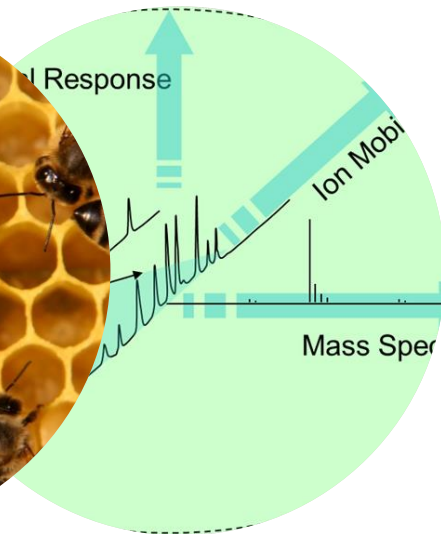


NAVIGATING THE COMPLEX LANDSCAPE OF PLANT TOXINS: REGULATORY FRAMEWORKS AND SCIENTIFIC ADVANCES

Josipa Grzetic Martens



Natural toxins as food contaminants @WFSR

- Mycotoxins
- **Plant toxins**
- Marine Biotoxins

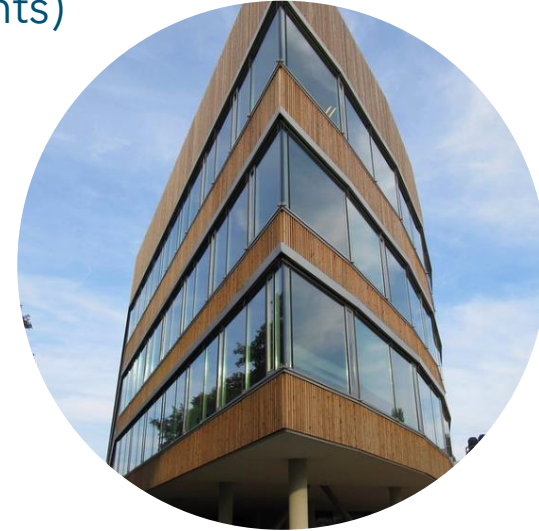


At WFSR

- Research
- Protocol development
- Sample analysis (surveys, enforcement, incidents)
- NRL
- EURL - as of 2018



Netherlands Food and Consumer
Product Safety Authority
*Ministry of Agriculture,
Nature and Food Quality*



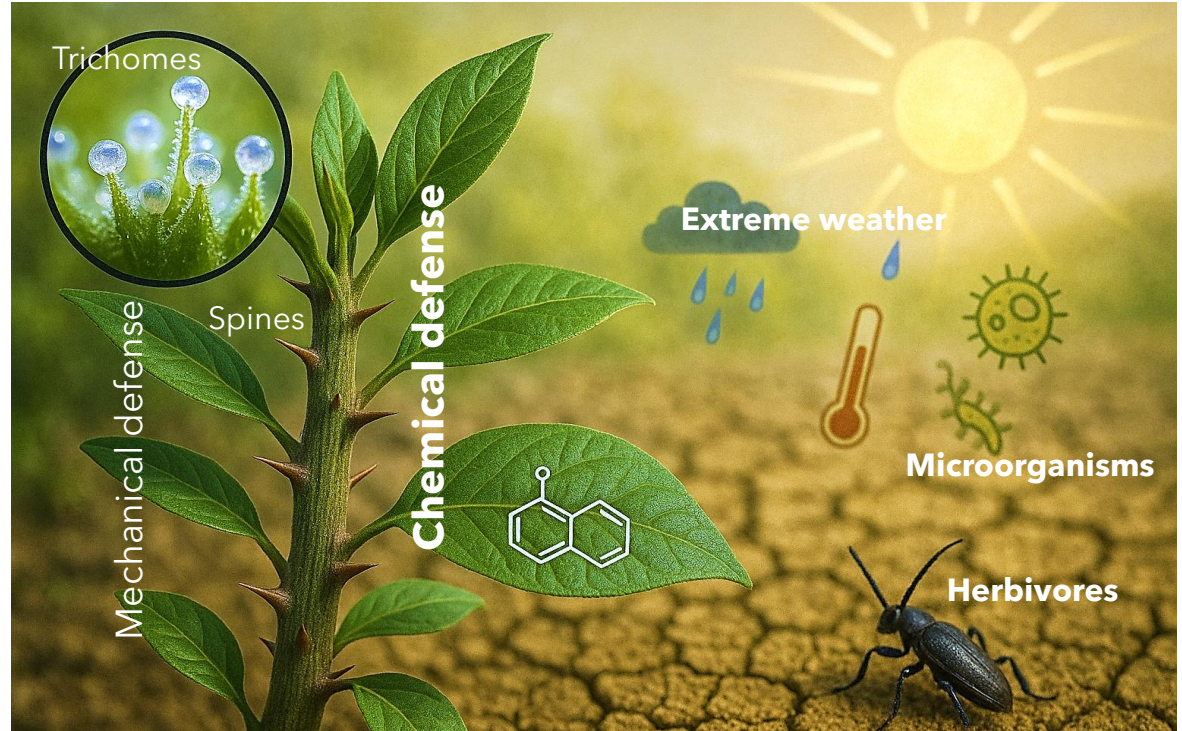
Outline

- What are plant toxins and how do they end up in our plate
- Regulatory frameworks
- Analytical methods and related advances



Plant toxins: biological roles

- Defense against **herbivores**
- Allelopathy: inhibit **competing plants**
- Protection from **microbial infections**
- Regulation of plant growth & development



Plant toxins in food (and feed) products

Inherent



Contamination



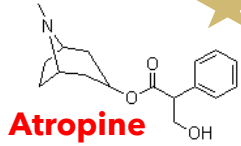
Transfer



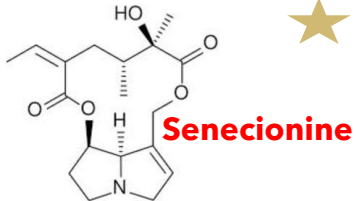
Classes of plant toxins....

★ Free gossypol (phenolic aldehyde)
★ Hydroxyanthracene derivatives

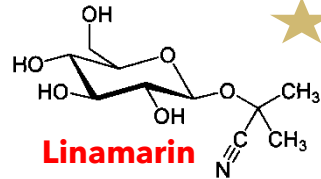
Tropane alkaloids



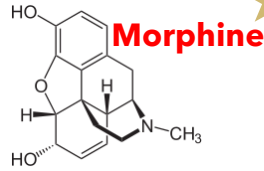
Pyrrolizidine alkaloids



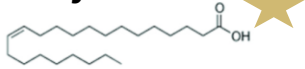
Cyanogen glycosides (HCN)



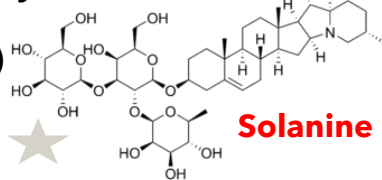
Opium alkaloids



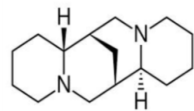
Fatty acids



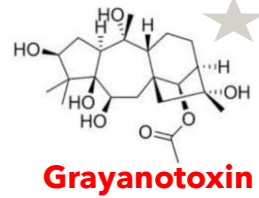
Glycoalkaloids



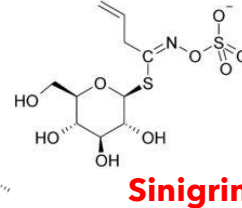
Quinolizidine alkaloids



Diterpenes



Glucosinolates

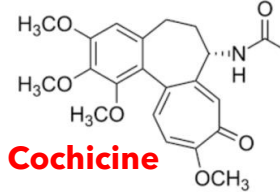


Lectins

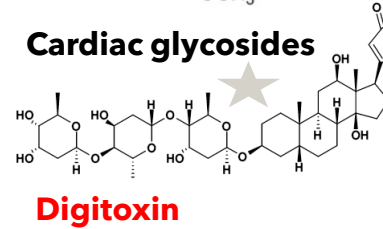


Alkenyl benzenes
Isoflavones

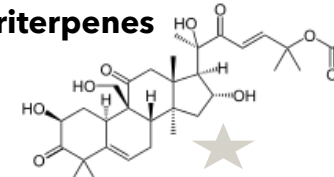
Cochicine alkaloids



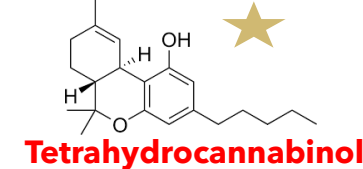
Cardiac glycosides



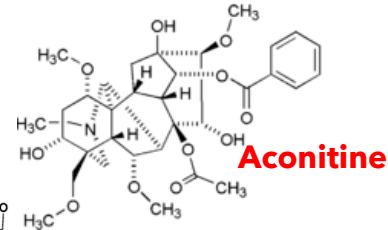
Triterpenes



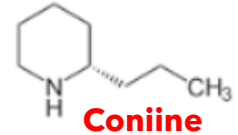
Cannabinoids



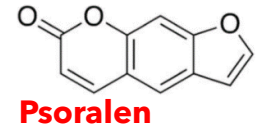
Aconitine alkaloids



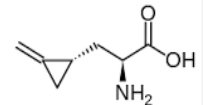
Piperidine alkaloids



Furanocoumarins

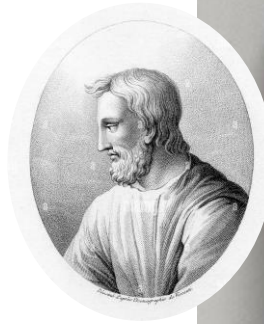


Amino acids



Botanical preparations - *De materia medica*

- Between 50 and 70 AD, Greek physician Pedanius Dioscorides, wrote a five-volume book, ***De materia medica***.



Excerpts on the opium poppy

- "...they cause sleepand if boiled with honey to make a **cough mixture**.
- A little of it is a **pain-easer, a sleep-causer,** **Taken as a drink too often it hurts (making men lethargic) and it kills.**

Codeine

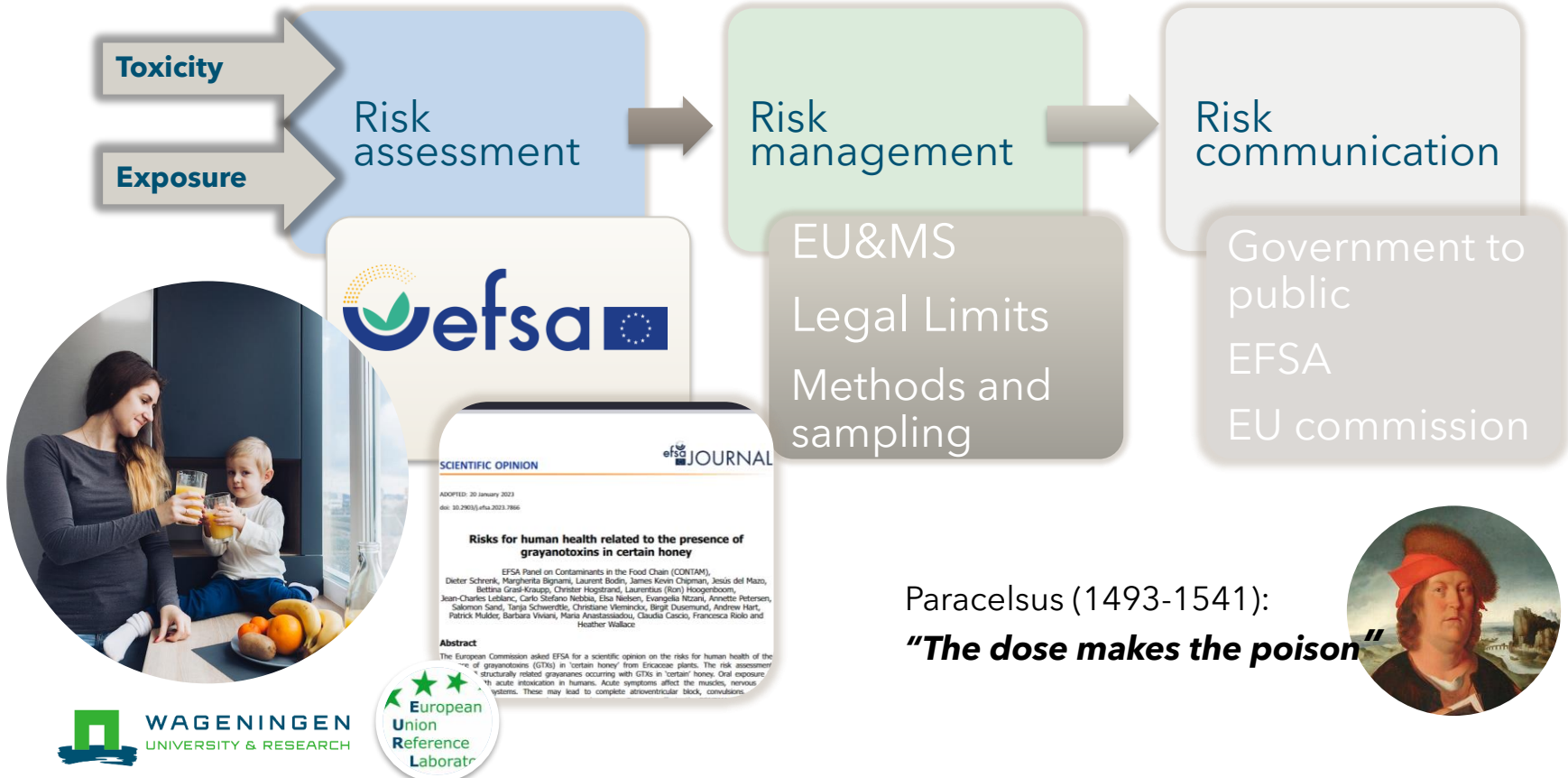
Morphine

The book became the principal reference work on pharmacology across Europe and the Middle East for over 1500 years, and was thus the precursor of all modern pharmacopoeias.



De materia medica
Turkey, Istanbul, mid 10th century, MS M.652 fol. 101v

Basis for regulation on contaminants in food and feed



Inherent plant toxins evaluated by EFSA

Compound/group	Relevant food/feed	Food	Feed	EFSA opinion
Alkenylbenzenes	Herbs, dietary supplements	Yes	No	2009:965
Cannabinoids	Food of animal origin	Yes	No	2015:4141
Cucurbitacins	Zucchini, squash, pumpkin	Yes	No	-
Cyanogenic glucosides (Cyanide)	Cassava, bitter almond, apricot kernels, linseed	Yes	Yes	2007:434; 2016:4424; 2019:5662
Erucic acid	Rapeseeds, mustard oils	Yes	Yes	2016:4593
Glucosinolates	Rapeseeds, mustard oils	No	Yes	2008:590
Glycoalkaloids	Potatoes	Yes	Yes	2020:6222
Glycoproteins	Beans, pulses	No	Yes	2008:726
Gossypol	Cotton seeds	No	Yes	2009:908
Hydroxyanthracenes	Aloe, Senna supplements	Yes	No	2018:5090
Opium alkaloids	Poppy seeds	Yes	No	2011:2405; 2018:5243
Phorbol esters	Jathropha seeds	No	Yes	2015:4321
Quinolizidine alkaloids	Lupine seeds	Yes	Yes	2019:5860
Theobromine	Cacao	No	Yes	2008:725

Plant toxins from co-harvesting/transfer evaluated by EFSA

Compound/group	Relevant food/feed	Food	Feed	EFSA opinion
Grayanotoxines	Honey	Yes	No	2023:7866
Pyrrolizidine alkaloids	(Herbal) tea, honey, milk, herbal supplements, forage, honey	Yes	Yes	2007:447; 2011:2406; 2016:4572; 2017:4908
Tropane alkaloids	(Herbal) tea, herbal supplements, cereals, grains	Yes	Yes	2008:691; 2013:3386; 2018:5160

However...



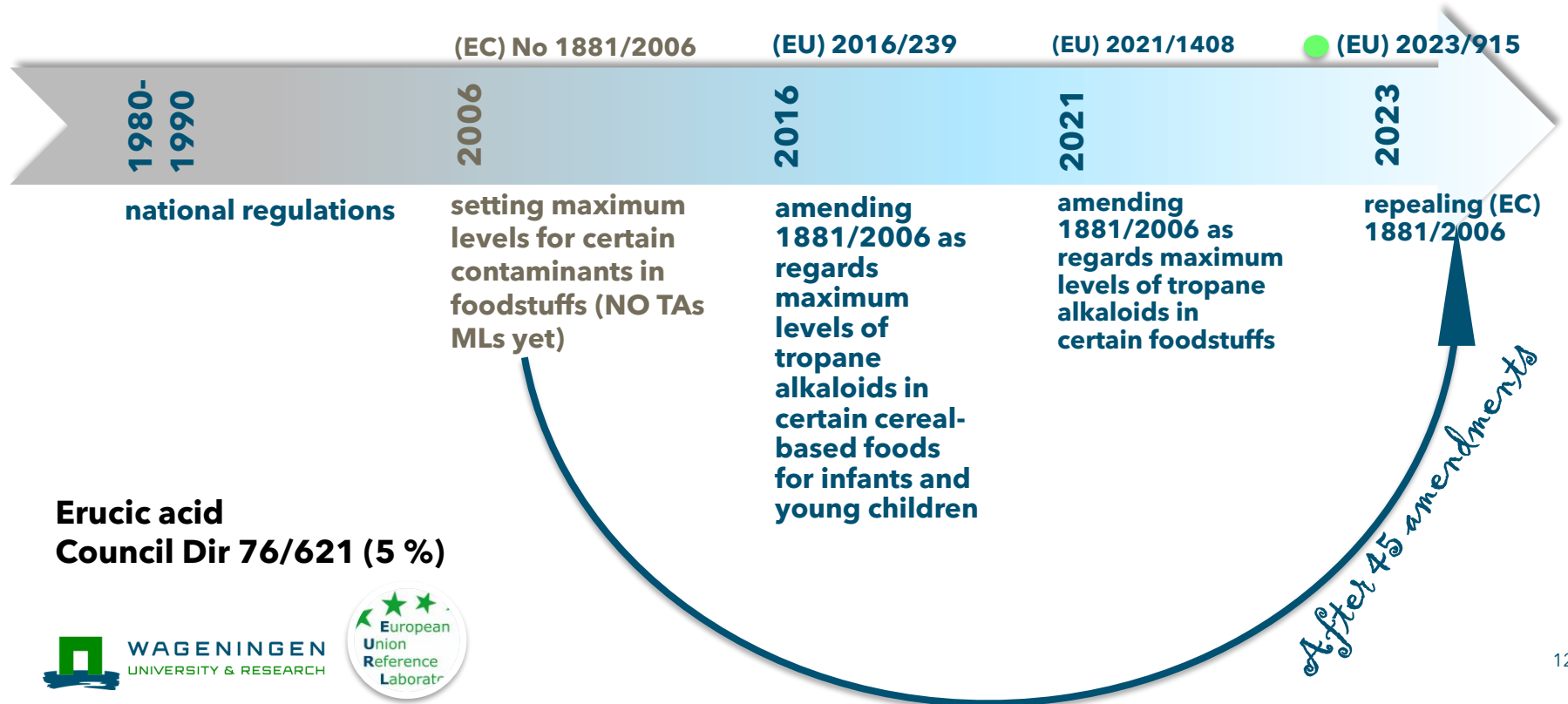
... often lack of toxicological data as compared to for example pesticides or veterinary medicines ...

Natural toxins often been regulated because of (incidence of) food-safety outbreaks (e.g., tropane contamination of grains, or bakery products containing poppy seeds).

Pesticides and medicines have been regulated from their inception as deliberate commercial products. Before market approval, they typically undergo GLP-compliant toxicology studies (acute, subchronic, chronic, reproductive/developmental, genotoxicity, etc.) in multiple species, plus pharmacokinetic (ADME)

EU 2023/915 Legal limits in food

MLs tropane alkaloids (TAs) evolution



Current legal limits in food Regulation EU 2023/915

Toxin Category Foods

(EU) 2022/1364 **HCN / Cyanogenic Glycosides** linseed, almonds, apricot kernels, cassava

(EU) 2020/2040 **Pyrrolizidine Alkaloids (PAs)** borage leaves, dried herbs, herbal teas/infusions

(EU) 2022/1393 **THC and THCA (Cannabinoids)** hemp seeds, hemp seed oil, hemp seed flour, infusions

Tropane Alkaloids cereals, infant cereal-based foods, dried pulses, snacks, herbal teas

(EU)2021/2142 **Opium Alkaloids** poppy seeds, bakery products with poppy seeds

Erucic Acid edible oils, mustard seeds

OUTLOOK

- **COMMISSION RECOMMENDATION (EU) 2022/561 of 6 April 2022 on monitoring the presence of glycoalkaloids in potatoes and potato-derived products**
- **Quinolizidine alkaloids**
- Grayanotoxins
- **Lectins**

<http://data.europa.eu/eli/reg/2023/915/oj>

5.5.2023

EN

Official Journal of the European Union

L 119/103

COMMISSION REGULATION (EU) 2023/915

of 25 April 2023

on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006

(Text with EEA relevance)

Current legal limits in feed Directive 2002/32

Toxin/Plant parts	Feed Categories
Free gossypol	cotton seeds, complete feed
Hydrocyanic acid (HCN)	feedingstuff
Theobromine	
Volatile mustard oil (glucosinolates)	
Weed and plant seeds	
Some weed and plant parts	

OUTLOOK

- **Tropane alkaloids**
- **Cannabinoids (MLs under discussion)**
- **Pyrrolizidine alkaloids (Recommendation for monitoring under discussion)**
- Quinolizidine alkaloids

<http://data.europa.eu/eli/dir/2002/32/oj>

L 140/10 EN Official Journal of the European Communities 30.5.2002

DIRECTIVE 2002/32/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 7 May 2002
on undesirable substances in animal feed

Regulation (EU) 2023/2783

- **Scope:** Establishes harmonised **methods of sampling and analysis** for the official control of **plant toxin** levels in food.
- **Repeals:** Regulation (EU) 2015/705 (erucic acid in foodstuffs).
- **Key changes:**
 - Adopts the sampling framework **from Reg 2023/2782 (mycotoxins)** to account for heterogenous distribution of plant toxins.
 - Sets general **performance criteria** for analytical methods (recovery, precision, LOQ).
 - Providing criteria for selecting the appropriate **sampling procedure**.
- **Timeline:** Enters into force 20 days after publication (15 Dec 2023), applies from **1 April 2024**, with existing validated methods permitted until **1 July 2028**

http://data.europa.eu/eli/reg_impl/2023/2783/oj

Regulation (EU) 2023/2783 – performance criteria

Recovery: the average recovery should be between 70 and 120 %.

The average recovery is the average value from replicates obtained during validation when determining the precision parameters RSD_r and RSD_{wR} . The criterion applies to all concentrations and all individual toxins, with the exception of ergot alkaloids.

For ergot alkaloids the criterion applies to the sum of each epimer-pair.

In exceptional cases, average recoveries outside the above range can be acceptable but shall lie within 50-130 %, and only when the precision criteria for RSD_r and RSD_{wR} are met.

Precision

RSD_r shall be ≤ 20 %.

RSD_{wR} shall be ≤ 20 %.

RSD_R should be ≤ 25 %.

These criteria apply to all concentrations.

LOQ: shall be $\leq 0,5*ML$ and should preferably be lower ($\leq 0,2*ML$).

In case the maximum level applies to a sum of toxins, then the LOQ of the individual toxins shall be $\leq 0,5*ML/n$, with n being the number of toxins included in the ML definition.

Identification

For identification, the criteria as laid down in the Guidance document on identification of mycotoxins and plant toxins in food and feed (1) shall be applied.

Guidance document on identification of mycotoxins and plant toxins in food and feed

Implemented by 01/01/2023

https://food.ec.europa.eu/document/download/f16cac78-9318-4f1f-b2fa-efb25d2f1880_en

Regulation (EU) 2024/885 am. 2023/2782

Regulation (EU) 2024/885 laying down the methods of sampling and analysis for the control of the levels of mycotoxins in food as regards the method of sampling for **dried herbs, herbal infusions (dried product), teas (dried product) and powdered spices**

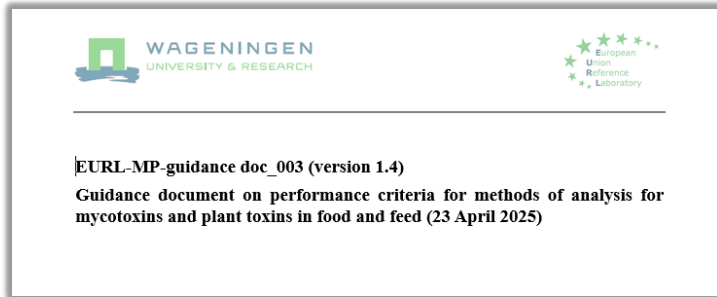
BfR and EURL MP - studies on homogeneity of the samples

- ➔ **Doubled the aggregate sample size (by increasing the increment sample size)**
- ➔ **That needs to be full homogenized prior the analysis**



Methods - food and feed (confirmatory)

- ➔ (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed
- ➔ CEN (feed - TC 327/WG 5 and food - TC 275/WG5)
- ➔ EURL MP - methods
- ➔ EURL MP - guidance document

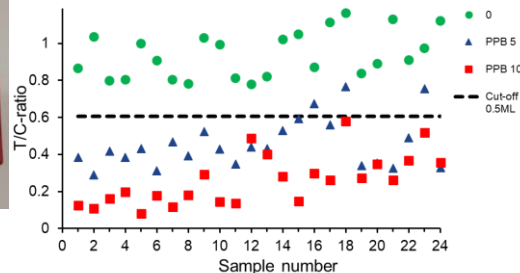
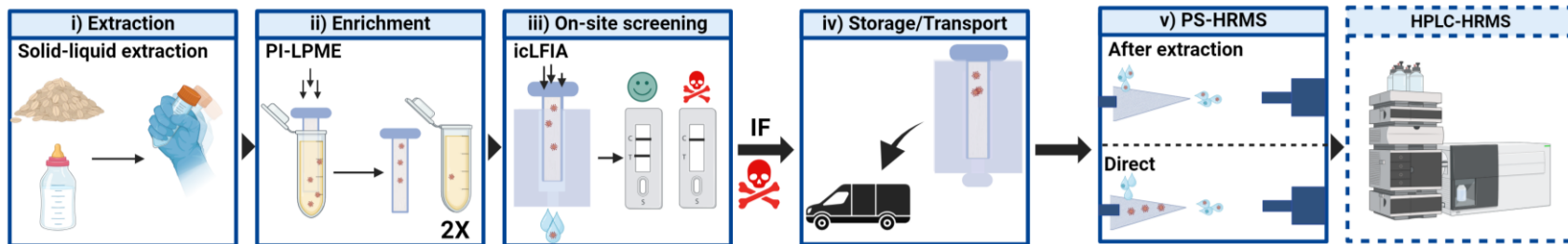


<https://www.wur.eu/EURLMP>

EURLMP Methods

- > EURLMP-method 001 DON and related compounds by LC-MS/MS v1
- > EURLMP-method 002 Pyrrolizidine alkaloids by LC-MS/MS v3
- > EURLMP-method 003 Ergot alkaloids by LC-MS/MS v2
- > EURLMP-method 004 Tropane alkaloids by LC-MS/MS v1
- > EURLMP-method 005 Erucic acid by GC-FID v1
- > EURLMP-method 006 Ergot sclerotia by visual screening v1
- > EURLMP-method 007 Opium alkaloids in food by LC-MS/MS v1
- > EURLMP-method 008 Citrinin in red yeast rice food supplements by LC-MS/MS v1
- > EURLMP-method 009 Cannabinoids in food by LC-MS/MS v2
- > EURLMP-method 010 Cyanogenic glucosides (CNGs) in food and feed by LC-MSMS v1
- > EURLMP-method 011 Hydrocyanic acid in feed by HPLC-FLD v1
- > EURLMP-method 012 Quinolizidine alkaloids in food and feed by LC-MS/MS v1
- > EURLMP-method 014 Glycoalkaloids in potato by LC-MS/MS v1
- > EURLMP-Method 016 Ochratoxin A in meat and cheese by LC-MSMS v1

Fast Tests for Plant Toxins – TAs in buckwheat



Ids Lemmink
PhD candidate



analytical
chemistry

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Article

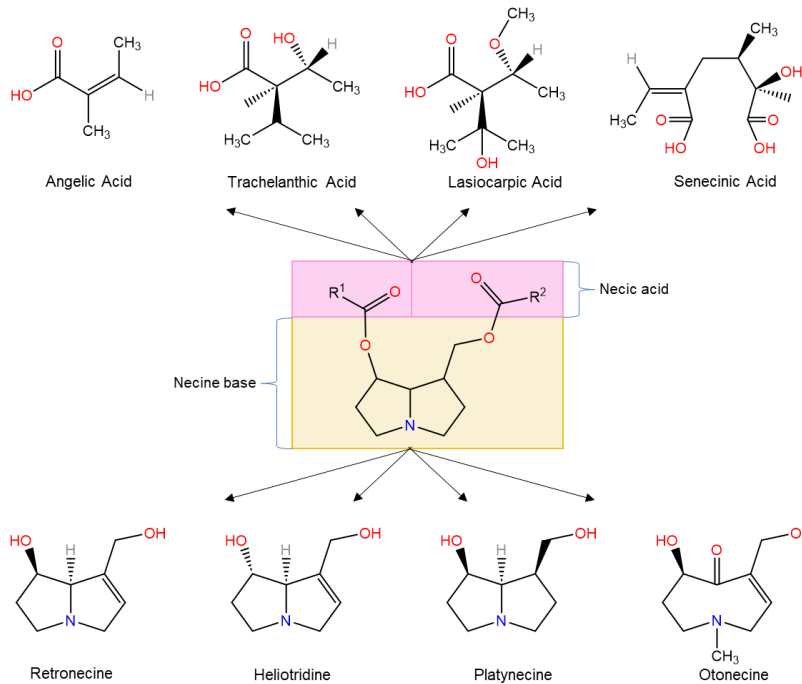
Modular Point-of-Need Tropane Alkaloid Detection at Regulatory Levels: Combining Solid–Liquid Extraction from Buckwheat with a Paper-Immobilized Liquid-Phase Microextraction and Immuno-Detection in Interconnectable 3D-Printed Devices

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

Going further: Pyrrolizidine Alkaloids HRMS DB



Leonie Straub
PhD candidate

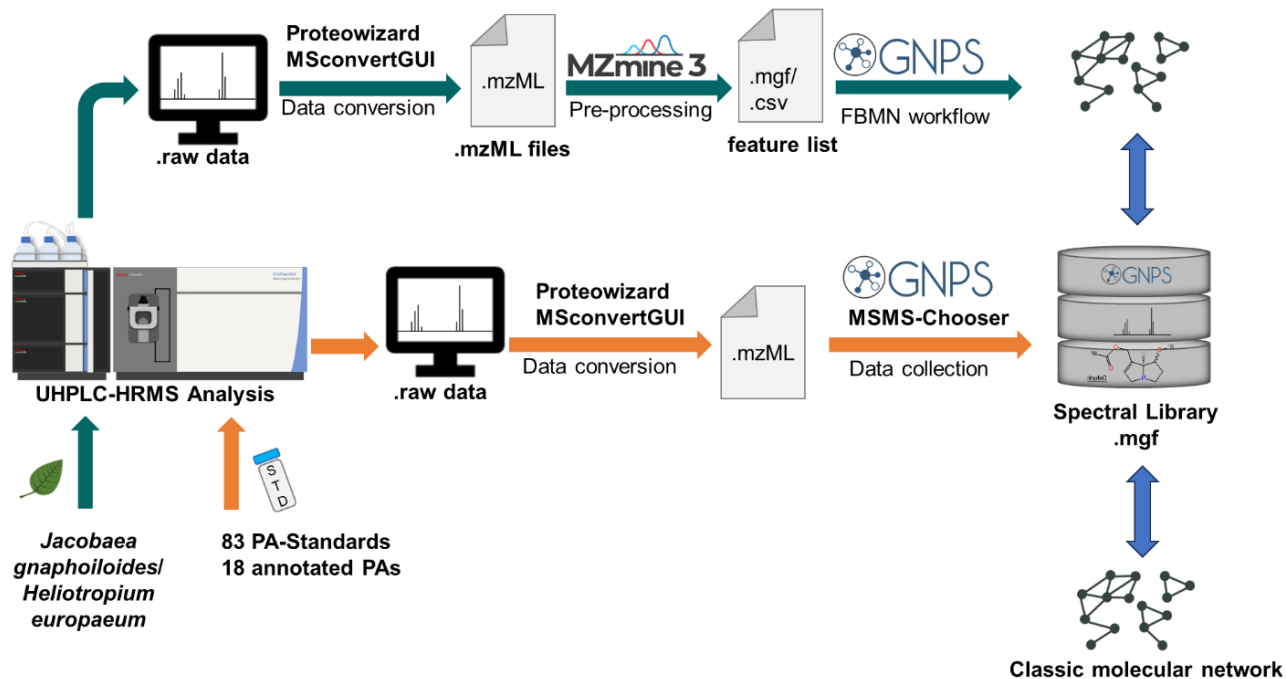


- hepatotoxic, mutagenic and hepatocarcinogenic
- 6000 species are producing PAs
- ~80 available standards
- **~1000s of different PAs in plants**

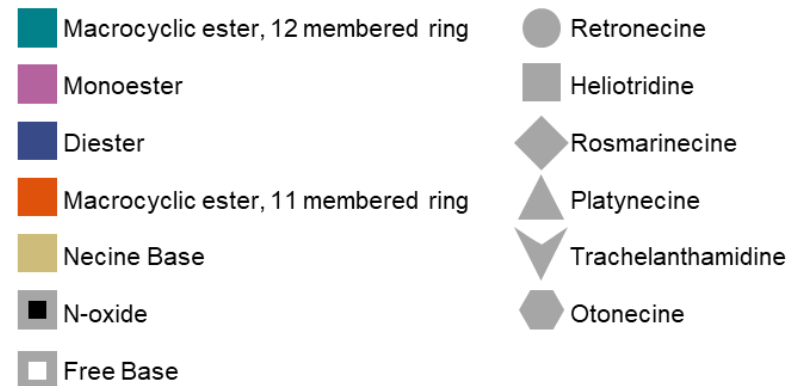
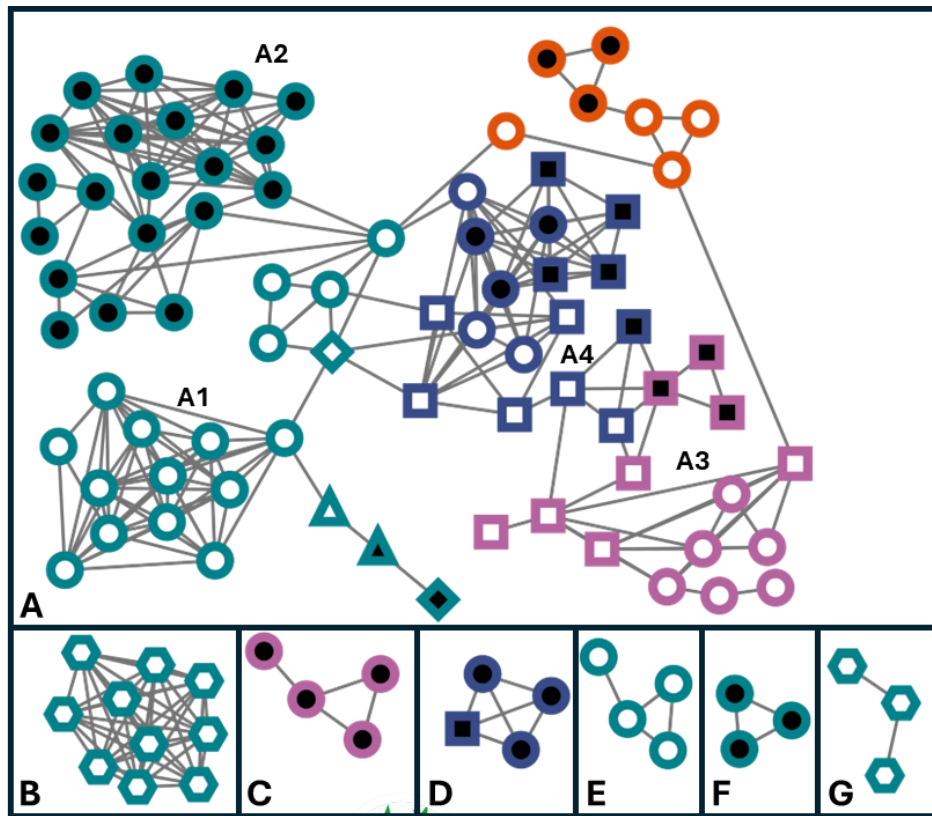
Going further: Pyrrolizidine Alkaloids HRMS DB



Leonie Straub
PhD candidate



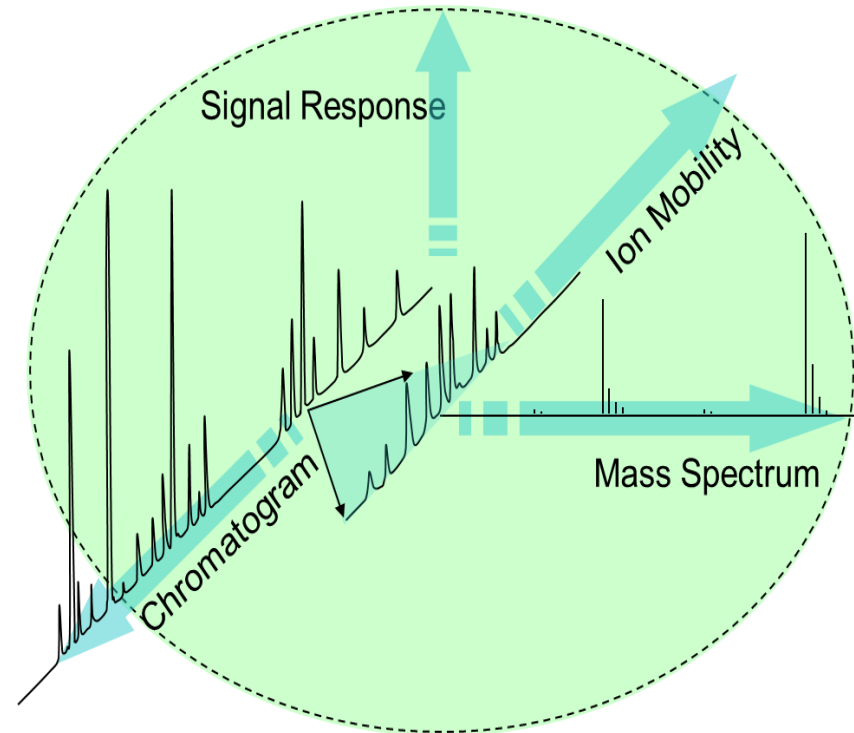
Going further: Pyrrolizidine Alkaloids HRMS DB



➔ **Unexpected known**
Expected unknown

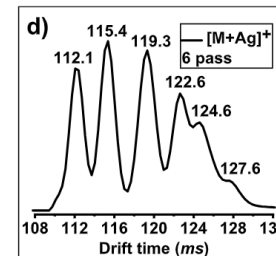
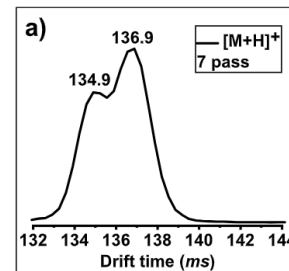
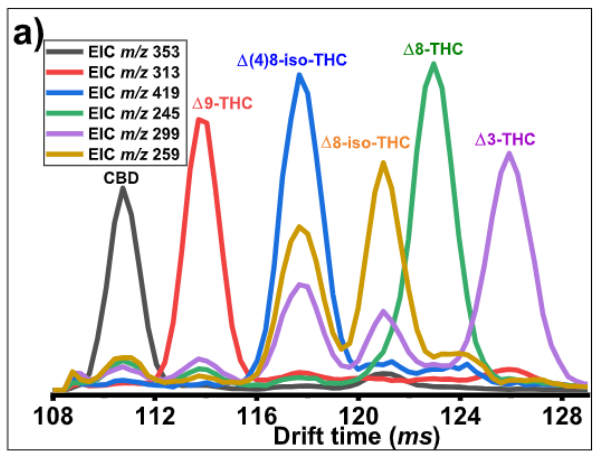
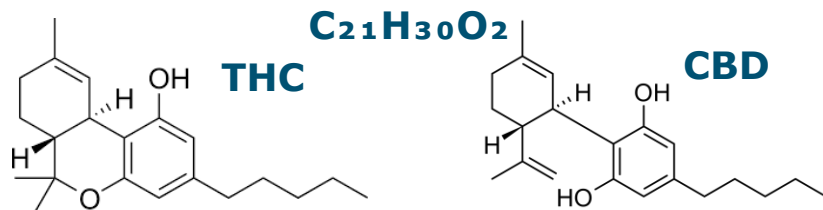
Going faster: Ion Mobility

- **Chromatography** offers separation by polarity and size
- **MS** offers separation in m/z space
- **(Cyclic) ion mobility spectrometry** offers separation by conformation (spatial shape)



Picture Source: Agilent Technologies

Going faster: Ion Mobility



Si Huang

analytical
chemistry

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Article

Ultrafast, Selective, and Highly Sensitive Nonchromatographic Analysis of Fourteen Cannabinoids in Cannabis Extracts, $\Delta 8$ -Tetrahydrocannabinol Synthetic Mixtures, and Edibles by Cyclic Ion Mobility Spectrometry–Mass Spectrometry

Si Huang, Laura Righetti,* Frank W. Claassen, Akash Krishna, Ming Ma, Teris A. van Beek, Bo Chen,* Han Zuilhof,* and Gert IJ. Salentijn*



Poster 78

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Exploring the potential of using ion mobility-mass spectrometry to separate matrix interferences from analytes in food control

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196, 10170–10181

Read Online

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cannabinoid isomers and complex-significant challenges for analytical developed a method to analyze 14 diverse samples within milli-

minuted cannabinoid isomers

Cannabis (derived)

- ✓ 14 cannabinoids
- ✓ Milliseconds separation
- ✓ Collision cross section
- ✓ Characteristic fragments

EURL MP team



Sylvia Kalli



Marta Sopel



Hans Mol



Ilaria Di Marco
Pisciottano



Ruud van Dam



Alwin Kruijt



Laura Righetti



Josipa Grzetic
Martens

Plant toxins

Sylvia Kalli
Ilaria Di Marco
Pisciottano

Mycotoxins

Ruud van Dam
Alwin Kruijt
Marta Sopel
Hans Mol

AQC

Hans Mol

Routine control

Ruud van Dam
Alwin Kruijt

Novel approaches and methodologies

Ilaria Di Marco Pisciottano
Laura Righetti

Proficiency tests

Diana Pereboom-de Fauw
Ingrid Elbers



Thank you for your attention!



josipa.grzeticmartens@wur.nl
eurl.mycotoxins-planttoxins@wur.nl
<https://www.wur.eu/EURLMP>

Typical characteristics inherent food toxins

- Long history of use and possible side effects (human case reports)
- Mode of action generally well known
 - CNS, anticholinergic, muscarinic receptors, inhibition of ATP
- Concentrations of concern typically > 1-100 mg/kg
 - GSLs, EA > CGs, GAs, QAs > OAs > THC, HADs
- Analysis seems relatively easy
 - Relevant substances are known
 - Concentrations are high
 - Wide variety of analytical techniques used
 - including fast screening/effect assays



Plant toxins: modes of action

- Disruption of ion channels & neurotransmission (e.g., tropane alkaloids, cannabinoids, grayanotoxins)
- Enzyme inhibition (cardiac glycosides inhibit Na^+/K^+ -ATPase)
- Membrane disruption (saponins)
- Photosynthesis interference (juglone)
- Nucleic acid synthesis disruption (tannins)
- Protein synthesis inhibition (ricin)
- Acetylcholinesterase inhibition (pyrrolizidine alkaloids)
- Inhibition of oxidative phosphorylation (cyanogenic glycosides)
- Often multimodal and species dependent

