Euroopa Maaelu Arengu Põllumajandusfond: Euroopa investeeringud maapiirkondadesse

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Research & Development

… one of the cornerstones of BIOMIN

Campus Tulln

Research & Development

Leading through science

> 20	> 120	> 200	> 35	> 125
Peer reviewed papers/year	R&D staff > 200,000 research hours/year	R&D cooperation partners worldwide	Research projects funded by the EU (8) & national agencies	Feeding trials per year 12,000 samples (organ, feces & blood)

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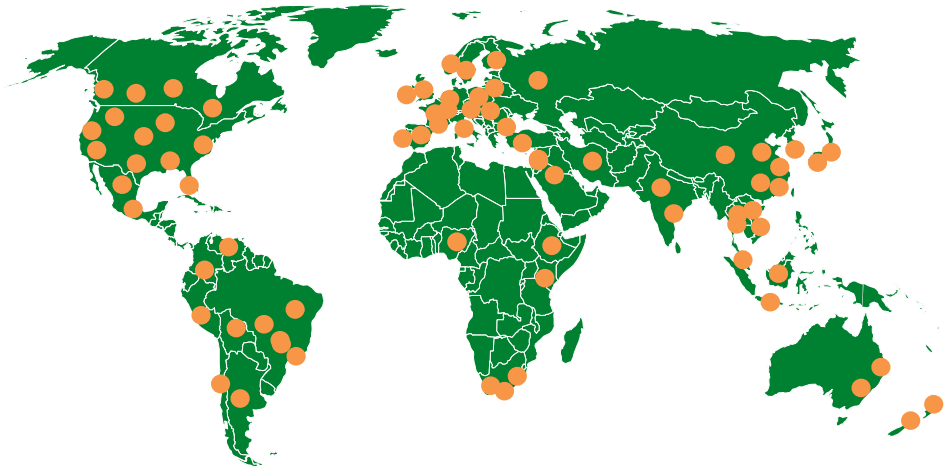
International R&D cooperation partners

Collaborations with more than **200 Universities & Research Institutes** during the last past 5 years



...

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Research & Development

Strong in-house capabilities

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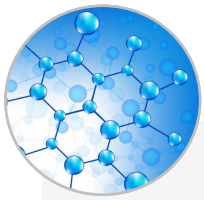
R&D Process at BIOMIN



from the molecule…

…to the application

R — New ingredients, New technologies

& — Formulation, Application

D — *in vivo/ex vivo*, Performance & Biomarker

Research Center Tulln

Headquarter Getzersdorf

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Trial Facilities

Center of Applied Animal Nutrition

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Mycotoxins…

Fusarium sp.

Aspergillus sp.

Penicillium sp.

Claviceps sp.

Alternaria sp.

etc.

… are toxic, secondary metabolites produced by fungi

- produced on almost all agricultural commodities worldwide!
- High stability:
  - chemically and heat stable
  - persistent during storage
  - resistant to processing methods
- > 1000 mycotoxins and bacterial secondary metabolites identified

Competitive advantage against other organisms…

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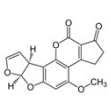
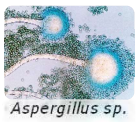
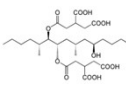
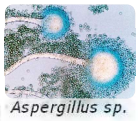
Mycotoxins…

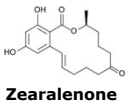

Aflatoxins


Aspergillus sp.

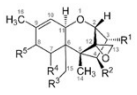

Fumonisins

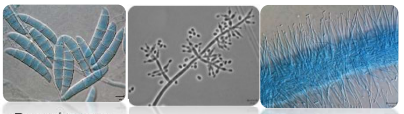

Fusarium sp.

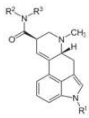

Aspergillus sp.


Zearalenone

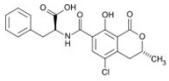

Fusarium sp.

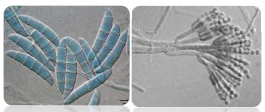

Trichothecenes


Fusarium sp. *Trichoderma sp.* *Acremonium sp.*

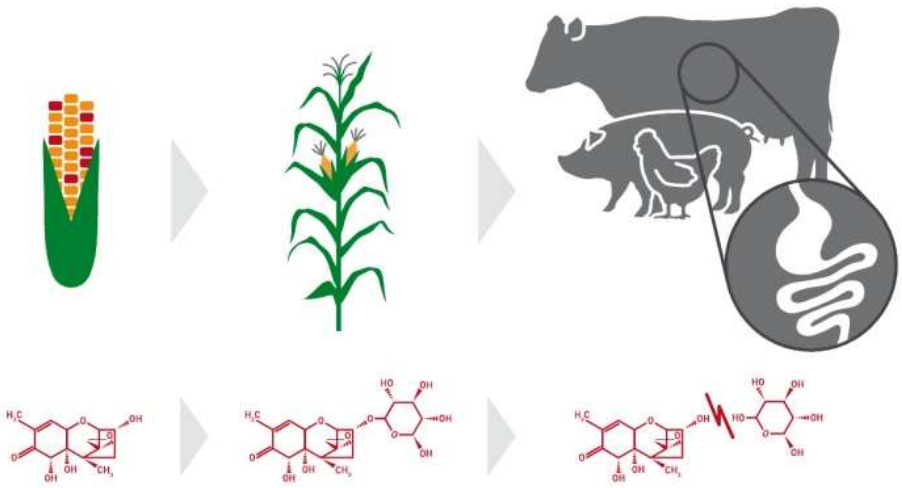

Ergot alkaloids


Claviceps sp.


Ochratoxin A


Fusarium sp. *Penicillium sp.*

Masked mycotoxins



Emerging mycotoxins
Beyond traditionally determined contaminants

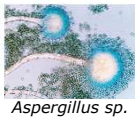
Fusarium metabolites…
- Enniatins (ENNs)
- Beauvericin (BEA)
- Moniliformin (MON)
- Fusaproliferin (FS)
- Fusaric acid (FA)
- Culmorin (CUL)
- Butenolide (BUT)

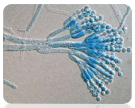
Aspergillus metabolites…
- Sterigmatocystin
- Emodin

Penicillium metabolites…
- Mycophenolic acid

Alternaria metabolites…
- Alternariol
- Alternariol monomethyl ether
- Tenuazonic acid


Fusarium sp.


Aspergillus sp.


Penicillium sp.


Alternaria sp.

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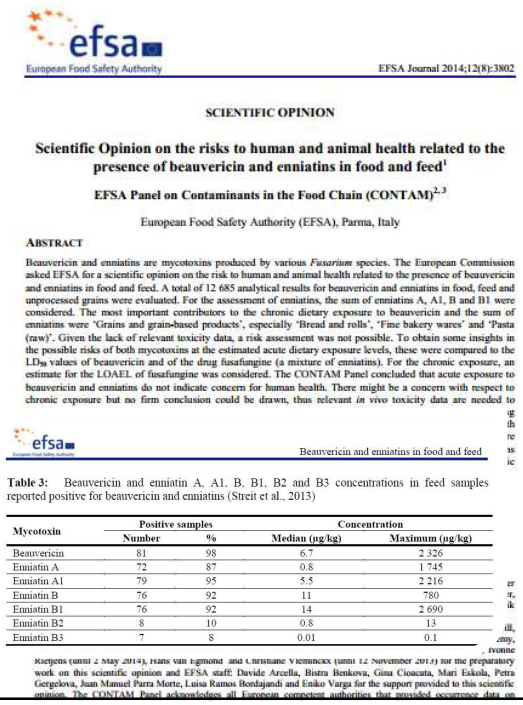
Emerging mycotoxins
Growing interest by official authorities

efsa
European Food Safety Authority

- Chronic Exposure of Enniatins & Beauvericin…

  … present a possible concern to animal health, but data are still lacking.

- Opinion includes data on the occurrence of enniatins and beauvericin from Streit *et al* 2013!



efsa
European Food Safety Authority

EFSA Journal 2014;12(8):3802

SCIENTIFIC OPINION

Scientific Opinion on the risks to human and animal health related to the presence of beauvericin and enniatins in food and feed[1]

EFSA Panel on Contaminants in the Food Chain (CONTAM)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Beauvericin and enniatins are mycotoxins produced by various *Fusarium* species. The European Commission asked EFSA for a scientific opinion on the risk to human and animal health related to the presence of beauvericin and enniatins in food and feed. A total of 12 685 analytical results for beauvericin and enniatins in food, feed and unprocessed grains were evaluated. For the assessment of enniatins, the sum of enniatins A, A1, B and B1 were considered. The most important contributors to the chronic dietary exposure to beauvericin and the sum of enniatins were 'Grains and grain-based products', especially 'Bread and rolls', 'Fine bakery wares' and 'Pasta (raw)'. Given the lack of relevant toxicity data, a risk assessment was not possible. To obtain some insights in the possible risks of both mycotoxins at the estimated acute dietary exposure levels, these were compared to the LD_{50} values of beauvericin and of the drug fusafungine (a mixture of enniatins). For the chronic exposure, an estimate for the LOAEL of fusafungine was considered. The CONTAM Panel concluded that acute exposure to beauvericin and enniatins do not indicate concern for human health. There might be a concern with respect to chronic exposure but no firm conclusion could be drawn, thus relevant *in vivo* toxicity data are needed to

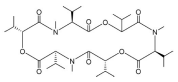
Table 3: Beauvericin and enniatin A, A1, B, B1, B2 and B3 concentrations in feed samples reported positive for beauvericin and enniatins (Streit et al., 2013)

Mycotoxin	Positive samples		Concentration	
	Number	%	Median (µg/kg)	Maximum (µg/kg)
Beauvericin	81	98	6.7	2 326
Enniatin A	72	87	0.8	1 745
Enniatin A1	79	95	5.5	2 216
Enniatin B	76	92	11	780
Enniatin B1	76	92	14	2 690
Enniatin B2	8	10	0.8	13
Enniatin B3	7	8	0.01	0.1

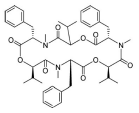
Kuiper (until 2 May 2014), Hans van Egmond and Christiane Vleminckx (until 12 November 2013) for the preparatory work on this scientific opinion and EFSA staff: Davide Arcella, Bistra Benkova, Gina Ciaccata, Mari Eskola, Petra Gergelova, Juan Manuel Parra Morte, Luisa Ramos Bordajandi and Eniko Varga for the support provided to this scientific opinion. The CONTAM Panel acknowledges all European competent authorities that provided occurrence data on

Emerging mycotoxins
Beyond traditionally determined contaminants

Fusarium metabolites…

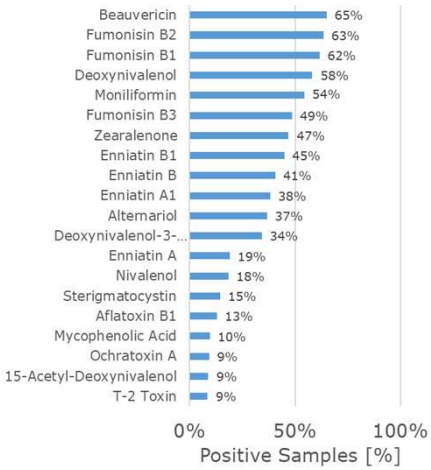


Enniatin B1 (ENN): (45% prevalence in 2019)
In vitro: cytotoxic at low concentrations
In vivo: chronic exposure cannot be assessed due to lack of data
Accumulation in meat, skin and liver of broilers



- **Beauvericin (BEA):** (65% prevalence in 2019)
  In vitro: cytotoxic
  In vivo: chronic exposure cannot be assessed (lack of data)
  Effects on immune system and on the bioavailability of pharmaceuticals
  Accumulation in fat-rich tissue: meat, liver and in eggs

- **Moniliformin (MON):** (54% prevalence in 2019)
  In vitro: toxic
  In vivo: poultry are the most affected species
  Main target organ: heart



Mycotoxin production

Production of mycotoxins by fungi depends on:

- Temperature
- Relative humidity
- Insect attacks

-> physical damage of the crop

- Stress conditions of the plants

-> weakening of the plant`s natural defenses

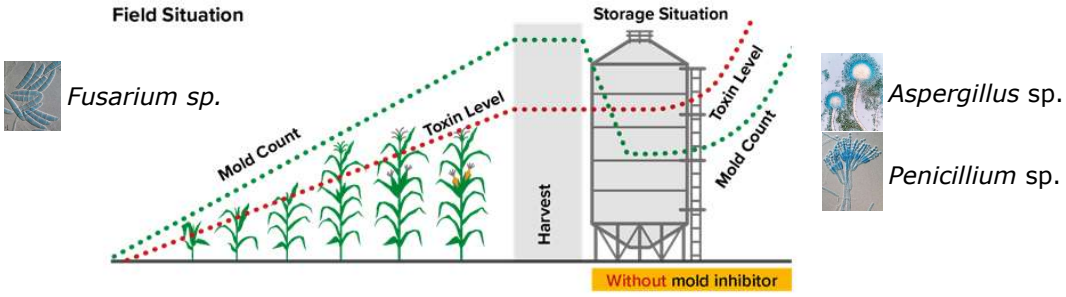
→ promotes colonization by fungi

Where do mycotoxins come from?

Field: 95% of mycotoxins in feed are produced on the field
typical field fungi: *Fusarium* sp. (Fumonisins, Trichothecenes, OTA, Zearalenone)

Harvest: toxin level remains steady

Storage: toxin level may increase depending on activity & colonization levels of fungi
typical storage fungi: *Aspergillus* sp. (Aflatoxins, Fumonisins, OTA, Patulin)
Penicillium sp. (Patulin, Ochratoxin A)



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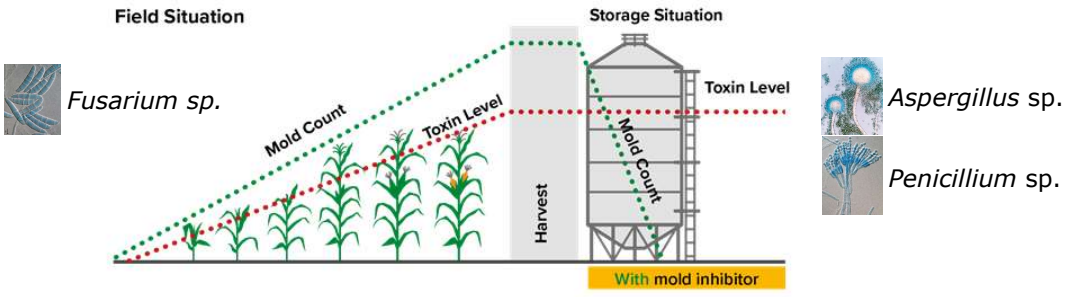

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BIOMIN Mycotoxin Survey

BIOMIN Survey: started in 2004
21 287 samples in 2019

Samples:


Maize


Maize Gluten Meal


Soybean


Wheat


Rice


Compound Feed


DDGS

others

Mycotoxins:

Aflatoxins (Afla)	Fumonisins (FUM)
Zearalenone (ZEN)	Ochratoxin A (OTA)
Deoxynivalenol (DON)	T-2 toxin (T-2)

Methods:

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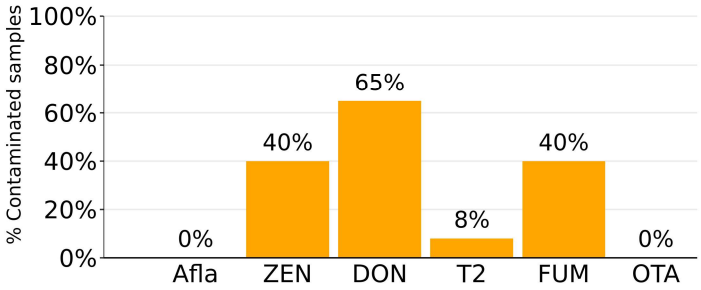
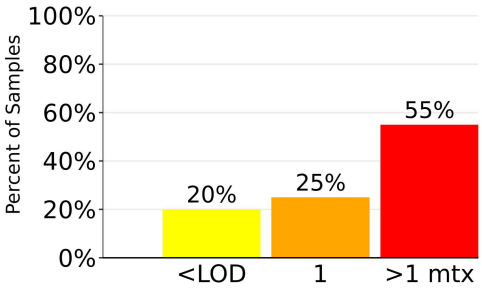
Summary for All commodities in Baltic Countries from Jan 2020 to Dec 2020

Parameter	Afla	ZEN	DON	T2	FUM	OTA
Number of samples	11	20	20	13	20	15
% Contaminated samples	0%	40%	65%	8%	40%	0%
% Above risk threshold	0%	5%	30%	0%	5%	0%
Average of positives (ppb)		57	305	3	103	
Median of positives (ppb)		21	143	3	26	
Maximum (ppb)	0	302	2062	3	643	0

Prevalence of Mycotoxins Detected



No. of Mycotoxins per Sample



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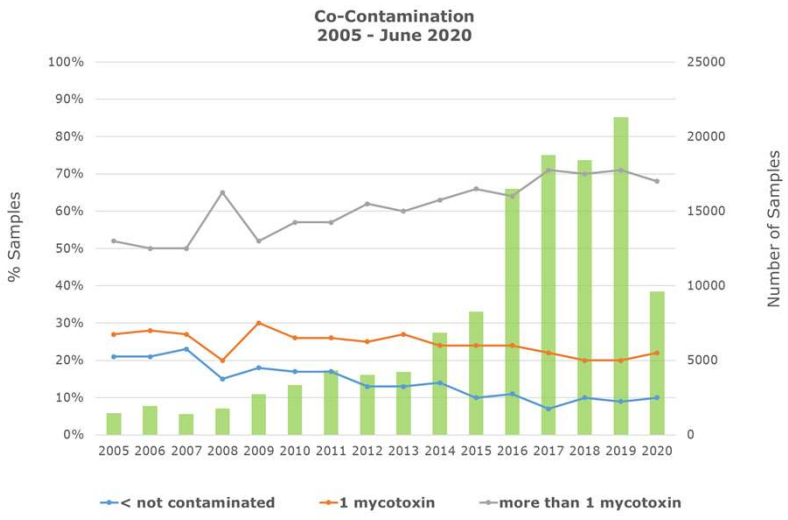
Trend for All commodities in Baltic Countries from Jan 2016 to Dec 2021

Trend for All commodities & Sugar beet in Baltic Countries from Jan 2016 to Dec 2021

Join us

Overview of Mycotoxin Co-Contamination 2005 – June 2020



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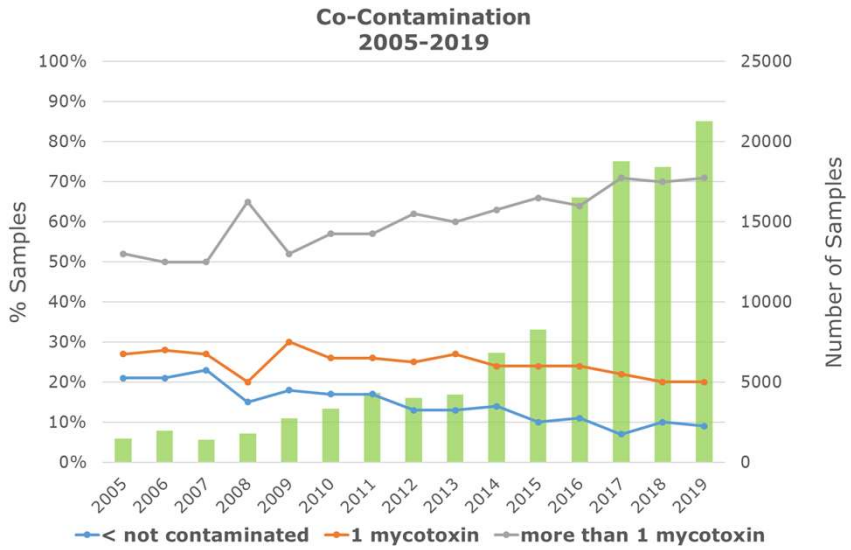
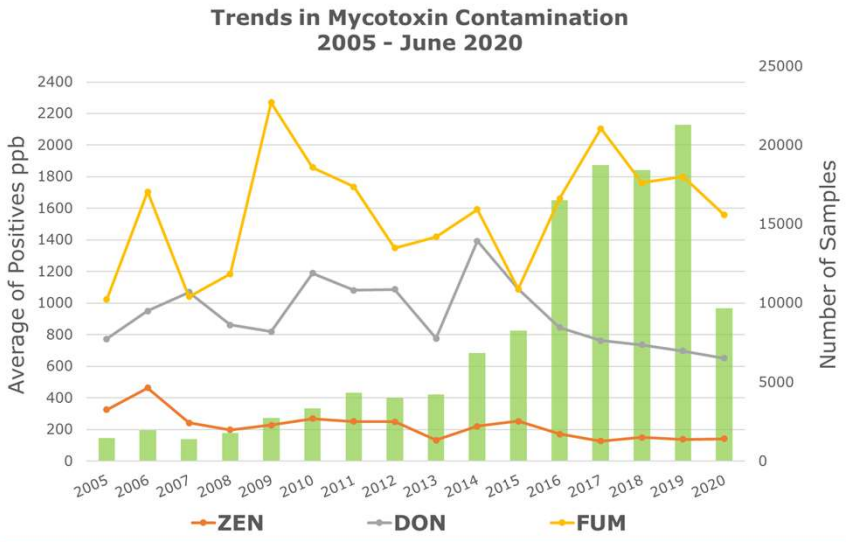
Overview of Mycotoxin Co-Contamination 2005 – 2019

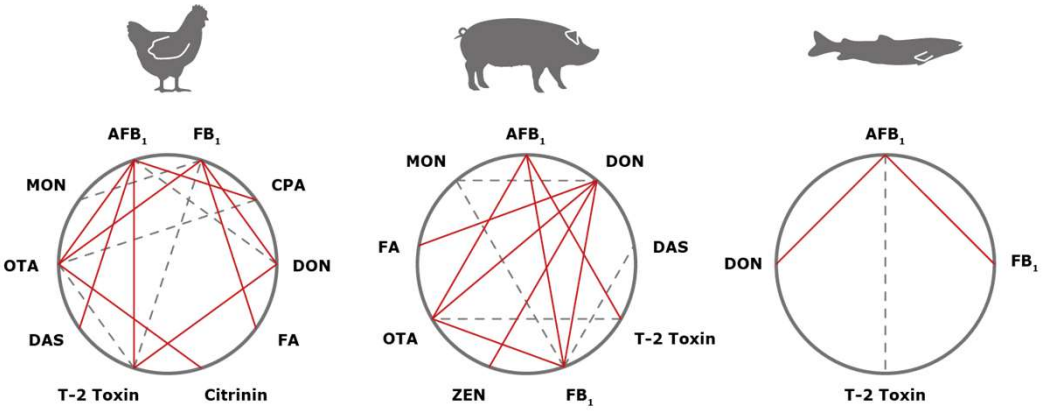


Overview of Mycotoxin Occurrence 2005 – June 2020: ZEN, DON, FUM



Co-Contamination → Synergism

The severity of one mycotoxin can be increased by the presence of others:
Additive (– – –) and synergistic (——) effects can occur



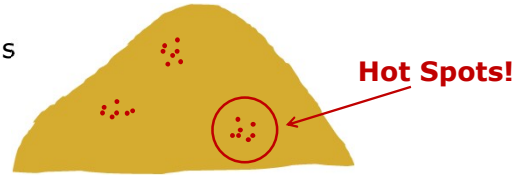
Can we see mycotoxins?

Mycotoxin Analysis

- Representative sampling is essential
- Masked mycotoxins are not detected by conventional analytical methods
- Co-occurrence → synergistic effects
- Selecting the proper method is a key step

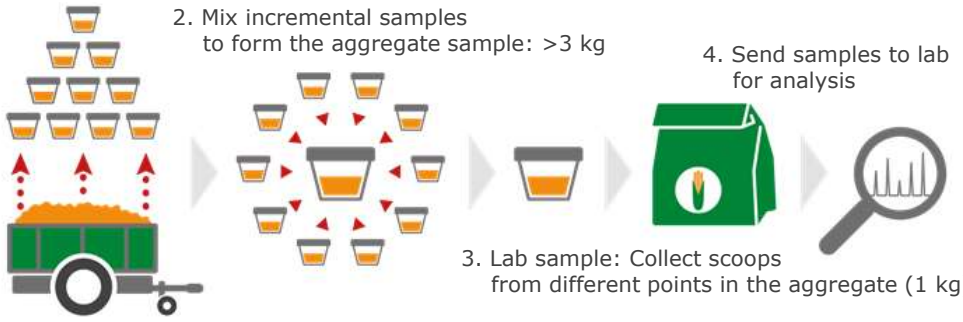
Mycotoxin Analysis - Sampling

Uneven distribution of **MYCOTOXINS** in grains



Based on EU regulations:

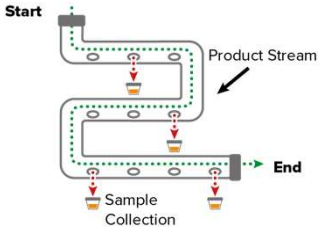
1. Collect incremental samples (1 kg) according to lot weight
2. Mix incremental samples to form the aggregate sample: >3 kg
3. Lab sample: Collect scoops from different points in the aggregate (1 kg)
4. Send samples to lab for analysis



Sampling procedure for mycotoxin analysis

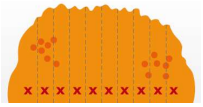
- **Solid feed**: grains, seeds, beans, pellets, meals and powders

during transfer

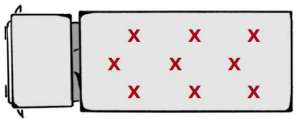


Loading spout: scoop

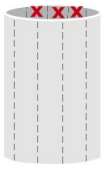
Loading bucket: use a scoop



Grain heap



Truck



Tower silo

Number of samples required for analysis depending on lot size (EC No 401/2006).

Lot weight (metric tons)	Number of Samples	Aggregate Sample Weight (kg)
Up to 1	10	1
Up to 10	40	4
Up to 20	60	6
More than 50*	100	10

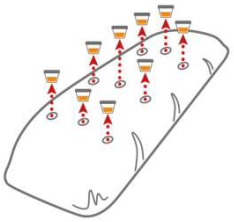
* For lots weighing more than 50 tons, calculate the number of samples using the following formula: √ (20 x lot weight in tons) = number of incremental samples.

Sampling procedure for mycotoxin analysis

- **Roughages**: fresh and ensiled or straw

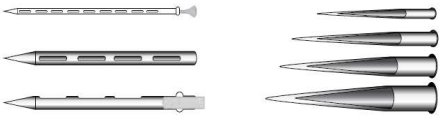
fresh
by hand, best in motion (unloading truck), leaves & stems unevenly distributed

bunker and bag silos
collect incremental samples by puncturing the plastic cover with a sharp coneshaped sampling device;
evenly distributed & carefully refilled



silage/straw bales
15-20 incremental samples from each bale
-> aggregate samples

grain probes and triers used for manual sampling



The minimum number of increments required when sampling roughages.

Lot weight [metric tons]	Minimum number of increments
≤ 5	10
≥ 5	√ (40 x metric tons) (max 50 metric tons)

Leaves and stems are distributed unevenly in a truckload with more leaf material along the edges. Collect incremental samples by hand as the truck is unloading.

X XXX XXX million

reduction of:

- seed germination (Hell,1994; Negedu et al., 2010),
- energy and nutritional value changes in terms of loss of carbohydrates,
- proteins,
- amino acids and vitamins and increases in fatty acids may also occur

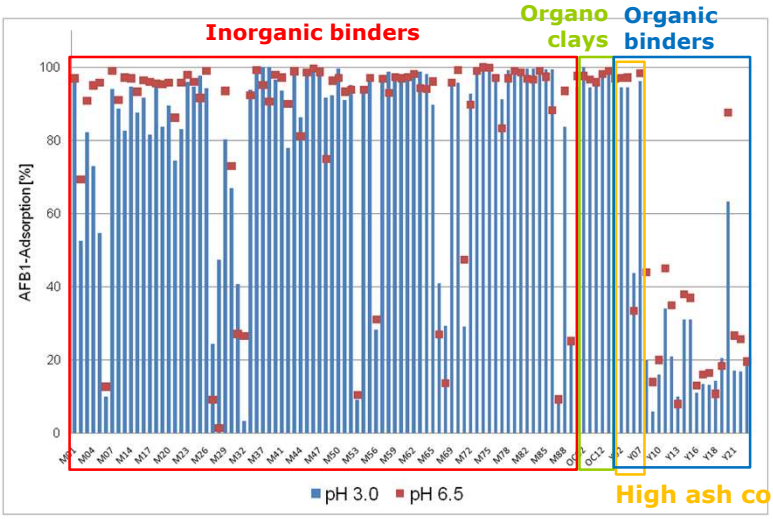
(Ominski et al., 1994; Negedu et al.,2009).

Overview on group of binders

Inorganic binder	Organoclay	Organic binder	(Activated) charcoal
Clay minerals Aluminosilicates Bentonite Montmorillonite Smectite HSCAS Illite Zeolite	Organophilic clays Organozeolite Montmorillonite with organic compounds Bentonite with quaternary amines	Yeast Yeast cell wall extracts Mannan oligo-saccharides (MOS) Glucomannans beta-Glucans Algae	Active carbon Vegetable charcoal

Adsorption of 200 ppb aflatoxin B_1 by 0.2 % binder

IFA TULLN



What adsorption is ?

Mycotoxin-adsorbing agents are large molecular weight compounds that should be able to bind the mycotoxins in contaminated feed without dissociating in the gastrointestinal tract of the animal.

In this way the toxin-adsorbing agent complex passes through the animal and is eliminated via the faeces.

This prevents or minimizes exposure of animals to mycotoxins.

SCIENTIFIC REPORT submitted to EFSA

Review of mycotoxin-detoxifying agents used as feed additives: mode of action, efficacy and feed/food safety[1]

ZEA, OTA and FB1:

- in acidic conditions, may all be bound with moderate to high affinities (log K_d typically between 2 and 4, i.e. BC_{50} between 0.1 and 10 g/L) by various adsorbing agents, mostly through hydrophobic interactions
- are ionized at higher pH (pH > 7.6, 4.4 and c. 5 respectively). The expected consequences are reduced adsorption on neutral or anionic adsorbing agents and inversely enhanced adsorption on cationic adsorbing agents such as anion-exchange resins. Such pH effects are indeed observed in some cases, but in other cases they are apparently negligible.

ISPA (Istituto di Scienze delle Produzioni Alimentari): Giuseppina AVANTAGGIATO - *ITALY*

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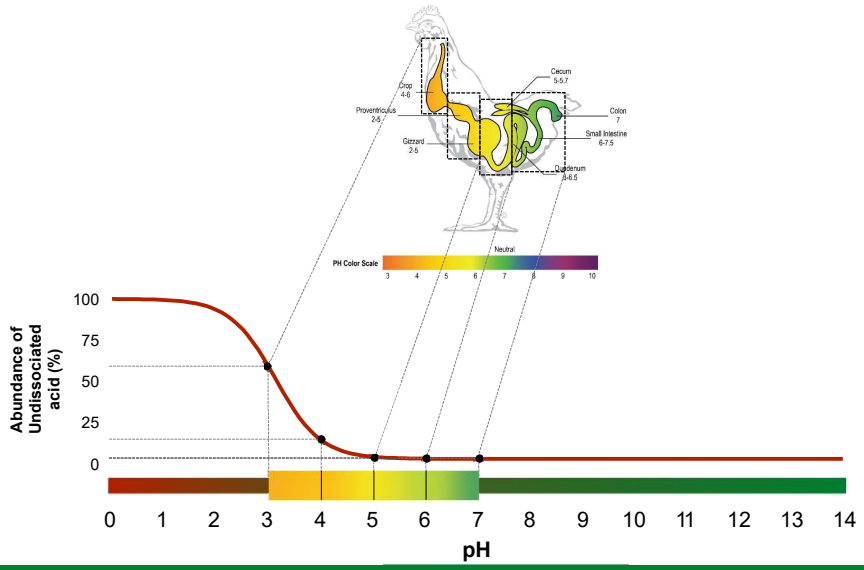
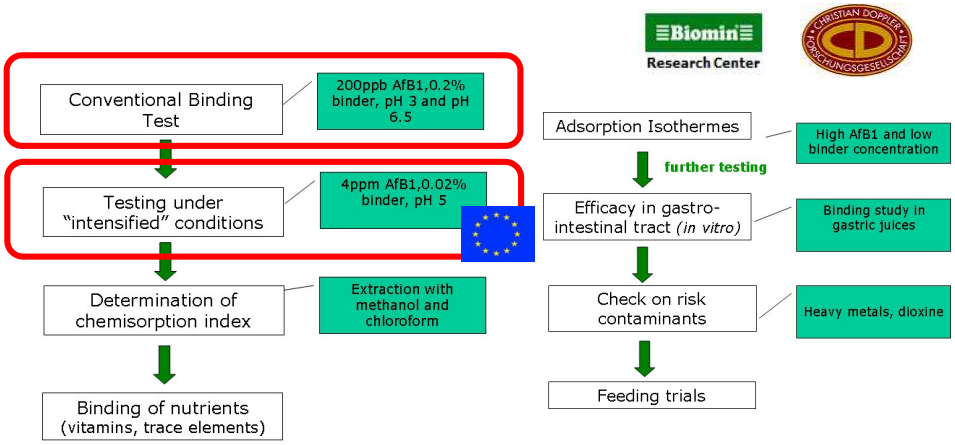
Why pH matters ?



Biomin evaluation scheme for MTX binders

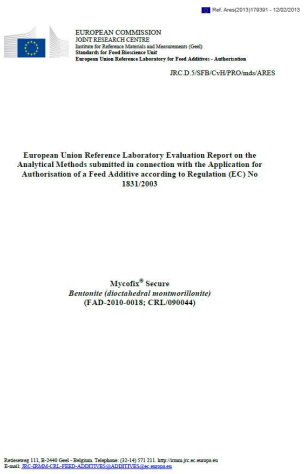


Biomin binder study published in Vekiru et al, 2007

The EURL method to test adsorption efficacy of aflatoxins

EURL-Method:

- 0.02% tested product
- 4,000 ppb AfB_1
- in buffer solution pH 5,0
- incubation for 1h at 37°C
- analyses by HPLC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Standards for Food Bioscience Unit
European Union Reference Laboratory for Feed Additives - Authorisation

JRC.D.5/SFB/CvH/PRO/mdu/ARES

European Union Reference Laboratory Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003

Mycofix® Secure
Bentonite (dioctahedral montmorillonite)
(FAD-2010-0018; CRL/090044)

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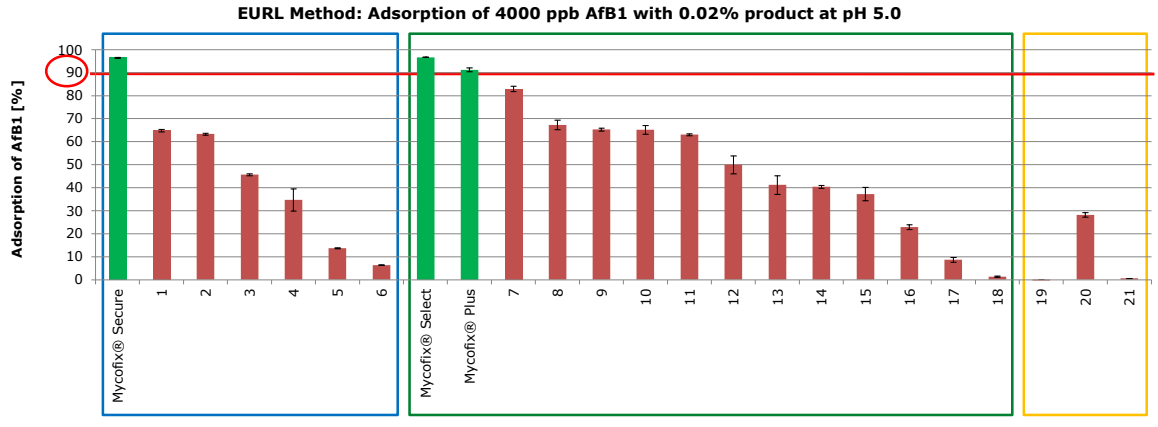
The EURL Method



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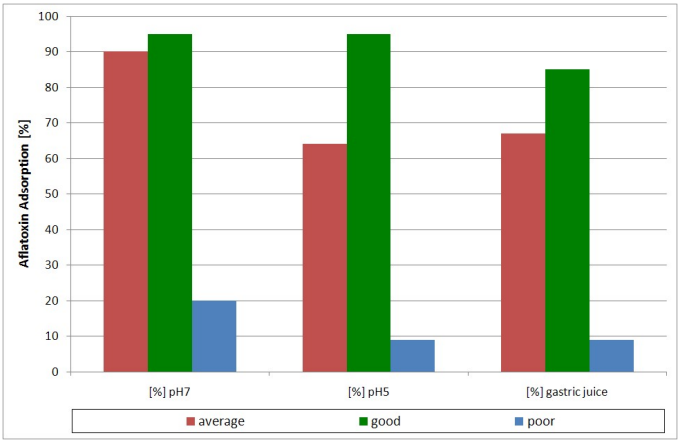
Comparrison of an avarage, good, and bad binder *in vitro*

- 4 ppm AFB1, 0.02% binder, 1h incubation at 37°C in buffer at pH 7, pH 5 and in gastric juice



Critical points when conducting adsorption tests

- **ELISA is NOT a suitable method** to test the mycotoxins in the supernatant after the incubation with the binder → due to the matrix effects of the binder but also the gastric fluid that can lead to false results.
- **HPLC or LC-MS/MS** it the method to use, but it needs to be adapted and **validated** for gastric fluid. Method needs to be studied and false conclusions need to be avoided (eg. pH changes might lead to assumption of bound aflatoxin when in reality it changed to a different metabolite)
- Only an **experienced lab** which is routinely performing this experiments will be capable to deliver reliable results.

Factors which can strongly influence the results are:

- Concentration of the mycotoxin
- Use of natural contaminated feed instead of mycotoxin
- Inclusion rate of binder
- Precise weighing of the binder
- Identical and strong enough mixing of the sample in a proper tube
- Incubation time
- pH!!!
- Temperature
- Identical preparation or source of gastric fluid
- Constant concentration of mycotoxin standard for analysis
- Use of the same binder as positive control for each experiment
- Triplicates
- Use of HPLC method established for detection of mycotoxins in the matrix of gastric fluid
- Well trained personnel

EU registration – benchmark for quality!

- For the first time an official authority imposes demanding and rigid requirements on identity, safety and efficacy of a mycotoxin deactivation product.
- The registration demonstrates the capacity of such products in a standardized and fair process.
- The requirements of scientific biomarkers to directly prove the deactivation of mycotoxins *in vivo.*

1m558

Category of technological additives. Functional group: substances for reduction of the contamination of feed by mycotoxins: aflatoxin B1

1m558	Bentonite	*Additive composition* Bentonite: ≥ 70 % smectite (dioctahedral montmorillonite)	Ruminants Poultry Pigs	*Analytical method* (1) For the determination of bentonite in feed additive: X-ray diffraction (XRD)	
		Characterisation of active substance Bentonite: ≥ 70 % smectite (dioctahedral montmorillonite) < 10 % opal and feldspar < 4 % quartz and calcite AfB_1-binding capacity (BC $_{AfB1}$) above 90 %		For the determination of BC $_{AfB1}$ of the additive: adsorption test carried out in a buffer solution at pH 5,0 with a concentration of 4 mg/l for AfB_1 and 0,02 % (w/v) for the feed additive.	

Efficacy of the product: *In vitro* data are not enough - biomarkers?

Significant effects must be proven **by relevant biomarkers** in different studies - with sufficient number of animals/ replicates for statistical analysis of data.

Improved 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).

Strategies to mitigate mycotoxin risk

Mycotoxin Risk Management

Mycotoxin	Mycofix® Plus	Mycofix® Select
Aflatoxins	+	+
Fumonisins	+	+
Ochratoxins	+	+
Zearalenone	+	
DON (Vomitoxin)	+	+
Nivalenol	+	+
T-2 toxin	+	+
DAS	+	+
Other trichothecenes (3-AcDON, 14-AcDON, Fus X, HT-2 toxin etc.)	+	+
Ergot Alkaloids	+	+
Endotoxins	+	+

Quality Management

We meet highest quality standards

ISO 9001 CERTIFIED

HACCP APPROVED

ISO 14040 Approved Life Cycle Assessment

ISO 14001 CERTIFIED

Feed Safety GMP+ Assurance

QS. Quality scheme for food.

FAMIQS

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