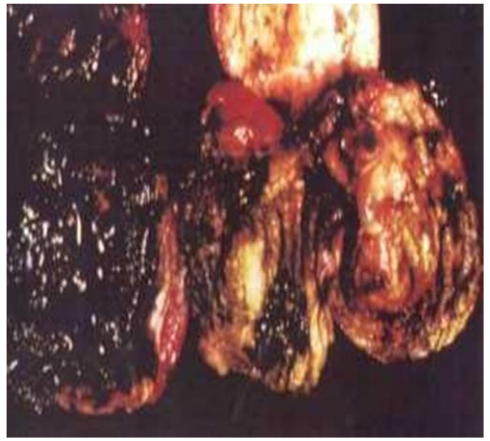


Hamilton, 1984

Consequences of immune suppression

- increased risk of infections
- more severe disease processes
- therapies become more difficult
- impaired vaccination response

Symptoms



acute – sub acute – chronic,

systemic – local – organ specific,

often non-specific (reduced productivity)

depend on **toxin-**, **animal-** and **environmental**-related factors

structure of mycotoxin(s), their distribution

exposition pattern / duration

animal species (ruminants, monogastric animals)

breed, sex, age, general health, immune status

farm management / infections

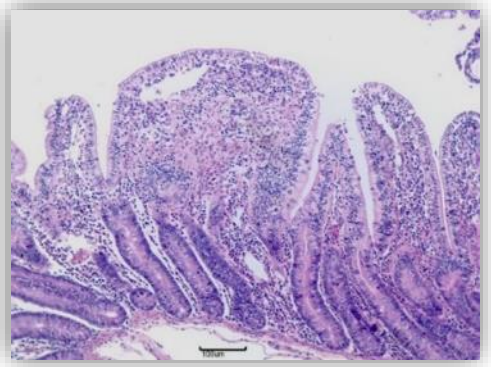
etc.

Subclinical effects

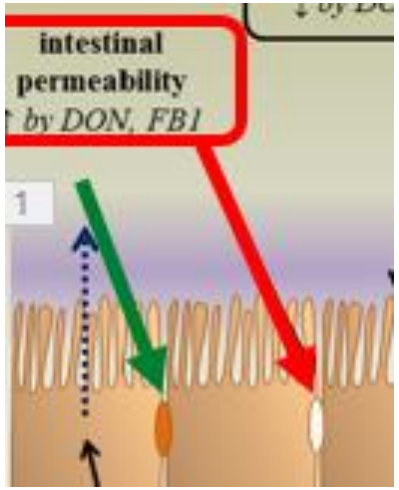
Mycotoxins ….

- ✓ Reduce the surface area for nutrient absorption and inhibit the transport of nutrients and minerals
- ✓ Reduce the barrier function of the GI tract increasing permeability opening for pathogens (and more toxins!)
- ✓ Interfere with the immune system
- ✓ Act as a cofactor in the development of diseases (NE, coccidiosis, pneumonia)

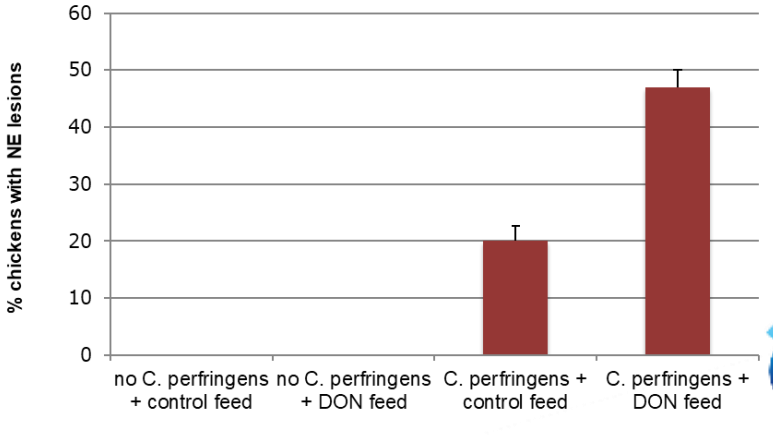
… resulting in impaired productivity and health



Bracarense et al., 2011; Grenier et al., 2011



Grenier & Applegate, 2013; Antonissen et al., 2014



Antonissen et al., 2013

Out of +500, only 3 ingredients are authorized in the EU

COMMISSION IMPLEMENTING REGULATION (EU) No 1060/2013

of 29 October 2013

concerning the authorisation of bentonite as a feed additive for all animal species

COMMISSION IMPLEMENTING REGULATION (EU) No 1016/2013

of 23 October 2013

concerning the authorisation of a preparation of a micro-organism strain DSM 11798 of the *Coriobacteriaceae* family as a feed additive for pigs

COMMISSION IMPLEMENTING REGULATION (EU) 2017/930

of 31 May 2017

concerning the authorisation of a preparation of a microorganism strain DSM 11798 of the *Coriobacteriaceae* family as a feed additive for all avian species and amending Implementing Regulation (EU) No 1016/2013

COMMISSION IMPLEMENTING REGULATION (EU) 2018/1568

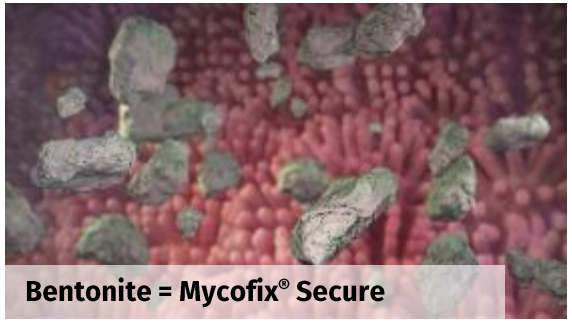
of 18 October 2018

concerning the authorisation of a preparation of fumonisin esterase produced by *Komagataella phaffii* (DSM 32159) as a feed additive for all pigs and all poultry species

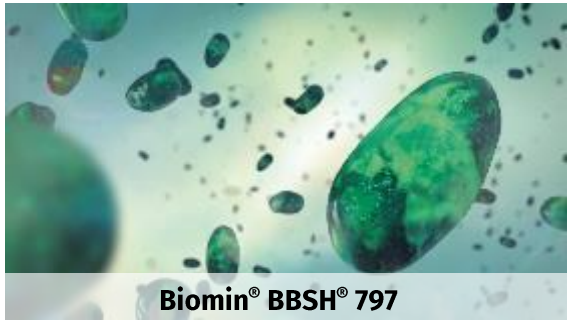
COMMISSION IMPLEMENTING REGULATION (EU) 2021/363

of 26 February 2021

concerning the authorisation of a preparation of fumonisin esterase produced by *Komagataella phaffii* DSM 32159 as a feed additive for all animal species



Bentonite = Mycofix® Secure



Biomin® BBSH® 797

FUMzyme®

Strict Requirements

EFSA set up rigid parameters for assessment of safety and efficacy of products.

- **Target mycotoxin(s)** must be specified.
- Mycotoxin binders/inactivators must not **interfere** with the **analytical determination** of mycotoxins in feed.
- **Safety** of the additive and the resulting metabolites, residues on the animal and consumer must be shown. Interaction with other feed components ascertained.
- Scientific **biomarkers** to **directly** prove the deactivation of mycotoxins *in vivo* are required

➢ The registration demonstrates the capacity of such products in a **standardized** and **fair process.**

Why do we need Biomarkers?

Efficacy of the product: *In vitro* data are not enough!

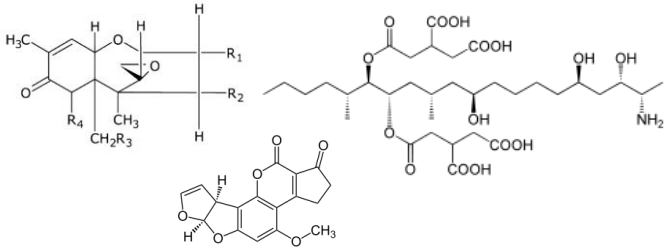
- Significant effects must be proven **by relevant biomarkers** (as most relevant end-points for substances reducing the contamination of feed by mycotoxins) in different studies - with sufficient number of animals/ replicates for statistical analysis of data.

- **Improved general animal performance:**
  Can be due to an indirect effect of the additive, e.g. **compensation of toxic effects** by antioxidants, immune stimulators, pharmacological substances (different group of additive).

Mycotoxin biomarkers

Which biomarkers?

EXPOSURE



Mycotoxins/Metabolites
in suitable biological matrix

- DON/metabolites in blood
- FUM/metabolites in feces
- ZEN/metabolites in urine
- …

EFFECT

Only one scientifically recognized biomarker of effect!

Sa/So ratio ↑ in presence of FUMs

Mycotoxin biomarkers

What for?

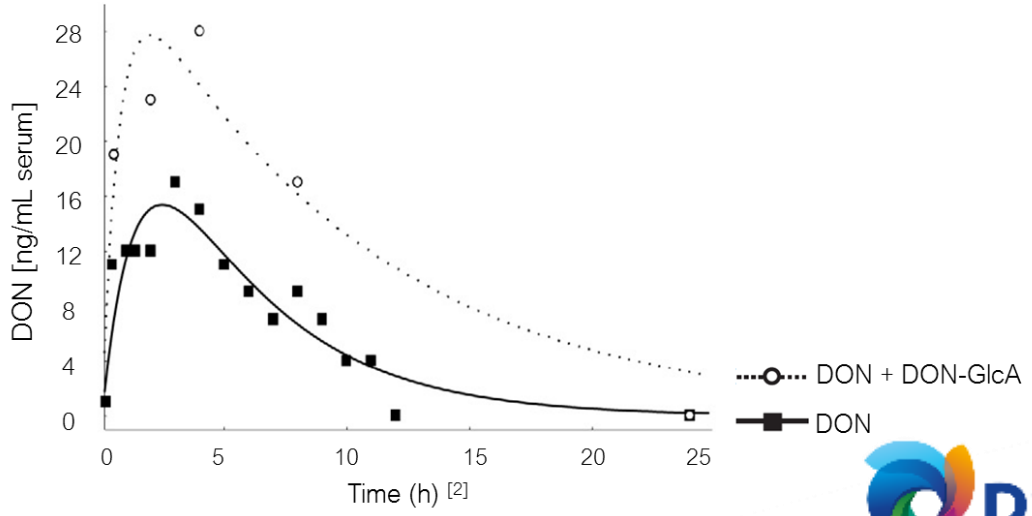
Diagnostic tool

for mycotoxin exposure or
to explain poor performance

- Analytical challenges
- Species-specific TOXICOkinetics
- Mycotoxin-specific TOXICOkinetics

Very low bioavailability [1]

High bioavailability
fast elimination [2] t_{max}: 1.7 – 4 h



[1] Voss et al., 2007. Anim. Feed Sci. Technol. 137; [2] Dänicke & Brezina 2013. Food Chem. Toxicol. 60; [3] Goyarts & Dänicke 2006. Toxicol. Lett. 163

Mycotoxin biomarkers

What for?

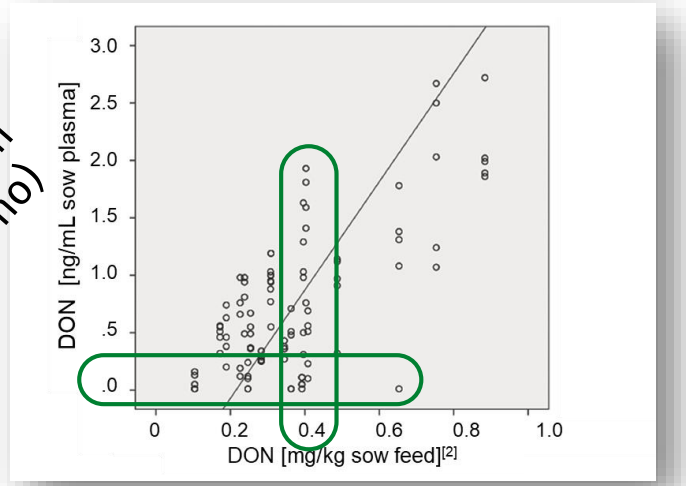
Diagnostic tool

for mycotoxin exposure or
to explain poor performance

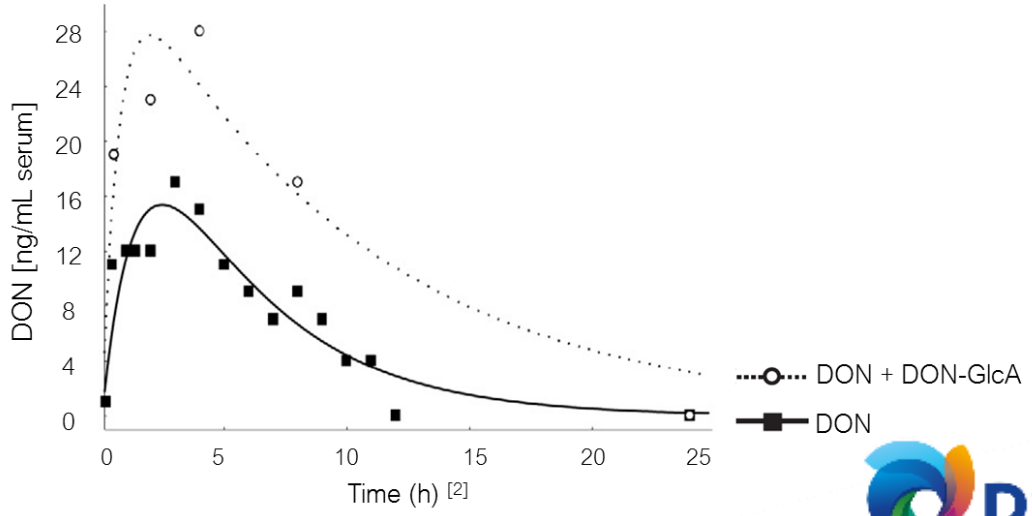
- Analytical challenges
- Species-specific TOXICOkinetics
- Mycotoxin-specific TOXICOkinetics

What does DON in blood tell about DON in feed?

No correlation
rough indication on exposure (yes/no)



Van Limbergen et al., 2017. Vet. Rec. 181



DSM

Mycotoxin biomarkers

What for?

Diagnostic tool

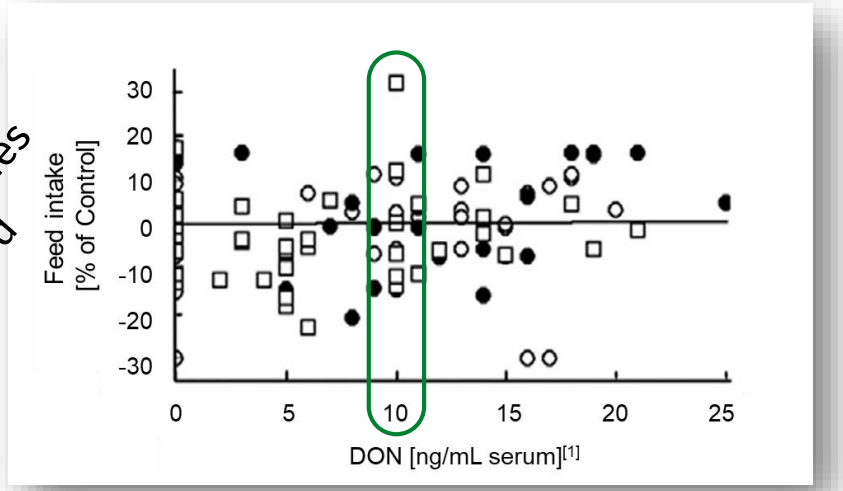
for mycotoxin exposure or
to explain poor performance

?

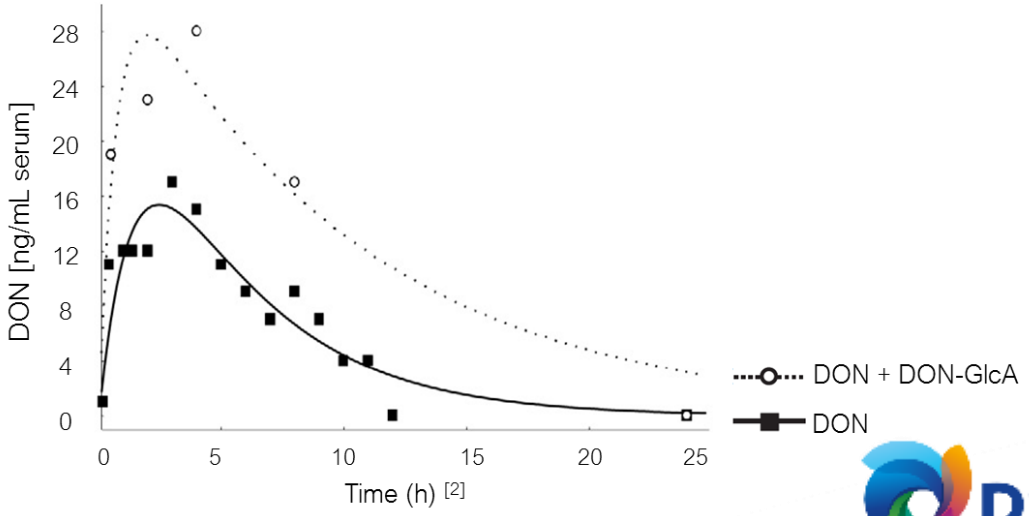
- Analytical challenges
- Species-specific TOXICOkinetics
- Mycotoxin-specific TOXICOkinetics

What does DON in blood tell about pig performance?

No correlation
no reference values established



Dänicke & Brezina 2013. Food Chem. Toxicol. 60



DSM

Mycotoxin biomarkers

What for?

Diagnostic tool

Not recommendable

Be skeptical when offered

Research purpose

- Novel insights into metabolism of mycotoxins in fungi, plants and animals
- Prove efficacy of mycotoxin detoxifying feed additives as requested by efsa European Food Safety Authority

Be skeptical when not available


Thank you for your attention!