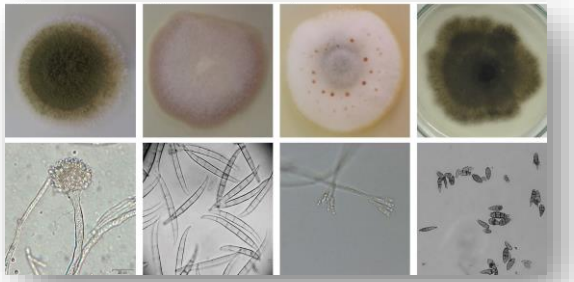
Challenge of Analyzing Contaminants in Food in Daily Routine

16th Waters Users´ Meeting
Slovenia, November 15th, 2019

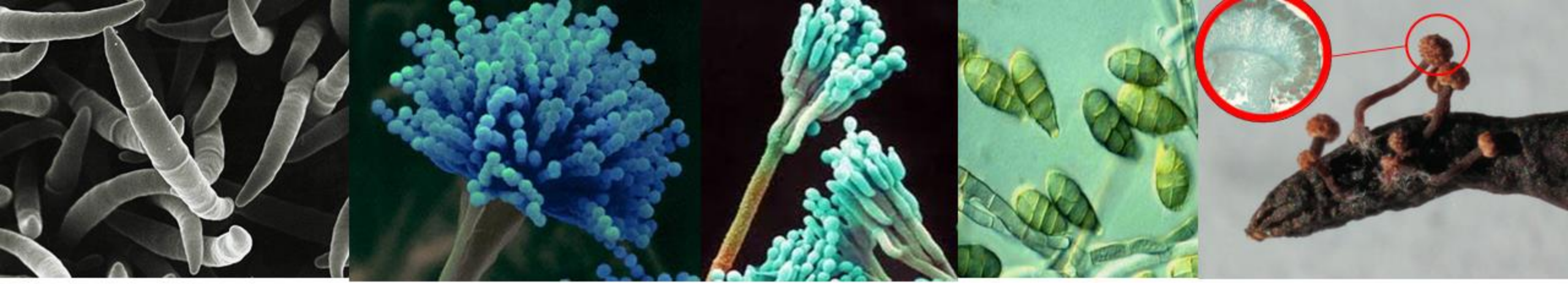
MARIJA KOVAČ

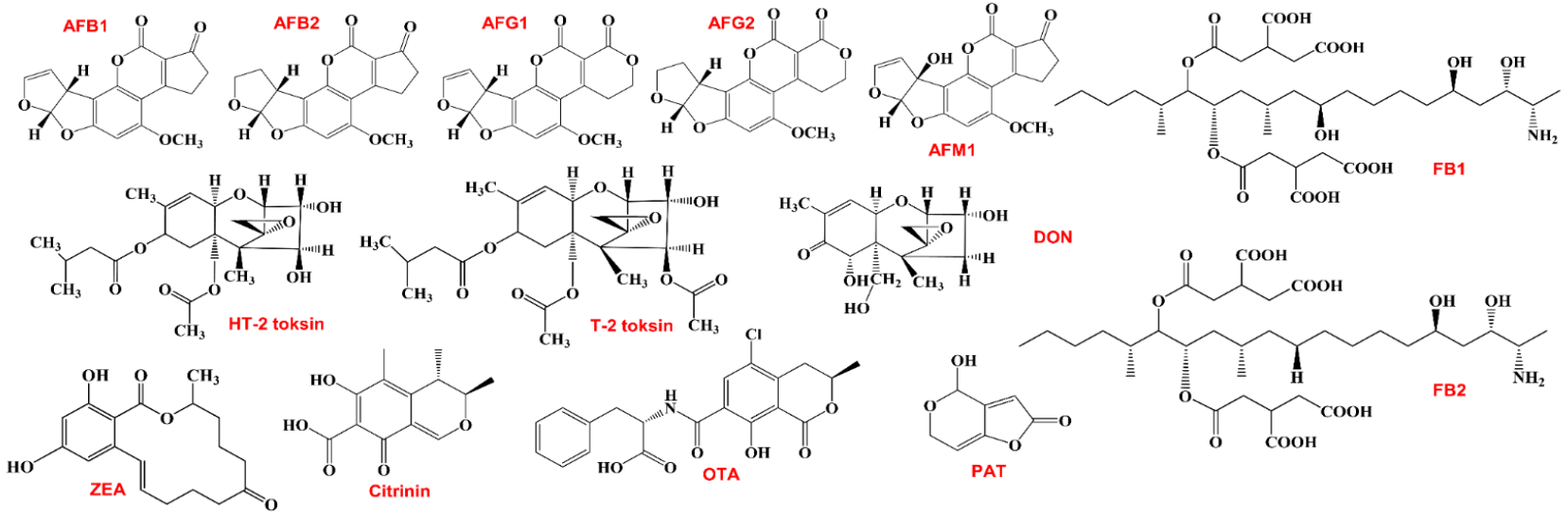


Inspecto Lab

EUROPSKI STRUKTURNI I INVESTICIJSKI FONDOVI

Europska unija
Zajedno do fondova EU





Mycotoxin analysis

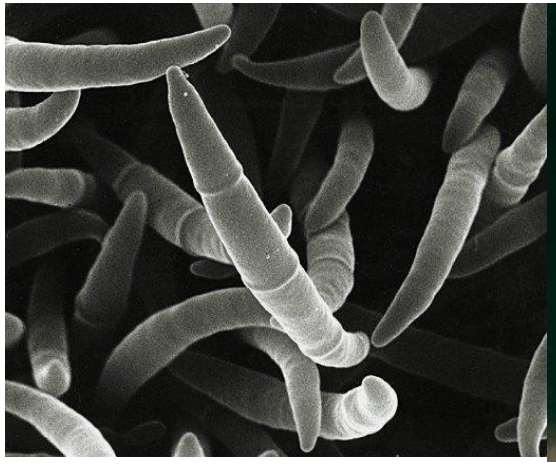
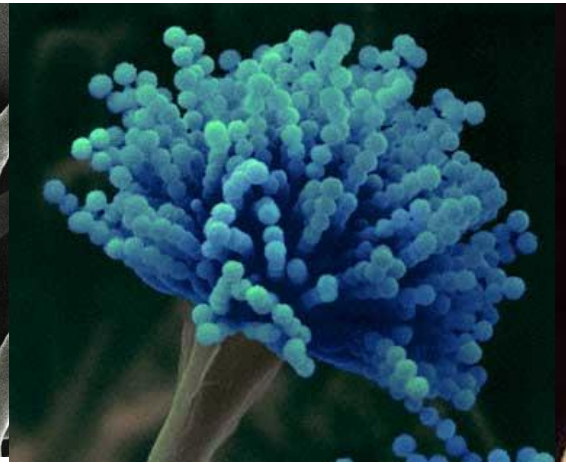
inspecto

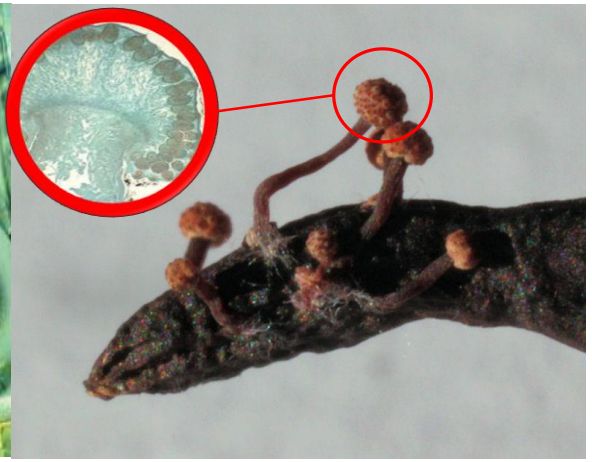
Mycotoxins

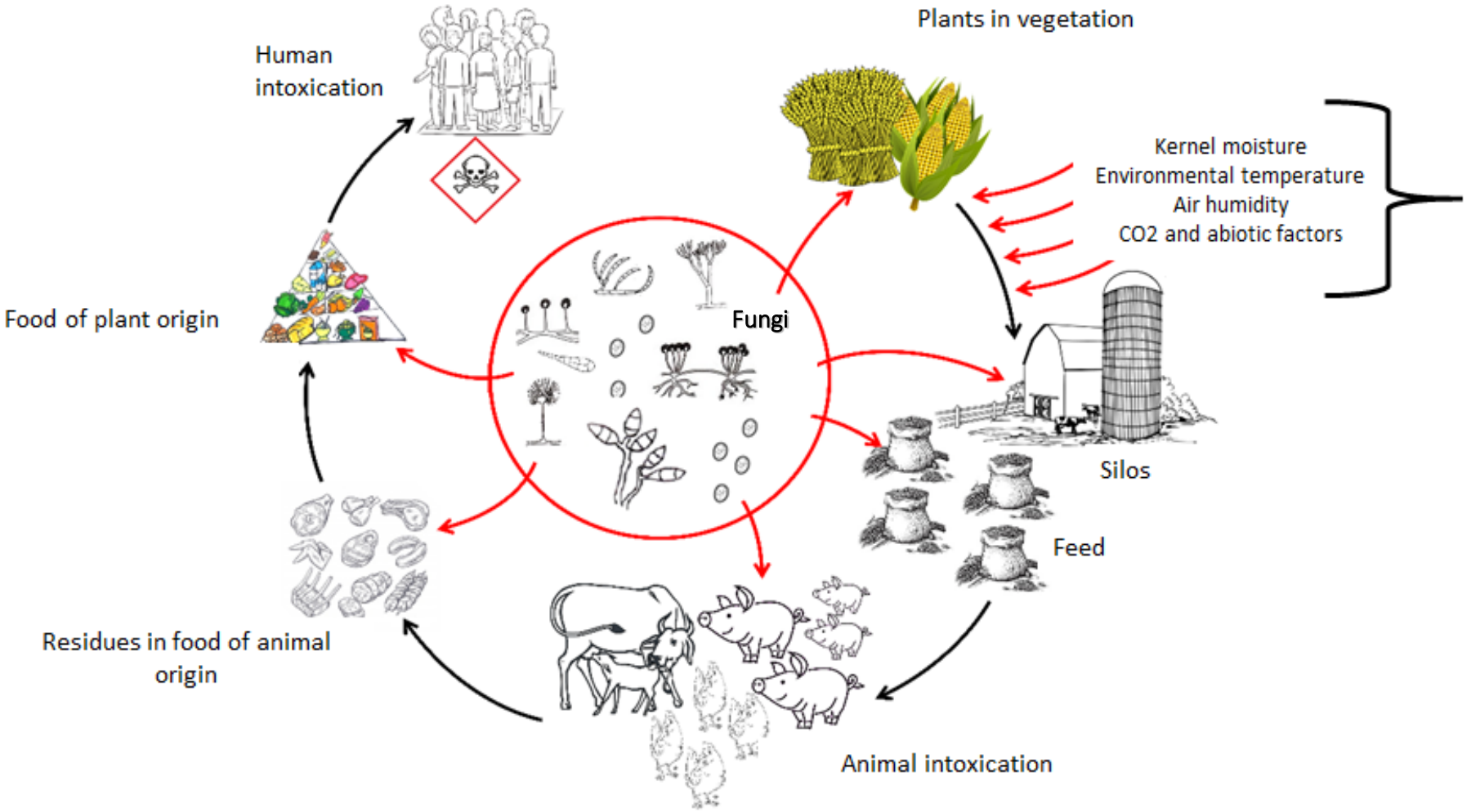
mykes (grč.) = fungi
toxicum (lat.) = toxin

mycotoxin

Toxic secondary metabolites produced by fungi - low molecular weight (700 Da)
Main producers*: Fusarium* spp.*, Aspergillus* spp.*, Penicillium* spp.*, Alternaria* spp.

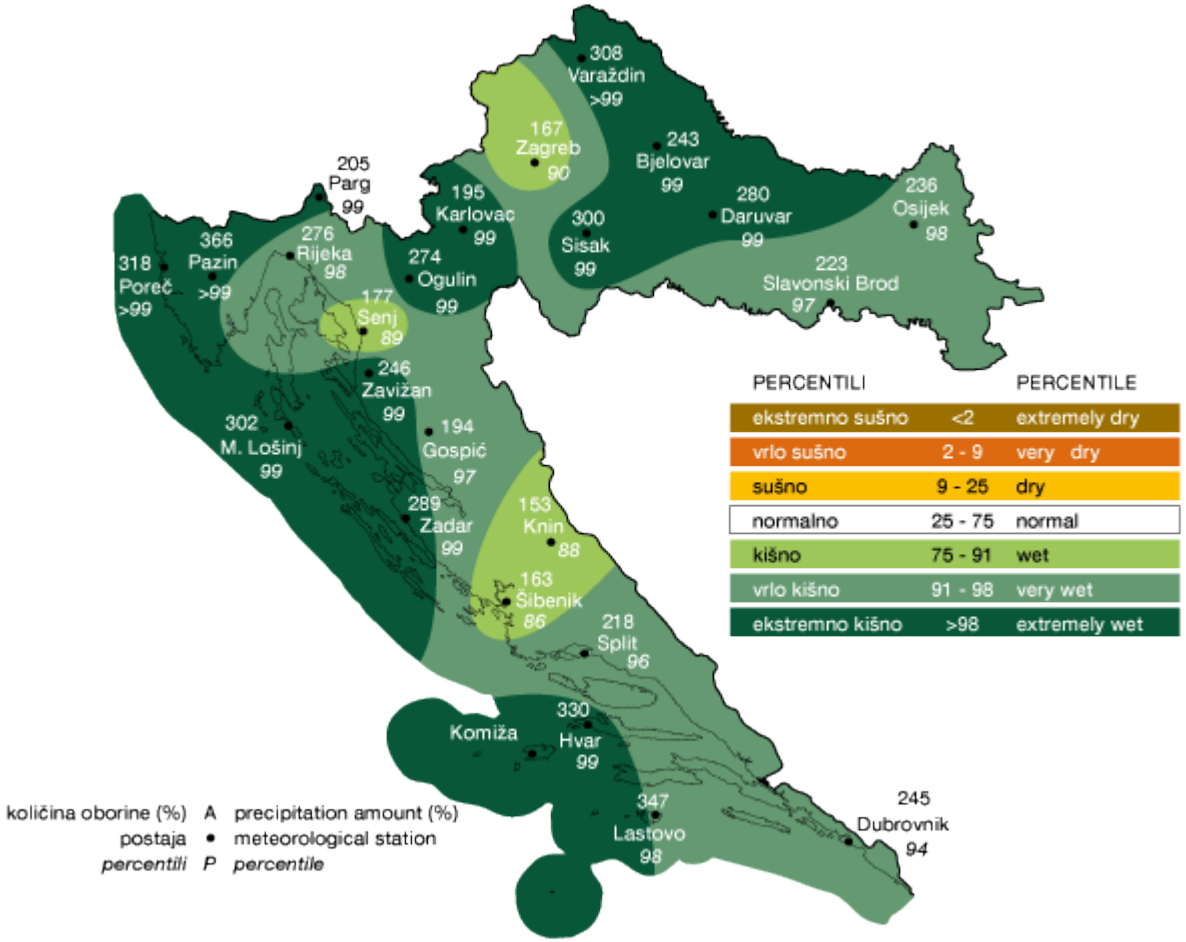


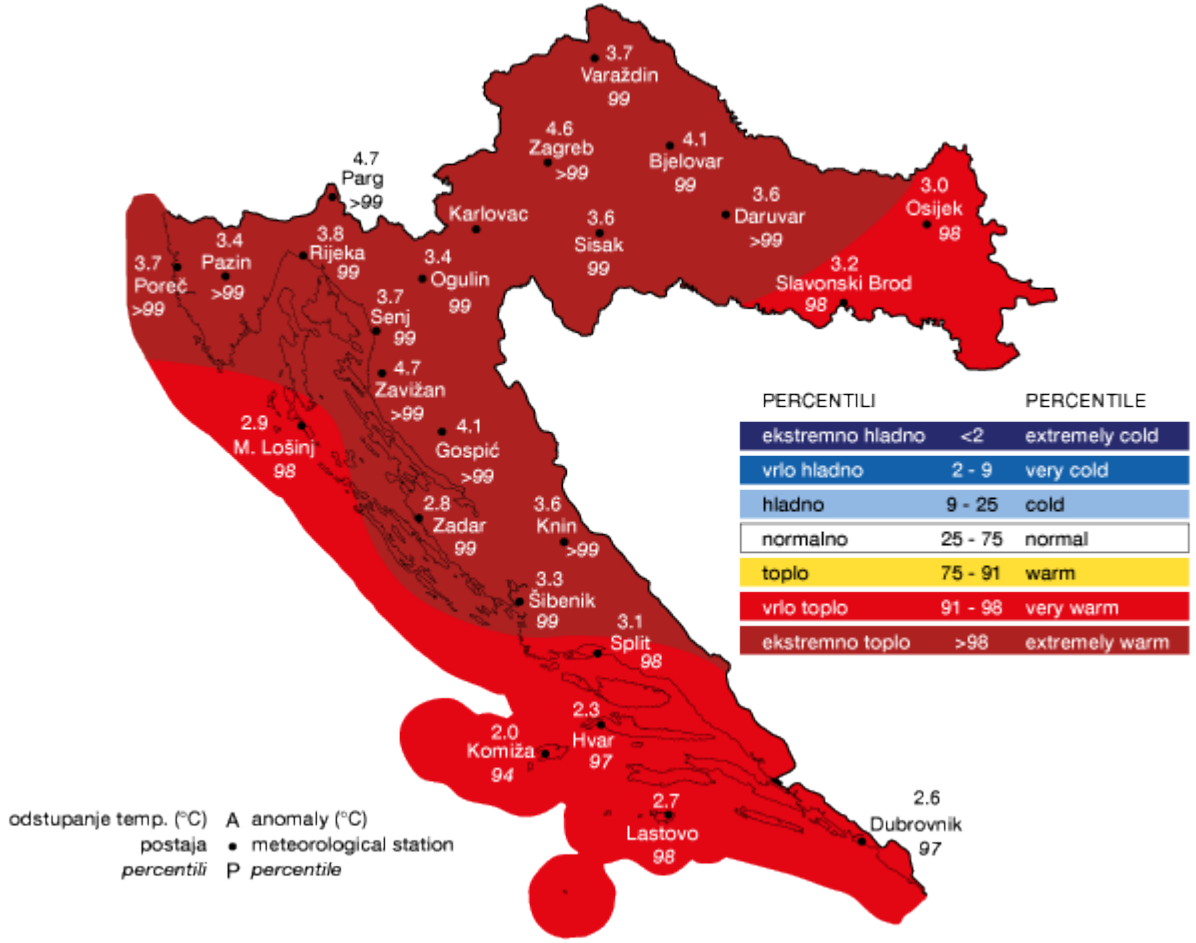


Climate and weather conditions

https://meteo.hr/klima.php?section=klima_pracenje&param=ocjena

https://meteo.hr/klima.php?section=klima_pracenje&param=ocjena





Climate change

nature climate change

LETTERS

PUBLISHED ONLINE: 1 SEPTEMBER 2013 | DOI:10.1038/NCLIMATE1990

Crop pests and pathogens move polewards in a warming world

Daniel P. Bebber[1], Mark A. T. Ramotowski[2] and Sarah J. Gurr[1]*

Pest and pathogens shift towards the Equator at rate of 2.7±0.8 km/yr

SCIENTIFIC REPORTS

OPEN

Aflatoxin B_1 contamination in maize in Europe increases due to climate change

Received: 09 December 2015
Accepted: 24 March 2016
Published: 12 April 2016

P. Battilani[1], P. Toscano[2], H. J. Van der Fels-Klerx[3], A. Moretti[4], M. Camardo Leggieri[1], C. Brera[5], A. Rortais[6], T. Goumperis[6] & T. Robinson[6]

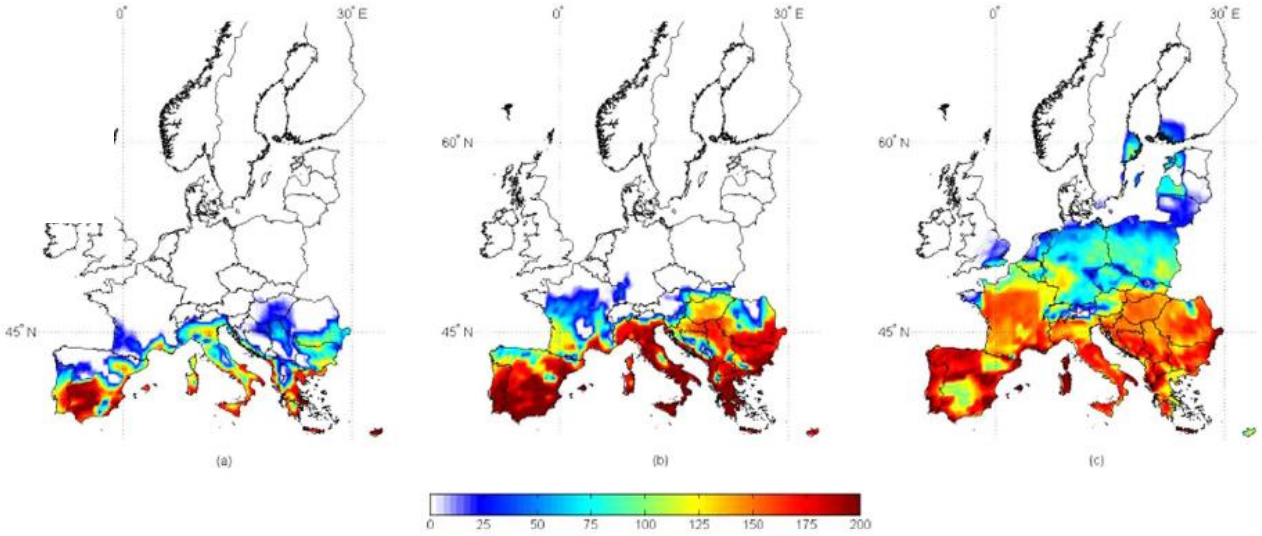
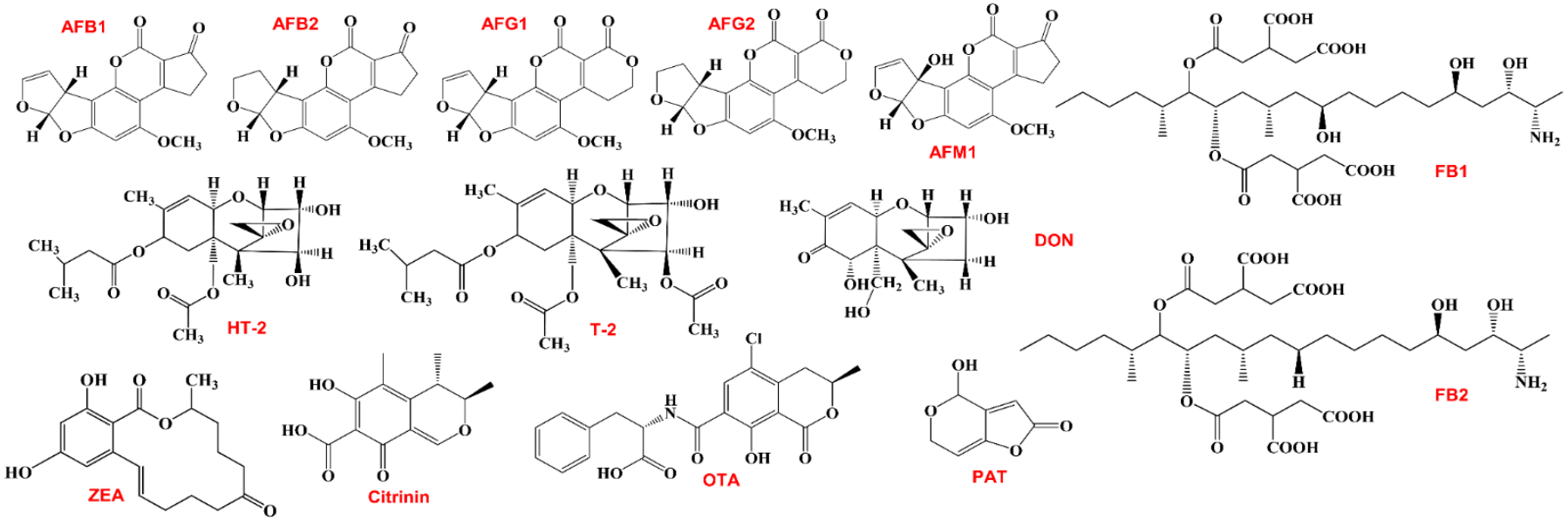


Figure 1. Risk maps for aflatoxin contamination in maize at harvest in 3 different climate scenarios, present, +2 °C, +5 °C. Mean daily data used as input result from 100-year run of the predictive model AFLA-maize in 2254 geo-referenced points throughout Europe, in the 3 scenarios. The scale 0–200 refers to the aflatoxin risk index (AFI), output from the predictive model; increasing the (present (a), +2°C (b), +5°C (c)) number, the risk of contamination increases. Maps generated using Mathworks, Matlab. Computer Program, 2012 http://it.mathworks.com/.

Mycotoxin legislation



- Food

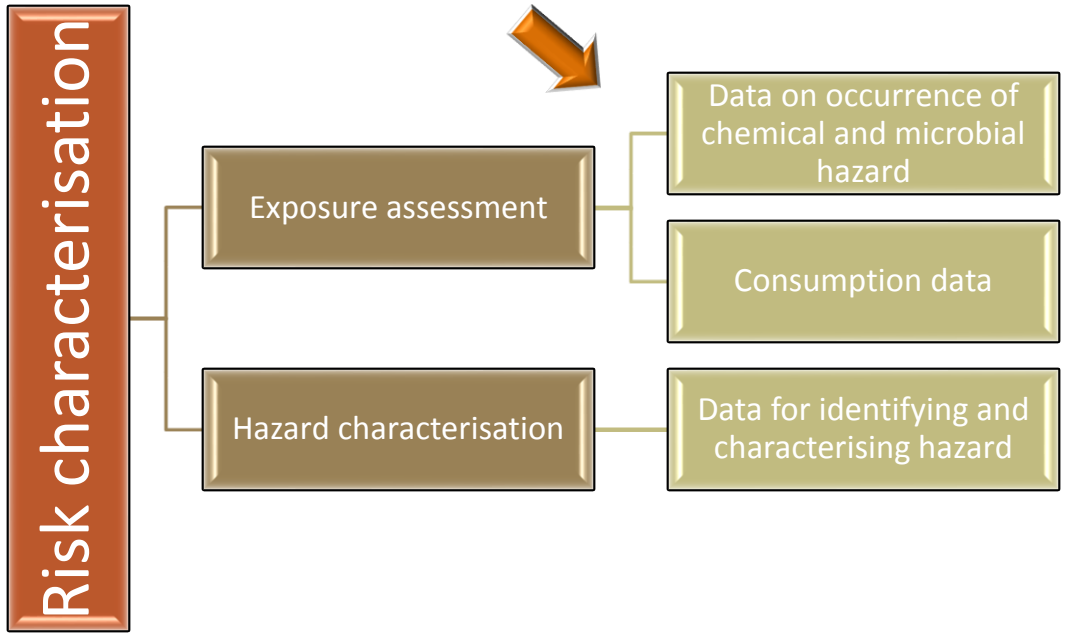
COMMISSION REGULATION (EC) No 1881/2006
COMMISSION RECOMMENDATION 2013/165/EU

- Feed

DIRECTIVE 2002/32/EC
COMMISSION RECOMMENDATION 2006/576/EC

(Multi)Mycotoxin analysis

- Need for multimycotoxin method in food safety control – proof of compliance to legislation – real threat of multimycotoxin contamination - one fungus can produce multiple mycotoxins

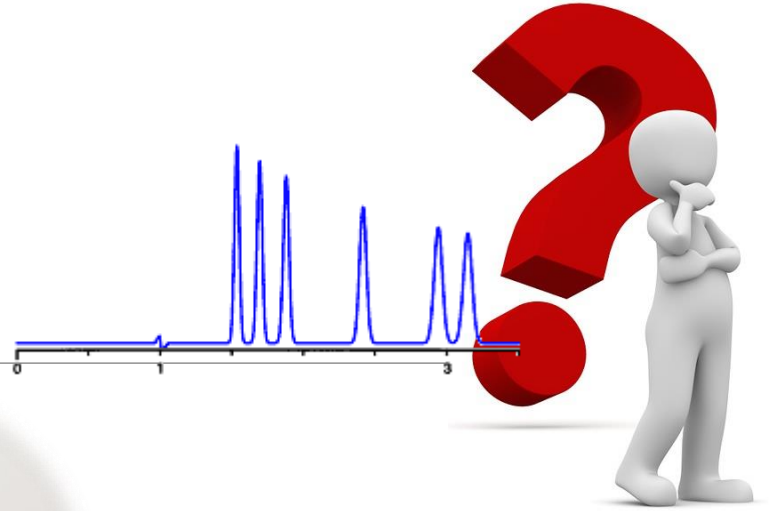


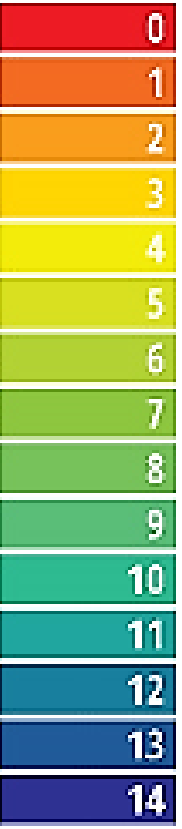
- ~~25% of crops are contaminated by mycotoxins (FAO, 1998)~~
- 90% of crops are contaminated by mycotoxins (Pinotti et al., 2016)
- 30-100% of food and feed samples contain more than one mycotoxin (Pinotti et al., 2016)
- Co-occurrence of mycotoxins – additive, synergistic or anatagonistic toxicological effects – not enough informations for exposure assessment

Acquity H-class Xevo TQD

Acquity H-class Xevo TQ-S micro

Method development





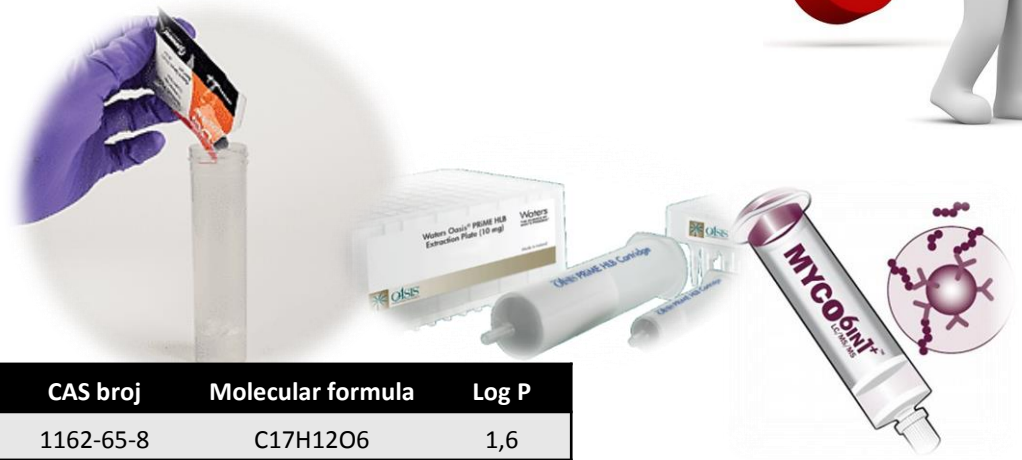
Ionization - Matrix effect

Chromatographic separation

Clean-up

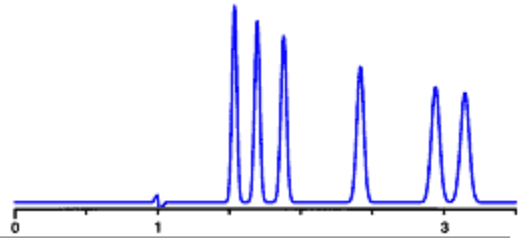
Extraction

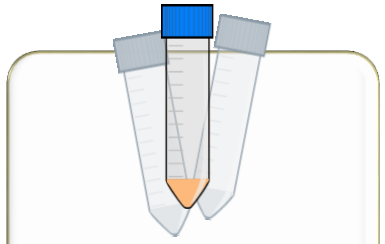
Physico-chemical diversity



Mycotoxin	CAS broj	Molecular formula	Log P
AFB1	1162-65-8	C17H12O6	1,6
AFB2	7220-81-7	C17H14O6	1,3
AFG1	1165-39-5	C17H12O7	1,8
AFG2	7241-98-7	C17H14O7	1,5
DON	51481-10-8	C15H20O6	-0,7
FB1	116355-83-0	C34H59NO15	-0,5
FB2	116355-84-1	C34H59NO14	1,2
ZEA	17924-92-4	C18H22O5	3,6
T-2	21259-20-1	C24H34O9	0,9
HT-2	26934-87-2	C22H32O8	0,4
OTA	303-47-9	C20H18ClNO6	4,7

Dilute and Shoot Approach

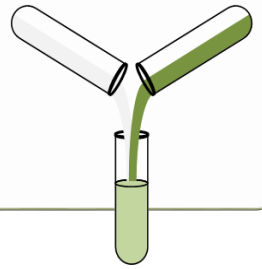




Extraction

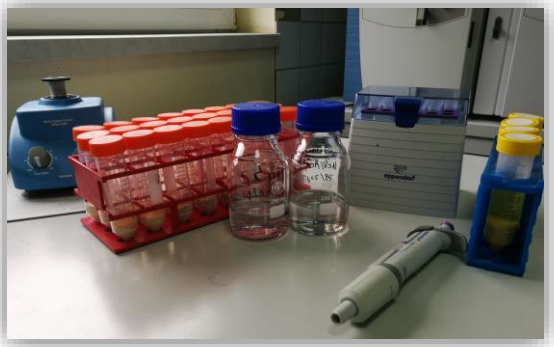
79/20/1
$CH_3CN/H2O/FA$
90 min

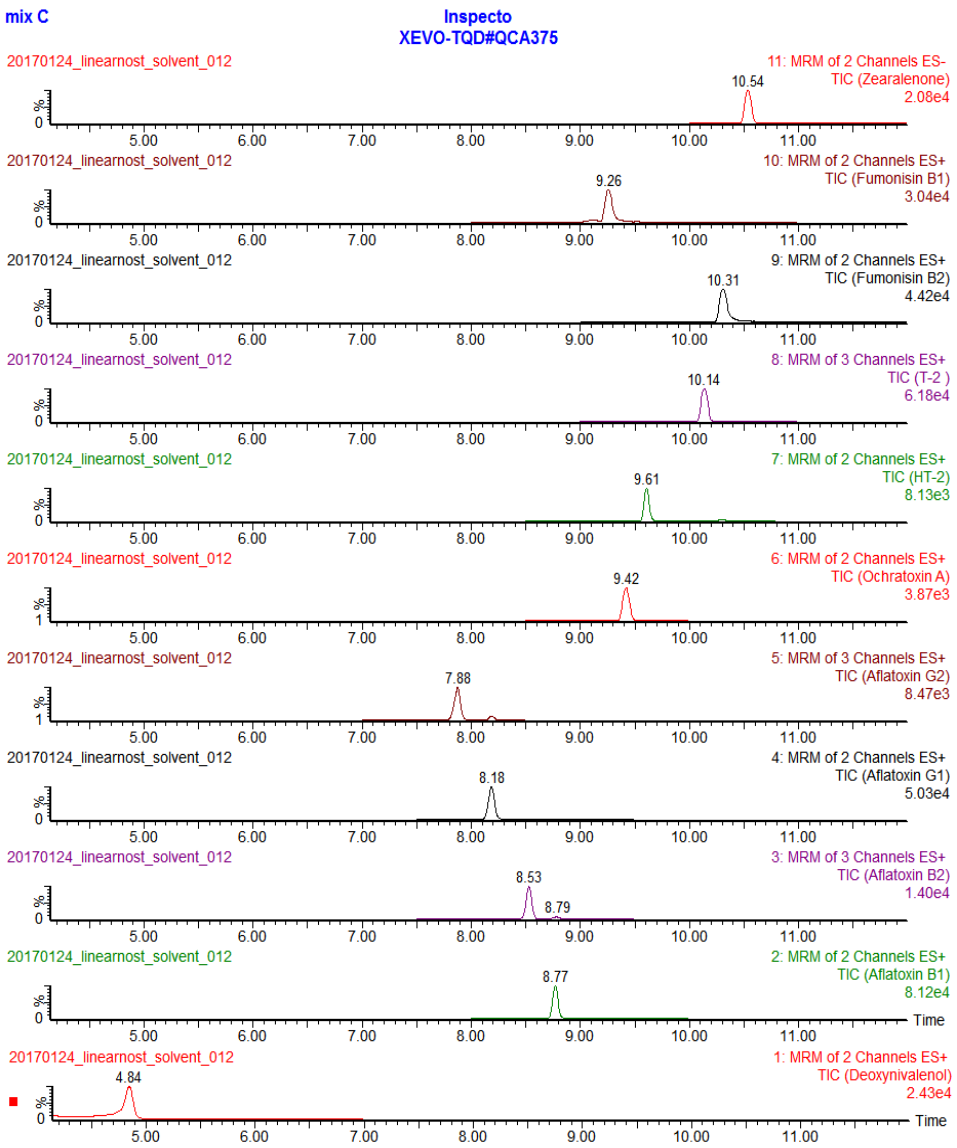
Dilution



20/79/1
$CH_3CN/H2O/FA$
1:1

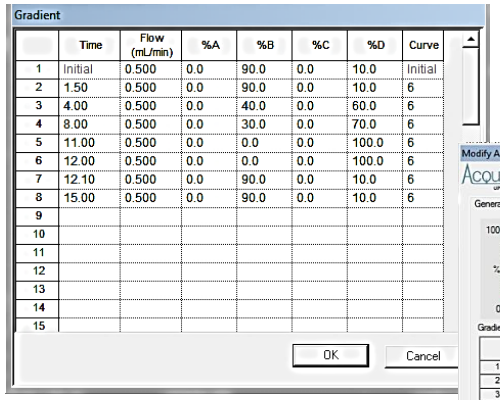
LC-ESI-MS/MS





LC

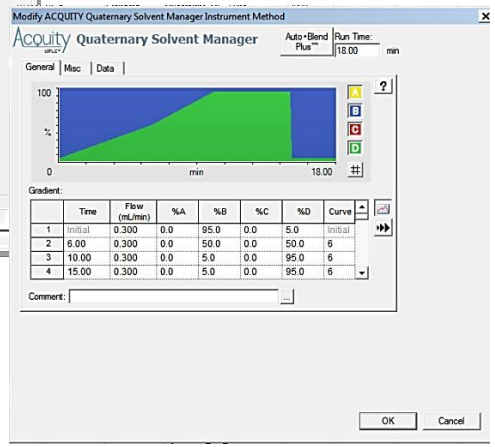
System: ACQUITY UPLC H-Class
Column: ACQUITY HSS T3 1,8µm, 2.1x100mm
Mobile phase A: 5mM AF/H2O
Mobile phase B: MeOH
Flow rate: 0,3 mL/min
Injection volume: 10 µL
Column temperature: 40°C
Sample temperature: 10°C
LC gradient:



Gradient

	Time	Flow (mL/min)	%A	%B	%C	%D	Curve
1	Initial	0.500	0.0	90.0	0.0	10.0	Initial
2	1.50	0.500	0.0	90.0	0.0	10.0	6
3	4.00	0.500	0.0	40.0	0.0	60.0	6
4	8.00	0.500	0.0	30.0	0.0	70.0	6
5	11.00	0.500	0.0	0.0	0.0	100.0	6
6	12.00	0.500	0.0	0.0	0.0	100.0	6
7	12.10	0.500	0.0	90.0	0.0	10.0	6
8	15.00	0.500	0.0	90.0	0.0	10.0	6
9							
10							
11							
12							
13							
14							
15							

OK Cancel



MS

System: XEVO TQD Tandem Quadrupole Mass Spectrometer
Ionization: Electrospray (+/-)
Capillary voltage: +1,5/-1,0kV
Source temperature: 150°C
Desolvation temperature: 400°C
Desolvation gas flow: 650 L/Hr
Cone gas flow: 0 L/Hr
Collision gas: Argon
Cone voltages: Various
Collision voltage: Various
Adducts: [M+H]+, [M–H]-, [M+NH4]+

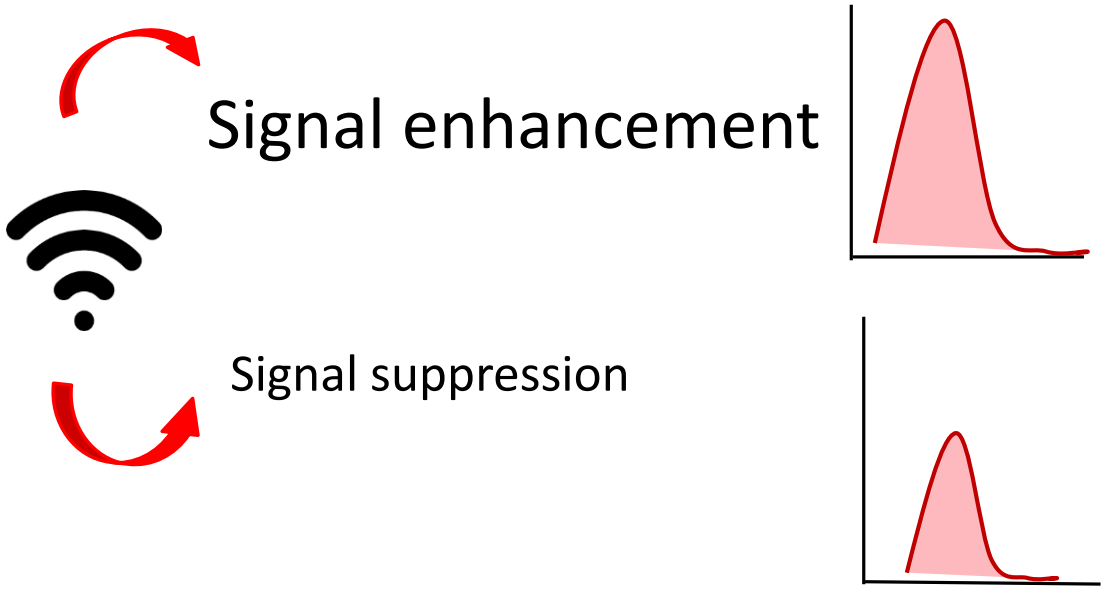
Matrix effect

Reducing the amount of matrix introduced into the system

- diluting the extract or reducing the injection volume
- optimizing the sample preparation procedures - stronger purification techniques

Matrix compenzation using different calibration approaches

- matrix-matched calibration
- standard addition method
- internal standard approach



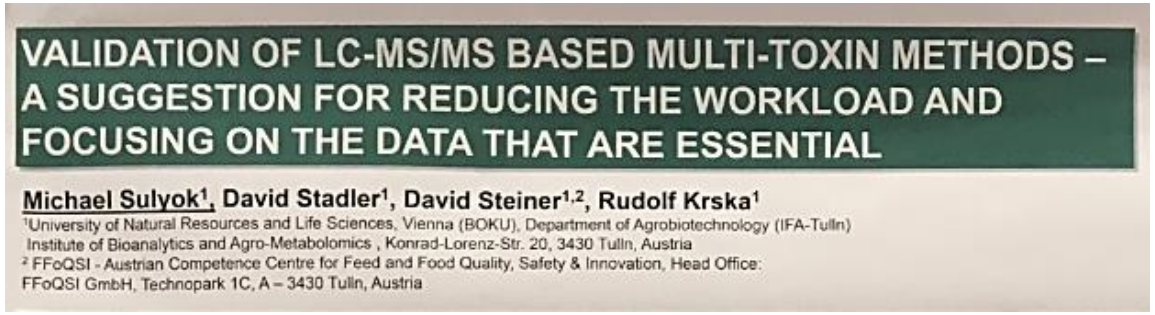
Recovery – term harmonization

COMMISSION DECISION 2002/657/EC

Recovery - the percentage of the true concentration of a substance recovered during the analytical procedure

IUPAC 2002

Apparent recovery – observed value derived from an analytical procedure by means of a calibration graph divided by a reference value


VALIDATION OF LC-MS/MS BASED MULTI-TOXIN METHODS – A SUGGESTION FOR REDUCING THE WORKLOAD AND FOCUSING ON THE DATA THAT ARE ESSENTIAL

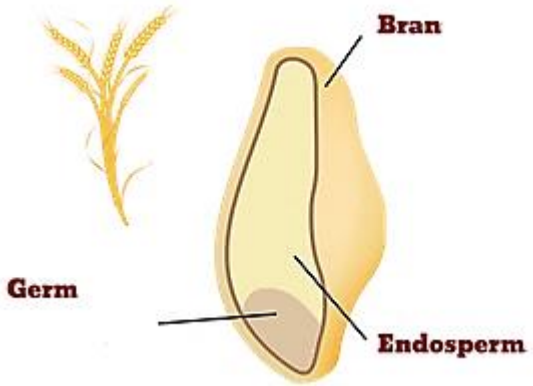
Michael Sulyok[1], David Stadler[1], David Steiner[1,2], Rudolf Krska[1]

[1]University of Natural Resources and Life Sciences, Vienna (BOKU), Department of Agrobiotechnology (IFA-Tulln)
Institute of Bioanalytics and Agro-Metabolomics , Konrad-Lorenz-Str. 20, 3430 Tulln, Austria
[2] FFoQSI - Austrian Competence Centre for Feed and Food Quality, Safety & Innovation, Head Office:
FFoQSI GmbH, Technopark 1C, A – 3430 Tulln, Austria

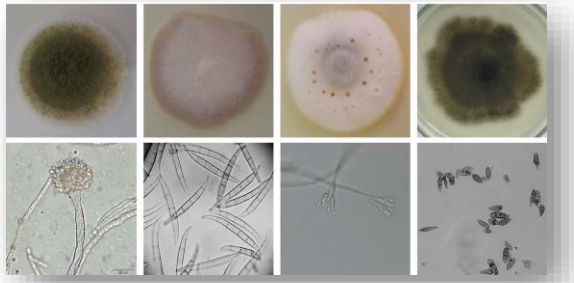
- **Difference** - suppression (or enhancement) of the analytical signal due to co-eluting matrix components contributes to apparent recovery but not recovery

- Is it apparent recovery or recovery to which related performance criteria are applied to?

Sample homogeneity



- Heterogeneous distribution within the batch (Cheli et al. 2014; Krska et al. 2008) - major impact on the accuracy of quantification of individual mycotoxins - **sampling is the largest source of error**
- Commission Regulation (EC) No 401/2006 - standardizing the sampling and analysis procedures for the determination of mycotoxins



►B 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 foodstuffs

(Text with EEA relevance)

Table 2

Number of incremental samples to be taken depending on the weight of the lot of cereals and cereal products

Lot weight (tonnes)	Number of incremental samples	Aggregate sample weight (kg)
≤ 0,05	3	1
> 0,05-≤ 0,5	5	1
> 0,5-≤ 1	10	1
> 1-≤ 3	20	2
> 3-≤ 10	40	4
> 10-≤ 20	60	6
> 20-≤ 50	100	10

InspectoKOM

Commercialization of Innovation of Laboratory Methods and Services of Inspecto Ltd

Total project value:
6.563.190,85 HRK

Non-refundable aid:
2.795.477,93 HRK

Implementation period:
07/2018 - 09/2021

Labtim

inspecto

EUROPSKI STRUKTURNI I INVESTICIJSKI FONDOVI

Europska unija
Zajedno do fondova EU

Innovative laboratory methods in the segment of mycotoxin analysis:

- Multicomponent services that will enable customers faster and technologically more exact analytical testing methods
- Regulated + unregulated mycotoxin determination

Acquity H-class Xevo TQD

Acquity H-class Xevo TQ-S micro

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https://www.inspecto.hr/

Thank you for your attention!