



Advanced analytical methods for chemical foodborne hazards

Rudolf Krska

G. Salentijn, Ids Lemmink, A. Schamann, M. Suman
+ FoodSafeR consortium

22 Members of the FoodSafeR Advisory Board representing EU and global multi-actors:



.....eminent outreach is given through an international Advisory Board spanning global dimensions, which will also serve as hubs for the FoodSafeR to anticipate and mitigate future food crises.

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Views

1,2k

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Rudolf Krška

A FoodSafeR perspective on emerging food safety hazards and associated risks

John F. Leslie¹, Chibundu N. Ezekiel², Martin Wagner^{3,4},
Chris Elliott⁵, Oonagh McNerney⁶, Mieke Uyttendaele⁷,
Wu Yongning⁸, Songxue Wang⁹, Sheila Okoth¹⁰,
James Lindsay¹¹, Dorothea F. K. Rawn¹², Sheot Harn Chan¹³,
Kai Zhang¹⁴, Veronica M. T. Lattanzio¹⁵, Felicia Wu¹⁶,
Ranajit Bandyopadhyay¹⁷, Eleonora Dupouy¹⁸, Steve Wearne¹⁹,
Samuel Godefroy^{20,21}, Michele Suman²² and Rudolf Krška^{2,23*}

TABLE 1 Food safety hazards discussed at the FoodSafeR workshop.

Type of risk	Risk agent	
	Natural toxins	Mycotoxins (e.g., <i>Aspergillus</i> toxins including aflatoxins and ochratoxin A, ergot alkaloids, and <i>Fusarium</i> toxins including deoxynivalenol, fumonisins, T-2, HT-2 and zearalenone), phycotoxins (e.g., ciguatera toxins), plant toxins (e.g., pyrrolizidine alkaloids and tropane alkaloids)
	Toxic elements	Arsenic, cadmium, lead, mercury and other heavy metals
	Chemicals	Allergens, bisphenol A, drug residues, environmental inhibitors, ethylene oxide, flame retardants, food additives, food processing contaminants (e.g., acrylamide), micro- and nano-plastics, mineral oils, plasticizers, per- and polyfluoroalkyl substances (PFAS), perfluorooctane sulfonate (PFOS), persistent organic pollutants (e.g., dioxins), pesticide residues (e.g., fipronil and neonicotinoids), and volcanic ashfall

Identification & managing **emerging hazards/risks**: **digital hub**

FOODSAFER

(EMERGING) HAZARD IDENTIFICATION

WP4 OPEN DIGITAL HUB FOR ENDURING DELIVERY OF PROJECT OUTCOMES & TRANSFORMATION

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Created in the FoodSAFE Project, which received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 101000000

* Case Study



FoodSafer
Framework





Other relevant case studies in Chemical hazard/risk emergence

- **Combatting illegal addition of toxic chemicals:** case **lead chromate** and **metanil** yellow dye in **turmeric** using portable spectroscopy devices
- **Advanced mycotoxin monitoring** in view of **climate change** and improved **prediction tools**
- **Reduction** of **furans** and **mycotoxins** through mechanical & thermal processing at industrial scale
- Advancing **(bio)analytical sensing** and diagnostic tools, for the on-site testing of
 - **pyrrolizidine alkaloids** (21 regulated) in herbal teas
 - **tropene alkaloids** in cereals



Drivers of (emerging) food safety hazards/risks

based on participatory workshop with stakeholders, interviews and International Advisory Board (in STEEP categories)



Social Drivers



Consumer behaviour:

- gluten free, high fibre, ...



Environmental Drivers

Environmental contamination:

- Pesticides, heavy metals
- Bioaccumulation of toxins



Technological Drivers



Technologies in food production:

- Novel food sources (insects)
- Thermal degradation of toxins



Political Drivers

Legislation, policies and governance:

- Food safety monitoring (RASFF)



Economic Driver

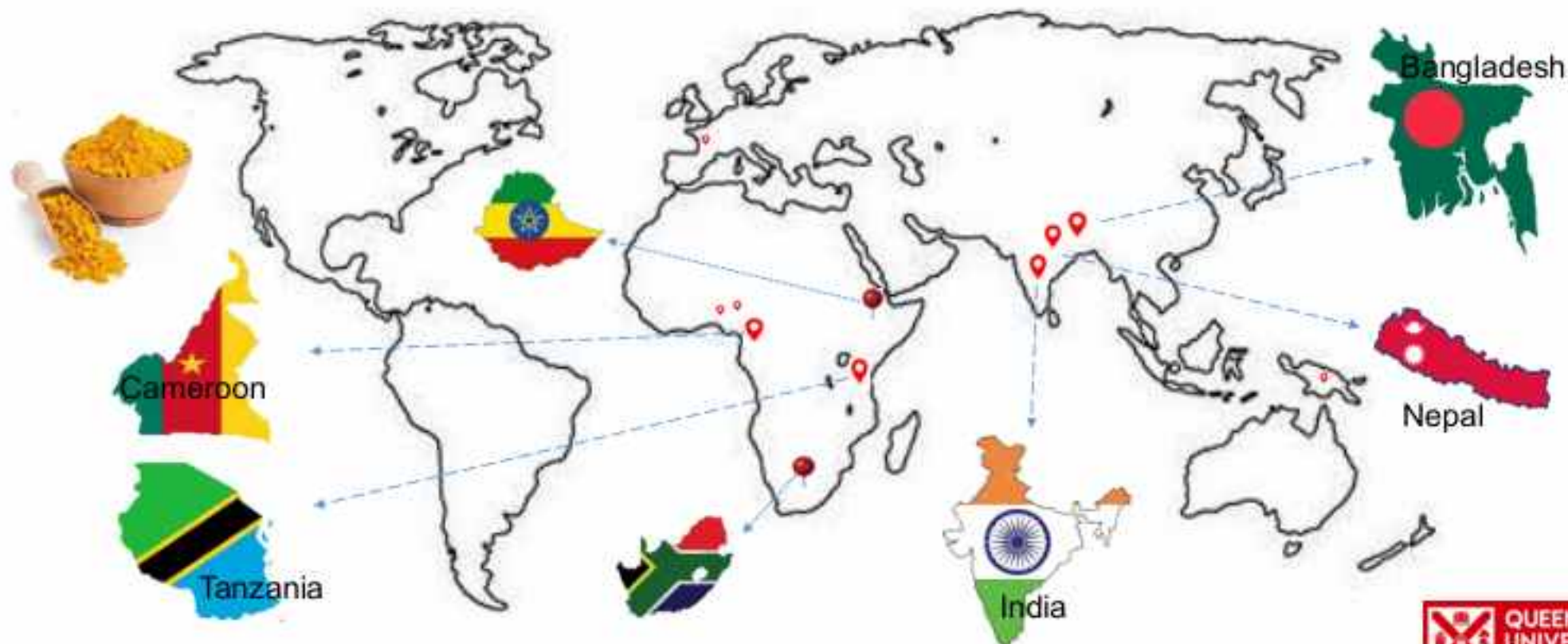


Distribution:

- Food fraud, Authenticity
- Proper Storage



Illegal addition of toxic chemicals to food: the turmeric - lead chromate case - sources & emerging markets of a popular spice



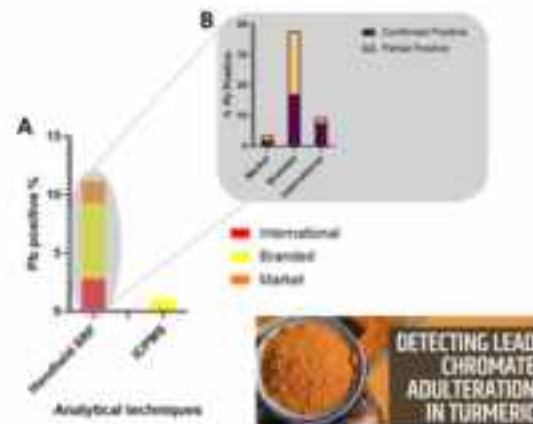


Food fraud: Combatting the illegal addition of toxic chemicals to food: the **tumeric-lead chromate** case.

- **Turmeric** = spice with numerous claimed health benefits => addition of **lead chromate** and **metanil** to give it a more vibrant yellow colour
- **Portable spectroscopy devices** e.g. NIR, FTIR, Hyperspectral imager, coupled with chemometrics and AI and XRF devices



bing.com



Multi-elemental Measurement

Field Test



Handheld XRF

LR

PLSR

RFR

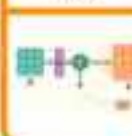


Machine Learning

LR

PLSR

RFR



Laboratory Test



Benchtop XRF

LR

PLSR

RFR



Multi-instrumental calibration



Mean (max) Pb levels: 1.89 – 3.62 (10.90) mg/kg (5-30 %)

Metanil yellow levels: 0.007 – 0.463 in 5.5 % of total samples

Stable C and N
Pb Isotope Ratio
Pb & Cr distribution map

Non-compliant samples -
Suspicious: Not turmeric powder



Paul William et al., IGFS, QUB

Drivers of (emerging) food safety hazards/risks



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Legislation, policies and governance:

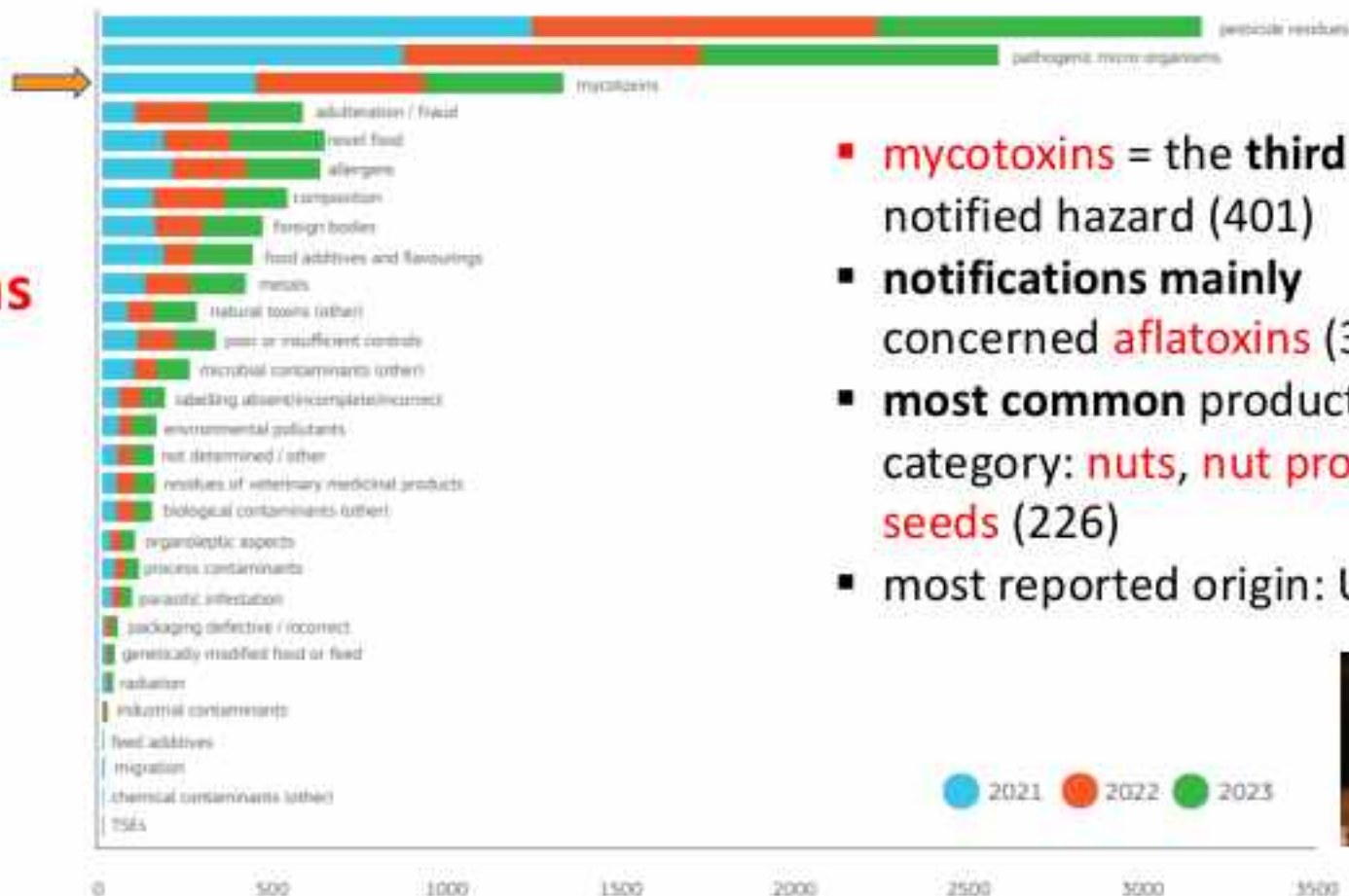
- Food safety monitoring (RASFF)



- From both, a **food systems** and a **hazard analysis** perspective
- In total: 39 subdrivers

EU Rapid Alert System for Food and Feed (RASFF)

Recent RASFF notifications by food hazard category



- **mycotoxins** = the **third** most notified hazard (401)
- **notifications** mainly concerned **aflatoxins** (331)
- **most common** product category: **nuts, nut products, seeds** (226)
- **most reported origin**: US (85)



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Economic Driver



Distribution:

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- Proper Storage





Reviews

Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate' of 25%

Mari Eskola , Gregor Kos , Christopher T. Elliott , Jana Hajšlová, Sultan Mayar & Rudolf Krska

Pages 2773–2789 | Published online: 03 Sep 2019

Download citation

<https://doi.org/10.1080/10408398.2019.1658570>

Check for updates

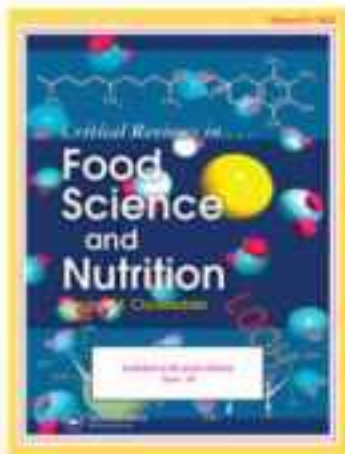
Based on
EFSA data from
175.000 food samples
& Biomin data from
1.900 feed samples

48,461

Views

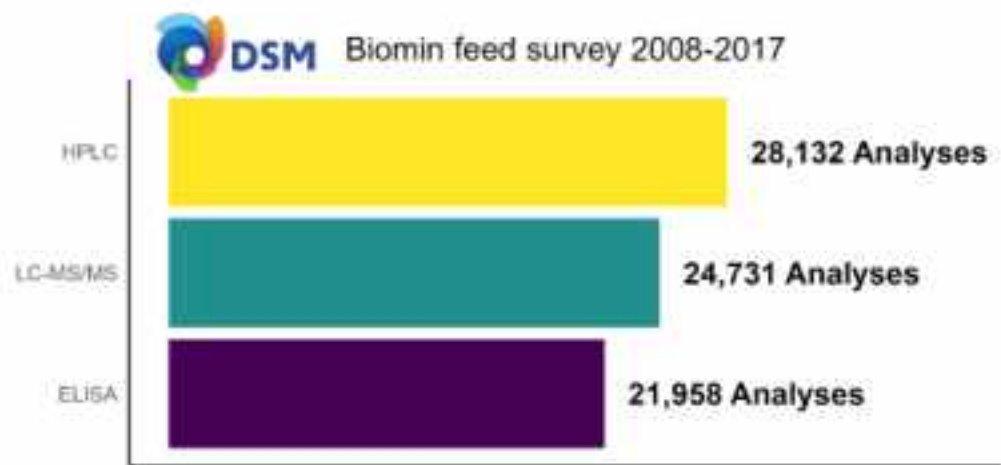
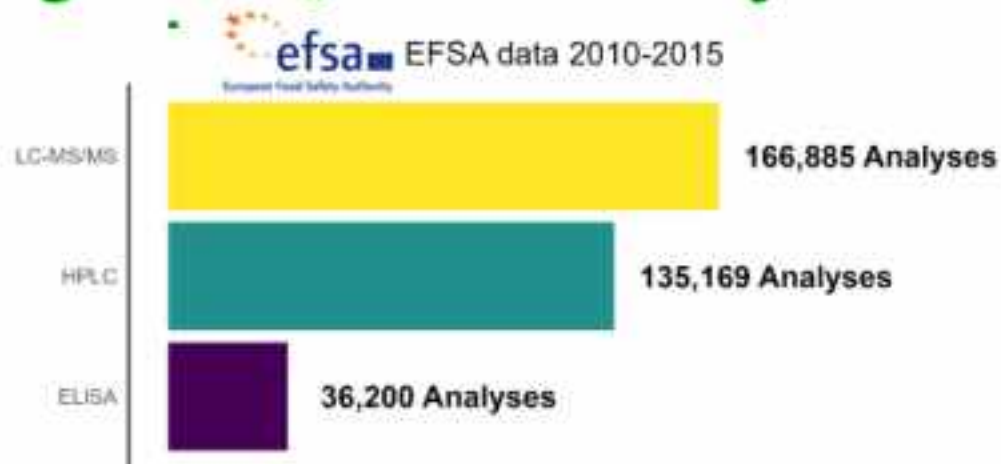
1,192

CrossRef
citations to date



*Current mycotoxin occurrence above the EU and Codex limits confirm the FAO 25% estimate, while this figure **greatly underestimates the occurrence above the detectable levels of (up to 80%)**. The high occurrence is likely explained by a combination of the **improved sensitivity of analytical methods** & **impact of climate change**.*

Targeted mycotoxin analysis – employed routine



Advancing Mycotoxin Detection in Food and Feed: Novel Insights from Surface-Enhanced Raman Spectroscopy (SERS)

Natasha Logan,* Cuong Cao, Stephan Freitag, Simon A. Haughey, Rudolf Krška, and Christopher T. Elliott



WAGENINGEN
UNIVERSITY & RESEARCH

Received: 19 May 2021 | Revised: 18 August 2022 | Accepted: 6 September 2022

DOI: 10.1002/1541-4337.13054

COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

Comprehensive
REVIEWS
in Food Science and Food Safety

WILEY

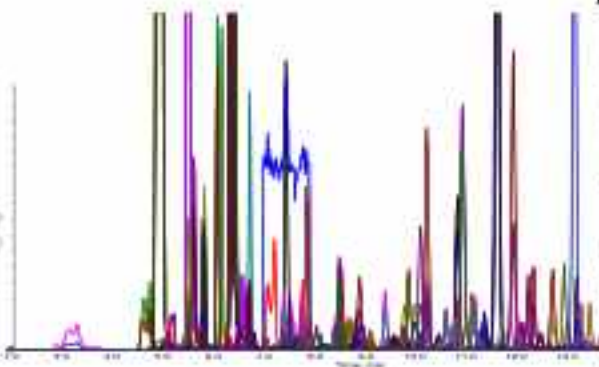
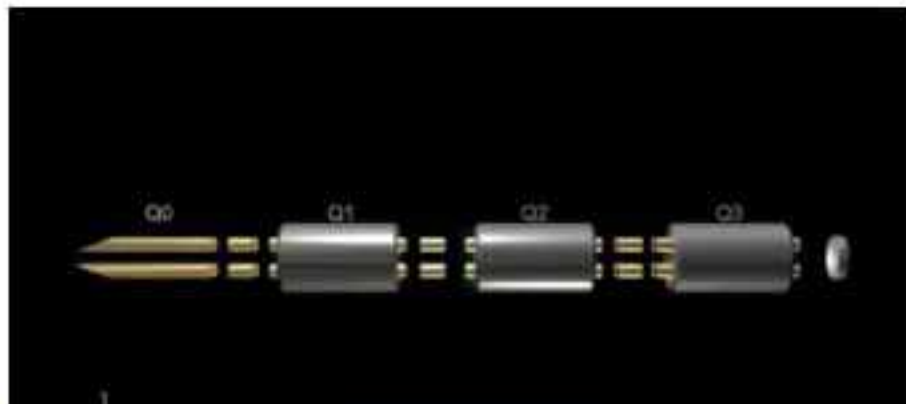
The potential and applicability of infrared spectroscopic methods for the rapid screening and routine analysis of mycotoxins in food crops

Stephan Freitag^{1,2} | Michael Sulyok^{1,2} | Natasha Logan¹ |
Christopher T. Elliott¹ | Rudolf Krška^{1,2,3}





Multi-Class-Analysis by LC-MS/MS: Dilute & shoot for 500 mycotoxins



Realizing the simultaneous liquid chromatography-tandem mass spectrometry based quantification of >1200 biotoxins, pesticides and veterinary drugs in complex feed

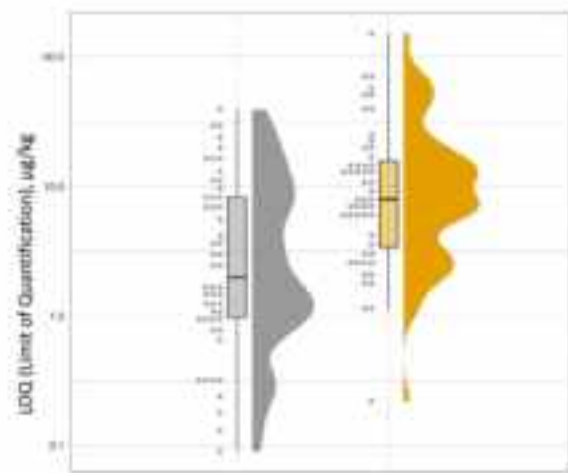
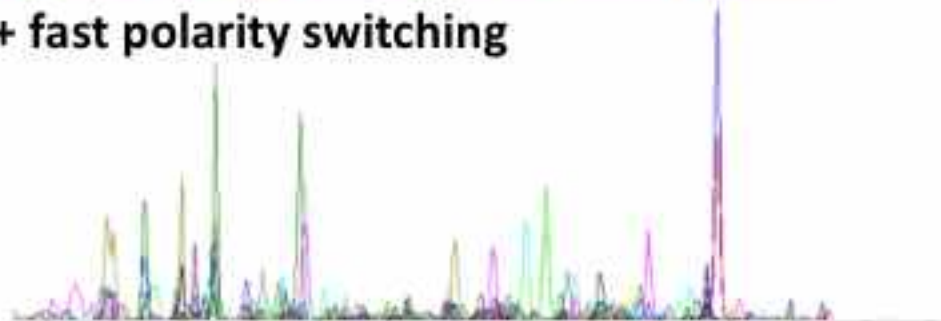
David Steiner^a, Michael Sulyok^{b,c}, Alexandra Malachová^d, Anneliese Moeller^d, Rudolf Krska^{b,c}

>1.200 contaminants in 45 min



Accelerating multi-toxin LC-MS/MS

UHPLC + fast polarity switching



Initial method Ultra-fast method

regulated Not regulated Regulated

➤ Quantification of
931 secondary
metabolites
in 11 min

➤ LOQ_{AFB1}: 1.7 µg/kg



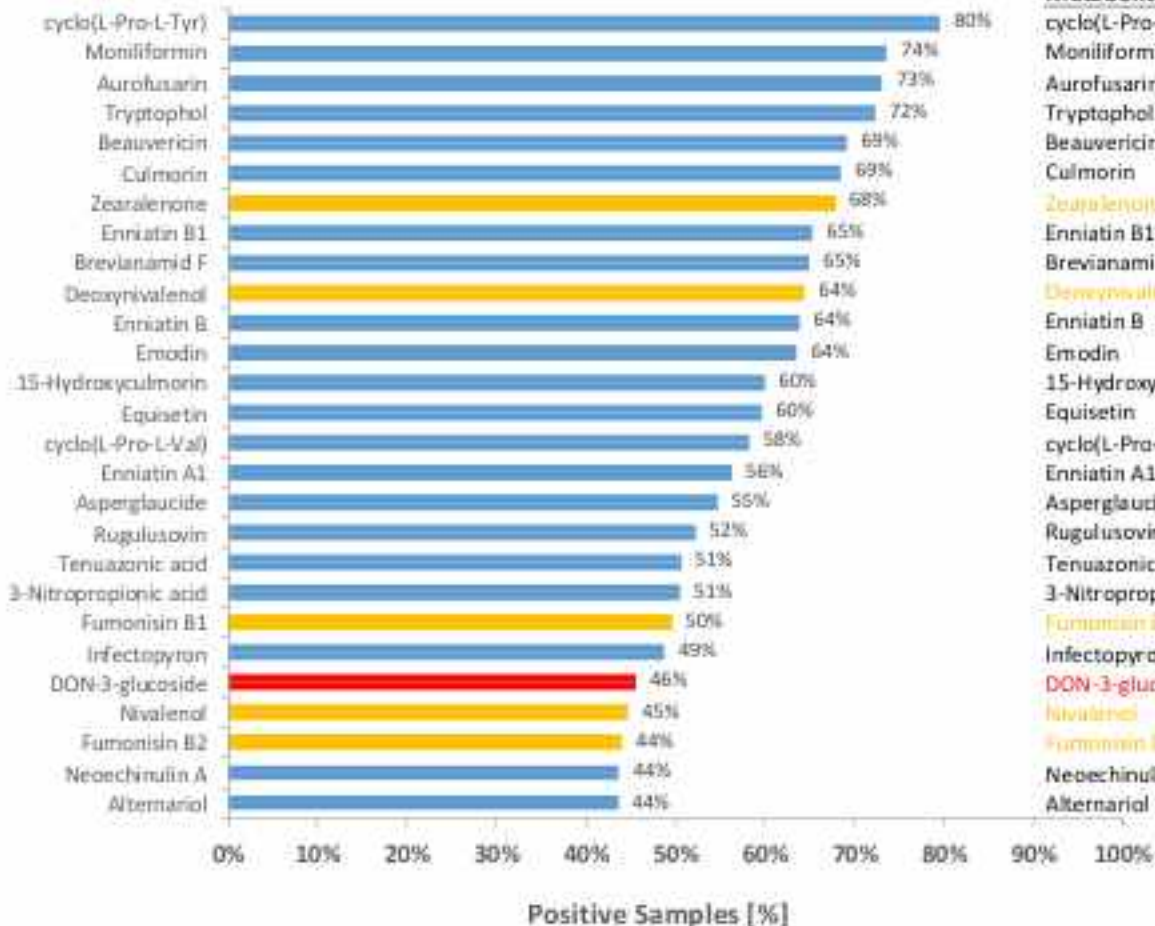
Journal of Food Composition and Analysis
Volume 131, March 2024, 108908



Exploring the limits of UHPLC-MS/MS with polarity switching towards the quantification of 931 mycotoxins and other secondary metabolites in cereal-based food

Lidija Kenjeric ¹, Michael Sulyok ², Christoph Bueschl ³, Alexandra Malochova ⁴, Rudolf Krska ^{1,5}

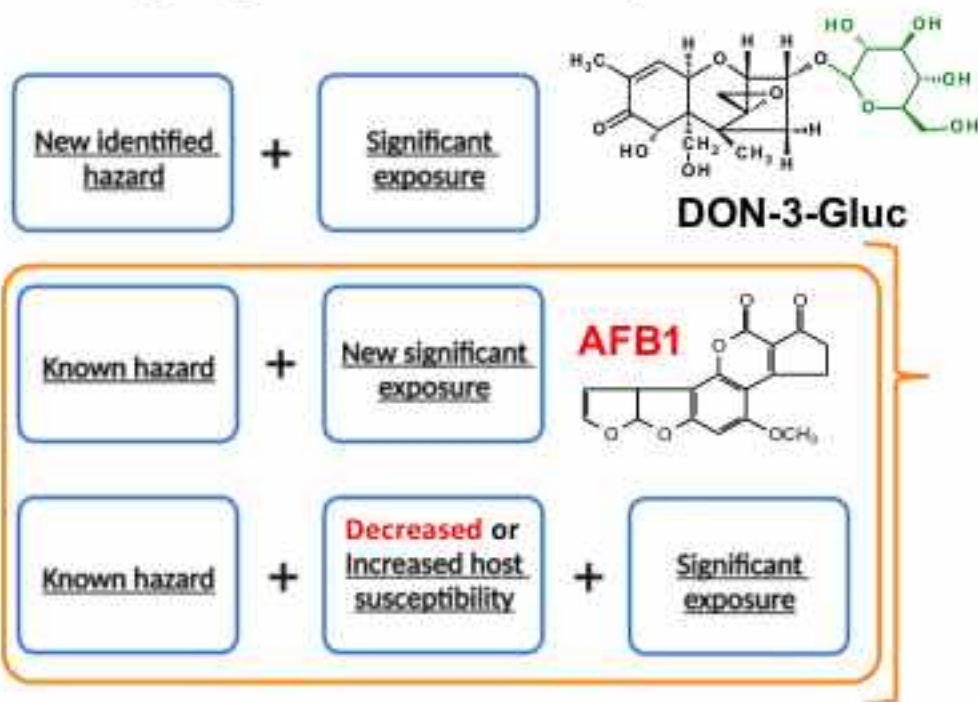
4.300 samples analysed with a multi-mycotoxin approach



Metabolite	Average [µg/kg]	Maximum [µg/kg]	Rank
cyclo(L-Pro-L-Tyr)	632	34,910	1
Moniliformin	95	2,900	2
Aurofusarin	954	319,920	3
Tryptophol	392	39,190	4
Beauvericin	28	1,610	5
Culmorin	525	157,114	6
Zearalenone	108	11,192	7
Enniatin B1	55	5,507	8
Brevianamid F	89	2,614	9
Deoxynivalenol	610	34,861	10
Enniatin B	53	7,519	11
Emodin	57	4,155	12
15-Hydroxyculmorin	419	19,320	13
Equisetin	34	2,081	14
cyclo(L-Pro-L-Val)	1,016	30,360	15
Enniatin A1	23	2,063	16
Asperglaucide	124	22,566	17
Rugulosovin	77	1,658	18
Tenuazonic acid	203	8,115	19
3-Nitropropionic acid	25	4,068	20
Fumonisin B1	791	111,253	21
Infectopyron	1,091	101,408	22
DON-3-glucoside	89	4,516	23
Nivalenol	104	11,232	24
Fumonisin B2	305	16,213	25
Neoechinulin A	87	14,320	26
Alternariol	15	1,458	27

(Identification of) **emerging** hazards and associated **risks**

Emerging risk definition (EFSA)



Emerging Mycotoxins

Advanced LC-MS
methods



New findings
(e.g. masked toxins)

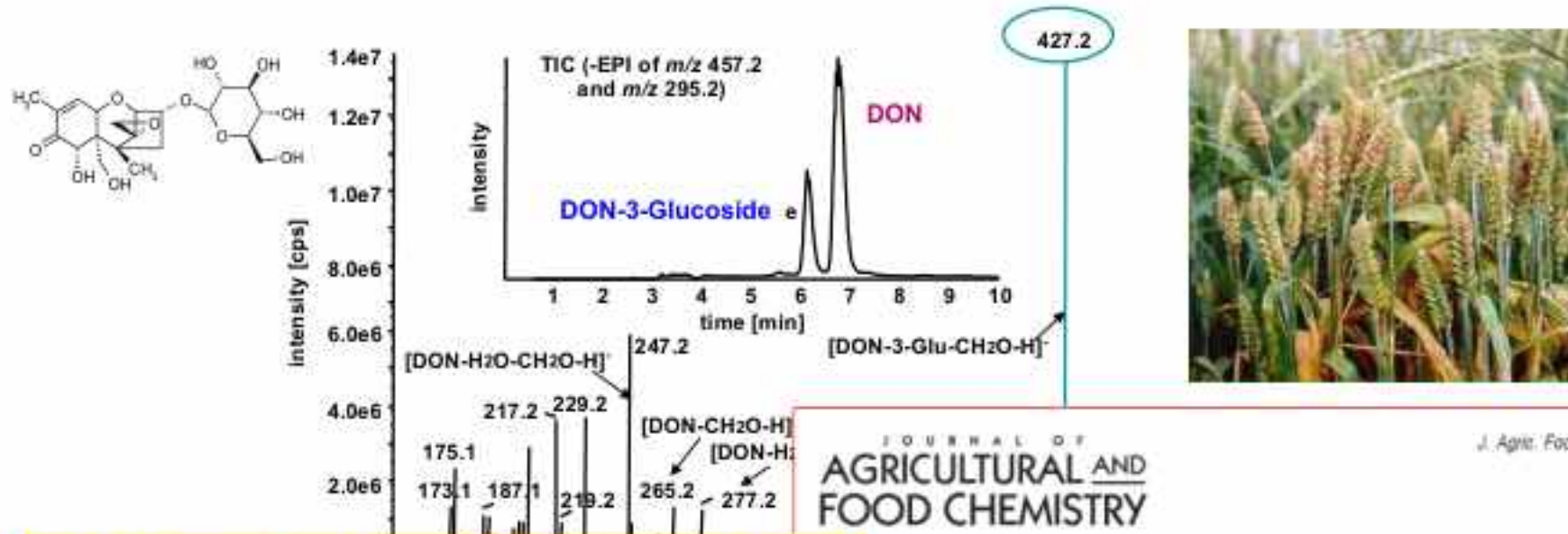
More frequent and
new/**unexpected**
occurrence
data

Climate
change

Plant
breeding



First report on the natural occurrence of conjugated (masked) DON in wheat (2005)



J. Agric. Food Chem. 2005, 53, 3421–3425 3421

EFSA conclusion, 2024: Group-TDI of 1 µg/kg bw per day for the sum of DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside.

Masked Mycotoxins: Determination of a Deoxynivalenol Glucoside in Artificially and Naturally Contaminated Wheat by Liquid Chromatography–Tandem Mass Spectrometry

FRANZ BERTHILLER,[†] CHIARA DALL'ANTA,[†] RAINER SCHUBDMACHER,^{*,†} MARC LEMMENS,[‡] GERHARD ADAM,[§] AND RUDOLF KRISKA[†]

RESEARCH PAPER

Culmorin inhibits detoxification of the mycotoxin deoxynivalenol by plant UDP-glucosyltransferases

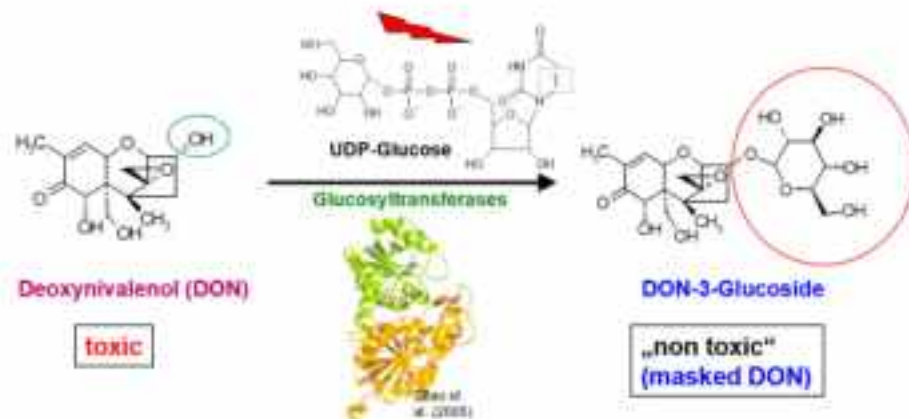
Herbert Michlmayr^{1,2,*}, Gerlinde Wiesenberger^{1,2}, Katrin Rehak¹, Marta Sopol^{3,†}, Kristina Funtak¹, Alexandra Malachová^{3,4}, Philipp Fruhmann^{5,6}, Julia Weber^{5,8}, Marie Dufresne⁶, Rudolf Krska^{3,7}, Franz Berthiller², and Gerhard Adam¹

Archives of Toxicology (2019) 93:1729–1743
https://doi.org/10.1007/s00204-019-02459-w

BIOLOGICS

The *Fusarium* metabolite culmorin suppresses the in vitro glucuronidation of deoxynivalenol

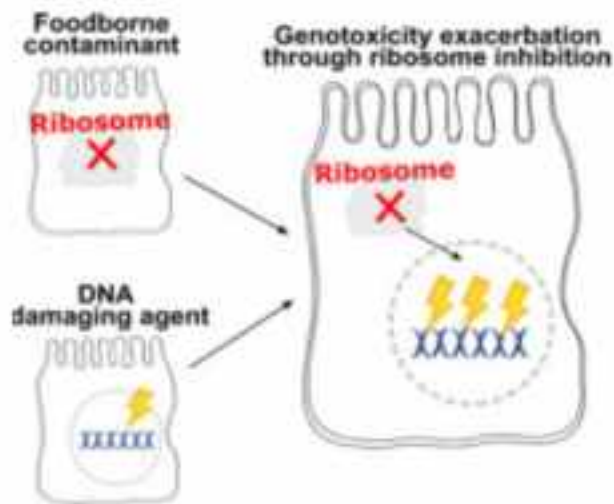
Lydia Woelflingseder¹ · Benedikt Warth¹ · Immina Vierheilig¹ · Heidi Schwartz-Zimmermann² · Christian Hametner³ · Veronika Nagl⁴ · Barbara Novak⁴ · Bojan Šarkanj⁵ · Franz Berthiller² · Gerhard Adam⁶ · Doris Marko¹





The foodborne contaminant deoxynivalenol exacerbates DNA damage caused by a broad spectrum of genotoxic agents

Marion Garofalo ^{a, b}, Delphine Payros ^b, Eric Oswald ^{b, c}, Jean-Philippe Nougayrède ^b ,
Isabelle P. Oswald ^a



DON increases **genotoxicity** induced by **colibactin**, a bacterial toxin produced by certain strains of *E. coli* bacteria in the large intestine

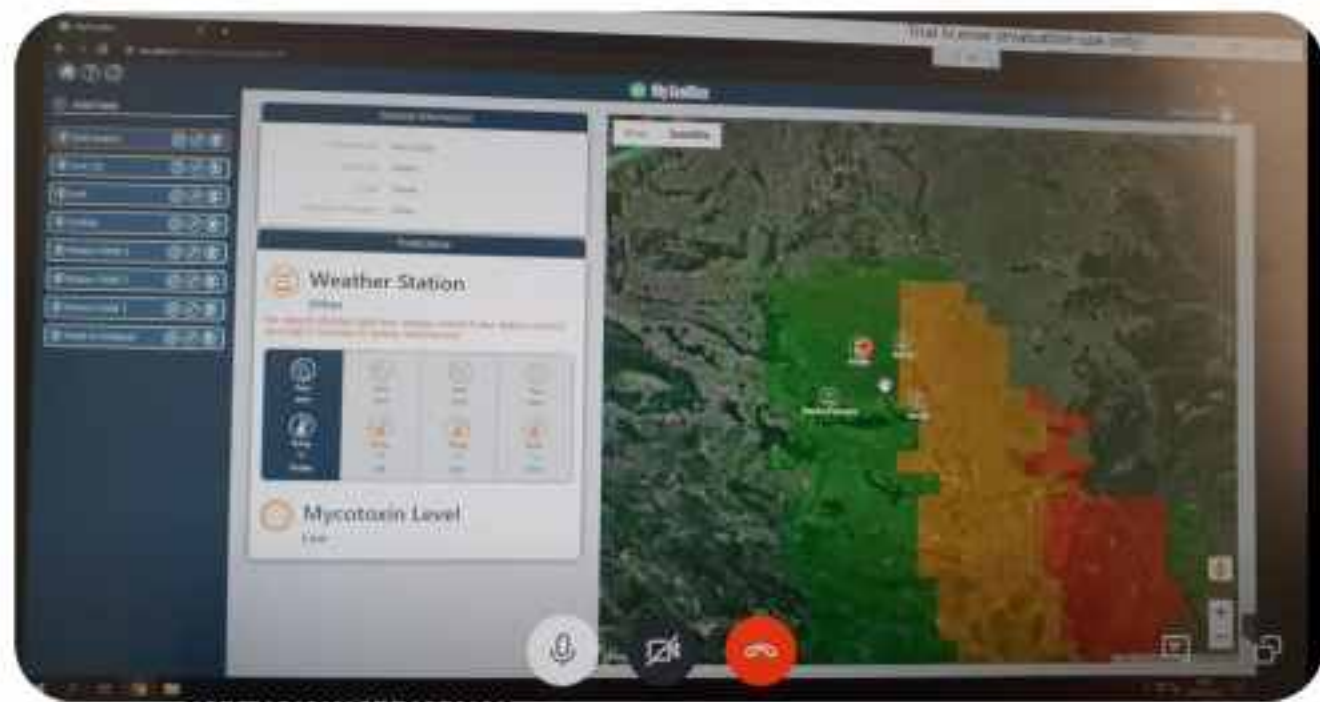
Emerging risk definition
to be extended:

Known hazard

+

New evidence
for
combined toxicity

Stressor climate change: Monitoring the occurrence of (emerging) secondary metabolites in food crops (Case)



- leads to **accurate forecasting**





Mycotoxin field trial uncovers first aflatoxin B1 occurrence in maize in Lower Austria

Patrick Rennhofer^{a,*}, Michael Sulyok^{a,*}, Dedy Leonardo Nadeak^{a,*}, Hermann Buerstmayr^b, Rudolf Krska^{a,c}, Stephan Freitag^{a,c}

^a BOKU University, Institute of Food Safety and Food Quality, Department of Agricultural Sciences, Kieselgasse 12, 231, Tulln, 3400, Austria

^b BOKU University, Institute of Crop Breeding and Genetics, Department of Agricultural Sciences, Kieselgasse 12, 231, Tulln, 3400, Austria

^c Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, University Road, Belfast, Northern Ireland, BT7 1NN, United Kingdom





Forscher an der Universität für Bodenkultur in Tulln entdeckten einen Schimmelpilz, der zu



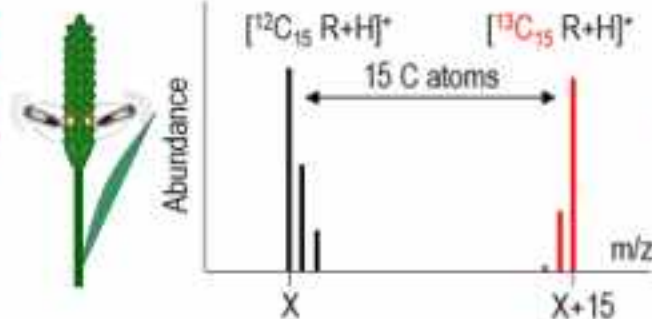
Für Aufregung sorgte die Entdeckung eines Forscherteams der Universität für Bodenkultur, das im Mais auf einem Versuchsfeld in Tulln das Gift Aflatoxin nachwies. Von der Landwirtschaftskammer heißt es, es werde seit Jahren ständig kontrolliert.



How to identify emerging hazards against the backdrop of climate change and plant breeding?

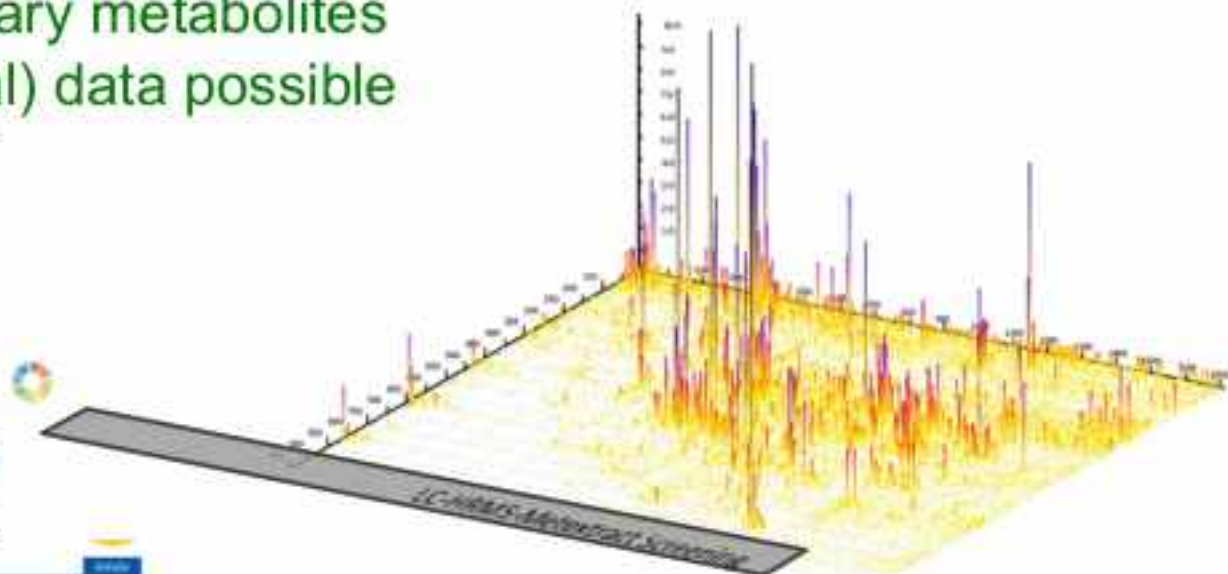
LC-High Resolution Mass Spectrometry

- full scan of all/new secondary metabolites
- reinterrogation of (historical) data possible



analytical
chemistry

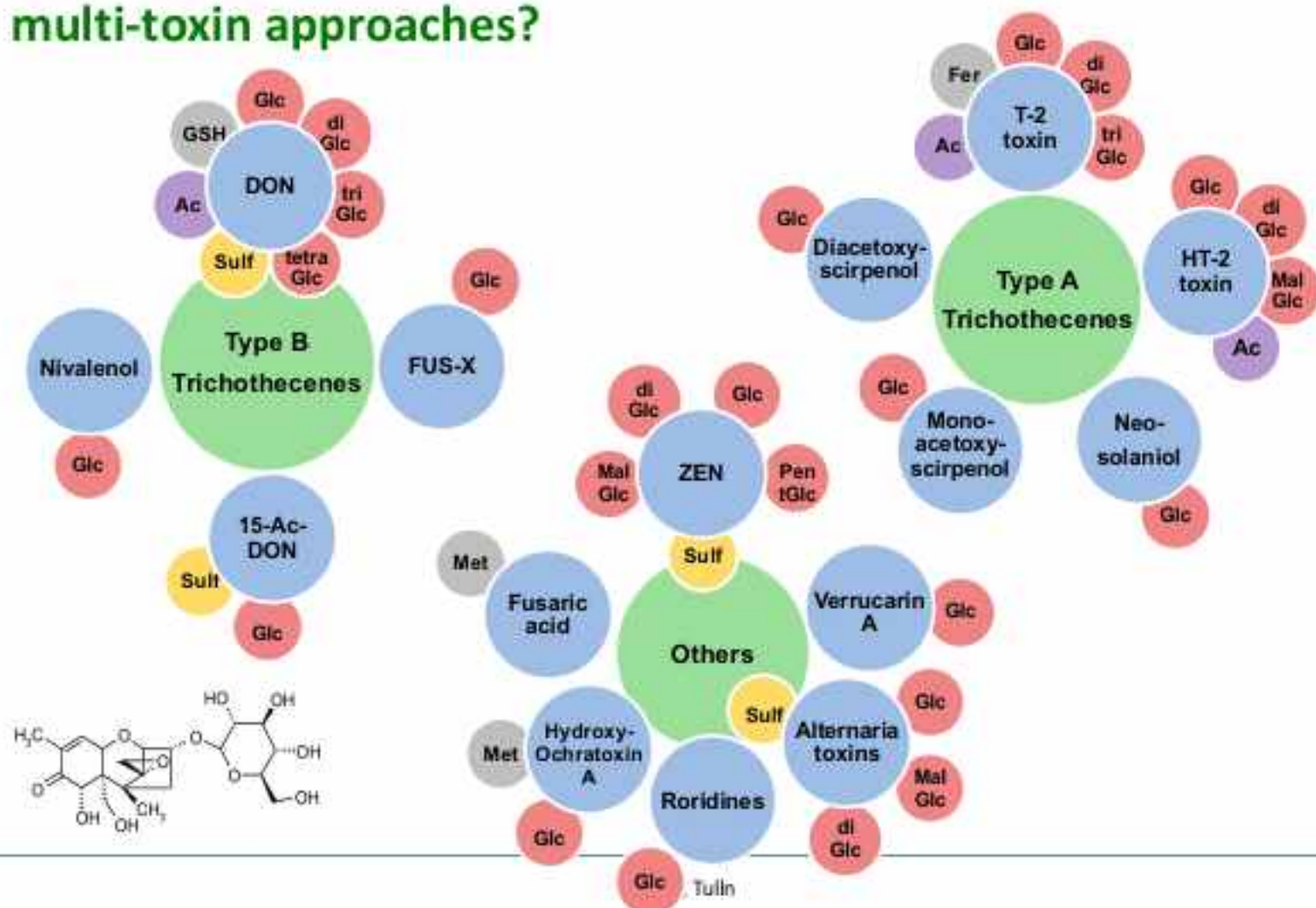
DOI: 10.1021/acs.analchem.7b02518
Anal. Chem. 2017, 89, 9518–9526



MetExtract II: A Software Suite for Stable Isotope-Assisted Untargeted Metabolomics

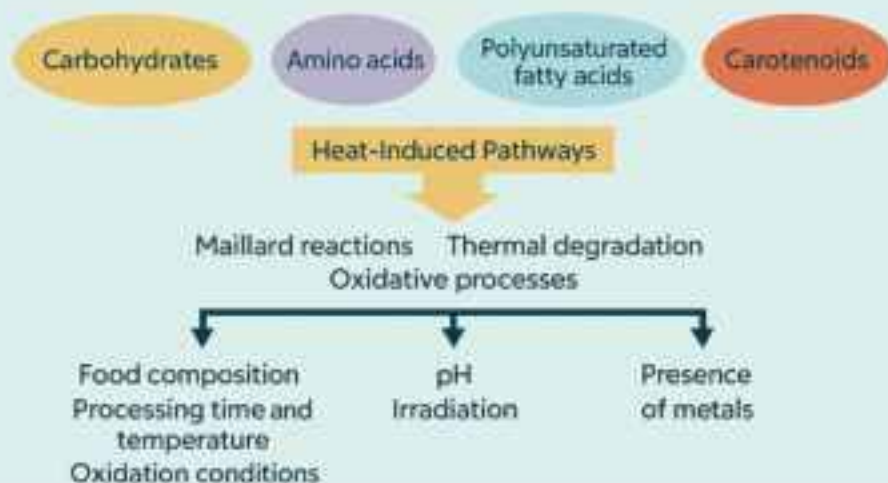
Christoph Bueschl,¹ Bernhard Kluge,¹ Nora K. N. Neumann,¹ Maria Doppler,¹ Valentina Maschietto,² Gerhard G. Thallinger,^{1,2} Jacqueline Meng-Retzer,^{1,2} Rudolf Krka,¹ and Rainer Schubert^{1,2}

What can only be seen by LC-MS based multi-toxin approaches?



Furan in Foods

Furan is widely present in foods because it can be formed from multiple precursors through different heat-induced pathways.



Furan



2-Methylfuran



3-Methylfuran



2,5-Dimethylfuran



2-Z-Butenedial

IARC classified furan as **"possibly carcinogenic to humans"** (Group 2B)

Pilot plant / industrial scale DoE on different food sauces production



Bolognese Sauce
Market Samples

	furan	furans sum
average:	52	97
max:	71	131

State-of-the-art:

HS-SPME-GC-MS/MS method with stable isotope dilution analysis



Plant-based products

Cereal harvesting



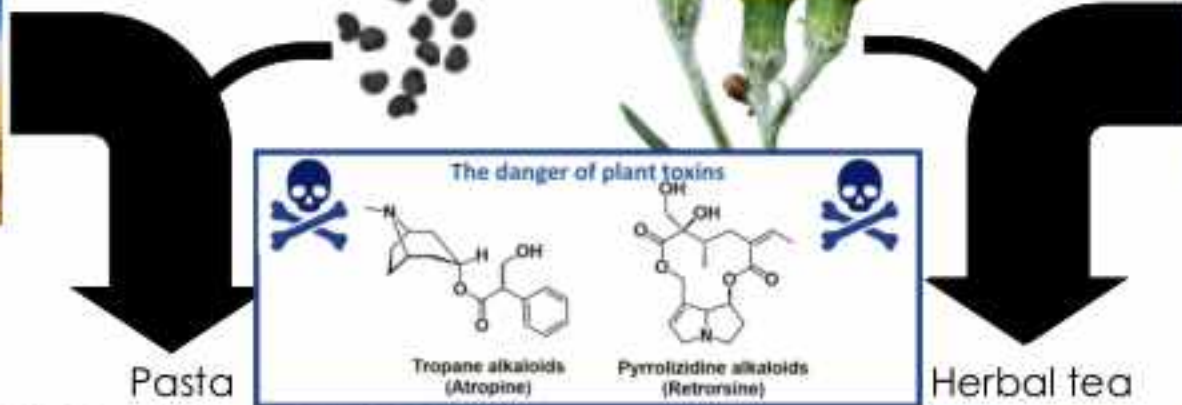
Datura stramonium



Senecio angustifolius



Rooibos harvesting



Pasta



Herbal tea

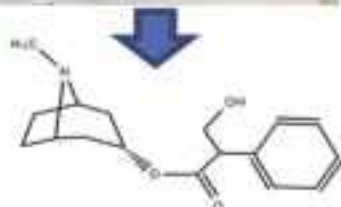


Stressor Climate Change & plant toxins: 200 tropane alkaloids: decreased heartrate, hallucinations,...



Datura in soybean and maize

Especially a problem in organic farming



- most frequent toxin: **atropine** (EU limit for grain-based baby food) & **scopolamine**
- **Recall of Tortilla chips**





Rapid detection of tropane alkaloids (TAs)

- Sample preparation using paper immobilized liquid-phase micro-extraction
- Pre-validated for buckwheat cereal samples at 5 to 10 $\mu\text{g}/\text{kg}$ contamination levels of atropine

analytical
chemistry

pubs.acs.org/ao

Modular Point-of-Need Tropane Alkaloid Detection at Regulatory Levels: Combining Solid-Liquid Extraction from Buckwheat with a Paper-Immobilized Liquid-Phase Microextraction and Immuno-Detection in Interconnectable 3D-Printed Devices

Ids B. Lemmink, Linda Willemssen, Erik Beij, Toine F. H. Bovee, Han Zuilhof, and Gert IJ. Salentijn*



Cite This: Anal. Chem. 2025, 96, 16452–16460

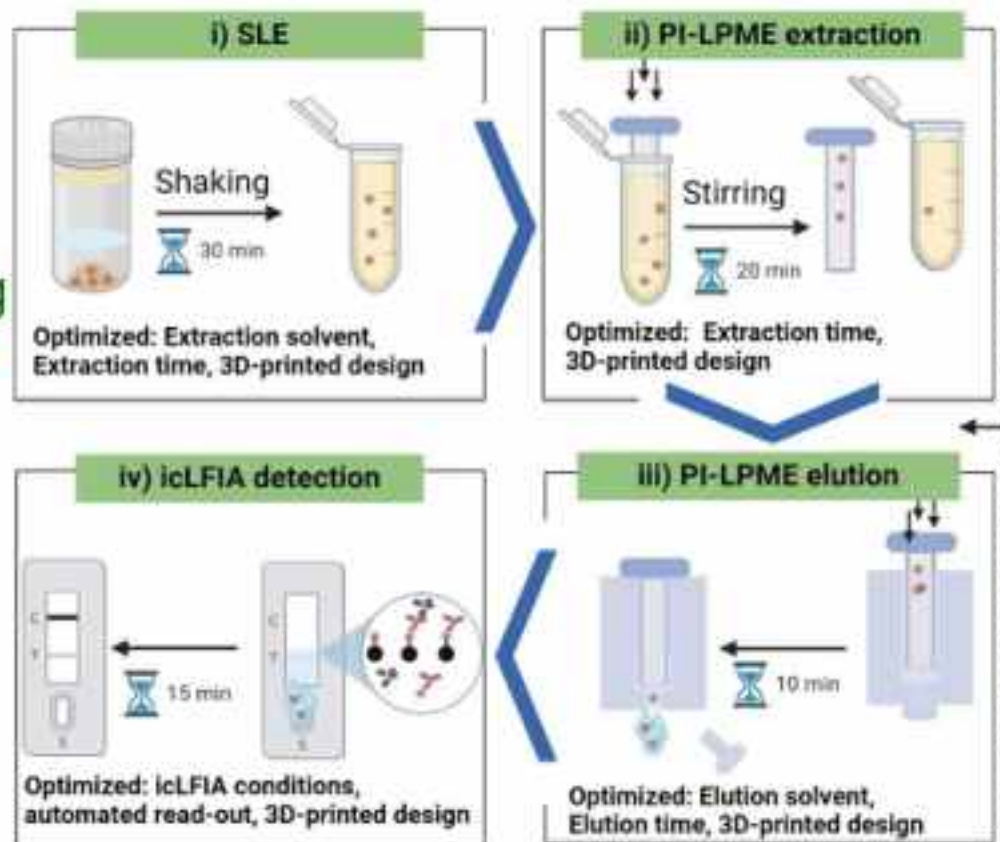


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Article

A) Workflow: development & optimization





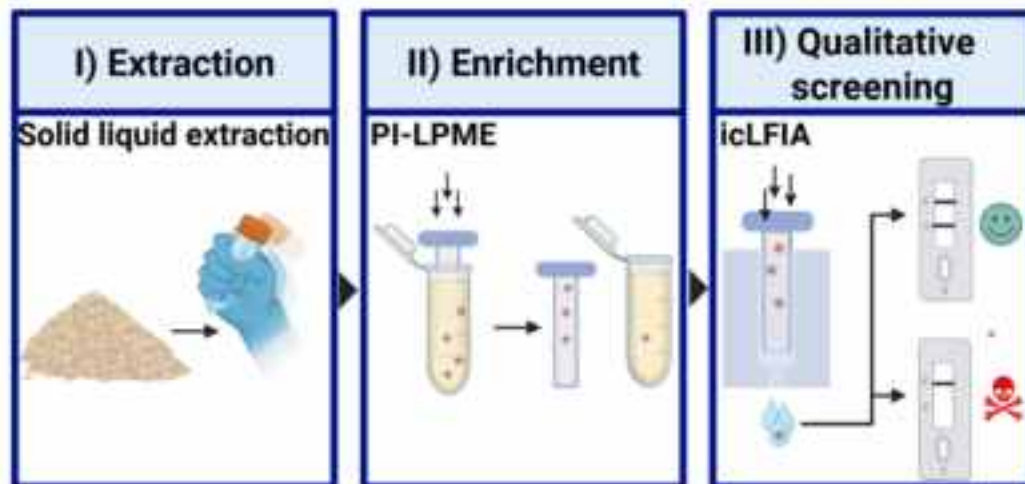
Validation of the novel test kit for tropane alkaloids

Development & validation of a rapid test for tropane alkaloids



Lemmink et al, Point-of-Need Tropane Alkaloid Detection. Analytical Chemistry
Lemmink et al, Paper-immobilized liquid phase microextraction. Analytica Chimica

Workflow of the 3D-printed biosensing toolkit



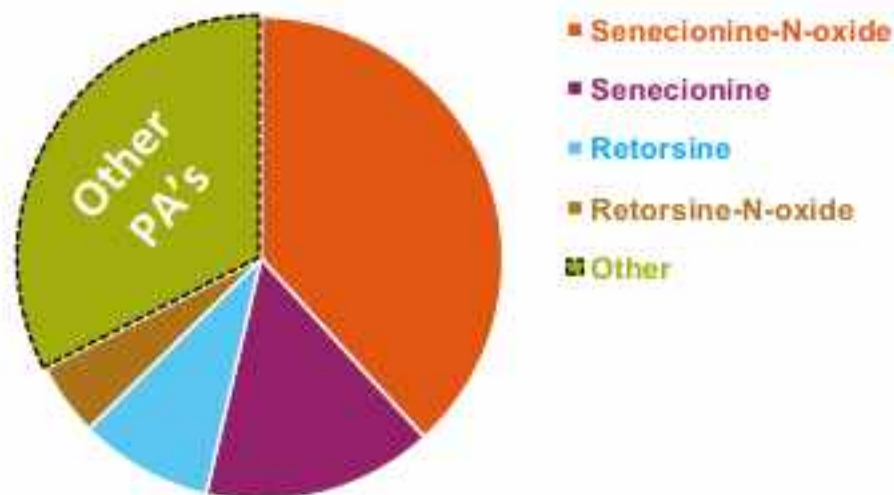


Pyrrolizidine alkaloids in Rooibos

Co-harvesting of *Senecio Angustifolius*



Distribution of pyrrolizidine alkaloids (PAs) in Rooibos
Four substances account for 70% of PAs in Rooibos

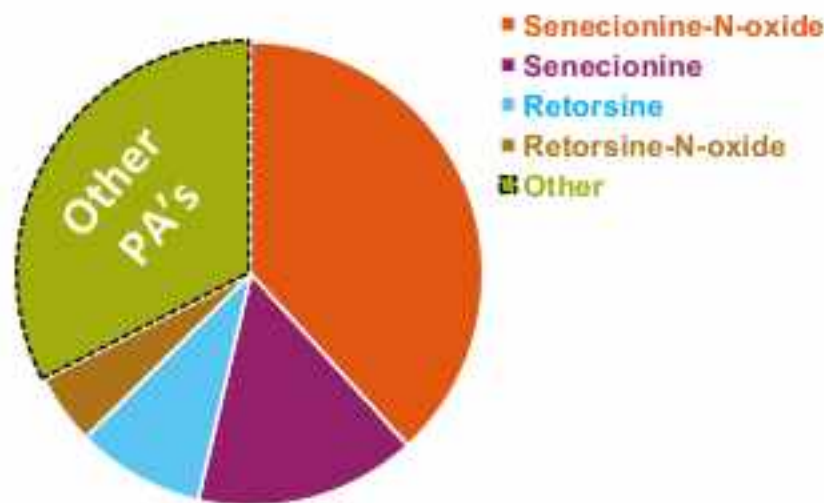




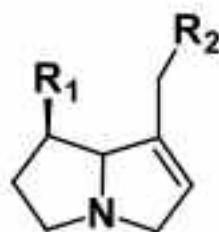
Towards rapid **pyrrolizidine alkaloid** detection

Development and validation of a rapid test for pyrrolizidine alkaloids

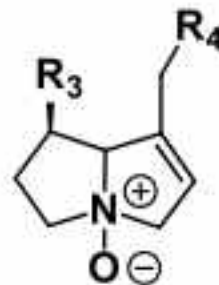
Distribution of pyrrolizidine alkaloids in rooibos



Pyrrolizidine alkaloids



Pyrrolizidine alkaloid



**Pyrrolizidine alkaloid
N-oxide**

Legislated as the sum of 35 pyrrolizidine alkaloid-
(N-oxides) at 400 $\mu\text{g kg}^{-1}$ in rooibos



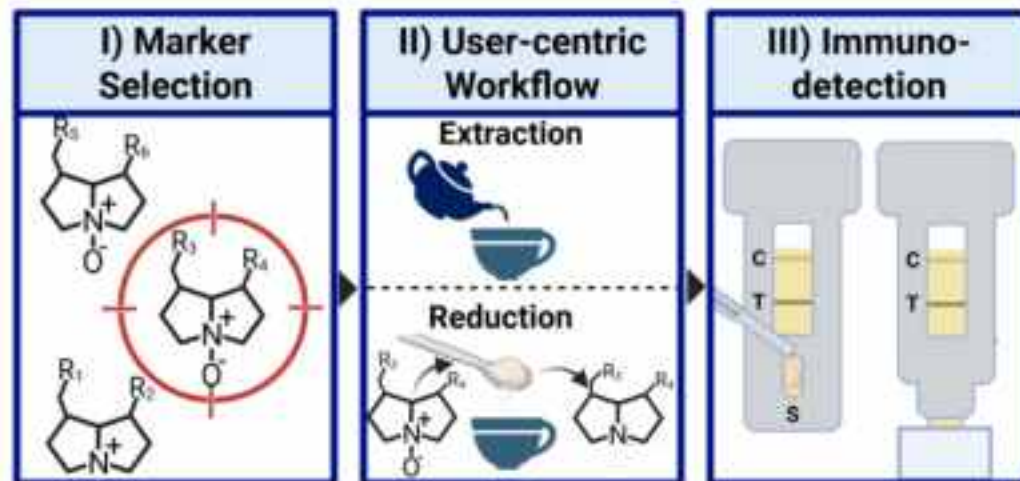
Towards rapid pyrrolizidine alkaloid detection

Development and validation of a rapid test for pyrrolizidine alkaloids



Lemmink et al. Point-of-need immunoassay for pyrrolizidine alkaloid. *In preparation*

Workflow of the 3D-printed biosensing toolkit



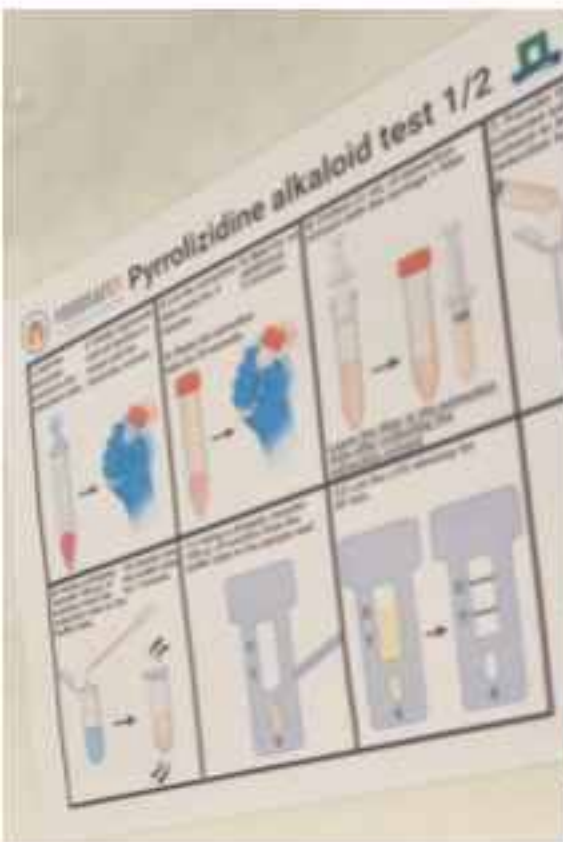


Validation exercise

International user-validation: production

Test the pyrrolizidine alkaloid test kit in
≥6 EU-member states



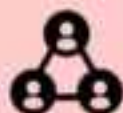


FFoQSI and BOKU/IFA-Tulln – head office members **validating pyrolizidine test kits** in the chemical lab (Case 4)

Drivers of (emerging) food safety hazards/risks



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Consumer behaviour:

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Economic Driver



Distribution:

- Food fraud, Authenticity
- Proper Storage



Environmental Drivers

Environmental contamination:

- Pesticides, heavy metals
- Bioaccumulation of toxins



Political Drivers

Legislation, policies and governance:

- Food safety monitoring (RASFF)





Gluten-free pasta: thermal degradation of mycotoxins

Attractive Opportunities in the Gluten-Free Products Market

5.6

USD BILLION
2020-4

8.3

USD BILLION
2025-9

CAGR
8.1%

The gluten-free products market is at 5.6 billion in 2020 and is projected to reach 8.3 billion through 2025.



Growing categories of gluten-free products and increasing inclination of consumers toward gluten-free products are expected to drive the market over the next five years.

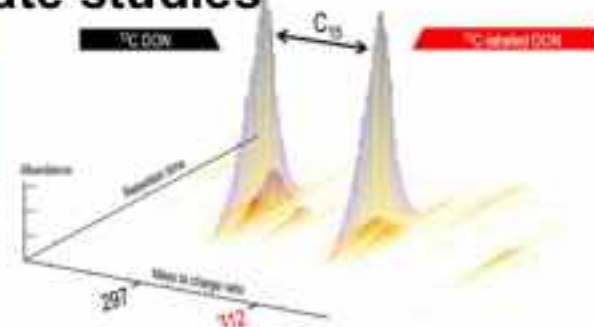
The North American region is projected to dominate the global gluten-free products market due to factors such as increasing awareness about celiac disease among consumers and growing inclination of consumers toward nutritional free-from products.

The market in Europe is the fastest-growing due to changing lifestyles of consumers.



Aflatoxins, Fumonisin & T2/HT2 Toxins are ??? degraded during commercial pasta making in an industrial environment

- in gluten free (=maize) pasta
- Using LC-HRMS based C12/C13 tracer fate studies



Thermal degradation of mycotoxins during thermal processing under semi-industrial conditions

Source:

Market Interviews, Government Publications, and



Conclusions and future needs

- For each of the **5 major drivers of (emerging) food safety hazards** **analytical chemistry** is crucial to manage the challenges:
 - **LC-MS/MS** for the **ultra-fast quantification** of **multiple classes** of chemical substances. Provides **selectivity**, **sensitivity** and **speed** even in complex food and feed
 - **SIL assisted LC-HRMS** approaches reveal **novel conjugated metabolites**, also ideal for **long term monitoring** against the backdrop of climate change
 - **Stable isotope assisted LC-MS based tracer fate studies** provide novel insights into the **fate of mycotoxins during thermal processing**
- **Indirect competitive lateral flow immunoassay for atropine** has been developed
- Novel approach for the **determination of pyrrolizidine alkaloids** in **Rooibos** based on a **3D-printed biosensing toolkit** introduced
- **Portable spectroscopy** devices e.g. XRF (combined with ML) as a new tool to **determine illegal lead chromate in turmeric** developed and tested





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tailored to your role in the
food safety ecosystem.

my.foodsafer.com/site/training



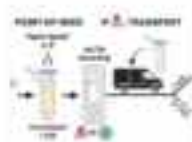
Targeted Stakeholder Group



Topic



Content Format



Paper-immobilized liquid-phase microextraction for direct paper...

Atropine is a strictly regulated natural toxin. Monitoring for atropine is thus important, but often expensive and time-consuming. Moreover, the range of relevant matrices, and corresponding differences in required detection limits for atropine vary. Therefore, we developed a more simplified and affordable method, combining immunodetection and...



Article

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A FoodSafer perspective on emerging food safety hazards and...

Scientific Publication. Both new and old microbial and chemical hazards were identified for inclusion in a central database. The database could be used to develop artificial intelligence (AI) models to explain existing and predict emerging food safety risks. The goal of this paper is to alert the global food safety community of the availability of this...



Article

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Review report on available models for fungal infection and...

An overview of the currently available prediction models for fungal infection and mycotoxin production in grains and tomatoes.



Report

DOWNLOAD



Predictive modelling for early warning mycotoxins

Introduction to predictive modelling for early warning mycotoxins



Infographic

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FOODSAFER IS MAKING FOOD SAFETY EVERYBODY'S BUSINESS

Curated by the scientific community, FoodSafer aims to be a trusted source of information, guidelines and advice in relation to emerging hazards that are posing a risk for food safety.

<https://foodsaferr.com>



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On behalf of the general conference chairs, Prof. Rudi Krska and Prof. Chris Elliott, **WMF2027** warmly invites researchers from universities, research institutes, and industry to submit abstracts for ORAL and/or POSTER presentations.