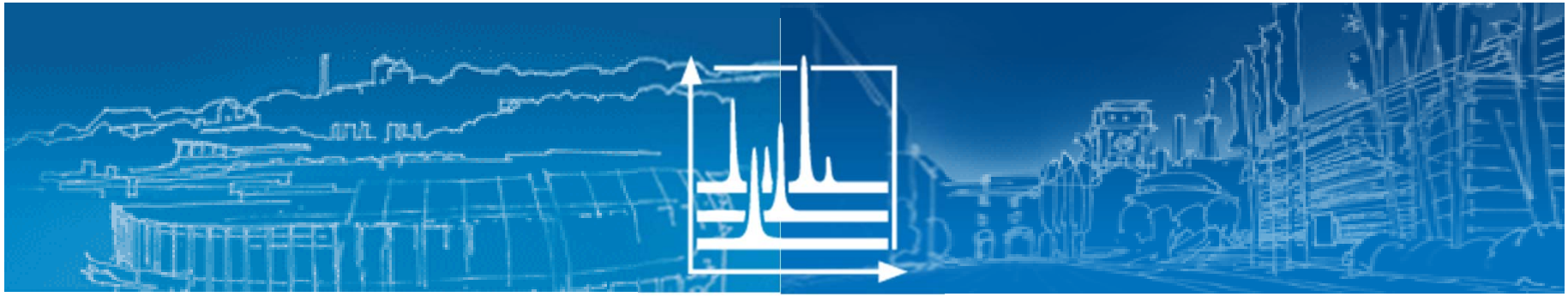
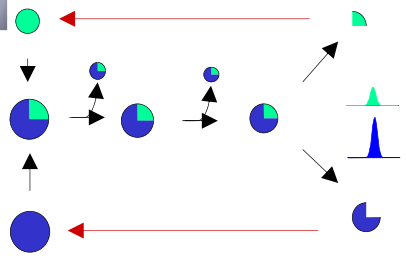


Analysis and Risk Assessment of “New” Mycotoxins

Michael Rychlik
Chair of Analytical Food Chemistry
Technical University Munich, Germany
University of Queensland, Australia

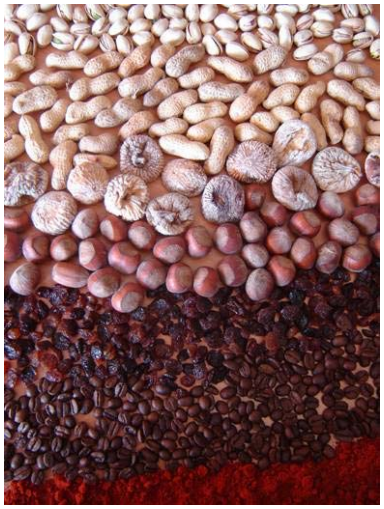
Outline

1. Introduction
   „Old Mycotoxins“
2. Mycotoxin Analysis
   State-of-the-art
3. „Emerging“ Mycotoxins:
   „New“ Genus: Alternaria
4. „Modified“ Mycotoxins-
   „Old“ Genus: Fusarium



Mouldy Foods

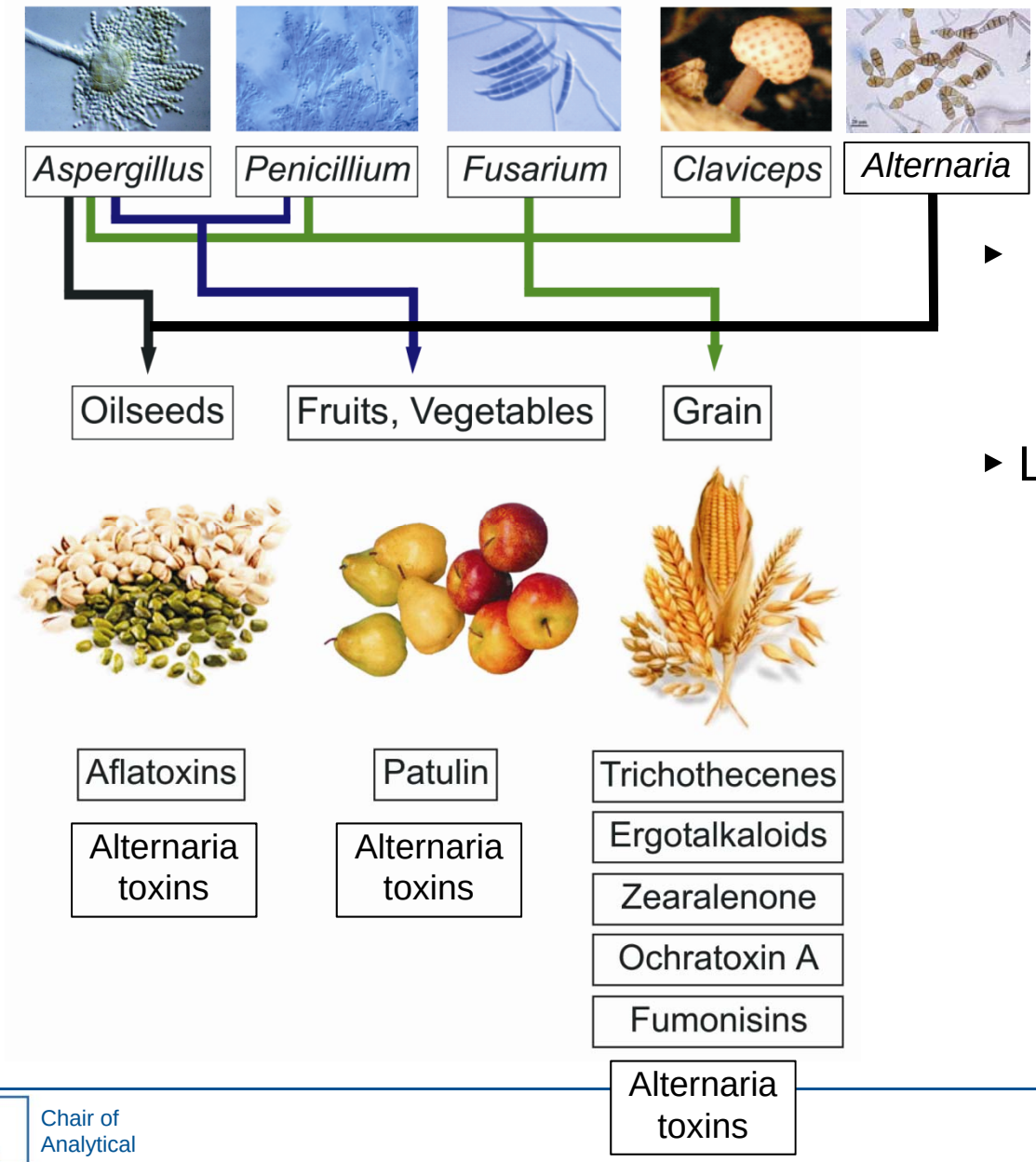
Sometimes obviously spoiled
and probably contaminated with
mycotoxins



but in most cases
not visibly contaminated

According to FAO 25 % of all foods
are contaminated with mycotoxins (2010)

Introduction: Mycotoxins



- 300 – 400 secondary mould metabolites are recognized as mycotoxins
- Legal limits (EU):

  Aflatoxins B1, B2, G1, G2
  Ochratoxin A
  Patulin
  Zearalenone
  Deoxynivalenol (DON)
  Fumonisins B1, B2
  (T2-toxin, HT2-toxin)

Emerging and Modified Mycotoxins

„New“ mycotoxins: recently discovered, lack of reference compounds

- **„Emerging“ mycotoxins**: „New“ mycotoxins

  lack of analytical methods,
  exposure data,
  toxicity data

- **„Modified“ mycotoxins**:

  max. regulatory limits apply for „free“ forms

  comprehensive definition including the term

  „Masked“ mycotoxins

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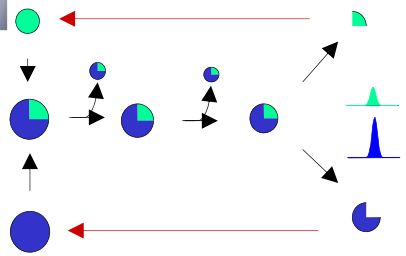
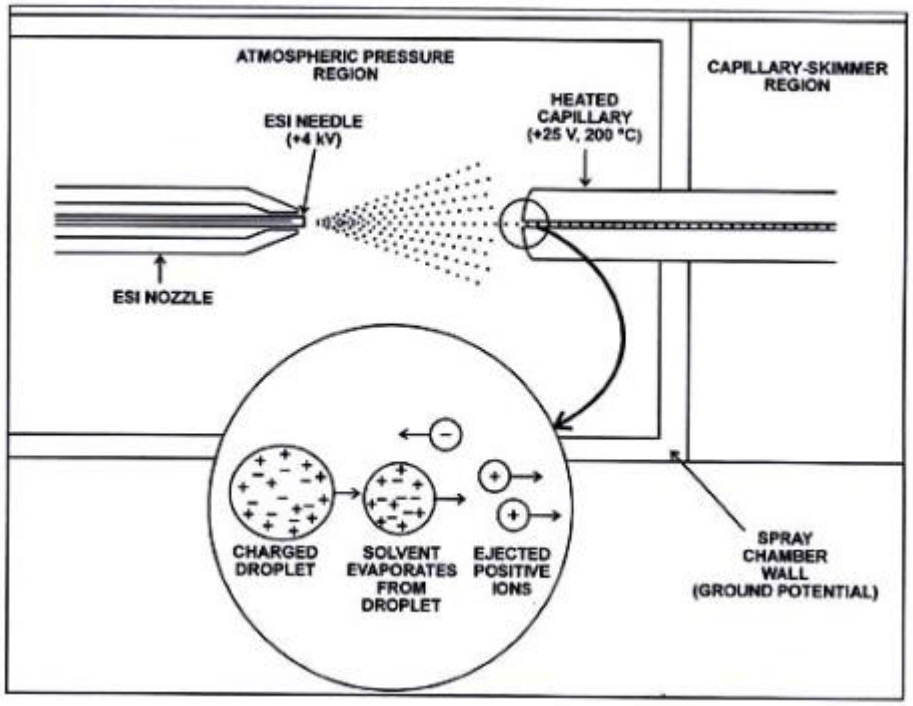


Electrospray Ionisation (ESI) in LC-MS

LC-ESI-MS: Nobel prize in chemistry, 2002, John B. Fenn

ESI Needle

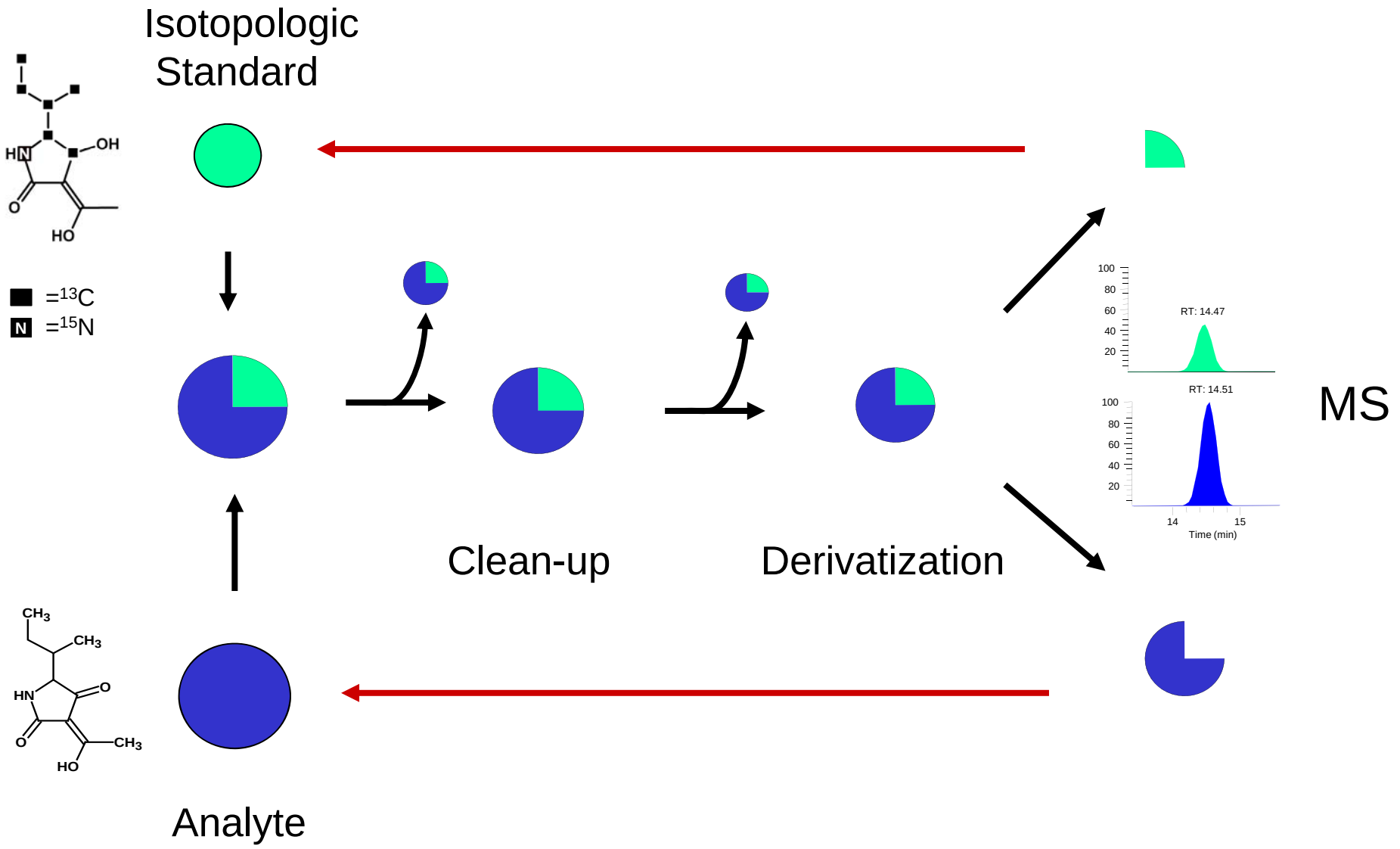


Coeluting matrix and gradient influence ionisation efficiency of analytes

- Ion suppression or enhancement

Need of suitable internal standards with same retention time:
stable isotopologues for compensation

Prinziple of the Stable Isotope Dilution Assay (SIDA)



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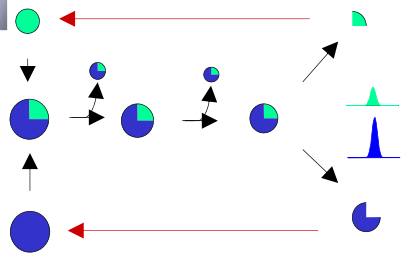
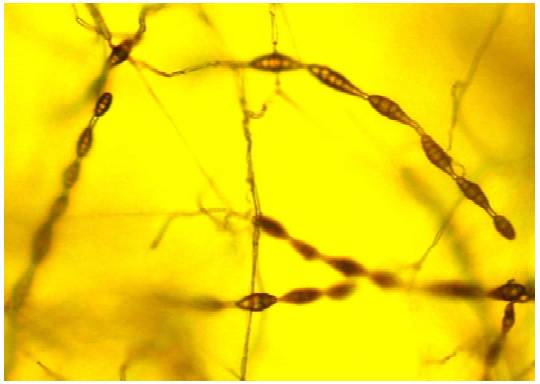


Alternaria toxins as „Emerging Mycotoxins“

© TUM



© B. Rücker TUM

Genus *Alternaria*:

© TUM

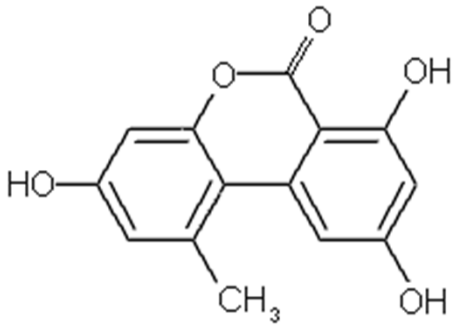
→ Characteristic black spores („Black Fungi“)

→ Species: *A. alternata, A. citri, A. dauci*, … (in total approx. 40)

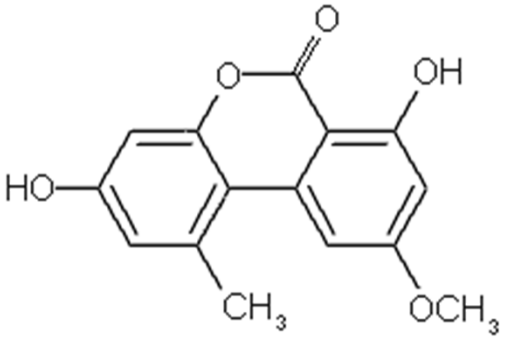
→ known to invade vegetables

→ approx. 70 mycotoxins known with a high structural variety

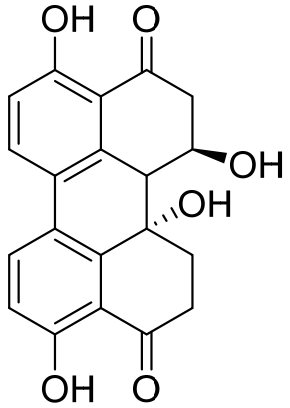
Alternaria toxins



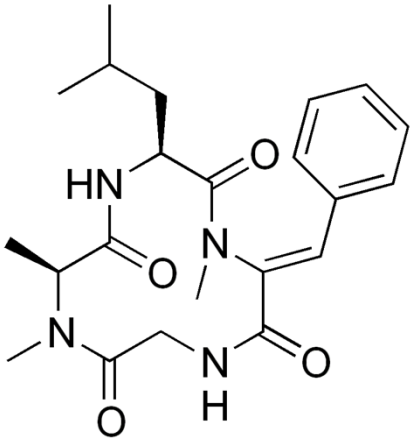
Benzopyrones: Alternariol (AOH)



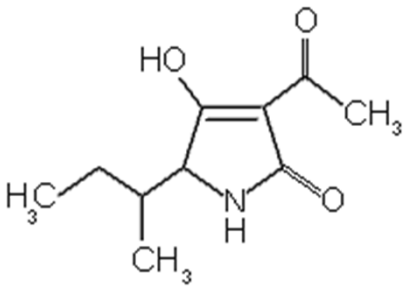
Alternariol methylether (AME)



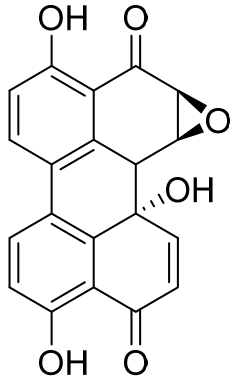
Altertoxin I (**ATX-I**)



Tentoxin



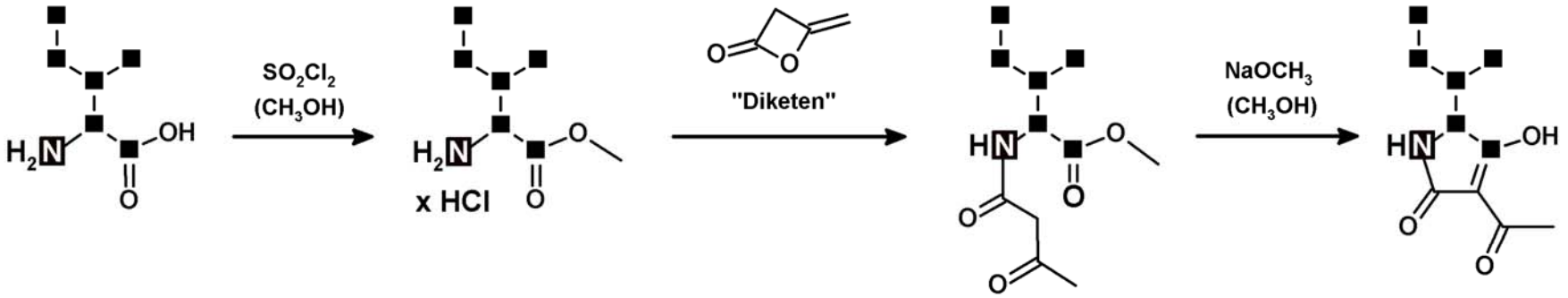
Tenuazonic acid (TA)



Stemphylotoxin III (**Ste-III**)

Labelled Standard for Tenuazonic Acid and Derivatization

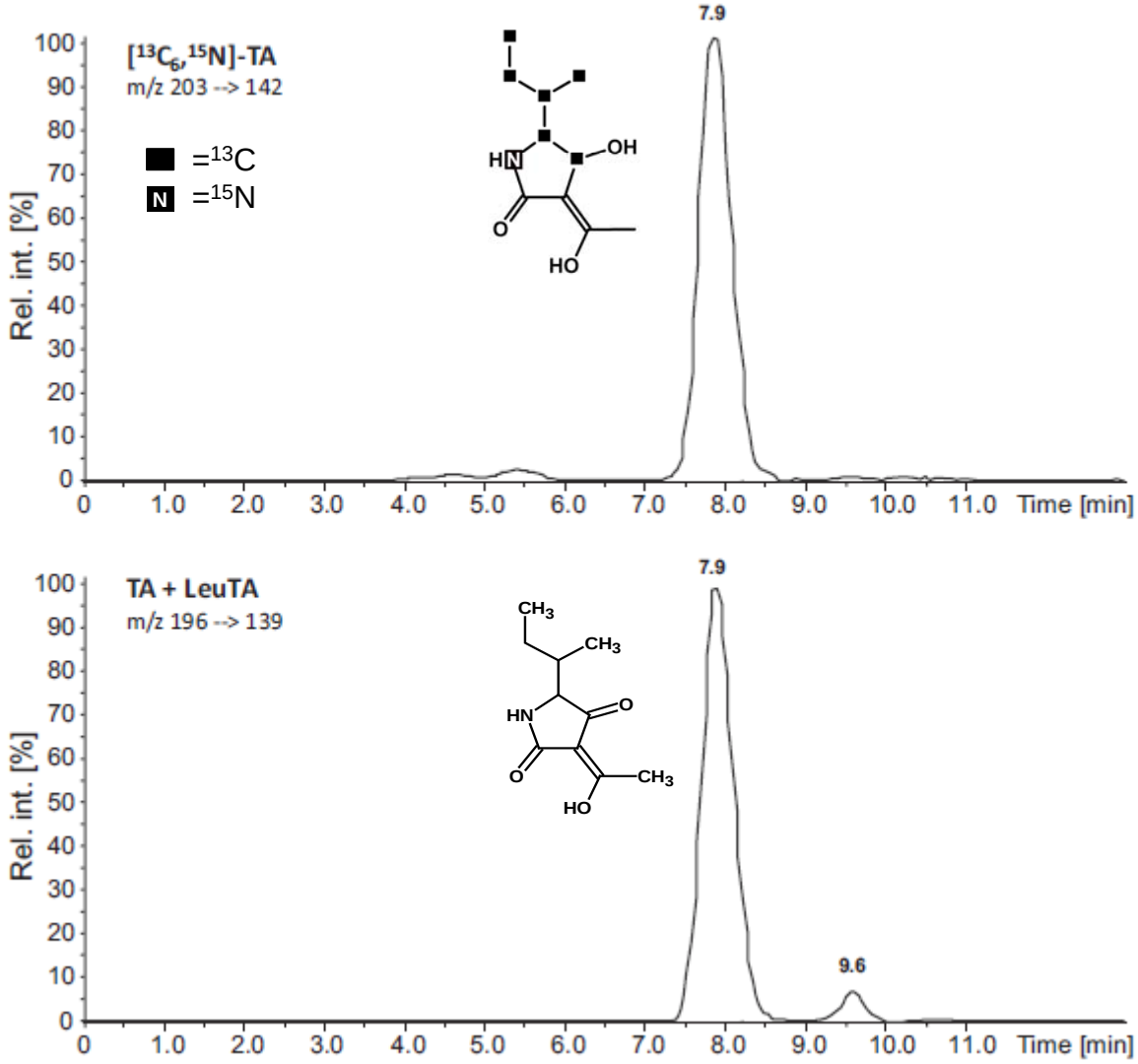
Synthesis of [$^{13}C_6$,^{15}N]-TA



[$^{13}C_6$,^{15}N]-isoleucine

[$^{13}C_6$,^{15}N]-tenuazonic acid

LC-MS/MS of Tenuazonic Acid



Contents of Alternaria Toxins in Foods (µg/kg)

Food	samples	AOH	AME	TA	Tentoxin	ATX1
Tomato products	20	1.4 - 8	0.2 - 3	10 - 250	3.2 [a]	n.d.
Paprika powder	12	31 [a]	21 [a]	2900 [a]	47 [a]	3.7 [a]
Millet	30	n.a.	n.a.	8 - 1200	n.d. – 4.1	n.d.
Sorghum feed	7	521 [a]	164 [a]	n.a.	55 [a]	43 [a]

[a] mean value n.a. not analysed

highest contamination in millet from Germany

Risk Assessment of Alternaria Toxins in Foods

Exposure ng/kg b.w. x d	AOH	AME	TA	Tentoxin	ATX1
Average population	20 (EFSA 2011)	2.5 (EFSA 2011)	<13 (EFSA 2011)	90 (EFSA 2011) 5.6 Liu (2014)	
high exposure group (95% perc.)	45 (EFSA 2011)	9 (EFSA 2011)	Infants: max. 3700		organic cereals ~ 1.2 Liu (2014)
TTC ng/kg b.w. x d	2.5	2.5	1500	1500	2.5

EFSA approach „Threshold of Toxicological Concern“

- in case of lacking toxicological data, concern is based on exposure data
- excess of TTC requires substantial toxicological data and decreasing the exposure with high priority

Conclusions and Outlook

Bavarian Official Food Authority:
„substantial doubts about harmlessness of these products“
⇒ „unfit for human consumption“ as it is unacceptable for reasons of contamination (Art. 14, 2. (b) Regulation (EC) 178/2002)
as „contaminant levels shall be kept as low as can reasonably achieved by following good practices“ (Art. 2, 2. Council Regulation 315/93)

Producers were called on reduction of TA contents
„warning limit“ 500 µg/kg

Need for more toxicity data:
chronic toxicity, NOAEL

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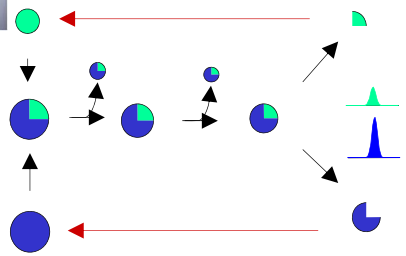


Fusarium species

- ***Fus. graminearum/culmorum*:** type-B Trichothecenes
- ***Fus. langsethiae/sporotrichioides*:** type-A Trichothecenes
- ***Fus. avenaceum/tricinctum*:** Enniatins + Beauvericin

(Emerging Mycotoxins)

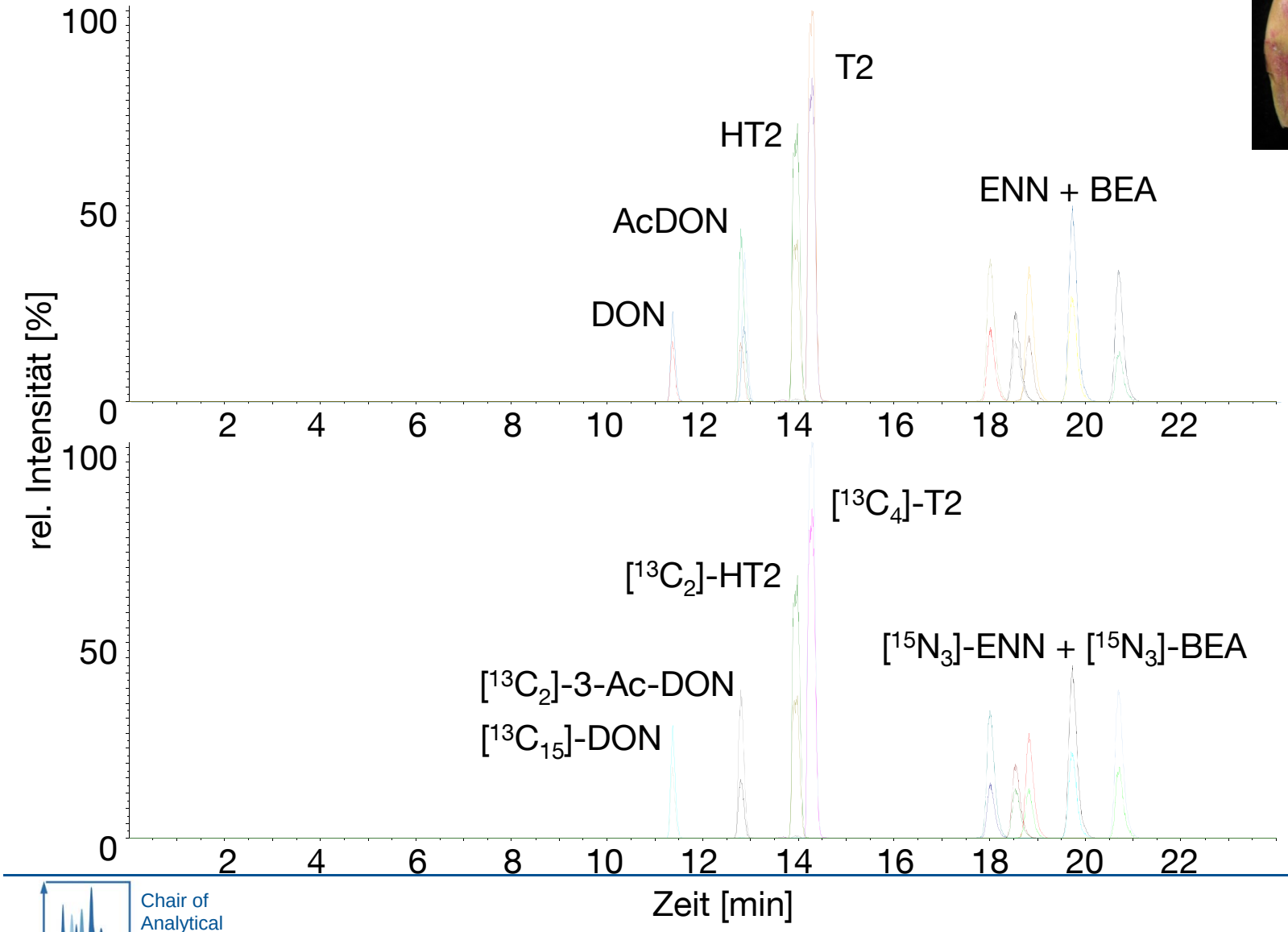
visually sound malt

Fusarium toxins

- toxic metabolites of plant pathogenic fungi
- Harming human and animal health
- Losses in yield, grade, end-use quality of cereals
- Impairment of solving, malting and brewing properties

red and black kernels

LC-MS/MS Analysis: Multi SIDA

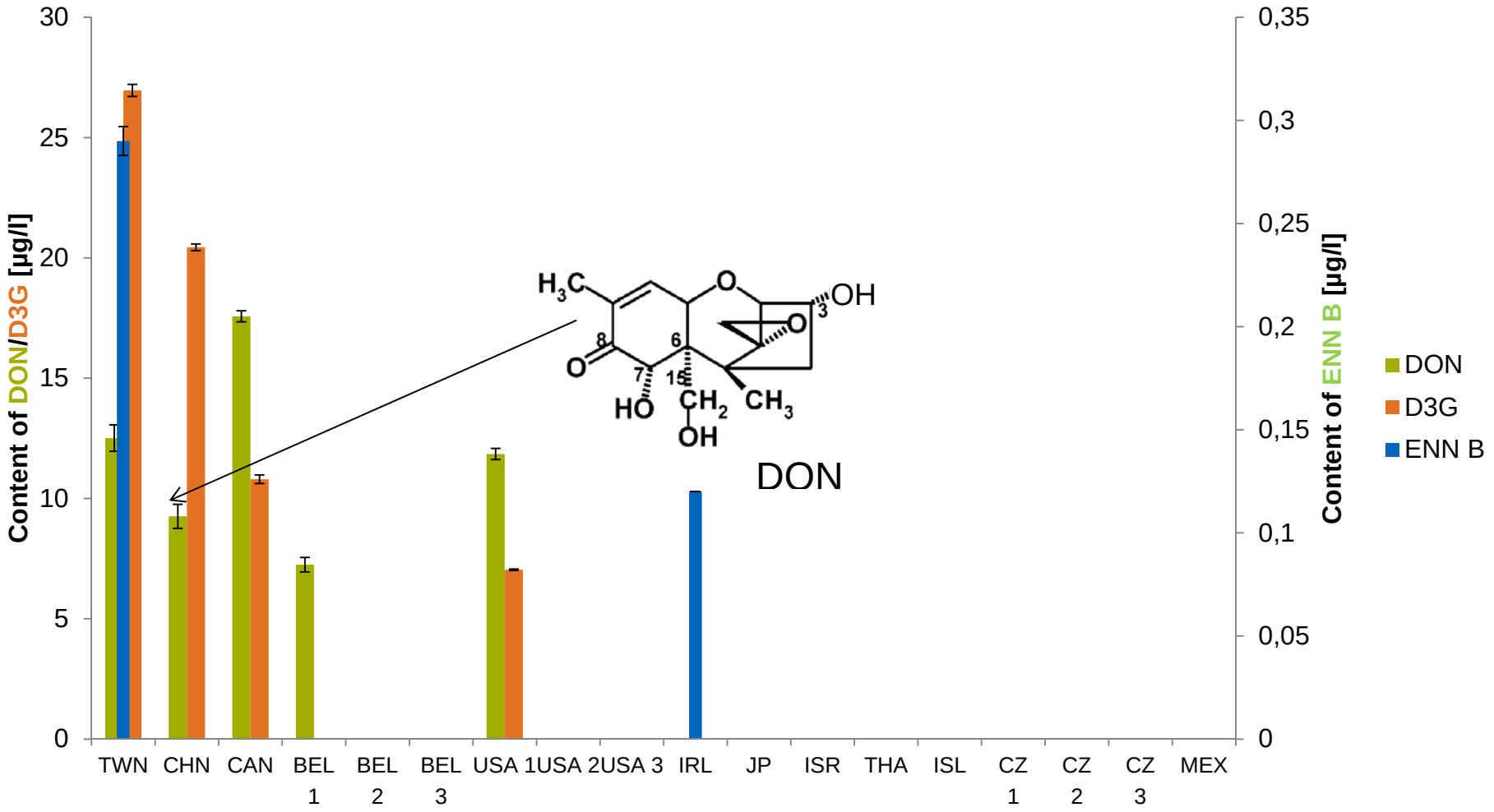


New definition of modified mycotoxins including “masked” mycotoxins

1st level	2nd level	3rd level	4th level	example
Free mycotoxins				Aflatoxin B1, DON, 3-acetyl-DON
Matrix-associated mycotoxins	Complexes, physically dissolved or trapped			
	Covalently bound			Fumonisines bound to starch, OTA- and DON-oligosaccharides
Modified mycotoxins	Biologically modified	Functionalised (phase 1-metabolites)		Aflatoxin B1-epoxide
		Conjugated (phase 2-metabolites)	Conjugated by plants (= masked mycotoxins according to ILSI)	DON-3-glucoside
			Conjugated by animals	DON-3/8/15-glucuronide, HT2-3/4-glucuronide
			Conjugated by fungi	ZEN-14-sulfate
		Differentially modified		Deepoxy-DON (= DOM-1)
	Chemically modified	Thermally formed		norDON A-C, N-carboxymethyl-FB1
		Non-thermally formed		Hydrolysed fumonisines, DON-sulfonate

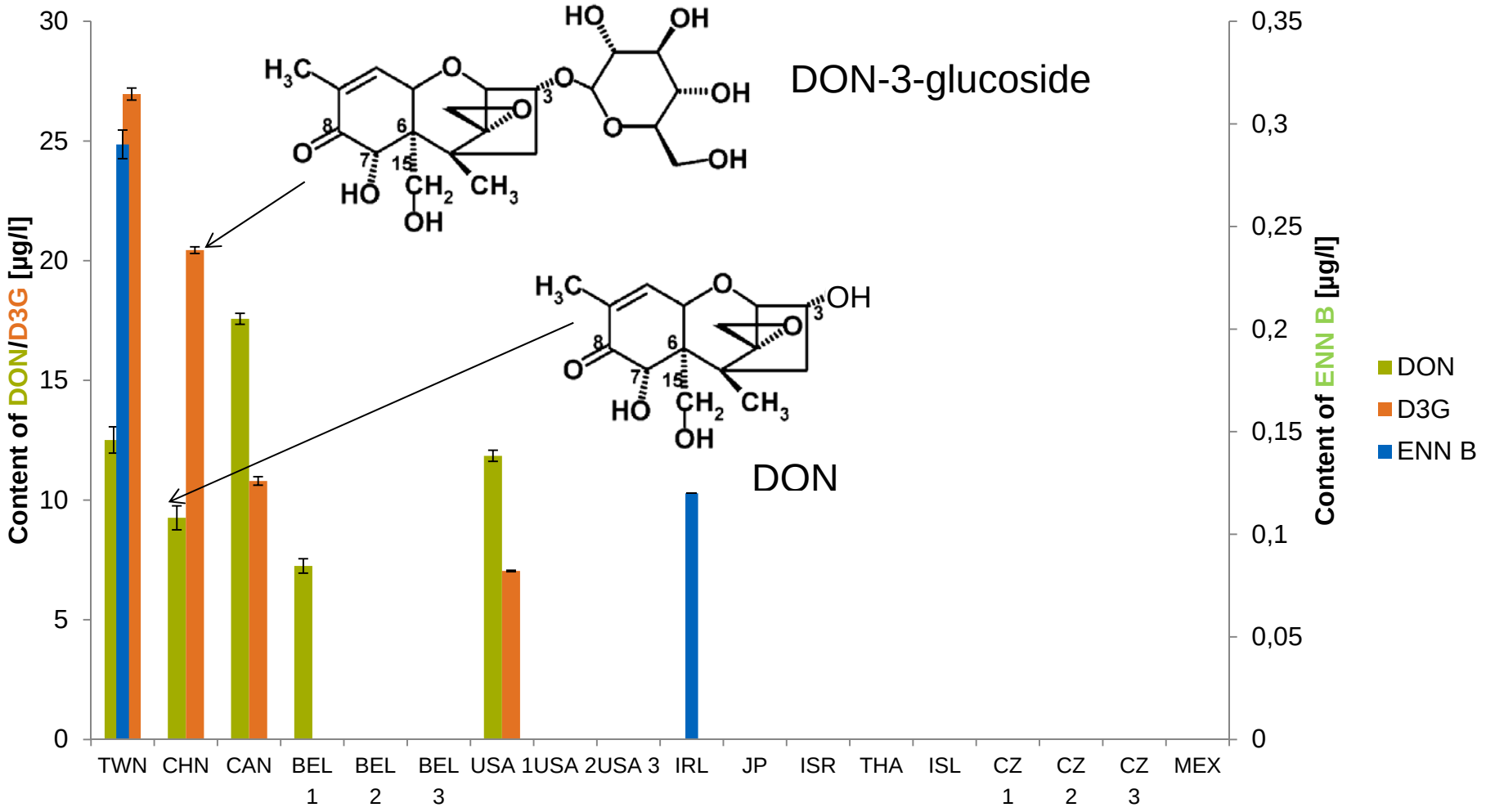
Source: Rychlik et al. (2014) Mycotoxin Research 30: 197-205

Fusarium Toxins: Occurrence in International Beers



TWN=Taiwan, CHN=China, CAN=Canada, BEL=Belgium, USA=United States of America, IRL=Ireland, JP=Japan, ISR=Israel, CZ=Czech Republic, MEX=Mexico

Fusarium Toxins: Occurrence in International Beers



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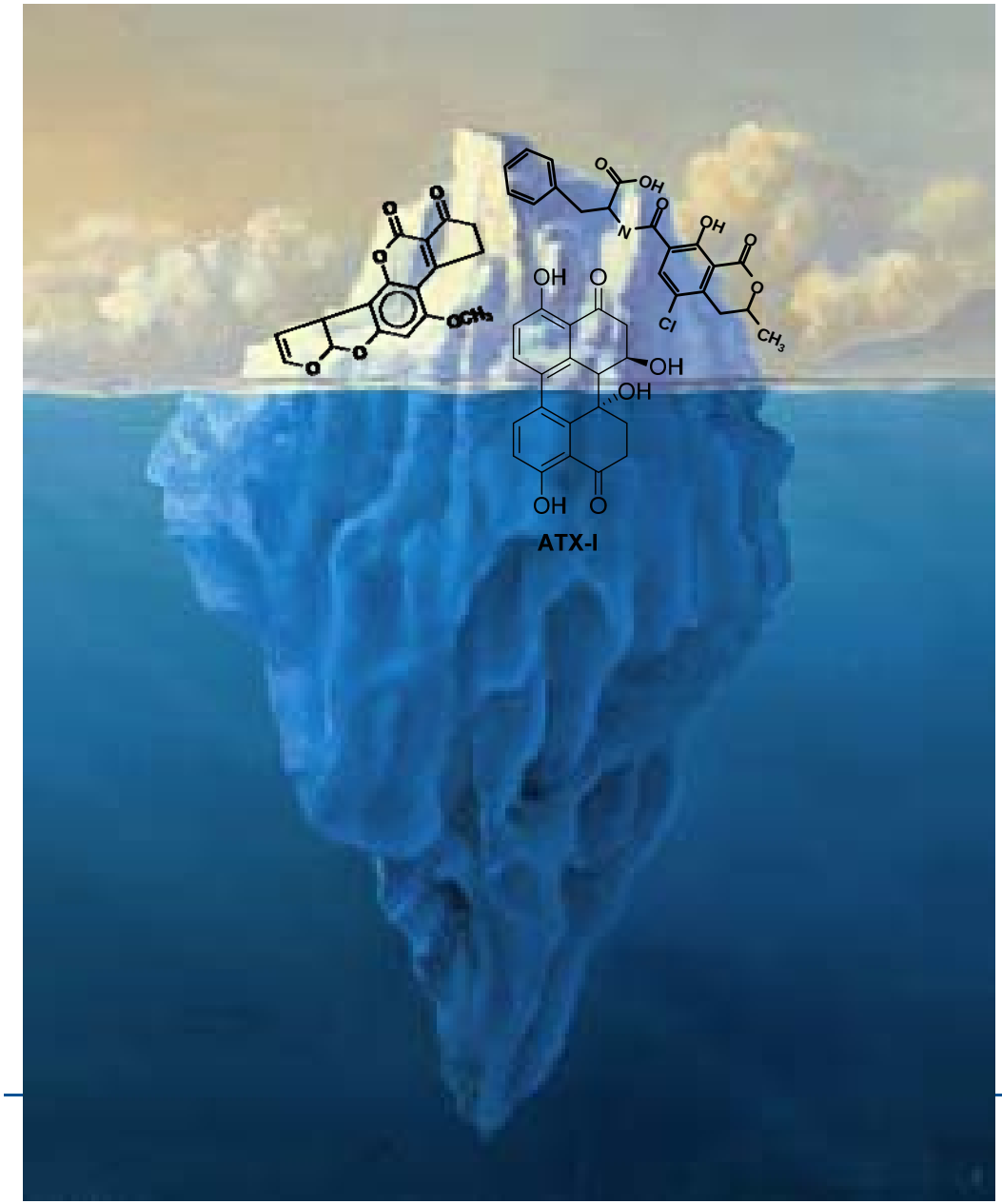
Fusarium toxins: Exposure of beer consumers

- DON, D3G, 3AcDON and ENN B found in beer samples
- Contamination level of analyzed beer samples rather low (below regulatory limits)
- Even heavy drinkers moderately exposed

Current analysis only sees the tip of the iceberg ?



Outlook 2017:

Rychlik et al. (2017)
Foodomics as a Promising Tool to Investigate the Mycobolome,
Trends in Analytical Chemistry
In press

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DAAD
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DFG

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Otto von Guericke

FEI

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BfR
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