Plant toxins as an emerging risk for food safety

From the perspective of an analytical chemist

Patrick Mulder

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Parma Summer School 2018
Emerging Risks for Food Safety and Public Perception
15-17 May 2018

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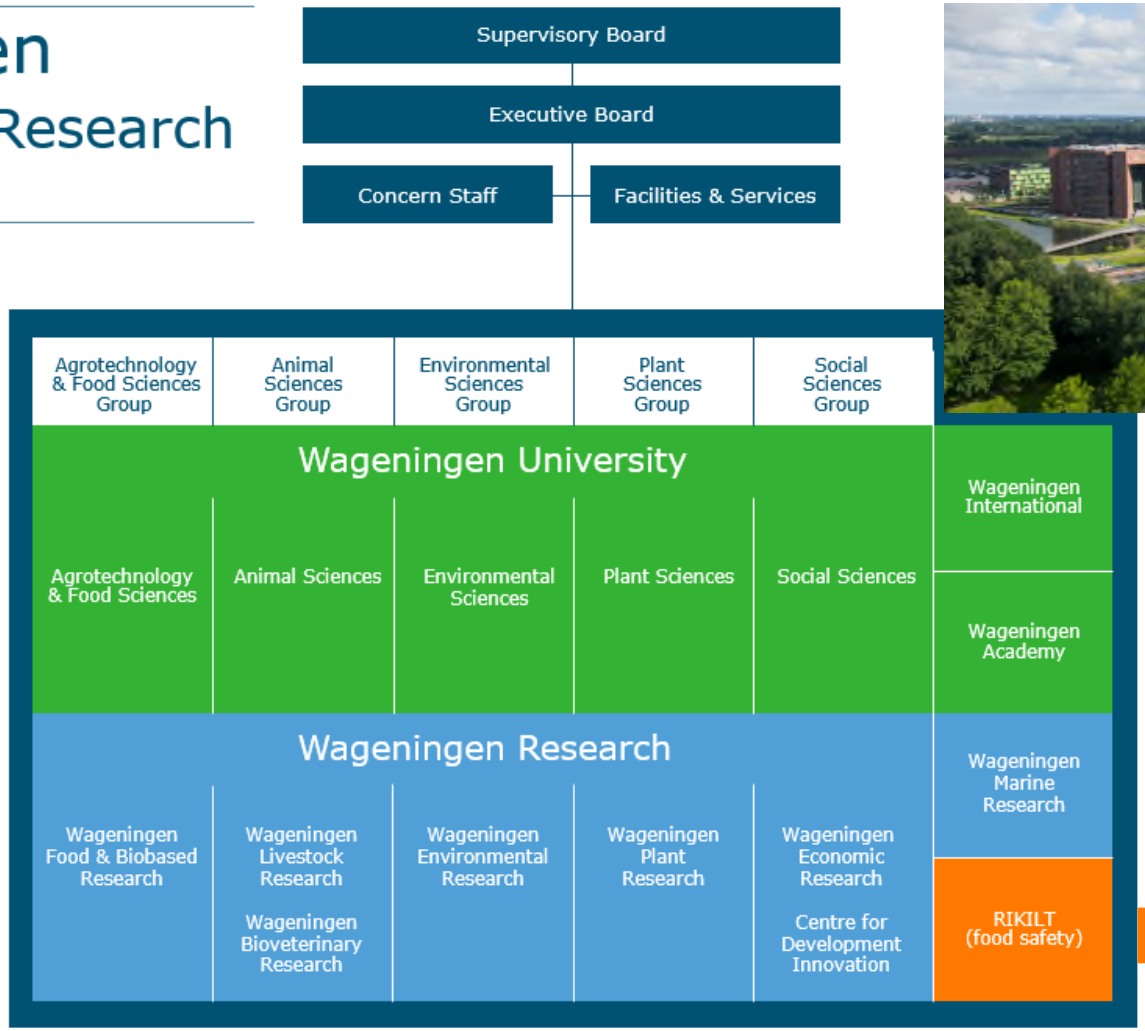

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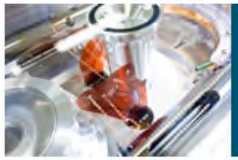
RIKILT Wageningen University & Research

Mission: Safe and reliable food for everyone

Reference institute

Measuring and detecting substances



Method development

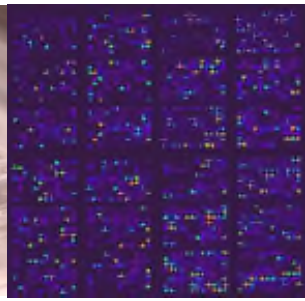
Training and consultancy

24/7

Effects substances on humans and animals

Safe food production

Food fraud and composition



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Overview

Plant toxins

Analytical methods

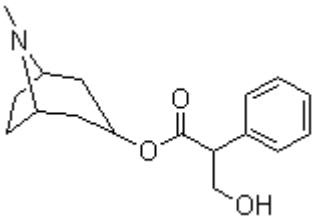
Risk assessment

PAs as emerging toxins

The (near) future

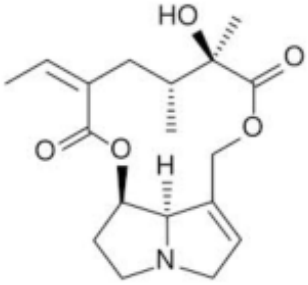
Classes of Plant toxins

Tropane alkaloids



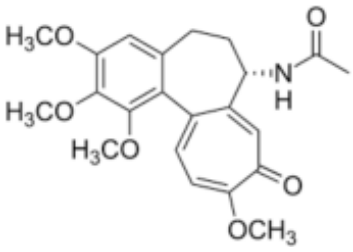
Atropine

Pyrrolizidine alkaloids



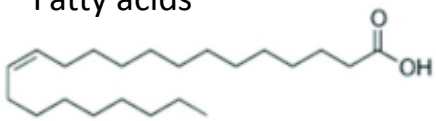
Senecionine

Cochicine alkaloids



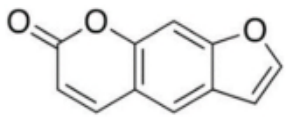
Cochicine

Fatty acids



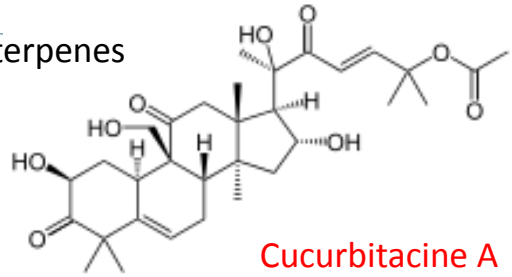
Erucic acid

Furanocoumarins



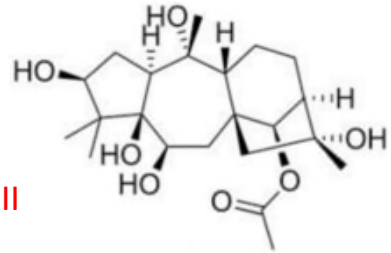
Psoralen

Triterpenes



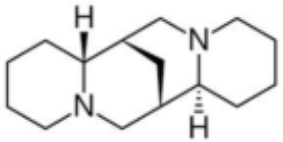
Cucurbitacine A

Diterpenes



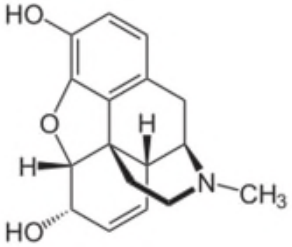
Grayanotoxin III

Quinolizidine alkaloids



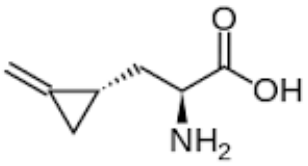
Sparteine

Opium alkaloids



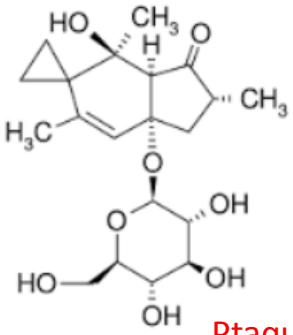
Morphine

Amino acids



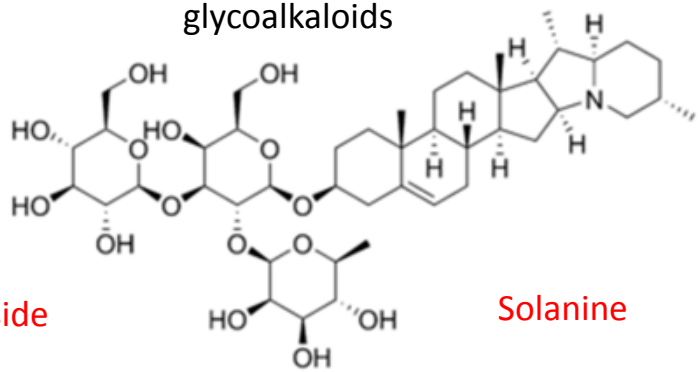
Hypoglycine A

sequiterpene glycosides



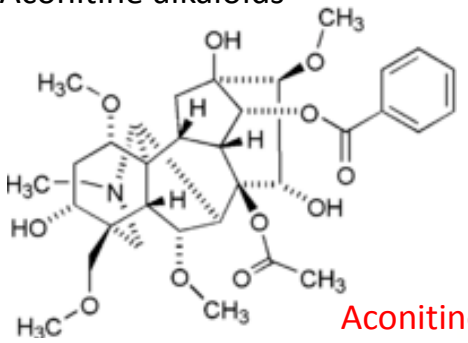
Ptaquiloside

glycoalkaloids



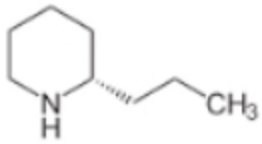
Solanine

Aconitine alkaloids



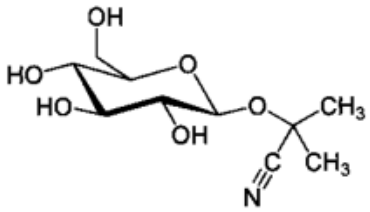
Aconitine

Piperidine alkaloids



Coniine

Cyanogen glycosides

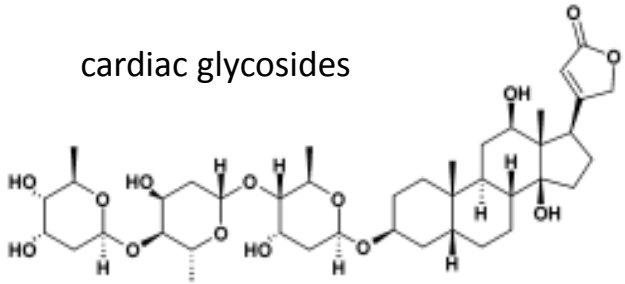


Linamarin

Lectins

Ricin

cardiac glycosides



Digitoxin

Plant toxins in food products

Inherent

Contamination

Transfer

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Inherent plant toxins evaluated by EFSA

Compound/group	Relevant food/feed	Food	Feed	EFSA opinion
Alkenylbenzenes	Herbs, dietary supplements	Yes	No	2009:965
Cannabinoids	Dietary supplements	Yes	No	2015:4141
Cyanogenic glucosides (Cyanide)	Cassava, bitter almond, apricot kernels, linseed	Yes	Yes	2007:434; 2016:4424
Erucic acid	Rapeseeds, mustard oils	Yes	Yes	2016:4593
Glucosinolates	Rapeseeds, mustard oils	No	Yes	2008:590
Glycoalkaloids	Potatoes	Yes	Yes	2019 (in prep)
Glycoproteins	Beans, pulses	No	Yes	2008:726
Gossypol	Cotton seeds	No	Yes	2009:908
Opium alkaloids	Poppy seeds	Yes	No	2011:2405; 2018 (May)
Phorbol esters	Jathropha seeds	No	Yes	2015:4321
Quinolizidine alkaloids	Lupine seeds	Yes	Yes	2019 (in prep)
Theobromine	Cacao	No	Yes	2008:725

- In general no EU legislation (ML) established for food, partly for feed
- Guidance levels/national limits/self regulation may be used

Typical characterics inherent food toxins

- Long history of use and possible side effects (human case reports)
- Mode of action generally well known
  - CNS, anticholeric, muscarinic receptors, inhibition of ATP
- Concentrations of concern typically > 1-100 mg/kg in food
  - Toxicity CGs(HCN) > THC > OAs, GAs > QAs, EA
- Analysis can be relatively easy
  - Limited number of relevant substances
  - Wide variety of analytical techniques used

  including fast screening/effect assays

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Potential risks foods containing inherent toxins

- Food products are generally considered as safe, but…
  - New food products/supplements
  - Supplements prepared as concentrates
  - New varieties with higher toxin content (to increase pest resistence)
  - Growing conditions (potato, lupine)
  - ‘Sweet’ vs ‘Bitter’ varieties (lupine, almond/apricot kernels, zucchini)
  - Food collected in the wild





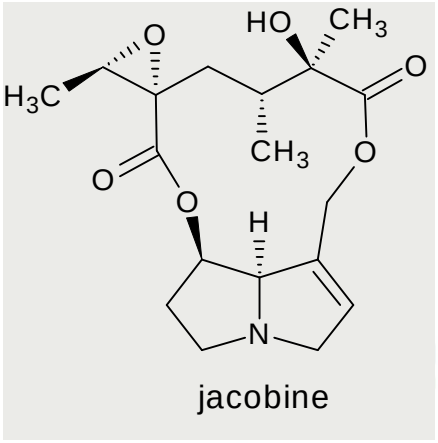
Plant toxins from contamination/transfer

Compound/group	Relevant food/feed	Food	Feed	EFSA opinion
Pyrrolizidine alkaloids	(Herbal) tea, honey, milk, herbal supplements, forage	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

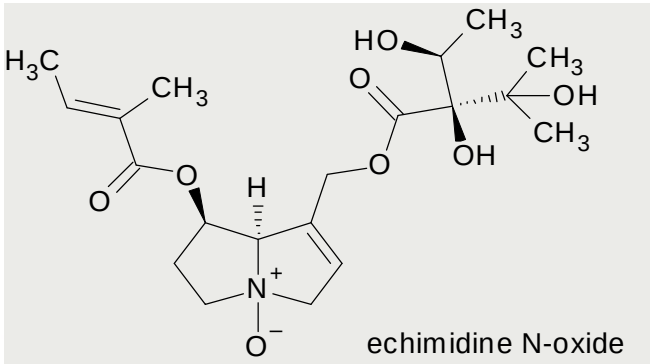
- EU legislation for feed based on plants rather than toxins
- Very limited EU legislation (ML) established for food (TAs in babyfood)
- Guidance levels/national limits are also lacking

Pyrrolizidine alkaloids

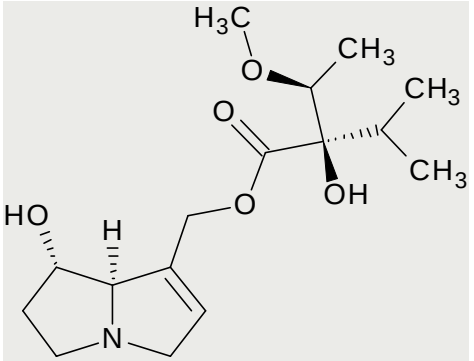
- 100s of structures known, wide variety
- 100-1000s of plant species, worldwide occurrence
- Major plant groups: *Senecio*, *Boraginaceae*, *Heliotropium*, *Crotalaria*



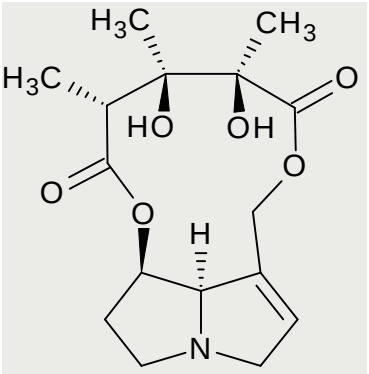
jacobine



echimidine N-oxide



heliotrine



monocrotaline

Pyrrolizidine alkaloids as emerging risk

- Toxic potential known for many years (since 1900)
  - Mostly affecting livestock in Africa, Australia, N. America
  - Several serious human outbreaks:
    - India, Tadzjikistan, Afghanistan, Ethiopia
    - Due to weed contaminated grains
    - Hepatic veno-occlusive disease

- Honey as potential source (known since 70s)

See: Wiedenfeld (2011), Food Add. Contam, 28, 282-292

EFSA - Scientific opinion on PAs 2007

The EFSA Journal (2007) 447, 1-51

OPINION OF THE SCIENTIFIC PANEL ON CONTAMINANTS IN THE FOOD CHAIN

ON A REQUEST FROM THE EUROPEAN COMMISSION RELATED TO

PYRROLIZIDINE ALKALOIDS

AS UNDESIRABLE SUBSTANCES IN ANIMAL FEED

(Question N° EFSA-Q-2003-065)

Adopted on 25 January 2007

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Main conclusions Opinion 2007

- **Analytical data** were **lacking**
- PAs are **possibly** carcinogenic genotoxins, but too limited data available
- Chronic exposure may be relevant
- **Impossible** to do a **risk assessment**

EFSA – Scientific Opinion PAs 2011

EFSA Journal 2011;9(11):2406

SCIENTIFIC OPINION

Scientific Opinion on Pyrrolizidine alkaloids in food and feed[1]

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

European Food Safety Authority (EFSA), Parma, Italy

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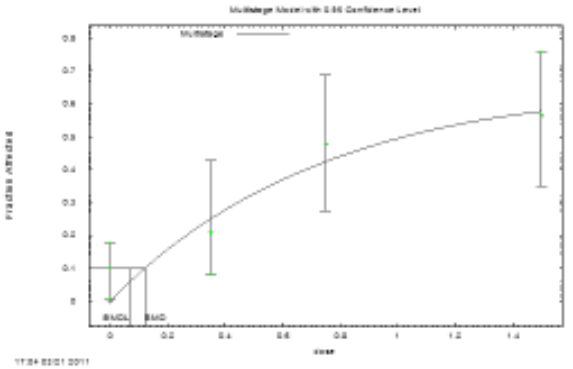
Main conclusions Opinion 2011

- CONTAM Panel concluded that 1,2-unsaturated PAs may act as **genotoxic carcinogens** in humans
- A **margin of exposure** (MOE) approach was applied for the risk characterisation of 1,2-unsaturated PAs
- Chronic exposure should not exceed **7 ng/kg bw/day**, or 500 ng PAs in the total daily diet (70-kg adult) (MOE of 10,000)
- **Data** available for **honey**: indicating a possible issue for **children/toddlers** that consume **honey**



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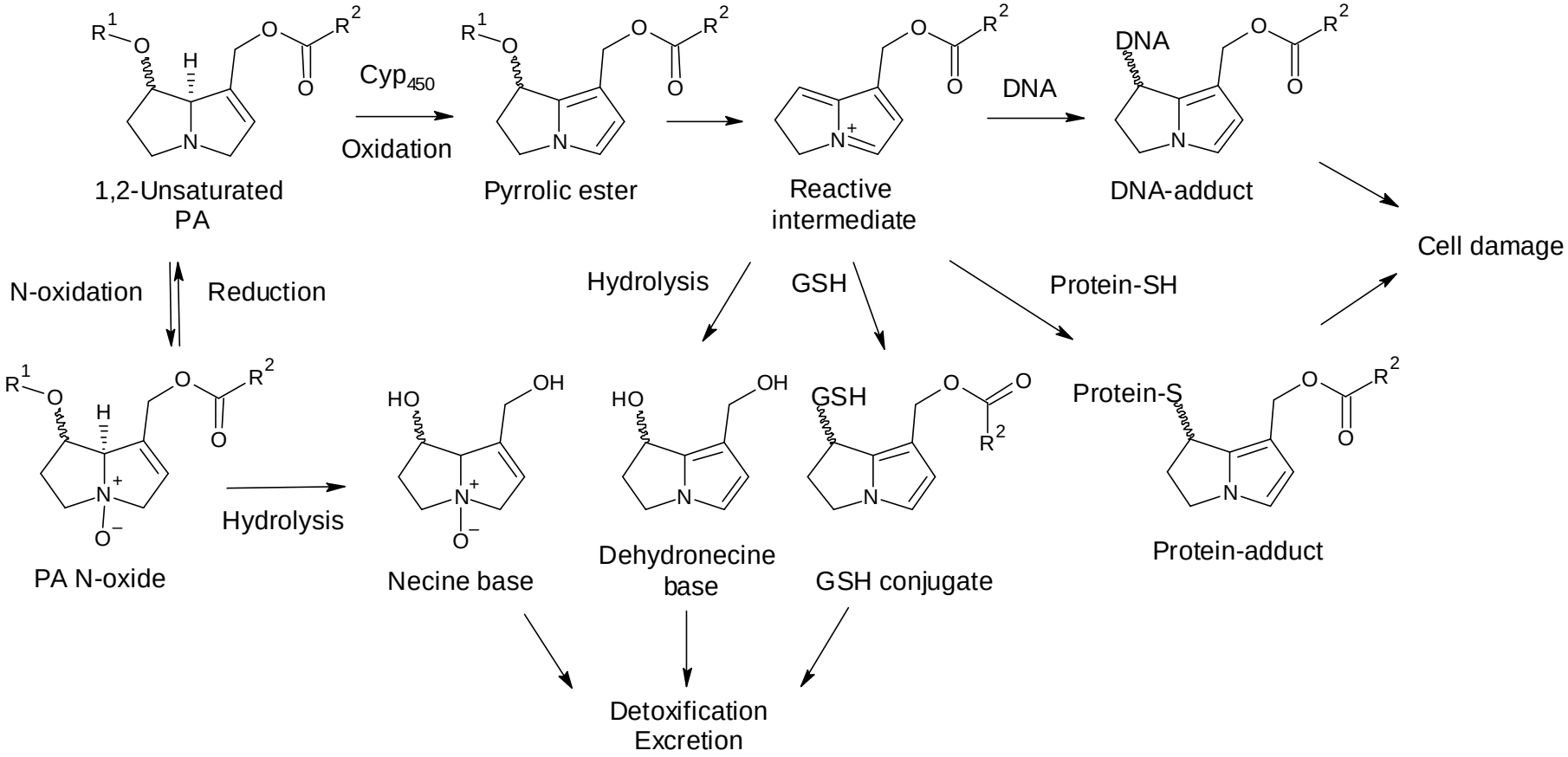
PAs - Metabolism and bioactivation



EFSA PA survey in food - 2015

EFSA supporting publication 2015:EN-859

EXTERNAL SCIENTIFIC REPORT

Occurrence of Pyrrolizidine Alkaloids in food[1]

Patrick P.J. Mulder[a], Patricia López Sánchez[a], Anja These[b], Angelika Preiss-Weigert[b], Massimo Castellari[c]

[a] RIKILT – Wageningen UR, Wageningen, the Netherlands

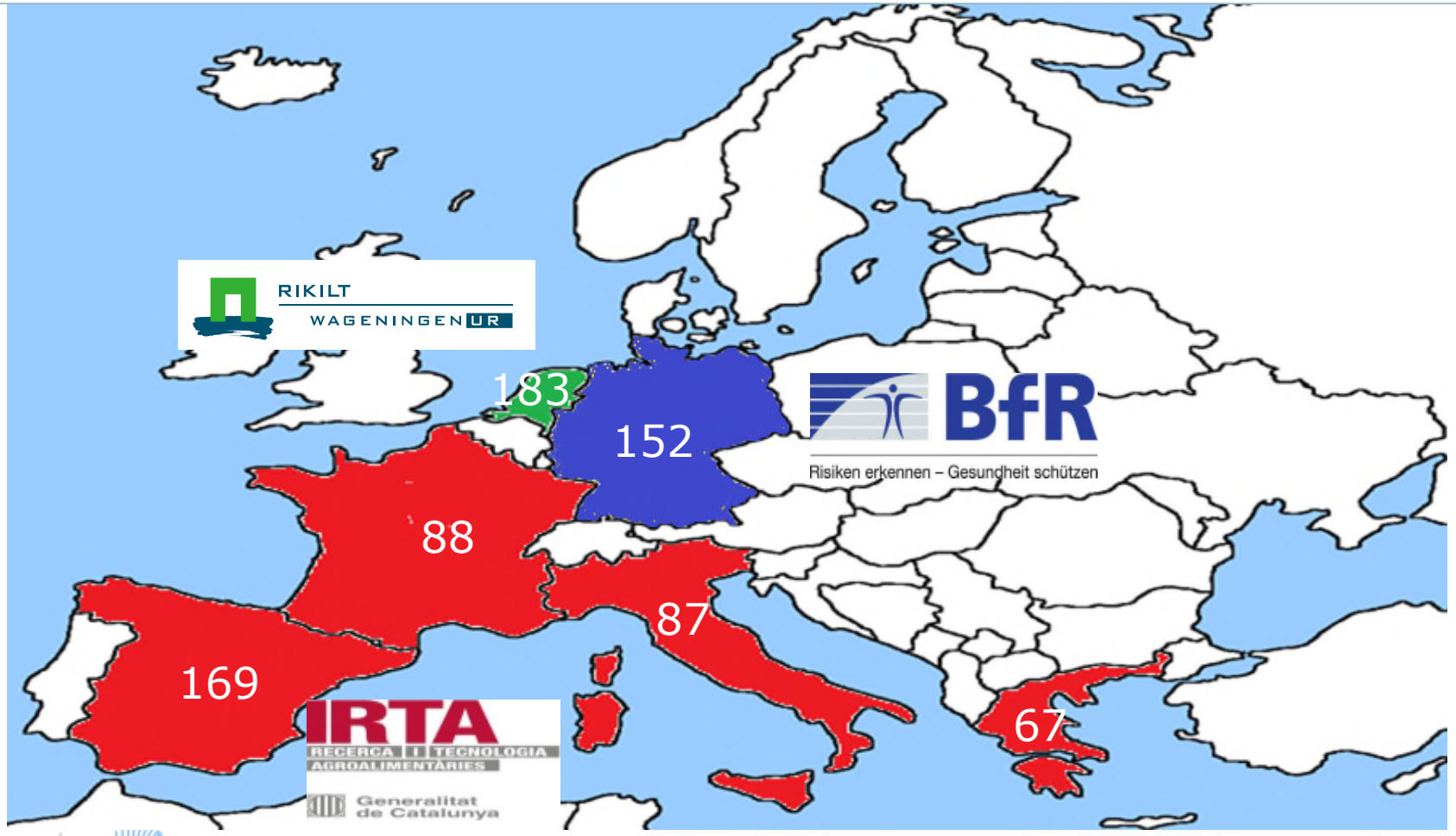
[b] Federal Institute for Risk Assessment (BfR), Berlin, Germany

[c] Institute for Research and Technology in Food and Agriculture (IRTA), Monells, Spain

See also: Mulder et al (2018), Food Add. Contam, 35, 118-133

Sampling of animal-derived products

Total: 746 samples



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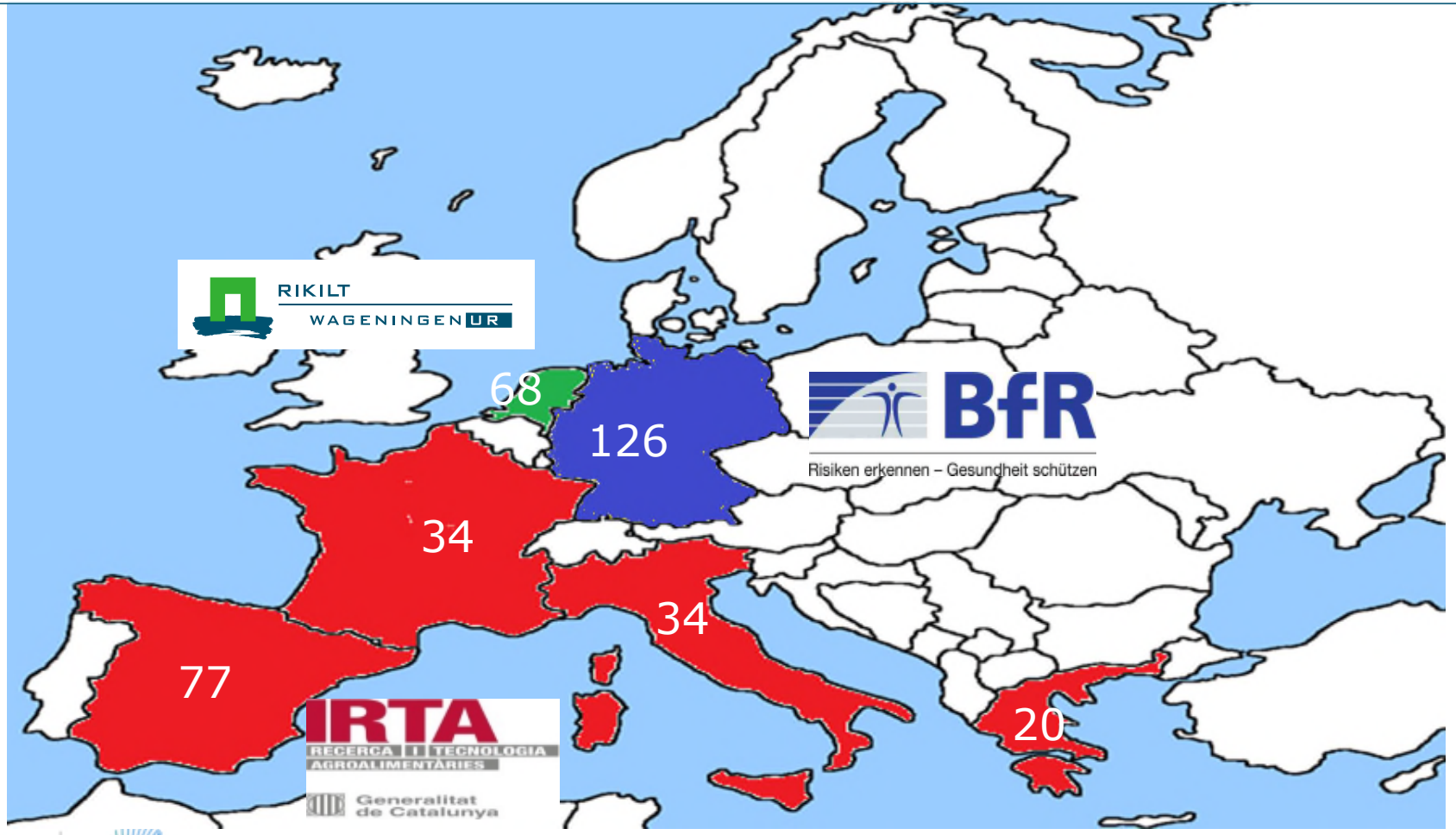
Sampling of plant-derived products

Total: 359 samples



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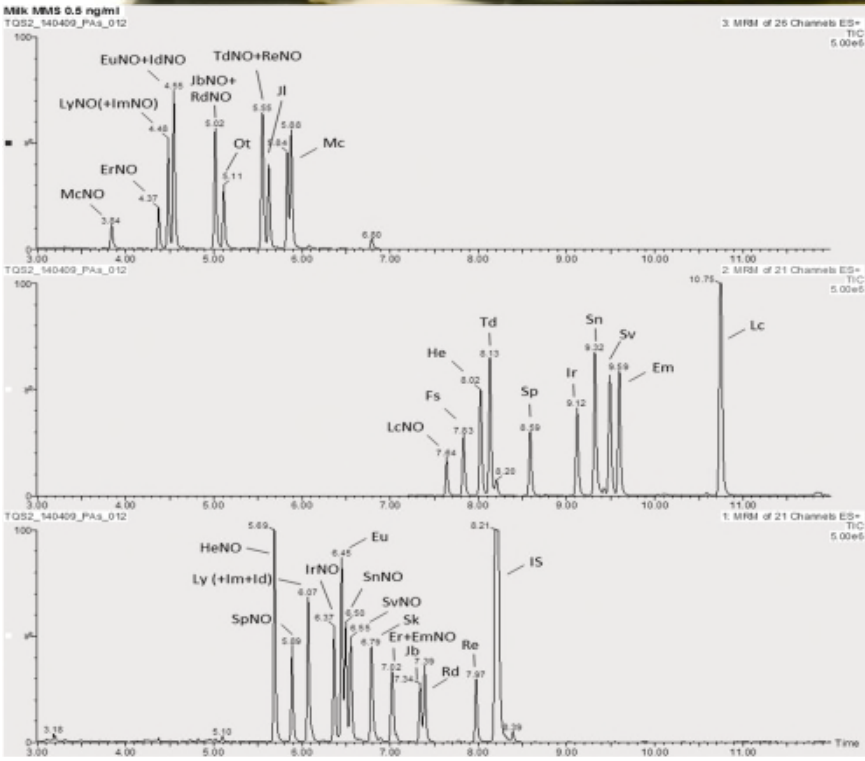
Analytical requirements

- LC-MS/MS technology

- Very low LOQs
  - 0,05-0,1 µg/L in milk
  - 0,25-0,5 µg/kg in meat, egg
  - 0,02-0,08 µg/L in tea infusion
  - 1-10 µg/kg in supplements

- Validated methods
  - Broad scope of PAs
  - Ca 30 PA standards



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Animal derived products

	Samples analysed	Samples> LOD	% > LOD	Highest conc. (µg/kg)
All animal-derived food products	746	13	1.7	0.17
Milk and milk products	268	11	4.1	0.17
Pasteurised and UHT milk	182	11	6.0	0.17
Yoghurt, cheese	61	0		
Milk powder (infant formula)	25	0		
Fresh eggs	205	2	1.0	0.12
Meat (beef, pork, poultry) and meat products (liver)	273	0	0.0	<0.1

- Only very limited exposure to PAs
- Exposure largely confined to milk

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Plant derived products

	Samples analysed	Samples> LOD	% > LOD	Max. conc. (µg/kg)	Tea infusion avg (µg/L)
All teas and food supplements	359	266	74.1		
Teas	166	151	91.0	4805	6.13
Black tea	33	31	93.9	4062	7.62
Green tea	26	22	85.2	3917	5.65
Rooibos tea	22	21	95.5	4805	7.99
Chamomile tea	35	30	85.7	1394	3.65
Peppermint tea	30	28	93.1	4401	6.68
Mixed herbal tea	22	21	95.2	1929	5.82
Food supplements	191	115	59.7	2410000	
non-PA producing plants	111	68	61.3	8488	
PA producing plants	51	26	51.0	2410000	
Bee products	29	20	69.0	1911	

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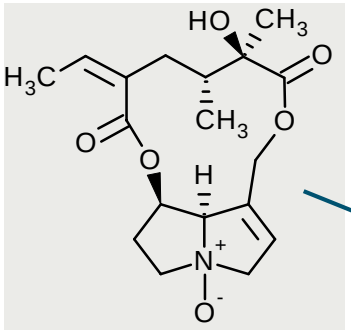
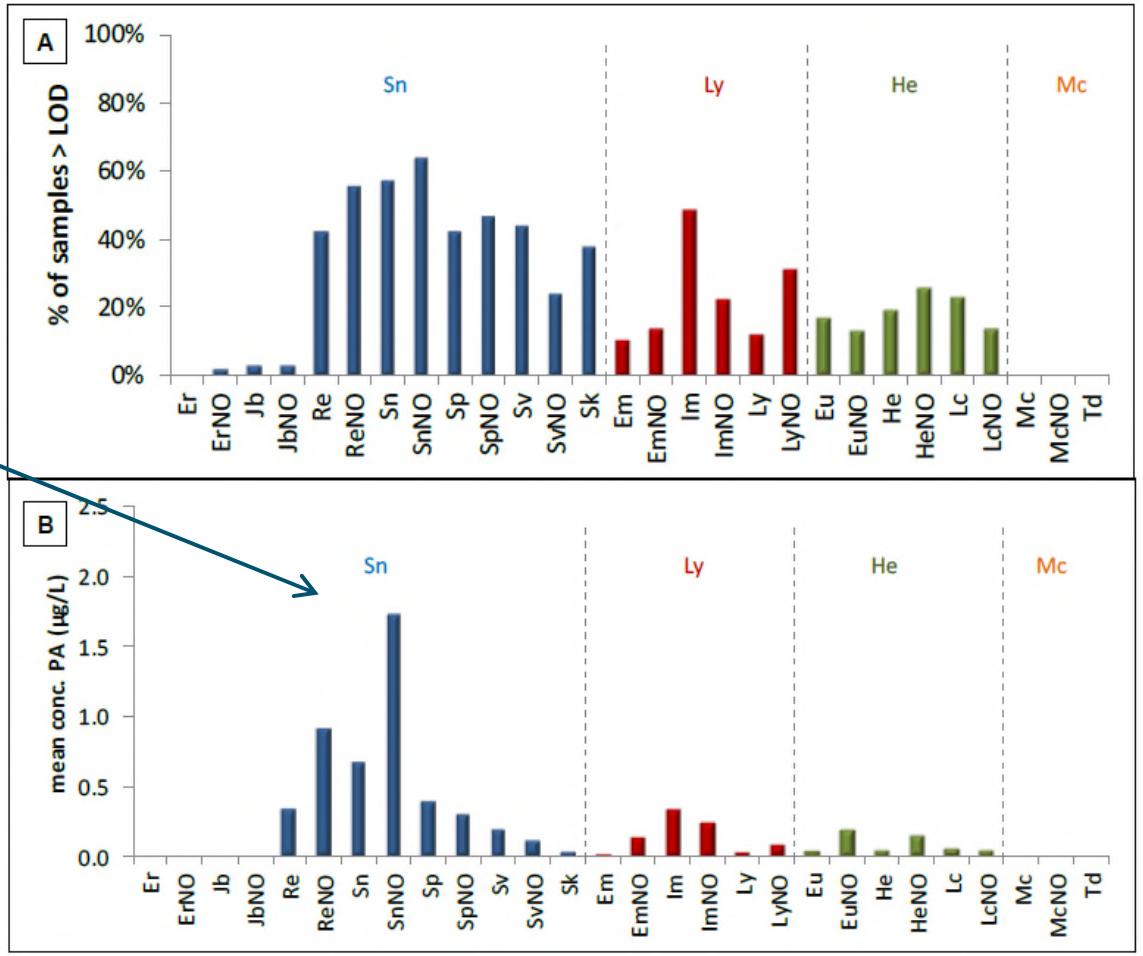
- Contamination with PAs is very common
- Substantial amounts found in tea and supplements

PAs in (herbal) tea





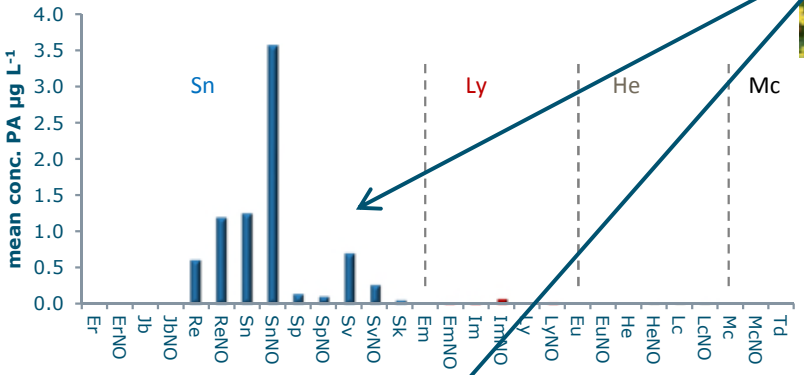
- 20 PAs are contributing to overall content

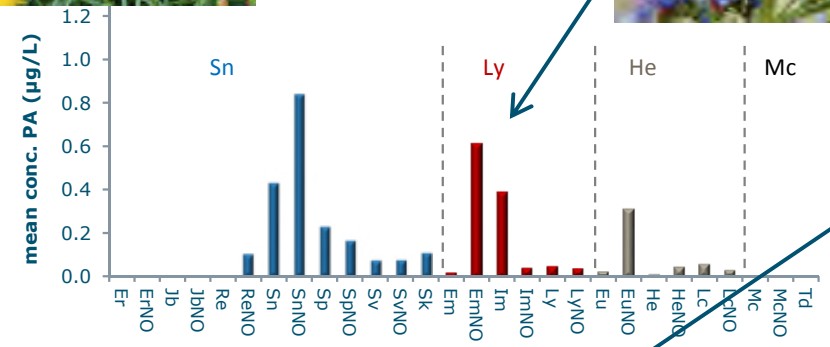
PAs in (herbal) tea

Senecio

Echium

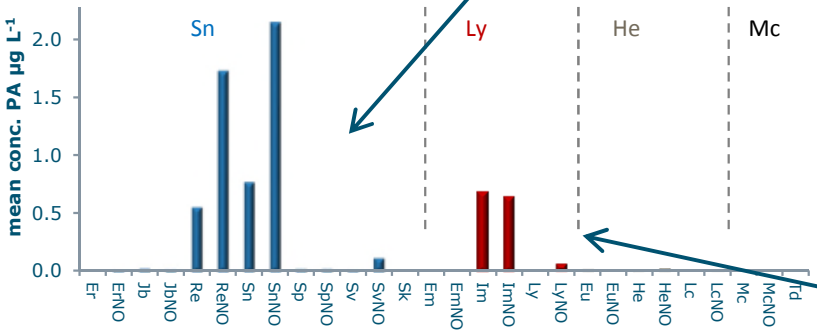
rooibos

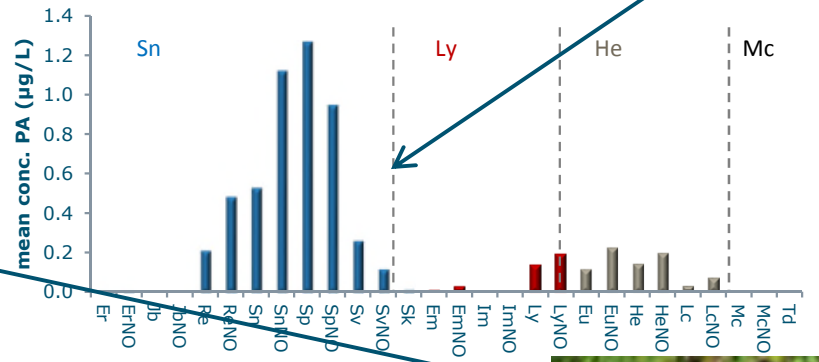




chamomile

black
green

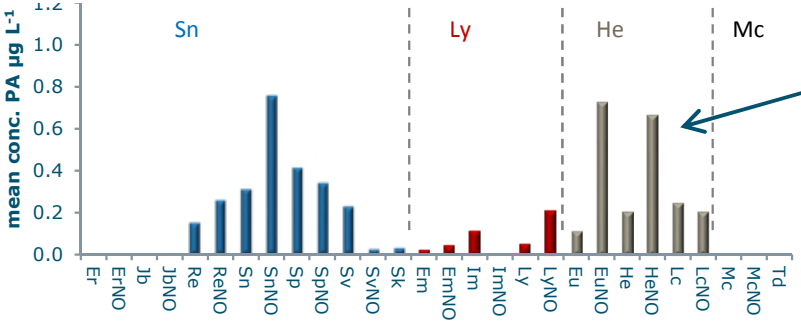




S. vulgaris

peppermint

mixed
herbal

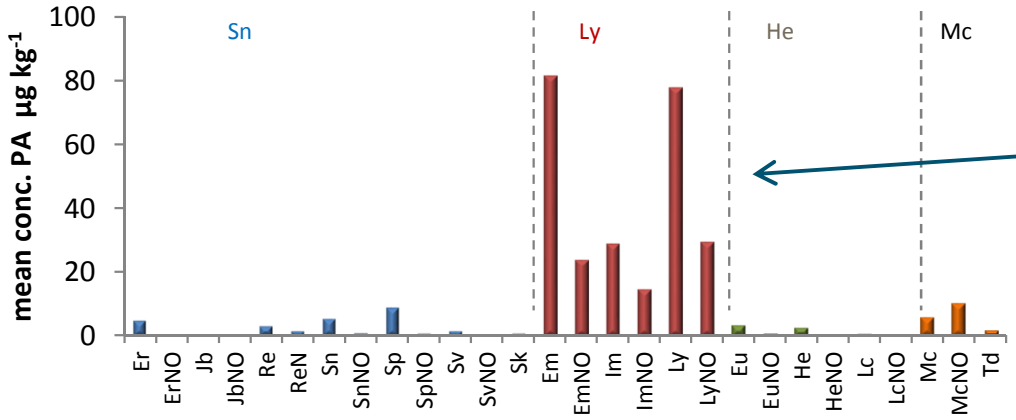


Heliotropium

Eupatorium

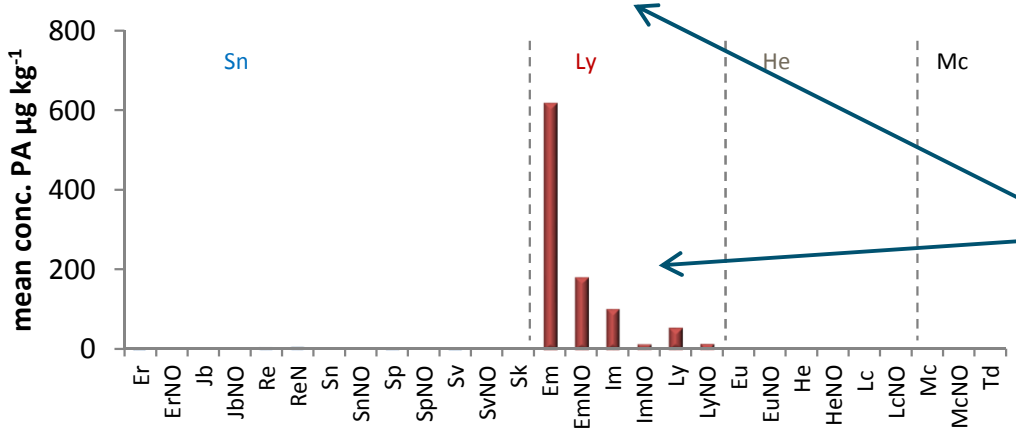
PA patterns in herbal supplements

All non-PA plants



Symphytum

St John's Wort



Echium

- Less variation in PAs

EFSA Risk Assessment - 2017

STATEMENT

EFSA Journal

ADOPTED: 21 June 2017

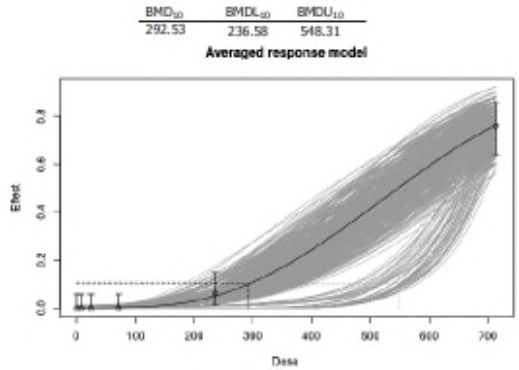
doi: 10.2903/j.efsa.2017.4908

Risks for human health related to the presence of pyrrolizidine alkaloids in honey, tea, herbal infusions and food supplements

EFSA Panel on Contaminants in the Food Chain (CONTAM),
Helle Katrine Knutsen, Jan Alexander, Lars Barregård, Margherita Bignami, Beat Brüschweiler, Sandra Ceccatelli, Bruce Cottrill, Michael Dinovi, Lutz Edler, Bettina Grasl-Kraupp, Christer Hogstrand, Laurentius (Ron) Hoogenboom, Carlo Stefano Nebbia, Isabelle P. Oswald, Annette Petersen, Martin Rose, Alain-Claude Roudot, Tanja Schwerdtle, Christiane Vleminckx, Günter Vollmer, Heather Wallace, José Angel Ruiz Gomes and Marco Binaglia

Main conclusions EFSA assessment 2017

- **Revised** margin of exposure (MOE) analysis
- Daily exposure should not exceed **23.7 ng/kg bw/day**, or 1660 ng PAs in the total daily diet (70-kg adult) (MOE of 10,000)
- Data available for honey, tea and supplements: indicating a possible issue for high consumers of **(herbal) teas**, in particular the **younger population**
- Set of **17 PAs** proposed



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PAs in tea: an emerging issue?

source: Wikipedia

- Mechanisation may result in increased co-harvesting of (toxic) weeds

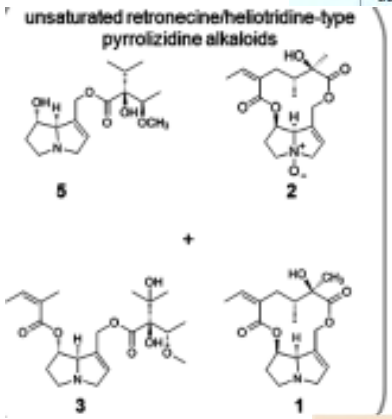
source: www.greentea.net

Under development…

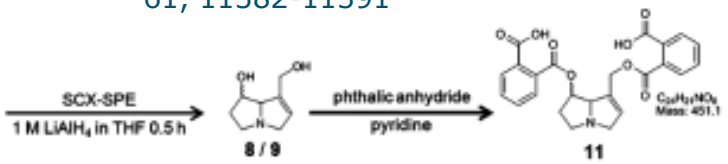
- Databases for PAs using LC-HR-MS
  - Profiling of PA plants
- Isolation of PAs from plants
  - Suppliers of standards (60 available)
- Sum methods for total PA content
  - Hydrolysis to necine bases
- In-vitro assays to assess genotoxicity of PAs
  - Effect of structure on activity

These et al (2013), Anal Bioanal Chem 405, 9375-9383

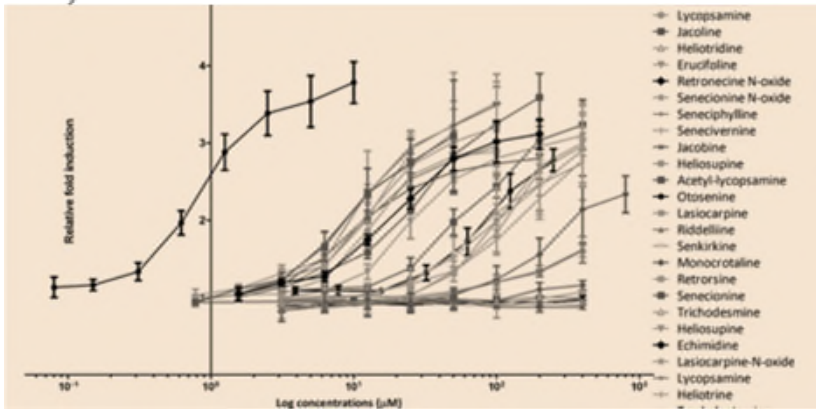
monoesters					open chained diesters				
PA	m/z PANO	no. of isomers[a]	sum formula	plant number[b]	PA	m/z PANO	no. of isomers[a]	sum formula	plant number[b]
	298	2	$C_{15}H_{24}O_5N$	6, 10, 12	322		2	$C_{17}H_{24}O_5N$	12, 13
306		1	$C_{17}H_{24}O_4N$	7	324		1	$C_{17}H_{26}O_5N$	12
308		1	$C_{17}H_{26}O_4N$	7	328		1	$C_{16}H_{26}O_6N$	9
312		4	$C_{16}H_{26}O_5N$	13, 14		338	2	$C_{17}H_{24}O_6N$	12, 13
324		1	$C_{17}H_{26}O_5N$	8	340		3	$C_{17}H_{26}O_6N$	11
328		1	$C_{17}H_{30}O_5N$	9, 13	356		2	$C_{18}H_{30}O_6N$	13
	328	3	$C_{16}H_{26}O_6N$	13, 14		356	4	$C_{17}H_{26}O_7N$	11, 12, 13
338		1	$C_{18}H_{28}O_5N$	6	362		2	$C_{20}H_{28}O_5N$	13
340		1	$C_{17}H_{26}O_6N$	1, 2		372	2	$C_{18}H_{30}O_7N$	13
	340	1	$C_{17}H_{26}O_6N$	6		378	2	$C_{20}H_{26}O_6N$	12, 13
342		1	$C_{17}H_{28}O_6N$	9	380		2	$C_{20}H_{30}O_6N$	12, 13
	344	1	$C_{16}H_{26}O_7N$	9		380	2	$C_{20}H_{30}O_6N$	12
	344	1	$C_{17}H_{30}O_6N$	13	382		1	$C_{19}H_{28}O_7N$	11
352		2	$C_{18}H_{28}O_6N$	3, 4, 7	384		2	$C_{19}H_{30}O_7N$	12
356		3	$C_{18}H_{30}O_6N$	5, 8, 10, 17	386		1	$C_{18}H_{28}O_8N$	12
	356	1	$C_{17}H_{26}O_7N$	2		388	1	$C_{18}H_{30}O_8N$	13



Cramer et al (2013), J Agric Food Chem 61, 11382-11391



Peijenburg et al (2018), in preparation



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The future in food safety analysis

Traditionally: Targeted sample analysis

Decide what you want to know,
then analyse

Past
SRM/group methods

OCP
GC-ECD

OPP
GC-FPD

PYR
GC-ECD

ONP
GC-MS

TBZ
LC-UV

CARB
LC-Flu

Present
MRMs

pesticides
GC-MS/MS

PCBs/PH
GC-MS/MS

mycotoxins
LC-MS/MS

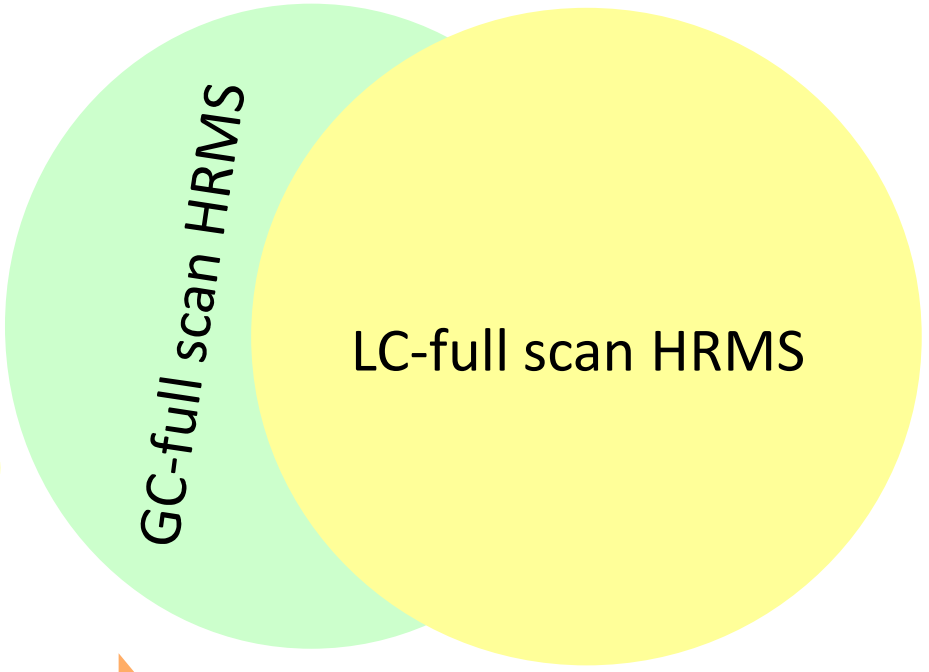
pesticides
LC-MS/MS

vet drugs
LC-MS/MS

Plant toxins
LC-MS/MS

1-20 analytes/method 20-150

(Near) future: Untargeted measurement



100-1000+

Analyse,
then decide what you want to know

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In conclusion

- In the past decade PAs have emerged as an important group of plant toxins
- Analytical developments have been an important driver
- Recognition of PAs as genotoxic carcinogens has been equally important
- Further refinement of the risk profiles of PAs, plants and food/feed products will be the next goal

Thank you for your attention

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RIKILT website

https://www.wur.nl/en/Expertise-Services/Research-Institutes/rikilt.htm

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