

**FINAL PROGRAM
&
ABSTRACTS OF LECTURES AND POSTERS**



Poisonous plants and their toxins:
New global challenges?

WELCOME

Welcome at the 2nd conference of **The World Plant Toxin Forum**. The conference will build on the success of the first edition held in Vienna, Austria (2014).

Plants contain a large number of biologically active metabolites. Some of these have been found to be extremely useful for treating various human and animal diseases. However, some plant constituents produce adverse human and animal health effects following exposure. These so-called plant toxins occur abundantly in, for example, aromatic plants used as food ingredients, scents and flavors, and traditional herbal medicines.

The World Plant Toxin Forum fills a gap in the plant toxin field by providing a unique platform for food and feed industry representatives as well as suppliers of botanicals to meet with people from universities and governments from around the world. The main objectives of The World Plant Toxin Forum are:

- to exchange information and experiences on the various aspects of plant toxins
- to review current knowledge related to plant toxins in food, feed and botanicals
- to discuss strategies for prevention and control of plant toxin contamination ensuring the safety and security of food, feed and botanicals supply
- to initiate approaches for the control of plant toxin contamination along the food, feed and botanicals supply chains.

The 2nd conference of **The World Plant Toxin Forum** will be an excellent way to network and to share ideas. You are invited to take part in the discussions with participants from different disciplines and to meet business relations in your area. We wish you an active and fruitful meeting!

Monique de Nijs
Jörg Stroka

Advisory committee

Dr. Monique de Nijs (chair)	RIKILT Wageningen UR, the Netherlands
Dr. Jörg Stroka (co-chair)	EC Joint Research Centre, Institute for Reference Materials and Measurement, Belgium
Prof.dr. Cristo Botha	University of Pretoria, South Africa
Ms. Zoe Gillespie	Health Canada, Canada
Dr. Leigh Henderson	Food Standards Australia New Zealand, New Zealand
Dr. Kip Panter	Poisonous Plant Research Laboratory, U.S. Department of Agriculture, USA

Secretariat The World Plant Toxin Forum

Bastiaanse Communication
P.O. Box 179
NL-3720 AD Bilthoven
the Netherlands
T +31 30 2294247
WPTF@bastiaanse-communication.com
www.PlantToxinForum.org

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Key to the abstracts of lectures and posters:

- abstracts of lectures and posters are grouped separately;
- the lectures are grouped according to the daily program;
- the posters are grouped in an alphabetical order according to the corresponding author.

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CANADIAN MUSEUM FOR HUMAN RIGHTS VISIT AND CONFERENCE DINNER

Wednesday, June 8, 2016

19:30 – 21:00 Visit to the museum

21:00 – 23:00 Conference dinner

Where:

Canadian Museum for Human Rights
85 Israel Asper Way
Winnipeg, MB R3C 0L5



CANADIAN MUSEUM FOR
HUMAN RIGHTS

As a special end to the 2nd conference of **The World Plant Toxin Forum** there will be a unique dinner in the Canadian Museum for Human Rights

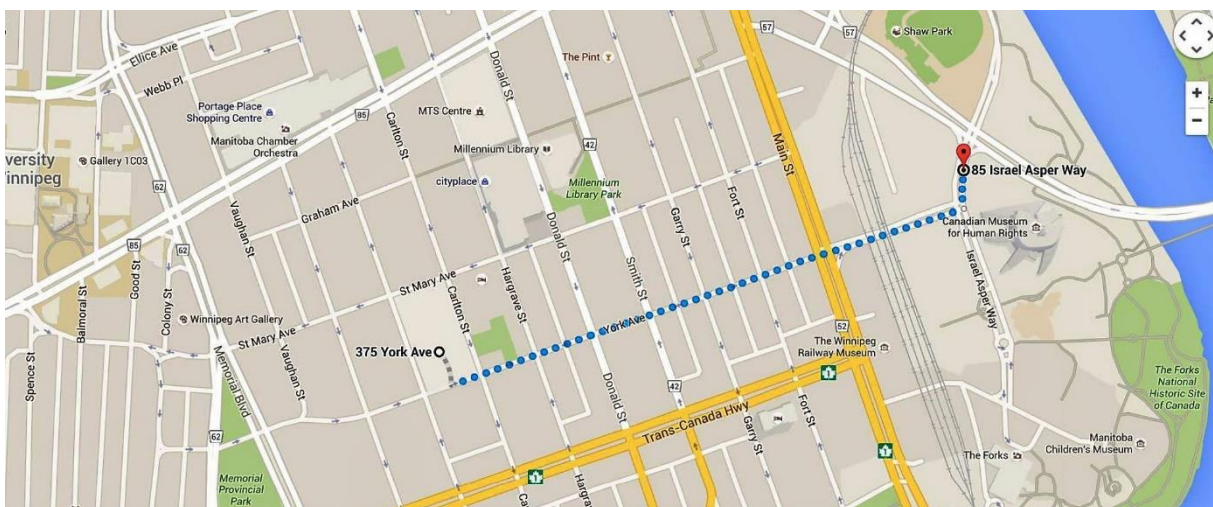
The Museum's building itself is a 'wow' experience. The unique architecture designed by Antoine Predock parallels a human rights journey – it requires some effort and has a few twists and turns, but can be very rewarding to complete.

Before dinner, you will have ample time to visit the Museum. You will enter the Museum at ground level, into a space that has a subterranean feel. You gradually work your way higher on a series of inclined ramps that reveal more and more daylight. Keep your camera ready – natural light combined with lit, alabaster-covered ramps is always a favorite for photographers.

From the RBC Convention Centre, it is just a 10-minute walk along York Avenue to the Canadian Museum for Human Rights. Shuttle buses will also run from the Convention Centre to the Museum starting at 19:00, and back from the Museum to the Convention Centre starting at 23:00.

IMPORTANT NOTE

The visit and dinner are only open to participants who registered in advance when they booked for the conference. You have found your ticket at the back of your badge that you have received upon arrival at the conference.



PROGRAM AT A GLANCE

Monday, June 6, 2016

17:30	Welcome reception sponsored by R-Biopharm Delta Grand Ballroom B Delta Winnipeg Hotel 350 St. Mary Avenue Winnipeg, MB R3C 3J2
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Tuesday, June 7, 2016

09:00 – 10:30	Session 1 <i>Introduction</i>	Exhibition
10:30 – 11:00	Networking break	
11:00 – 12:30	Session 2 <i>Occurrence of plant toxins</i>	
12:30 – 14:00	Lunch break Poster viewing	
14:00 – 15:30	Session 3 <i>Toxicology of plant toxins</i>	
16:00 – 17:30	Session 4 <i>Analysis of plant toxins</i>	
17:30 – 18:30	Poster viewing	
18:30	Wine tasting sponsored by Biomin and Romer Labs	

Wednesday, June 8, 2016

08:30 – 10:00	Session 5 <i>Risk assessment and management in the supply chain</i>	Exhibition
10:00 – 10:30	Session 6 <i>Speed presentations</i>	
10:30 – 11:00	Networking break	
11:00 – 12:00	Session 7 <i>Looking forward</i>	
19:30 – 23:00	Visit to the Canadian Museum for Human Rights Conference dinner	

CONFERENCE PROGRAM

TUESDAY, JUNE 7, 2016

SESSION 1: INTRODUCTION

Session 1: Introduction

Chairs: Dr. Monique de Nijs, *RIKILT Wageningen UR, the Netherlands*
Dr. Jörg Stroka, *EC Joint Research Centre, Institute for Reference Materials and Measurements, Belgium*

- 09:00 Welcoming remarks
- 09:10 Poisonous plants and their toxins: setting the scene
Dr. Jörg Stroka, *EC Joint Research Centre, Institute for Reference Materials and Measurements, Belgium*
- 09:30 Current use of herbal products in North America
Dr. Joe Betz, *U.S. National Institutes of Health, USA*
- 10:00 Challenges faced by food regulators in Australia and New Zealand relating to plant toxins
Dr. Leigh Henderson, *Food Standards Australia New Zealand, New Zealand*
- 10:30 Networking break & exhibition

SESSION 2: OCCURRENCE OF PLANT TOXINS

Chair: Dr. Leigh Henderson, *Food Standards Australia New Zealand, New Zealand*

- 11:00 Plant toxins in the water environment
Dr. Bjarne W. Strobel, *University of Copenhagen, Denmark*
- 11:30 Pyrrolizidine alkaloids in our daily food: occurrence and food safety implications
Dr. Patrick Mulder, *RIKILT Wageningen UR, the Netherlands*
- 12:00 Pyrrolizidine alkaloids in honey, tea and herbs – occurrence and challenges for routine laboratories
Arne Dübecke, *Quality Services International, Germany*
- 12:30 Lunch break
Exhibition & poster viewing

TUESDAY, JUNE 7, 2016

SESSION 3: TOXICOLOGY OF PLANT TOXINS

Chair: Dr. Leigh Henderson, *Food Standards Australia New Zealand, New Zealand*

- 14:00 *Indigofera linnaei* – the Jekyll and Hyde of Australian pasture legumes
Dr. Mary Fletcher, *University of Queensland, Australia*
- 14:30 Presence of the natural carcinogen ptaquiloside from bracken fern in food and drinking water – what is the evidence?
Dr. Lars Holm Rasmussen, *Metropolitan University College, Denmark*
- 15:00 Co-exposure: If one plant toxin is harmful to livestock, what about two?
Dr. Kevin Welch, *Poisonous Plant Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, USA*
- 15:30 Networking break & exhibition

SESSION 4: ANALYSIS OF PLANT TOXINS

Chair: Dr. Leigh Henderson, *Food Standards Australia New Zealand, New Zealand*

- 16:00 What are TAs? Tropane Alkaloids or Troublesome Analytes?
Susan MacDonald, *Fera Science Ltd., UK*
- 16:30 Analysis of fluoroacetate in plants
Dr. Stephen Lee, *Poisonous Plant Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, USA*
- 17:00 How reliable are current analytical methods to determine tropane alkaloids in food and tea infusions at levels of interest?
Dr. Carlos Goncalves, *EC Joint Research Centre, Institute for Reference Materials and Measurements, Belgium*
- 17:30 Poster viewing
- 18:30 **WINE TASTING SPONSORED BY BIOMIN AND ROMER LABS**

WEDNESDAY, JUNE 8, 2016

Session 5: Risk assessment and management in the supply chain

Chair: Dr. Kip Panter, *Poisonous Plant Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, USA*

08:30 Pyrrolizidine alkaloids in animal forage and impacts on feed safety
Dr. Christoph Gottschalk, *Ludwig Maximilians University Munich, Germany*

09:00 A New Zealand collaborative industry approach to manage and mitigate the risk of pyrrolizidine alkaloids in honey
Jane Lancaster, *Catalyst Ltd., New Zealand*

09:30 Pyrrolizidine alkaloids in natural products – a new and enormous challenge for the tea and herbal infusions industry
Maximilian Wittig, *Tea & Herbal Infusions Europe (THIE), Germany*

SESSION 6: SPEED PRESENTATIONS

Chair: Dr. Kip Panter, *Poisonous Plant Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, USA*

10:00 Short presentations by selected poster presenters to provide an overview of their research and inspire the audience to visit their posters
For details, see page 18

10:30 Networking break & exhibition

SESSION 7: LOOKING FORWARD

Chairs: Dr. Monique de Nijs, *RIKILT Wageningen UR, the Netherlands*
Dr. Jörg Stroka, *EC Joint Research Centre, Institute for Reference Materials and Measurements, Belgium*

11:00 Can unmanned aircraft systems reduce plant poisoning risk in livestock?
Prof.dr. Deon van der Merwe, *Kansas State University, USA*

11:30 Poisonous plants and their toxins – new global challenges? Overview and conclusions
Dr. Monique de Nijs, *RIKILT Wageningen UR, the Netherlands*

12:00 End of The World Plant Toxin Forum – 2nd conference

19:30 **VISIT TO THE CANADIAN MUSEUM FOR HUMAN RIGHTS & CONFERENCE DINNER**
For details, see page 4

LECTURES

TUESDAY, JUNE 7, 2016 – Session 1

Poisonous plants and their toxins: setting the scene

Jörg Stroka

EC Joint Research Centre, Institute for Reference Materials and Measurements, Belgium;
joerg.stroka@ec.europa.eu

Current use of herbal products in North America

Joe Betz

Office of Dietary Supplements, U.S. National Institutes of Health, USA; betzj@od.nih.gov

In 1994, the U.S. Food, and Cosmetic Act was changed to create a category of foods called dietary supplements (DS). Similarly, in 2004, Canada created a class of over the counter products called Natural Health Products (NHP). There are similarities as well as differences in the two systems. Both permit sale of products that contain vitamins and minerals, botanicals and botanical extracts, amino acids, and other natural substances. However, NHP require pre-market approval while DS do not. Various estimates indicate there may be >85,000 products on each market. The perception that natural products are inherently safe and healthful survives despite the experience of Socrates and the practices of the Borgias. Natural toxins can occur in DS constitutively or as contaminants and can originate in plants (phytotoxins), fungi (mycotoxins), algae (phycotoxins) and microorganisms (cyanotoxins). In general, adverse events associated with botanicals may be placed into categories: accidental or deliberate adulteration, misuse, and inherent toxicity. An additional category involves ingredients that would not normally be expected to pose safety issues and confounds efforts to predict adverse events. Enhanced pharmacovigilance has demonstrated that serious adverse events associated with botanical products do occur, and the number of cases has risen with increased reporting. For example, hepatotoxicity has been reported for a number of botanicals, including *Microcystis* spp. (microcystins), germander (teucrin A), *Usnea* spp. (usnic acid), chaparral (NDGA), *Senecio* spp. (dehydroPA), and pennyroyal oil (*Hedeoma pulegioides*, *Mentha pulegium*) (pulegone). More recently, ingredients that do not contain obvious toxins (green tea extract, kava, black cohosh) have been associated with hepatotoxicity. Other potential sources of natural toxins include mycotoxins from poorly processed materials, co-harvested weeds, misidentified plants, and materials that have been subjected to economically motivated adulteration. Finally, not all adverse health effects manifest in a timeframe that permits immediate assignment of causality, and this must be taken into account when safety is considered.

Challenges faced by food regulators in Australia and New Zealand relating to plant toxins

Leigh Henderson

Food Standards Australia New Zealand, New Zealand; leigh.henderson@foodstandards.gov.au

Consumers expect food to be safe – the primary objective of food regulators is to mitigate the risk of unsafe foods in the food supply. Whilst conducting a comprehensive safety assessment of new foods entering the food supply can rely on an adequate safety data package, the consideration of adverse effects arising from natural plant toxins in foods, already in the food supply, can be more challenging. This presentation addresses issues of data paucity, consumer views on safety, impacts on industry and the appropriate level of intervention in relation to risks arising from foods with plant toxins in Australia and New Zealand. Examples such as the risk management of cassava products and apricot kernels and pyrrolizidine alkaloids will be used to illustrate these regulatory challenges. Whilst the risk management of existing foods which may contain toxins is reactive, the introduction of new plant materials into the food supply is managed via novel food regulations in many jurisdictions. Future developments in the risk management of novel foods in Australia and New Zealand will be sign-posted.

Plant toxins in the water environment

Bjarne W. Strobel

Environmental Chemistry and Physics, University of Copenhagen, Environmental Chemistry and Physics, Denmark; bjwe@plen.ku.dk

Plant toxins or phytotoxins are essential components in many plants and the presence almost everywhere in the ecosystems. The function as active compounds giving advance to the plant producing the compounds is multiple, and initiation of formation is either continuous or tricked by interactions from the surroundings, e.g., tissue damage or insect attack. The species density in an area and concentration of the compounds define where or not it is harmful, and the local hydrology defines the flux of potentially harmful compounds to nearby aqueous ecosystems. Categories of chemical properties for plant toxins and ecosystem hydrology parameters of concern are presented. Solubility, mobility, and chemical stability are key parameters that will be compared with equivalent properties for other known pollutants supporting the categories of compounds of concern in comparison with bigger data for xenobiotics.

The ecosystem characteristics influence the likelihood of transport to groundwater and surface waters, and species density and seasonal biochemical formation combined with the local climate determine the transport potential. Model estimates for transport of the categories of plant toxins are presented to illustrate the conditions of safe and unsafe systems with respect to risk of plant toxins in the aqueous environment in local scale and catchment scale. Precautionary considerations are proposed for avoiding water contamination of freshwaters. Exposure and hazard to freshwater organisms is given as overview for the categories of plant toxins and summarising the current knowledge in comparison with selected common xenobiotics. The plant toxins are continuously formed and based on the local conditions generating constant or pulsed transport, the aquatic environment experience season long exposure in pulses or in almost constant doses causing a different impact to freshwater organisms compared to usual pollution pattern in ecosystems in natural and rural districts not exposed to urban waste water.

Plant toxins in crop, pasture and forests are developing for the purpose of intelligent multifunctional plant tissues, and environmentally friendly production systems, and the presentation will illustrate pros and cons in this area of bioeconomy, and what to consider for the safety of new plant products used as a beneficial resource and balanced with the environmental concerns related to water environment.

Pyrrrolizidine alkaloids in our daily food: occurrence and food safety implications

Patrick Mulder¹, P. López Sánchez¹, A. These², A. Preiss-Weigert² and M. Castellari³

¹RIKILT Wageningen UR, the Netherlands, ²Federal Institute for Risk Assessment (BfR), Germany and ³Institute for Research and Technology in Food and Agriculture (IRTA), Spain; patrick.mulder@wur.nl

1,2-Unsaturated pyrrolizidine alkaloids (PAs) are secondary metabolites produced by a wide variety of plants from the families of *Asteraceae* (*Senecio*, *Eupatorium*), *Boraginaceae* and *Fabaceae* (*Crotalaria*). PAs are regarded as highly undesirable substances in food and feed, due to their genotoxic and carcinogenic properties, and for that reason have been the subject of two EFSA opinions (2007, 2011). Due to the limited availability of suitable occurrence data in food products (only substantial data for honey were available), in 2013 a large survey was initiated and funded by EFSA, to investigate the occurrence of PAs in animal-derived food products including milk, eggs and meat products, and in plant-derived food products including (herbal) teas and food supplements. Over 1100 samples were collected between January 2014 and April 2015 in 6 different European countries and analyzed for the presence of PAs. Two in-house validated LC-MS/MS methods were used to quantify 35 different PAs in animal-derived samples and 28 different PAs in plant-derived samples. LODs ranged from 0.03-0.25 µg/kg in the animal products and from 0.3-2.3 µg/kg for tea and herbal supplements. Analysis of the animal-derived products revealed the occasional presence (6%), albeit at low levels and mostly of single PAs, in milk samples. PAs were practically absent in egg and meat samples. In contrast, a high proportion of teas (91%) contained one or more PAs. The mean concentration for the sum of 28 PAs was 454 µg/kg tea, with a maximum of 4800 µg/kg. 20 different PAs contributed for at least 0.5% to the total PA

contamination in tea, the most prominent being senecionine, retrorsine and seneciphylline and their N-oxide forms. Food supplements were often contaminated with PAs (60%), but the concentrations were highly variable. As expected, the highest levels were found in herbal food supplements made from plant material of known PA producers. Lycopamine, intermedine and echimidine were the PAs most frequently found.

Based on limited available toxicological data, in 2011 a margin of exposure (MOE) of 1:10.000 was estimated by EFSA for a daily exposure of 7 ng PAs/kg body weight. Although the calculation was based on a worse case approach, it is evident from the collected data that in particular the contamination of teas presents a major issue, as daily consumption of a single cup (150 ml) in 40% of cases already could result in a MOE smaller than 1:10.000. The consequences for food safety and control will be discussed as well as suggestions made for further research.

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Pyrrolizidine alkaloids in honey, tea and herbs – occurrence and challenges for routine laboratories

Arne Dübecke, G. Beckh and C. Lüllmann
Quality Services International GmbH, Germany; arne.duebecke@qsi-q3.de

During the last few years, pyrrolizidine alkaloids (PAs) in food and feed turned out to be permanent hot topic in Europe. After discovery of parts of PA containing plants in a mixed salad, interest increased quickly. In 2009, leaves of the PA-plant *Senecio vulgaris* were found in rucola (*Eruca sativa*). In August 2011, the German Federal Institute for Risk Assessment (BfR) published a statement on PAs in honey, followed by an opinion of the European Food Safety Authority (EFSA) about PAs in food and feed in the same year. The occurrence of PAs in tea was reported in 2013 by the BfR and just last year a report on the 'Occurrence of pyrrolizidine alkaloids in food' was published by the EFSA. Research activities targeting PAs in food and feed also increased as can be seen by the number of papers published on that topic.

In the past years, several thousand samples of honey, tea and herbs of different origin have been analyzed. Analysis was done by means of LC-MS/MS after a cleanup step using solid phase extraction. Data evaluation revealed certain patterns of PAs found in honey as well as (herbal) tea. Furthermore, data demonstrates the necessity to reduce PAs in these matrices, e.g., by means as proposed by the Codex Alimentarius in the code of practice for weed control to prevent and reduce pyrrolizidine alkaloid contamination in food and feed.

The challenge for routine laboratories consists in the huge number of structurally closely related PAs. As reference material is only available for about 30 PAs, including their N-oxides, overlapping with other PAs may happen which complicates quantification. Reference material is expensive and availability is variable. Moreover, laboratories include different PAs in their scope. As usually the sum of the analyzed PAs is assessed, comparison of data between different laboratories has to be done with great care.

***Indigofera linnaei* – the Jekyll and Hyde of Australian pasture legumes**

Mary T. Fletcher

Queensland Alliance for Agricultural and Food Innovation, The University of Queensland, Australia;
mary.fletcher@uq.edu.au

The toxicity of poisonous plants containing acute toxins is generally reasonably apparent. Cumulative, non-acute toxins are however often harder to identify and their effects can be more insidious. *Indigofera linnaei* is native across the Indian subcontinent through Southeast Asia to Papua New Guinea and northern Australia, and contains 2 toxins, the neurotoxin 3-nitropropionic acid (3-NPA) and the cumulative hepatotoxin indospicine. This deep-rooted legume (known in Australia as 'Birdsville indigo') has a seasonally variable prevalence in pasture, and can at times be a predominant plant in some arid regions of central and northern Australia. Horses consuming *Indigofera linnaei* suffer from a neurological syndrome (Birdsville horse disease), which is more common in the late spring-early summer when this plant is predominant in pasture. Affected horses exhibit a variety of signs, including general weakness and nervousness, particularly incoordination, swaying and toe dragging, with these neurological effects attributed to the presence of glycosides of 3-NPA in this plant. In ruminants, 3-NPA is metabolized and detoxified by ruminal bacteria, and cattle and sheep consuming *Indigofera linnaei* are seemingly not affected by the neurological syndrome.

Indospicine toxicosis manifests itself in two ways, acting as both a hepatotoxin and secondly as a teratogen and abortifacient. These effects were first observed in livestock consuming *Indigofera spicata* plant material in Hawaii, with indospicine subsequently identified as the responsible toxin through in vivo rodent toxicity studies. In Australia, livestock consuming *Indigofera linnaei* are not reported to suffer from indospicine hepatotoxicity, but this cumulative toxin has gained notoriety for its ability to form persistent residues in tissues of grazing animals. Ingestion of indospicine-contaminated horse meat and more recently camel meat over prolonged periods has resulted in the secondary poisoning of dogs which are particularly sensitive to indospicine hepatotoxicosis.

Presence of the natural carcinogen ptaquiloside from bracken fern in food and drinking water – what is the evidence?

Lars Holm Rasmussen

Department of Technology, Metropolitan University College, Denmark; lhra@phmetropol.dk

Bracken fern (*Pteridium* sp.) are worldwide problematic weeds due to their abundance, invasive properties and content of carcinogens, most notably ptaquiloside (PTA). Bracken is classified by WHO/IARC as "...possibly carcinogenic to humans. The exposure circumstance entails exposures that are possibly carcinogenic to humans". PTA degrades to a number of carcinogenic metabolites in aqueous environments. Intake of PTA and its metabolites may lead to gastric/esophageal cancer among humans and can take place directly via bracken-based food products (e.g., warabi mochi (Japan) or yucaï (China)), via traditional Chinese medicine or indirectly via contaminated milk, meat or drinking water. Bracken causes a number of syndromes among cattle and sheep, of which the acute bracken disease (internal bleedings) and bovine enzootic hematuria (cancer) are the most prevalent.

PTA is highly water soluble and mobile in soils. Thus, PTA is easily leached from living or dead biomass by mist/rain/snow from where it can cause contamination of drinking water. Degradation of PTA in ground and surface waters is a function of pH and microbiological activity (bacteria/fungi). PTA is most stable in water around pH 4.5. PTA has proven to be more stable in soil solution in laboratory experiments. The reason for this remains unresolved, but may be due to colloidal surface reactions. In 2014, PTA was reported in shallow groundwater below bracken stands in Denmark (up to 90 ng/l) while up to 2,500 ng/l has been found in small streams running out of bracken stands. Based on existing toxicological experiments, a tolerable concentration of 1.5 ng/l has been estimated according to Danish guidelines. The results suggest potential for contamination of drinking water in Denmark and elsewhere. PTA can also be inhaled as PTA is present in bracken spores. In addition, the carcinogenic properties of bracken can be transferred to dairy products and meat. Animal experiments have shown that the carcinogenic properties of PTA are carried into the bloodstream and further into milk with the

carcinogenic degradation products of PTA (i.e., ptaquilosin and/or the bracken dienone, but not PTA in itself). Accordingly, in 2015 PTA/metabolites were reported in the milk of free-roaming sheep and goats in Italy.

The toxicology of bracken fern has been studied intensively for decades. Now, current research proves presence of PTA in the human food chain. Not just in the form of bracken-based food products, but also in drinking water and dairy products, which calls for governmental action.

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Coexposure: If one plant toxin is harmful to livestock, what about two?

Kevin Welch

Poisonous Plant Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, USA; kevin.welch@ars.usda.gov

Many of the plants in a given rangeland provide valuable forage for livestock species. However, plants that can poison livestock are very much a part of our rangelands. In this regard, most rangelands contain more than one potentially poisonous plant. Historically, research on poisonous plants has focused on a single plant and/or the major toxin within the plant. Consequently for many plants, much is known regarding the toxicity of the individual plant and its effect on livestock. However, little is known regarding the effect of co-exposure to multiple toxic plants, or even the effect of multiple toxins from an individual plant. Mixture toxicology, or the study of the co-exposure to multiple toxins, can result in additive, synergistic, or antagonistic effects. This presentation will highlight some of the recent research from the Poisonous Plant Research Lab wherein the effect of co-administering multiple plant toxins from the same plant and the effect of co-administration of different poisonous plants has been evaluated. A better understanding of the effect of coexposure to multiple poisonous plants, and the mixture toxicology involved, will be useful in developing more beneficial management recommendations for livestock producers.

What are TAs? – Tropane Alkaloids or Troublesome Analytes?

Susan MacDonald

Fera Science Ltd., UK; susan.macdonald@fera.co.uk

Although more than 200 different Tropane Alkaloids (TAs) have been identified in various plants, data on their toxicity and occurrence in food are limited. In 2013, the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM Panel) published an opinion (risk assessment) that was restricted to two compounds. Only minimal occurrence data were available, and a reliable exposure assessment could only be carried out for the two so called *Datura* type TA ((-)-hyoscyamine and (-)-scopolamine)), and this covered only one food group. Consequently, the CONTAM Panel recommended the collection of more occurrence data for various TAs, besides (-)-hyoscyamine and (-)-scopolamine. Since June 2015, the EU Rapid Alert System for Food and Feed (RASFF; http://ec.europa.eu/food/safety/rasff/index_en.htm) has reported a few high-level occurrences of TAs in cereals (and their products) and a new EU Regulation was introduced in 2016 to limit levels of atropine and scopolamine in infant food. Levels were set at 1 µg/kg each alkaloid, which is analytically challenging.

Historically, methods of analysis have concentrated on atropine and scopolamine, however EFSA have stated there is a need to collect occurrence data on other TAs. Multi-analyte methods described in the literature for *Datura* type TA in food products are typically based on GC-MS(/MS) or LC-MS/MS. Thus far, isotopically-labeled internal standards have rarely been used in published methods for *Datura* type TA, however are now available for atropine and scopolamine. GC-MS/MS and LC-MS/MS methods have comparable sensitivity and, in principle, all relevant TAs can be measured with both methods. LC-MS/MS has, however, the advantage that simple sample preparation procedures can be used and derivatization of analytes is not necessary. TAs also have a wide range of molecular weights and analyte polarity. This causes many difficulties when developing extraction, cleanup and chromatography methods and it has proved to be particularly difficult to include the low molecular weight TAs in a multi-analyte method. This presentation will cover some of the technical issues and difficulties encountered in the analysis of TAs in food samples using a multi-analyte LC-MS/MS method and will highlight future issues and challenges associated with the control and monitoring of these naturally occurring plant toxins in food and feed.

Analysis of fluoroacetate in plants

Stephen T. Lee

Poisonous Plant Research Laboratory, Agricultural Research Service, United States Department of Agriculture, USA; stephen.lee@ars.usda.gov

Many plant species worldwide cause sudden death in livestock; a number of these species are suspected, or known, to contain the toxic compound monofluoroacetate (MFA). Clinical signs associated with sudden death are loss of balance, ataxia, labored breathing, muscle tremors, and recumbency leading to death. Plants associated with sudden death in livestock, and that have been shown to produce MFA, include *Dichapetalum* spp. native to southern Africa; *Acacia georginae* and *Gastrolobium* spp. in Australia; and *Tanaecium bilabiatum* and *Palicourea marcgravii* in Brazil. Numerous other plant genera in Brazil, including *Palicourea*, *Amorimia*, *Neidenzuella*, and *Fridericia*, are reported to cause sudden death in livestock and are suspected to contain MFA due to the similarity of clinical signs; however, the presence of MFA has not been verified in these species.

In this regard, a rapid HPLC-APCI-MS method to detect and quantify MFA in plant tissue was developed and was used to analyze plant material from field collections and herbarium specimens of plants throughout the world, which are known to cause sudden death in livestock and are suspected to contain MFA.

How reliable are current analytical methods to determine tropane alkaloids in food and tea infusions at levels of interest?

Carlos Goncalves and J. Stroka

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Belgium; carlos.goncalves@ec.europa.eu

Tropane alkaloids (TAs) are plant toxins that occur mainly in *Datura*, *Atropa* and *Hyocyamus* sp. belonging to the *Solanaceae* family, besides a variety of other families, such as *Erythroxylaceae*, *Brassicaceae*, *Proteaceae*, *Euphorbiaceae*, *Rhizophoraceae*, *Convolvulaceae* and *Cruciferae*. The class contains over 200 compounds, but the most common TAs are atropine and scopolamine. They may cause anticholinergic poisoning, e.g., blurred vision, pupil dilation, dry mouth, vomiting, clouded consciousness, muscle spasms, low body temperature, hallucinations, tachycardia, and even death. TAs occur in all parts of the plants and botanical impurities have been found in a range of crops due to accidental contamination during harvesting. A survey conducted in the Netherlands in 2011, 2012 and 2014 investigating the presence of TAs in cereal-based food for infants and young children resulted in average TA levels of 4.6, 4.4 and 0.5 µg/kg, respectively, with maximum levels of 80.8, 57.6 and 3.9 µg/kg. The EFSA CONTAM Panel established a group acute reference dose (ARfD) of 0.016 µg/kg body weight expressed as the sum of (-)-hyoscyamine and (-)-scopolamine. Such ARfD would actually have been exceeded for 8 of the 93 products sampled in 2011 and 2012.

In 2015, the European Commission has published a recommendation (Commission Recommendation (EU) 2015/976) to monitor for the presence of tropane alkaloids in food, in particular: cereals and cereal-derived products, gluten-free products, food supplements, teas and herbal infusions and, legume vegetables, pulses and oilseeds and derived products. Two proficiency tests (PT) were organized by the EURL-mycotoxins aiming to underpin and assess the measurement capability of member states (MS) laboratories concerning the recent legislation on TAs in cereal-based food for infants and young children (Commission Regulation (EU) 2016/239) as well as tea and herbal infusions. Special focus was given to levels relevant for legislation enforcement. Forty-eight datasets for cereals from 18 EU MS laboratories were received. Overall, 84% of the z-scores were in the range of [-2,2] and 91% were in the range of [-3,3]. At the lowest TA level, 78.6% of z-scores fell in an acceptable range while this figure improved to 89.9% at the highest level. These results support the assumption that atropine and scopolamine can be reliably determined at levels that ensure protection of baby and infants population. The results received so far for tea and herbs also show good measurement capability, though the envisaged levels were higher. Matrix complexity (peppermint leaves>black tea>fennel seeds) seems to play an important role on the methods performance. The wealth of data collected (similar to a method validation study) will allow correlating the results obtained with the origin of the standards, sophistication of the instrumentation used, experience and procedural aspects, which will be discussed in detail in this presentation.

Pyrrrolizidine alkaloids in animal forage and impacts on feed safety

Christoph Gottschalk¹, J. Ostertag², B. Biermaier¹, V. Schnittke¹, K. Meyer³ and M. Gareis¹

¹Chair of Food Safety, Veterinary Faculty, Ludwig-Maximilians-University Munich, ²Institute for Animal Nutrition and Feed Management, Bavarian State Research Center for Agriculture and ³Chair of Animal Hygiene, Technische Universität München, Germany;
christoph.gottschalk@ls.vetmed.uni-muenchen.de

Pyrrrolizidine alkaloids (PA) and their N-oxides (PANO) are a group of more than 600 compounds produced primarily by plants of the *Asteraceae*, *Boraginaceae*, and *Leguminosae* families. Altogether, more than 6,000 plant species worldwide are expected to contain these alkaloids. PA/PANO are mono- or diesters of 7-hydroxy-1-hydroxymethyl-pyrrrolizidine (necine base) and aliphatic mono- or dicarboxylic acids (necic acids). 1,2-dehydro-PA exhibit acute hepatotoxic and carcinogenic properties [1]. In the past few years, an increasing spread of PA containing plants, such as *Senecio* spp. (e.g., common groundsel, tansy ragwort, marsh ragwort) attracted the attention of farmers, food and feed industry, and responsible authorities.

Intoxications of livestock due to an (accidental) ingestion of PA containing plants or contaminated forage are known for decades. However, data on PA-contents in animal feed or critical doses of intake are very limited [1]. Stegelmeier reported a lethal daily dose of 2.5 mg PA/kg body weight in cattle after an administration of PA from *Senecio jacobaea* during 18 days [2]. Additionally, the stability of PA and PANO during forage preservation and the different susceptibility of animal species have strong impacts on the risk assessment of feed (and food of animal origin) but it is not yet thoroughly known whether a decrease of (measurable) PA-levels in feed actually equals a detoxification of PA [3].

In this lecture, we will give an overview on currently available data on PA/PANO contents in various feedstuffs and on the current knowledge as regards their behaviour during preservation. We also will discuss a first attempt to derive practical recommendations for farmers in the framework of an ongoing research project on marsh ragwort in Bavaria, Germany.

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A New Zealand collaborative industry approach to manage and mitigate the risk of pyrrrolizidine alkaloids in honey.

Jane Lancaster

Catalyst Ltd., New Zealand; jane.lancaster@catalystnz.co.nz

In 2012, the New Zealand Bee Products Industry commenced a comprehensive program to understand the risk to the industry from the occurrence of pyrrrolizidine alkaloids (PAs) in honey from the pollen/nectar of plants visited by bees. An integrated program was undertaken to develop analytical data on the occurrence of PAs in honey and data on the geographical and botanical origin of PA containing plants, including GIS mapping. Echimidine, a PA known to occur in some mono floral honeys, was purified to enable acute and long term toxicological studies to be carried out.

The program has delivered information to the industry to enable a proactive response so that New Zealand honey is positioned to meet future international regulations and that market access is maintained. The programs also provided information to Food Standards Australia New Zealand (FSANZ) and the Ministry for Primary Industries to play a leading role internationally in standards setting. The program shows the importance of taking a supply chain perspective on PAs.

Pyrrrolizidine alkaloids in natural products – a new and enormous challenge for the tea and herbal infusions industry

Maximilian Wittig

Tea & Herbal Infusions Europe (THIE), Germany; mwi@wga-hh.de

Pyrrrolizidine alkaloids (PAs) are secondary metabolites produced by a large number of plant species all over the world. The presence of traces of PAs in teas and herbal infusions first became an issue through studies recently published by the German Federal Institute for Risk Assessment (BfR) [1] and the European Food Safety Authority (EFSA) [2]. Prior to this a new highly sensitive analytical method still needed to be developed to determine trace levels of PAs in plant material. Consequently, based on these new insights, a comprehensive cause study was conducted under the leadership of the European association Tea & Herbal Infusions Europe (THIE), which represents the interests of producers and traders of tea (*Camellia sinensis*) and herbal infusions, as well as extracts thereof, in the EU. In the course of this, the unintentional co-harvesting of PA containing weeds was identified as a source of contamination that could now be uncovered by applying the new analytical method. The whole issue of PAs has turned out to show a dimension which by far surpasses all previous standards of good agricultural practice. Even a small number of PA containing plants, such as *Senecio* sp. or *Heliotropium* sp., is sufficient to generate considerable levels of PAs in the harvest and therefore also in teas as well as in herbal infusions. Analytical findings show that raw materials for fruit infusions are significantly less affected by the PA issue. Results from retention samples indicate that the PA issue is fundamentally not a new phenomenon. It is rather a new and important finding which could be discovered due to the rapid technical progress in the field of trace analysis.

In order to reduce PAs in their products, THIE members immediately set up an action plan along the process chain – from field to cup – to instantly eliminate PA contamination peaks and to gradually lower PA levels to the best possible extent following the ALARA principle ('as low as reasonably achievable'). Despite greatest efforts, it is not expected that a significant improvement in the situation can be achieved in a short run. Besides that, complete freedom from PAs in natural products, such as teas and herbal infusions, will be very hard to achieve, as far as can be judged today. It is reasonable to assume that the tea industry as well as producers/manufacturers from other areas of the food business are confronted with a new and enormous challenge, which has developed into a serious and currently underestimated problem of global dimensions.

By means of demonstrative examples and pictures from practice, the tremendous challenge for the tea industry in fighting this fundamental weed problem, the range of various measures taken, different approaches of research projects and first successes in the reduction of PAs in teas and herbal infusions, are to be presented.

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WEDNESDAY, JUNE 8, 2016 – Session 6

Short presentations by selected poster presenters to provide an overview of their research and inspire the audience to visit their poster.

P3

Metabolism of pyrrolizidine alkaloid ester types in liver tissue fractions of farm animals

Dorina Bodi

Department Safety in the Food Chain, Federal Institute for Risk Assessment, Germany

P5

Screening of pyrrolizidine alkaloids in Asian herbal teas

Wellyana Chua

Health Sciences Authority, Singapore

P11

Presence of plant toxins in food supplements

Hans Mol

RIKILT Wageningen UR, the Netherlands

P17

Detection of pyrrolizidine alkaloids in milk and honey on the Belgian market using UHPLC-MS/MS

Jean-François Picron

Food, Medicines and Consumer Safety Department, Scientific Institute of Public Health (WIV-ISP), Belgium

Can unmanned aircraft systems reduce plant poisoning risk in livestock?

Deon van der Merwe

College of Veterinary Medicine, Kansas State University, USA; dmerwe@vet.k-state.edu

Development of rapid inventory methods based on remote sensing of plants in pastures that are relevant to animal health, including toxic plants and sources of critical nutrients, could decrease the resources needed for assessment of risk and/or pasture value. Small unmanned aircraft systems (sUAS) have the potential to massively increase the efficiency and accuracy of pasture assessment at the individual farm and field level to assist in the identification and quantification of risks, and to support livestock management decisions. The key factor that differentiates this type of remote sensing from traditional methods is that sensors are positioned at relatively low altitude, resulting in ultra-high spatial resolution and accessibility to shorter wavelengths of light that are not available to high altitude sensors due to excessive light scatter. Important factors in the successful implementation of this assessment method include the use of appropriate sensors and aircraft, suitable flight plans aimed at reducing imaging and mapping artifacts, and a thorough understanding of the phenological progression of species that determine the time periods for adequate differentiation of species. Species differentiation enables more efficient risk assessment, and therefore more effective risk management.

Poisonous plants and their toxins – new global challenges? Overview and conclusions

Monique de Nijs

RIKILT Wageningen UR, the Netherlands; monique.denijs@wur.nl

The food safety community worldwide has come to realize that plant toxins from poisonous plants in our food, a topic on the horizon two years ago, are real hazards. These hazards can no longer be ignored, and have already resulted in establishment of legislation on the plant toxins in food in, among others, New Zealand, Australia and the EU.

Developments in analytical methods, mainly multimethods, enable the analysis of many different foods and plant toxins but there is lack of reference material and standards to chemically identify/quantify new threats. Research on poisonous plants has traditionally focused on plant toxins in food supplements, due to incidents in the past. However, surveys show that plant toxins are also present in daily food products, such as pyrrolizidine alkaloids in honey and tea in Western Europe; both pyrrolizidine alkaloids and tropane alkaloids in cereal-based foods and even ptaquiloside in our drinking water. Contamination results from either intentional or unintentional addition of poisonous plants or from unintentional co-harvesting of weeds containing plant toxins. Recent surveys focus on endogenous plant toxins in food plants, such as cyanogenic glycosides in cassava, alkenylbenzenes in herbs, quinolizidine alkaloids in lupine and calystegines in *Solanaceae*, such as potatoes and aubergines. A major gap in knowledge, however, lies in understanding the toxic effects of the plant toxins, which makes risk assessments difficult.

Societal changes, such as related to food security, introduce the trend of bringing agricultural by-products to value. This holds the risks for introducing new or more plant toxins in the food and feed chain, e.g., from the by-products of biofuel production such as from *Ricinus communis*. New trends in preparations and treatments of foods, such as raw food diets, may result in unsafe foods that were formerly safe due to proper processing. Economic changes may lead to more complex or quickly changing supply chains with the risk of loss of insight into food quality and food safety.

Despite many gaps in knowledge on exposure and toxic effects, agronomic management and quality control measures play a major role in preventing the problem. The increase in number and species of weeds in the field may result from the decreased use of herbicides, which in itself is a highly desired development. Quality assurance in the food supply chain will prevent mingling of undesired with desired plants, such as was the case in an incident in the Netherlands in 2013 where a tropane alkaloid containing plant was mistaken for marshmallow root tea. Treatment of animals with herbal medicines to reduce the

use of antibiotics, holds the risk for intoxications of the animals and carry-over of plant toxins to foods of animal origin.

It is clear that plant toxins in food are a global challenge which requires bringing knowledge and solutions together on an international base to come to control measures. Increased knowledge and regulation will have an effect on international trade. The presentation will give an overview on some of the examples and trends mentioned above in reflection to the topics discussed at the 2nd conference of The World Plant Toxin Forum.

POSTERS

- P1 *A fast dilute-and-shoot method for the determination of 30 pyrrolizidine alkaloids and related N-oxides in plant materials using LC-Q-Orbitrap-MS analysis*
J.S. Mänz, S. Staiger and **Scarlett Biselli**
Eurofins, Germany
- P2 *The course of pyrrolizidine alkaloid profile of *Senecio vernalis* – a greenhouse pilot study*
Dorina Bodi¹, A. Krähmer², A. Preiss-Weigert¹, M. Lahrssen-Wiederholt¹
¹Department Safety in the Food Chain, Federal Institute for Risk Assessment and ²Julius-Kühn-Institute, Federal Research Centre for Cultivated Plants, Germany
- P3 *Metabolism of pyrrolizidine alkaloid ester types in liver tissue fractions of farm animals*
F. Kolrep, I. Schirrmann, **Dorina Bodi**, A. Preiss-Weigert, M. Lahrssen-Wiederholt and A. These
Department Safety in the Food Chain, Federal Institute for Risk Assessment, Germany
- P4 *Development of a planar waveguide method for tropane alkaloids*
C. Deng, S. McNamee and **Katrina Campbell**
Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, UK
- P5 *Screening of pyrrolizidine alkaloids in Asian herbal teas*
Wellyana Chua and S.H. Chan
Health Sciences Authority, Singapore
- P6 *Determination of tropane alkaloids in buckwheat-based organic food*
M. Cirlini¹, T. Demuth^{1,2}, R. Bruni¹, A. Biancardi³ and **Chiara Dall'Asta**¹
¹Department of Food Science, University of Parma, Italy, ²Department for Analytical and Food Chemistry, Technical University of Munich, Germany and ³Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Italy
- P7 *Accumulation of indospicine in camels fed a diet containing Indigofera plant material*
E.T.T. Tan^{1,2}, R.A.M. Al Jassim¹, A.J. Cawdell-Smith¹, S.M. Ossedryver³, B.R. D'Arcy¹ and **Mary T. Fletcher**¹
¹The University of Queensland, Australia, ²Universiti Teknologi MARA, Malaysia and ³Queensland Department of Agriculture and Fisheries, Australia
- P8 *A UK survey of honey, teas and herbal supplements for pyrrolizidine alkaloids*
C. Crews, A. Lloyd and **Susan MacDonald**
Fera Science Ltd., UK
- P9 *Determination of linamarin and lotaustralin in cassava flour by LC-MS/MS*
P. López, D. van Horst and **Hans Mol**
RIKILT Wageningen UR, the Netherlands
- P10 *Determination of hypoglycin-A in maple tree species*
Hans Mol, R. van Leeuwen, L. van Raamsdonk and M. de Nijs
RIKILT Wageningen UR, the Netherlands
- P11 *Presence of plant toxins in food supplements*
R. van Dam¹, J. Biesterbos² and **Hans Mol**¹
¹RIKILT Wageningen UR, the Netherlands and ²Netherlands Food and Consumer Product Safety Authority (NVWA), the Netherlands
- P12 *Tropane and ergot alkaloids in grain-based products for infants and young children in the Netherlands 2011-2014*
Patrick Mulder¹, D. Pereboom-de Fauw¹, R. Hoogenboom¹, J. de Stoppelaar² and M. de Nijs¹
¹RIKILT Wageningen UR and ²Netherlands Food and Consumer Product Safety Authority (NVWA), the Netherlands

- P13 *Determination of quinolizidine alkaloids in lupin flour by LC-MS/MS: a promising novel way of analysis*
Patrick Mulder and M. de Nijs
RIKILT Wageningen UR, the Netherlands
- P14 *Opium alkaloids in poppy seeds and food products*
P. López, **Patrick Mulder**, D. Pereboom-de Fauw, H. Mol and M. de Nijs
RIKILT Wageningen UR, the Netherlands
- P15 *Potato glycoalkaloids in the Netherlands*
P. López, P. Mulder, E. de Vries and **Monique de Nijs**
RIKILT Wageningen UR, the Netherlands
- P16 *Development of method for determination of tropane alkaloids and pyrrolizidine alkaloids in herbal tea*
Ock Jin Paek, S.H. Kim, W.S. Nam, D.S. Kim and S. Suh
Food Contaminants Division, National Institute of Food and Drug Safety Evaluation, Ministry of Food and Drug Safety, Republic of Korea
- P17 *Detection of pyrrolizidine alkaloids in milk and honey on the Belgian market using UHPLC-MS/MS*
Jean-François Picron, S. Gosciny, M. Andjelkovic and E. Van Hoeck
Food, Medicines and Consumer Safety Department, Scientific Institute of Public Health (WIV-ISP), Belgium
- P18 *Presence of ptaquiloside in bracken-based food, food supplements and alternative medicine*
Lars Holm Rasmussen
Department of Technology, Metropolitan University College, Denmark
- P19 *Ptaquiloside in surface water – development of a versatile HPLC-based procedure for screening of water samples*
Lars Holm Rasmussen, J. Pedersen and D.N. Lindqvist
Department of Technology, Metropolitan University College, Denmark
- P20 *Degradation of the carcinogen ptaquiloside from bracken fern in rain and surface water*
Janni Sandersen, D.N. Lindqvist and L.H. Rasmussen
Department of Technology, Metropolitan University College, Denmark

P1: A fast dilute-and-shoot method for the determination of 30 pyrrolizidine alkaloids and related N-oxides in plant materials using LC-Q-Orbitrap-MS analysis

J.S. Mänz, S. Staiger and **Scarlett Biselli**

Eurofins, Germany. Corresponding author: scarlettbiselli@eurofins.de

Pyrrolizidine alkaloids (PAs) are a group of food contaminants which are exclusively biosynthesized by plants. So far, more than 600 different PAs and PA N-oxides are known and it has been estimated that approximately 6,000 plant species, representing 3% of all flowering plants, are able to express PAs. Typical plant families are *Boraginaceae*, *Asteraceae* and *Fabaceae*. PAs are derived from the chemical structure of pyrrolizidine and can be subgrouped into retronecine-, heliotridine-, crotanecine-, supinidine- and otonecine-type PAs. In particular, the 1,2-unsaturated compounds are known to expose hepatotoxic effects and they are under suspicion to be genotoxic and cancerogen. Common sources for PA intoxications of humans and livestock are phytopharmaceuticals, food and feed supplements, herbal teas, milk, honey and silage. In these commodities high concentrations of up to several mg/kg can be found. So far, no regulatory limits have been defined for this group of contaminants in food and feed. On this poster a fast dilute-and-shoot method followed by LC-Q-Orbitrap analysis for PA identification and quantification in plant materials will be presented. The sample preparation was carried out by a liquid-solid extraction of homogenized sample materials using a mixture of acidified water/methanol followed by shaking, centrifugation and dilution of the supernatant. For analysis, an Ultimate 3000 UHPLC system coupled to a Q-Exactive Orbitrap mass spectrometer was used. The mass spectrometer was operated in fullscan + data dependent MS2 acquisition mode (ESI+) with a resolving power of 70,000 FWHM. The analytes were identified by verification of their exact mass, isotopic pattern and MS/MS fragmentation. As there are no internal standards available on the commercial market so far, quantifications were performed using matrix matched calibrations. In order to assess the fitness-for-purpose of the method a validation was performed by PA fortification of blank herbal tea extracts in a concentration range of 0.5-10 ng/ml (representing 10-200 ppb sample concentration). The linearity was found to be very good with $r^2 \geq 0.9994$ for 29 out of 30 compounds. The precision and LOQs of the method was determined by a six-fold determination of fortified extracts at 10 and 40 ppb. The results show an RSD of 1.4 ± 0.7 % and LOQs of 2.6 ± 1.2 ppb. Further performance parameters regarding the analytical method will be presented on the poster.

P2: The course of pyrrolizidine alkaloid profile of *Senecio vernalis* – a greenhouse pilot study

Dorina Bodi¹, A. Krähmer², A. Preiss-Weigert¹, M. Lahrssen-Wiederholt¹

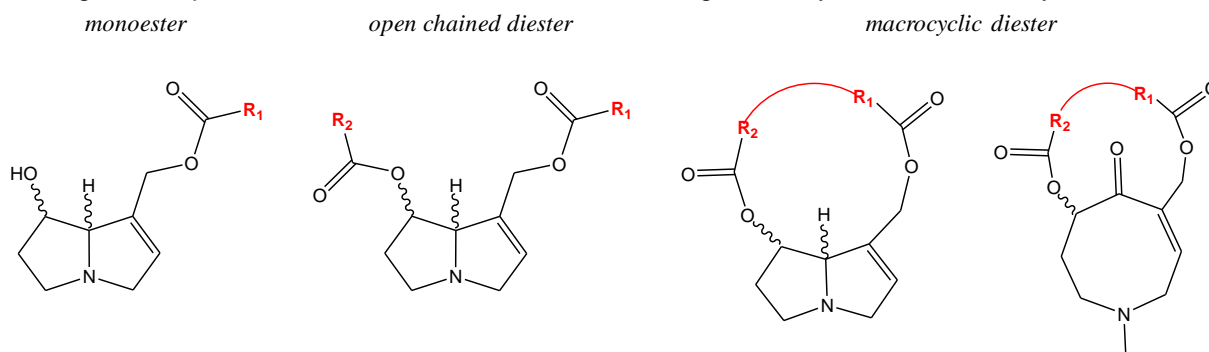
¹Department Safety in the Food Chain, Federal Institute for Risk Assessment and ²Julius-Kühn-Institute, Federal Research Centre for Cultivated Plants, Germany. Corresponding author: dorina.bodi@bfr.bund.de

Senecio vernalis is one of a large variety of pyrrolizidine alkaloid (PA) producing plant species. Those often toxic secondary plant metabolites are synthesized by 3% of the flowering plants as a matter of plant defense. Ingestion of high doses results in acute liver damage. In animal studies some PAs have proven to be genotoxic carcinogens, therefore, PA producing plants like *Senecio vernalis* are undesired material in feed and food (<http://www.bfr.bund.de/cm/349/pyrrolizidine-alkaloids-in-herbal-teas-and-teas.pdf>). There are numerous reports of losses in livestock due to feeding from pastures, hay or grass silage containing PA producing plants, demonstrating the need of appropriate pasture care. Knowledge on the dependence of PA contents on seasons, environmental conditions and plant age might help to develop pasture cutting strategies to minimize the PA content in derived feed products. As a first step, we recorded the course of the PA profile and content in the leaves of 60 *Senecio vernalis* plants grown in a greenhouse over 22 weeks. Samples of one leaf per plant were taken every week and analyzed individually. Qualitative and quantitative PA determination was accomplished by LC-MS/MS analysis (Bodi *et al.*, 2014) after a simple extraction with aqueous sulfuric acid and subsequent dilution. The scope of the LC-MS/MS-method covered 28 individual PA, while only 7 of them (retrorsine, retrorsine-N-oxide, seneciphylline, seneciphylline-N-oxide, senecionine, senecionine-N-oxide, senecivernine, senecivernine-N-oxide and senkirkine) were detected in the leaves. We observed remarkable variability of PA concentrations in the leaves of plant individuals collected at the same point in time. However, average concentrations of all 60 plants showed a similar course over the whole sampling period with the exception of retrorsine and retrorsine-N-oxide. The most prominent PA was senkirkine reaching average concentrations of up to 23 mg/kg. Total PA concentrations ranged between 11 mg/kg and 75 mg/kg. Based on these primary data received under artificial conditions free range approaches will be carried out in order to reveal the impact of multiple environmental factors on the PA concentration in *Senecio vernalis*.

P3: Metabolism of pyrrolizidine alkaloid ester types in liver tissue fractions of farm animals

F. Kolrep, I. Schirrmann, **Dorina Bodi**, A. Preiss-Weigert, M. Lahrssen-Wiederholt and A. These
Department Safety in the Food Chain, Federal Institute for Risk Assessment, Germany.
Corresponding author: dorina.bodi@bfr.bund.de

Pyrrolizidine alkaloids (PAs) are nitrogen-based secondary ester alkaloids produced by plant species of families within the angiosperms. The common structure moiety of all PAs consists of a few necine bases that are esterified with one or more necic acids. Some PAs are considered as genotoxic and acute liver damage was reported for animals and humans. PAs in silage and hay are unintentionally administered



to livestock. As soon as PAs are transformed, the targets for analysis in food become unknown, because to date no metabolite is structurally elucidated. The possibility that humans are exposed to PA-metabolites through consumption of food products of animal origin, such as milk or meat, prompted us to evaluate biotransformation of PA in farm animals. We investigated the metabolism of diverse PA structure types, in particular monoesters, open chained and macrocyclic diesters of the retronecine-, heliotridine- and otonecine type. PA reference substances were incubated at 37°C in an NADPH-regenerating system simulating phase I metabolism (S9-mix). The reactions were stopped at various points in time. Sensitivity to PAs is known to be different in various animals. Therefore, PA metabolism of a high number of species was investigated. PAs were incubated with S9 liver fractions of cow, horse, goat, sheep, pig, or rabbit and analyzed by liquid chromatography in combination with mass spectrometry. The metabolic degradation rates were detected for each individual PA within the respective species fraction. Furthermore, fractions were investigated for typical phase I metabolites. Results confirm the effective biotransformation of all PA structure types in animals. This highlights that methods in use for analysis of herbal food are not useful for the investigation of animal foodstuffs. Rather, the need for analytical methods that enable the detection of PA metabolite profiles is demonstrated. Furthermore, determined PA degradation rates varied and depended on PA structures as well as on animal species. Results show unexpected relations between metabolic conversion rates and reported species susceptibility and will provide new insight in PA metabolism.

P4: Development of a planar waveguide method for tropane alkaloids

C. Deng, S. McNamee and **Katrina Campbell**
Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, UK.
Corresponding author: katrina.campbell@qub.ac.uk

In 2014, the occurrence of tropane alkaloids in baby food raised media and public concern. The European Union just recently set limits for tropane alkaloids in cereal-based baby foods and toddler foods to lower exposure risks to the naturally occurring contaminant. The limit of quantification (LOQ) for atropine (racemic mixture of hyoscyamine enantiomers) and scopolamine should be preferably below 5 µg/kg and not higher 10 µg/kg for agricultural commodities, ingredients, food supplements and herbal teas and should preferably be lower than 2 µg/kg for finished foods (e.g., breakfast cereals) and 1 µg/kg for cereal-based foods for infants and young children. The regulatory data for tropane alkaloids was not clear until now and the clarification of which has caused analysts to reconsider the detection of tropane alkaloids and whereby investigations to identify the agricultural conditions resulting in the presence and prevention of tropane alkaloids should be considered. Therefore, a rapid, accurate, sensitive and highly selective detection method is needed for food and feed analysis of tropane alkaloids. The purpose of this investigation was the development and validation of a rapid immunoassay for the detection of tropane alkaloids. MBio planar waveguide technology was applied in this study for the detection of multiple tropane alkaloids and whereby limited sample preparation was required. Multiplexed calibration curves (matrix-normalized data curves) were produced with high sensitivity. For atropine and scopolamine, the preliminary studies show a linear range (IC₂₀-IC₈₀) of 0.8-5.0 ng/ml and 0.2-3.3 ng/ml,

respectively. These linear ranges correlate to lower than the new legislation. Contaminated samples require only simple sample dilution to bring positive samples within the range of quantification. Moreover, the recovery in this assay of atropine is 91% and of scopolamine is 89%. This could offer a rapid tool for analysis in the feed and food supply chain.

P5: Screening of pyrrolizidine alkaloids in Asian herbal teas

Wellyana Chua and S.H. Chan

Health Sciences Authority, Singapore. Corresponding author: wellyana@hsa.gov.sg

Pyrrolizidine alkaloids (PA) are secondary metabolites produced by estimated 6,000 species of plants, specifically the *Asteraceae*, *Boraginaceae*, *Fabaceae*, *Orchidaceae* and *Apocynaceae* families, as a defense mechanism against herbivores. More than 600 different PA (including their N-oxides) are known, and new PA continue to be identified on a regular basis in both new and previously studied plant species. PA have been identified by many regulatory bodies such as the FAO, EU as an emerging contaminant. However, there is a lack of occurrence data in the food supply chain. The PA content in plants varies widely, depending on factors such as plant species, plant organ and environmental conditions (climate, soil properties, etc). Based on scientific opinions and discussion papers on PA in food and feed published by EFSA (2011) and the Federal Institute for Risk Assessment (2011, 2013), the PA have been proven to be hepatotoxic, as they can lead to acute and chronic liver cirrhosis. 1,2-unsaturated PA, in particular, have been concluded by EFSA as being genotoxic carcinogens in humans. It was advised that dietary exposure to PA be minimized. The main route of PA exposure for consumers is from co-harvesting of crops intended for human and animal as well as carry-over into animal based products. Honey has been comprehensively analyzed and found to be one of the main food sources of human PA exposure. Recently, the potential human exposure of PA from teas has been gaining interests from the EU, due to high levels of PA found in commercially available teas in Germany and Switzerland. The objective of this study was to screen for the presence of PA in Asian herbal teas, such as chrysanthemum, jasmine, roselle, rose, oolong and pu-erh teas sold in the Singapore retail markets. This work presents an analytical method for identification of 19 different PA, including their N-oxides, in Asian herbal teas using LC-MS/MS. Results of the analysis are discussed in the view of the current discussion on PA in the food chain with special focus on Asian herbal teas widely found in the southeast Asian regions. The results are presented as part of a survey to ascertain the exposure of the population to PA through consumption of herbal teas.

P6: Determination of tropane alkaloids in buckwheat-based organic food

M. Cirlini¹, T. Demuth^{1,2}, R. Bruni¹, A. Biancardi³ and **Chiara Dall'Asta**¹

¹Department of Food Science, University of Parma, Italy, ²Department for Analytical and Food Chemistry, Technical University of Munich, Germany and ³Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Italy. Corresponding author: chiara.dallasta@unipr.it

Tropane alkaloids (TAs) are secondary metabolites which are naturally produced by different species of plants, including *Brassicaceae* and *Solanaceae* (i.e., *Datura stramonium* L.). More than 200 different TAs were identified in plants, but data about the toxicity and the occurrence of them in feed and food are very limited. The most studied TAs are scopolamine and atropine. In fact, TAs can influence the human central nervous system and bring to alteration of movement and of heart rate, secretion of salivary glands, dilatation of pupils and gastrointestinal problems. On the one hand, these compounds could naturally occur in plants; on the other side, there is the possibility of a carry-over in food or feed. In these case TAs plant producers (i.e., *Datura stramonium* L.) could infest crops and be harvested together with the other plants. In this way, seeds containing alkaloids may contaminate other mixed seeds and products thereof. In 2013, the European Food Safety Authority (EFSA) presented a Scientific Opinion about exposure to tropane alkaloids in humans, linked to the consumption of food containing TAs. As general conclusion, more data concerning the occurrence of TAs, in particular of atropine and scopolamine, are needed in order to better evaluate the actual human exposure. With the aim of getting an overview about the occurrence of TAs in food, a sensitive UHPLC-ESI/MS/MS method based on a 'dilute and shoot' approach was developed, and further tested in an international proficiency test scheme. The method showed very good QA analytical parameters, in particular sensitivity and accuracy. The developed method was then applied on cereal-based organic products containing buckwheat from the EU market.

P7: Accumulation of indospicine in camels fed a diet containing *Indigofera* plant material

E.T.T. Tan^{1,2}, R.A.M. Al Jassim¹, A.J. Cawdell-Smith¹, S.M. Ossedryver³, B.R. D'Arcy¹ and **Mary T. Fletcher¹**

¹The University of Queensland, Australia, ²Universiti Teknologi MARA, Malaysia and ³Queensland Department of Agriculture and Fisheries, Australia. Corresponding author: eddie.tan@uq.net.au

Indospicine (L-2-amino-6-amidinohexanoic acid) is a hepatotoxin produced by certain *Indigofera* plants, especially *Indigofera spicata* and *Indigofera linnaei*. It has caused mild to severe liver disease in animals including cattle, sheep, dogs and rats. Accumulation and distribution of indospicine in animal tissues appears universal but the magnitude of hepatotoxic effects is variable across species, and the effect in humans remains unknown. Camels in central Australia are known to consume *Indigofera* plants and secondary poisoning of dogs has resulted from prolonged dietary exposure to indospicine-contaminated camel meat. This raises industry concern about the accumulation of indospicine in camels harvested from *Indigofera* prevalent regions, and potential secondary health risks for consumers. However, no previous study has investigated the levels and distribution of indospicine in camel tissues after dietary exposure to *Indigofera* plants. In this study, the accumulation and distribution of indospicine in camels was investigated in a feeding trial. Six young camels weighing between 270-390 kg were fed 597 mg *Indigofera spicata* dm/kg bw/day to deliver 337 µg indospicine/kg bw/day for a duration of 32 days. The camels allowed *ad libitum* intake of a roughage based diet consisted of Rhodes grass hay (*Chloris gayana*) and Lucerne hay. Concentrations of indospicine in plasma, muscle biopsy and necropsy samples were quantitated by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). The highest indospicine concentrations were found in pancreas and liver; followed by skeletal muscle, heart and kidney tissues. This study confirms the consumption of *Indigofera spicata* plant by camels leads to indospicine accumulation in muscle at levels similar to that which previously caused secondary intoxication in dogs.

P8: A UK survey of honey, teas and herbal supplements for pyrrolizidine alkaloids

C. Crews, A. Lloyd and **Susan MacDonald**

Fera Science Ltd., UK. Corresponding author: susan.macdonald@fera.co.uk

Pyrrolizidine alkaloids (PAs) are found in many plant families, including *Senecio*, *Eupatorium*, *Symphytum*, *Cynoglossum*, *Heliotropium* and *Crotalaria*. These genera are widely distributed throughout different climates. Each plant species has a characteristic distribution of PAs and a typical ratio of free base to N-oxide. A survey was commissioned by the UK Food Standards Agency to determine the occurrence of 19 PAs in selected products in the UK. The samples analyzed were; 59 *Camelia sinensis* teas (including three fruit flavored instant teas), 69 herbal teas (including non-*Camelia* instant teas), 45 plant supplement preparations and 54 samples of honey. The German Federal Institute for Risk Assessment (BfR) methods were adapted as necessary and applied to measure the PA content of the survey samples (protocol BfR-PA-Tea-2.0/2014). Sample preparation involved extraction into aqueous acid and clean up/concentration on solid phase extraction columns (SPE), based on strong cation exchange (SCX), or C18. PAs were found in some teas and herbal infusions. High levels (echimidine and intermidine) were measured in comfrey tea. A small number of black teas contained detectable PAs (total PAs up to approx. 1mg/kg, comprising combinations of retrorsine, retrorsine N-oxide, senecionine and senecionine N-oxide), and herbal infusions also contained PAs, the highest level was approx. 1 mg/kg total PAs (retrorsine and senecionine compounds). This survey found higher contamination of honey from countries having a warmer climate and in particular a relatively high frequency of low level contamination of Manuka honeys, and higher levels of borage PAs in honey from bees foraging on borage. Four plant supplements were also found to contain low levels of PAs. This survey confirmed that the N-oxide forms were present at higher concentrations than the free base, that certain PAs, notably monocrotaline and its N-oxide were absent, and that lycopsamine, echimidine and senecionine N-oxide were the major contaminating PAs. The sampling of teas is significant for PA analysis due to the inhomogeneous distribution of the contamination. In this work tea bags were mixed well into a composite. Initial brewing experiments were carried out using intact tea bags but these did not correspond to the more homogenous samples taken to determine the PA content of the dry tea. These results highlight that further studies are required in this area as the contamination of individual tea bags are highly variable. Future work is needed to address sampling issues in this area.

P9: Determination of linamarin and lotaustralin in cassava flour by LC-MS/MS

P. López, D. van Horst and **Hans Mol**

RIKILT Wageningen UR, the Netherlands. Corresponding author: patricia.lopezsanchez@wur.nl; hans.mol@wur.nl

The root of cassava plant is considered staple food in many African countries. Cassava root is an excellent source for carbohydrates, but also contains secondary metabolites called cyanogenic glucosides. The most common cyanogenic glucosides in cassava are linamarin and lotaustralin. They can be hydrolyzed to hydrogen cyanide (HCN) by the action of enzymes present in separate compartments in the cell wall. Hydrolysis occurs when the cells are ruptured, e.g. during consumption. HCN poses a risk for health consumers, therefore WHO/FAO limits its content to 10 mg/kg in cassava flours. The European Union set limits for HCN in certain food commodities (Regulation 1334/2008), in spirits drinks (Regulation 110/2008) and in feed material (Directive 2002/32/EC). The aim of this work was the development a method for the determination of the intact cyanogenic glucosides linamarin and lotaustralin in cassava flours, based on liquid chromatography tandem mass spectrometry (LC-MS/MS). The method was successfully validated in terms of linearity, limit of quantification (0.5 mg/kg), selectivity, repeatability (3-7%) and recovery (78-100%) and applied to six cassava flour products. The direct determination of the intact cyanogenic glucosides is straightforward and potentially an easier and faster alternative to the determination of total HCN. To verify this, the cassava flours were also analyzed for total HCN according to an existing European standard (EN16160), and the results were compared to the LC-MS/MS method.

P10: Determination of hypoglycin-A in maple tree species

Hans Mol, R. van Leeuwen, L. van Raamsdonk and M. de Nijs

RIKILT Wageningen UR, the Netherlands. Corresponding author: hans.mol@wur.nl

Hypoglycin-A is a plant toxin present in ackee (*Blighia sapida*), a fruit that grows in West Africa and the Caribbean. Consumption of the unripe fruit is associated with an illness known as Jamaican vomiting disease which may be fatal to humans. Hypoglycin-A also occurs in the seeds of several species of maple (*Acer* spp.) and there are strong indications that the presence of this compound is related to equine atypical myopathy in Europe (Westermann *et al.*, 2016) (called seasonal pasture myopathy in North America), which is often fatal to horses. Hypoglycin-A is a highly polar toxic amino acid that is not retained in reversed phase LC, and cannot be detected by UV due to lack of a chromophoric group. Existing analytical methods therefore involve a derivatization step followed by LC with either UV, fluorescence or MS/MS detection. In this work, we developed a more straightforward method based on extraction with water and direct injection of the (diluted) extract onto an Obelisc R column. This is a reversed phase type column but has cationic groups close to the silica surface separated from anionic groups by a hydrophobic chain. This column provides adequate retention for direct analysis. Detection is by tandem mass spectrometry measuring two transitions (m/z 142.1→74.1 and 142.1→96.0). The method was successfully validated for seeds, leaves, and seedlings of *Acer* spp. at levels down to 0.2 mg/kg in leaves and 1 mg/kg in seeds. The method was applied to investigate the presence of hypoglycin-A in *Acer* species. In total, 24 different seed samples were collected, covering multiple species of four sections of *Acer* spp. In three sections, hypoglycin-A was present. The highest levels, median 970 mg/kg, were found in samples from *A. section Acer* (4 species, 6 samples). High levels (median 218 mg/kg) were also found in *A. section Negundo* (2 species, 2 samples). Much lower levels, median 7 mg/kg, were found in *A. section Palmata* (1 species, 3 samples). No hypoglycin-A was detected in all samples from *A. section Platanoidea* (3 species, 7 samples). The results of the survey indicate that the presence of hypoglycin-A is section related. **Acknowledgments.** Dedde Smid and Mirjam Lemmens of the Belmonte Arboretum, Wageningen, the Netherlands (<http://belmonte.arboretumexplorer.org>) are acknowledged for the collaboration on collection of seeds from various *Acer* species.

P11: Presence of plant toxins in food supplements

R. van Dam¹, J. Biesterbos² and **Hans Mol**¹

¹RIKILT Wageningen UR, the Netherlands and ²Netherlands Food and Consumer Product Safety Authority (NVWA), the Netherlands. Corresponding author: hans.mol@wur.nl

Many food supplements are herbal preparations, often containing mixtures of dried herbs. They are sold in the form of tablets, capsules or herbal tea. Plant toxins may occur in such food supplements as inherent secondary plant metabolite of the plant used, or as a result of cross-contamination with non-intended plants. In addition, food supplements may be enriched with either natural or synthetic bioactive

substances. The European Food Safety Authority (EFSA) has increased concerns regarding plant toxins and compiled a compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern (EFSA, 2012), but so far, at an EU level, plant toxins are hardly regulated. In the Netherlands, legislation on herbal preparations exists (Dutch Commodities Act 'Herbal preparations', 2001) which states that toxic pyrrolizidine alkaloids should not exceed 1 µg/kg, and that aristolochic acids and yohimbe alkaloids should be absent. Besides, herbal preparations should not contain plant material derived from certain plant species (exclusion list).

In this work we present the results of a survey of approximately 100 food supplements marketed as weight loss products, performance enhancers and sexual enhancers. Samples were extracted using a mixture of acetonitrile/water and then first analyzed by liquid chromatography with full scan high resolution mass spectrometry (LC-HRMS) Mol *et al.*, 2011). A targeted search of the raw data was performed against a database with plant toxins (and pharmaceuticals, beyond the scope of this presentation). Positive samples were confirmed and quantified by LC with tandem MS/MS.

In total 14 'substances of concern' were detected. Most frequently detected substances were icariin (up to 400 mg/daily dose), synephrine (up to 45 mg/dose), higenamine (up to 88 mg/dose), yohimbine (up to 13 mg/dose), and ephedrine (up to 6 mg/dose). In some cases, enrichment or addition of the synthetic substance was suspected. Other toxins included tropane alkaloids, pyrrolizidine alkaloids, strychnine and THC.

P12: Tropane and ergot alkaloids in grain-based products for infants and young children in the Netherlands 2011-2014

Patrick Mulder¹, D. Pereboom-de Fauw¹, R. Hoogenboom¹, J. de Stoppelaar² and M. de Nijs¹
¹RIKILT Wageningen UR and ²Netherlands Food and Consumer Product Safety Authority (NVWA), the Netherlands. Corresponding author: patrick.mulder@wur.nl

Mycotoxins and plant toxins can contaminate cereals during the field period and after harvest. Subsequently, produced foods can contain these contaminants in quantities harmful for human health. EFSA published in 2012 an opinion on ergot alkaloids (EAs) in which a tolerable daily intake (TDI) was proposed and in 2013 an opinion on tropane alkaloids (TAs) in which they set an acute reference dose (ARfD). Based on these opinions the European Union has recently introduced legislation for ergot alkaloids (Commission Regulation (EU) 2015/1940) and for atropine and scopolamine (Commission Regulation (EU) 2016/239). An LC-MS/MS multi-method was developed to simultaneously quantify 20 EAs and 6 TAs in cereal-based food products. Limit of detection was 0.1-0.2 µg/kg for EAs and of 0.2-0.5 µg/kg for TAs. The method was used to analyze 113 cereal-based food samples (breakfast cereals and biscuits) for young children (6-36 months) from the retail market in the Netherlands. Samples were collected in 2011, 2012 and 2014. EAs were detected in 54% and TAs in 22% of the samples; 15% of the samples contained both EAs and TAs. Mean EA levels were 10.6, 6.2 and 8.6 µg/kg in the respective sampling years, with a maximum level of 115.4 µg/kg. Mean TA levels were 3.9, 2.4 and 0.4 µg/kg in the respective years, with a highest level of 80.8 µg/kg. Based on these results and recommended use, exposure to EAs would not have exceeded the TDI proposed by EFSA. However, the results indicate that the ARfD for TAs could have been exceeded by up to a factor of 10 for young children consuming some of the products sampled in the years 2011-2012. Over the years the contamination of EAs in cereal-based food products for infants and young children remained at similar levels, but TA levels had decreased drastically in 2014. The latter may be the result of the scientific opinion published by EFSA in 2013, which may have prompted producers to take measures and install adequate quality control of their supplies. The results show the advantage of applying multi-methods in food analysis for detection of potential risks. **Acknowledgements.** This research was financed by the Ministry of Economic Affairs of the Netherlands, project no. WOT-02-001-018.

P13: Determination of quinolizidine alkaloids in lupin flour by LC-MS/MS: a promising novel way of analysis

Patrick Mulder and M. de Nijs
RIKILT Wageningen UR, the Netherlands. Corresponding author: patrick.mulder@wur.nl

Quinolizidine alkaloids in lupin flours and lupin containing food products are typically measured by GC-MS, GC-NPD or GC-FID. These methods have a long history of use and extensive databases are available to the researcher to assist in the identification of known or novel alkaloids. Main disadvantages of GC-based methods are the relatively complex and time-consuming sample preparation procedures required, including liquid-liquid partitioning (LLE) or solid phase extraction (SPE) techniques. Challenging matrices may require additional derivatization and polar or high-molecular weight metabolites may be lost due to limitations of the GC columns used. In principle, many of these

disadvantages can be circumvented by using LC-based methods, most notably LC-MS/MS. Surprisingly, thus far very little work has been conducted in this area and in practice no LC-MS methods are published that can be used for the routine analysis of quinolizidine alkaloids. Our aim was to develop a fast, efficient and reliable LC-MS/MS method for the detection and quantification of quinolizidine alkaloids in lupin flours and products. As no LC-MS methods have been published to date, the method was developed from scratch. Using commercially available analytical standards and lupin seed extracts, mass fragmentation spectra were generated under positive electrospray (ESI) conditions and a method was build using multiple reaction monitoring (MRM). A UPLC method using reversed phase chromatography was developed that allowed baseline separation of all relevant isomeric pairs (e.g., lupanine/isolupanine; sparteine/isosparteine). The quinolizidine alkaloids were extracted in high yield (>90%), repeatability (RSDs<10%) and sensitivity (LOQs<1 mg/kg) from the lupin matrix. Due to the high intrinsic sensitivity and selectivity of the LC-MS/MS instrument, the only clean-up required was dilution and filtration through a 0.45 µm membrane filter in the vial. **Acknowledgements.** This research was financed by the Ministry of Economic Affairs of the Netherlands, project no. WOT-02-001-018.

P14: Opium alkaloids in poppy seeds and food products

P. López, **Patrick Mulder**, D. Pereboom-de Fauw, H. Mol and M. de Nijs
RIKILT Wageningen UR, the Netherlands. Corresponding author: patrick.mulder@wur.nl

Poppy seeds (*Papaver somniferum* L.) are used as food ingredients in central Europe in bakery products, on top of dishes, in fillings of cakes and in desserts and to produce edible oil. Opium alkaloids (OAs) are present in the latex, but not in the seeds of the poppy plant. However, seeds can become contaminated with the alkaloids from the latex as a result of insect damage or through poor harvesting practices. Morphine and codeine are the two most well-known opium alkaloids. In addition to these two opiates, the latex may also contain thebaine, noscapine, papaverine, narceine and/or oripavine. EFSA estimated that the acute reference dose (ARfD) of 10 µg morphine/kg bw can be exceeded during a single serving by consumers, particularly by children. Consumption of foods containing poppy seeds contaminated with OAs may, therefore, lead to adverse health effects, and may therefore be of concern, particularly with respect to children. It is also known that consumption of poppy seed containing foods can lead to measurable concentrations of OAs in urine, sufficient to interfere with drug abuse testing. There is currently no harmonized EU legislation for OAs in poppy seeds intended for food. In 2005, the German Federal Institute for Risk Assessment (BfR) derived a provisional reference value of 4 mg/kg for morphine in poppy seed samples. This recommendation has been used as a basis for action in the EU but has not been incorporated in legislation. The aim of the present work was the development of a straightforward LC-MS/MS method to determine OAs in poppy seeds and food products containing poppy seeds. This method was validated and applied in a survey that was carried out in poppy seeds purchased from the retail market in the Netherlands at the end of 2015 and early in 2016. The outcomes will be presented. **Acknowledgements.** This research was financed by the Ministry of Economic Affairs of the Netherlands, project no. WOT-02-001-018.

P15: Potato glycoalkaloids in the Netherlands

P. López, P. Mulder, E. de Vries and **Monique de Nijs**
RIKILT Wageningen UR, the Netherlands. Corresponding author: monique.denijis@wur.nl

Potato tubers (*Solanum tuberosum*) contain glycoalkaloids (PGA), plant toxins naturally occurring in the potatoes. Low amounts of PGA are necessary for the taste, but high amounts can cause a bitter off-taste and health problems in humans. Potatoes produce a variety of PGAs of which α-chaconine and α-solanine are the major ones (95%). There are no EU legal limits on potato glycoalkaloids, although Sweden has a maximum limit of 200 mg PGA/kg for unpeeled fresh potatoes. In 2001, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded in their risk assessment that levels of up to 100 mg/kg fresh unpeeled potato are of no safety concern for adult consumers. The aim of the study was to develop a straightforward method based on dilute-and-shoot LC-MS/MS analysis to determine α-chaconine and α-solanine in fresh potato tuber samples. The method was in-house validated in the range of 0.5-50 mg/kg, with recoveries around 100% and good repeatability (RSD<10%) for each PGA. The limit of quantification was 0.1 mg/kg. The method was applied to 71 unpeeled fresh potato samples collected in retail stores in the Netherlands in the autumn of 2015. The levels of total potato glycoalkaloids ranged from 20-100 mg/kg, the range considered of no health concern for consumers. The concentrations of α-chaconine and α-solanine and their ratio were cultivar-dependent. In general, organically grown varieties showed higher amounts of PGA than the conventional ones, whereas the content of α-solanine in red varieties appeared to be slightly higher than that in the yellow varieties. This method can be easily used for routine purposes and food monitoring.

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P16: Development of method for determination of tropane alkaloids and pyrrolizidine alkaloids in herbal tea

Ock Jin Paek, S.H. Kim, W.S. Nam, D.S. Kim and S. Suh

Food Contaminants Division, National Institute of Food and Drug Safety Evaluation, Ministry of Food and Drug Safety, Republic of Korea. Corresponding author: ojpaek92@korea.kr

Pyrrolizidine alkaloids (PAs) are secondary plant metabolites with carcinogenic and genotoxic properties. Recently, the occurrence of PAs in herbal teas has gained increasing attention from the EU, due to the high levels of PAs found in commercially available herbal teas in Germany. Tropane alkaloids occur naturally in a number of plant species, including herbs. Plants that are known to potentially contain higher levels of tropane alkaloids must therefore not be used in herb mixes. A rapid, specific, and sensitive method based on liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI/MS/MS) in the positive and negative ion mode using selective reaction monitoring (SRM) has been developed and validated for the quantification of tropane alkaloids (TAs; atropine, scopolamine) and PAs (7) residues in herbal teas. Optimization of the simultaneous extraction of these alkaloids and the sample pretreatment procedure was performed. The results indicated that the solvent mixture of acetonitrile/water/formic acid (60:40:0.1, v/v) was the best compromise for extracting the analytes from food supplements. Limits of detection and quantification depend on the matrix and were also estimated, obtaining limits of quantification equal to 0.3 µg/kg for TAs and 1 µg/kg for PAs. The estimated recovery rates, at spiking levels 1 to 10 µg/kg, ranged from 78.4 to 116.6% with relative standard deviations <25%. The method proved to be sensitive and reliable and thus renders an appropriate means for residue analysis studies.

P17: Detection of pyrrolizidine alkaloids in milk and honey on the Belgian market using UHPLC-MS/MS

Jean-François Picron, S. Gosciny, M. Andjelkovic and E. Van Hoeck

Food, Medicines and Consumer Safety Department, Scientific Institute of Public Health (WIV-ISP), Belgium. Corresponding author: jean-francois.picron@wiv-isp.be

The objective of the research is to report occurrence levels of pyrrolizidine alkaloids (PAs) and N-oxide (PANOs) in targeted food items on the Belgian market to perform a dietary exposure assessment to those contaminants and evaluate if they can pose a health problem, hampering a data gap at Belgium's level. Diverse cases of human poisoning are documented and are mainly related to consumption of traditional herbal remedy, milk, dairy products or honey. As PAs are biosynthesized by a wide variety of plant species, intoxication could occur worldwide. Therefore, the development of efficient analytical methods is required to detect PAs at (very) low levels. Analytical methods based on UPLC separation and MS/MS detection were developed for the analysis of 16 PAs and 13 PANOs in honey and milk matrices at WIV-ISP. The sample preparation involves acidic extraction of the analytes followed by SCX-SPE purification to avoid loss of sensitivity due to matrix effect. Several parameters were optimized in order to obtain the best method sensitivity. The separation of PAs/PANOs was performed on a C18 stationary phase LC column. The analytical methods were validated in-house and the validation parameters met the critical validation criteria allowed by Directive 2002/657/CE. Moreover, the LOQs achieved (0.05 ng/g for honey, 5 pg/g for milk) were lower than those proposed by EFSA. A structured sampling plan has been established for more than 700 samples covering 7 food groups. To date, milks and a part of honey samples were analyzed. The research will also provide data for herbal products (teas and salads), meat, food supplements, dairy products and eggs. Presence of PAs/PANOs at ppt levels (5-17 pg/g) was detected in 20% among 104 milk samples. Two organic goat milks and two organic cow milks were the most contaminated samples by retrorsine (+N-oxide). Despite low levels in milk samples, particular attention must be paid to this food matrix during the future exposure assessment because of the widespread consumption of milk products, particularly by infants and children. Ninety percent among the 119 honey samples analyzed so far were contaminated by 1 or more PAs/PANOs. The highest PA levels were detected in Mediterranean honeys with concentrations of echimidine, heliotrine or europine up to 135 ng/g. However, the great majority of the samples contained less than 5 ng/g of individual contaminants. The results obtained fit well with the previously reported patterns and levels of pyrrolizidine contaminants for these 2 food matrices. **Acknowledgements.** The research that yielded these results was funded by the Belgian Federal Public Service of Health, Food Chain Safety and Environment through the contract RT 14/10 PASFOOD.

P18: Presence of ptaquiloside in bracken-based food, food supplements and alternative medicine

Lars Holm Rasmussen

Department of Technology, Metropolitan University College, Denmark. Corresponding author:
lhra@phmetropol.dk

Ptaquiloside (PTA) is a noxious toxin present in bracken (*Pteridium* sp.) and a number of other ferns found worldwide. Bracken ferns are classified by WHO/IARC as 'possibly carcinogenic to humans'. Consequently, bracken and PTA are placed on the WHO/IARC urgency list of potential carcinogenic compounds/products to be re-evaluated in 2018 and many countries has enforced a ban on the use of bracken in food, food supplements and alternative medicine. Denmark and the Netherlands are examples of such countries. Nonetheless, it is still possible to buy and import bracken-based products as these products are widely available at internet-based stores and, of course, it is still possible to pick fresh bracken yourself. The purposes of this study were to generate an overview of the use of bracken and to unveil the availability and toxicity of bracken-based products for the European consumer. The study was based on a literature review in combination with internet search and visits to Asian shops in Europe and China. A selection of products was imported to Denmark and analyzed for content of PTA and the main degradation product pterisin B (LC-MS and HPLC-DAD) at the Metropolitan University College. Bracken is widely used for consumption in Asia (e.g., Japan (warabi), Korea (gosari/kosari) and China (juecai/jue cai)). Different servings are made of the young fresh fronds (the croziers). Sometimes the fronds are raw, sometimes used as pickled products, or boiled/blanched. Dried and frozen products are also available. In Japan, dried rhizomes are used for making flour for the delicacy warabi mochi (a sort of sweet cake). Also, traditional blanching of croziers with wood ash or similar alkaline material are used to lower the toxicity. Bracken is also eaten in South America and in Africa, where it, e.g., is used for traditional medicine in the Eastern and Southern parts. The native population in North America are also users of the young fronds. In Europe, Asian bracken products are found in Asian stores and as food supplements or alternative medicine (homeopathic products – pills and extracts). The study showed that a wide range of these products can be imported from Asian or North American web stores. PTA and pterisin B were present in many products and in highly variable concentrations. Blanching of fresh bracken was not found to eliminate PTA in contradiction to previous reports. The food authorities are advised to take these findings into account.

P19: Ptaquiloside in surface water – development of a versatile HPLC-based procedure for screening of water samples

Lars Holm Rasmussen, J. Pedersen and D.N. Lindqvist

Department of Technology, Metropolitan University College, Denmark. Corresponding author:
lhra@phmetropol.dk

Bracken ferns (*Pteridium* sp.) cause cancer and other diseases among farm animals. The main toxin is the nor-sesquiterpene glycoside ptaquiloside (PTA), which is acute toxic, mutagenic and carcinogenic. PTA is found in all parts of the fern (up to 5% w/w, dry matter), and is highly soluble in water. Hence, it can be leached from fronds to surface waters, where PTA has been found in concentrations up to approx. 90 ng/l. PTA is relative stable at pH 5-6.5 and at temperatures up to 45°C. At higher/lower pH and at higher temperatures a series of different reactions takes place with water and other organic/inorganic compounds. The main hydrolysis product is however pterisin B (PtB). PTA is also subject to microbial degradation. This calls for fast analysis which is challenging due to the low expected concentration in surface waters when using routine HPLC equipment. The purpose of the project was to develop a versatile method for screening of water samples for content of PTA. PTA was quantified in water samples by HPLC-DAD after sample purification and reaction into PtB. Hence, water samples were filtered and 300 ml filtrate passed through a column dry packed with 3.5 g polyamide 6S resin (2.0x20 cm I.D. glass Econo-Column®). PTA pass the column together with cat/anions and hydrophilic compounds (recovery percentage, approx. 100%), while hydrophobic compounds, such as PtB, stay at the column. PTA in the eluate is converted to PtB by acid hydrolysis (7.5 ml 5M CF₃COOH) following heating and treatment with base (7.5 ml 1M NaOH; 35°C; 60 min). PtB was captured by passing the solution through a wet-packed polyamide 6S column (100 ml to 3.0 g polyamide; 10 ml/min; 2.0x20 cm I.D. glass Econo-Column®). PtB was recovered by AcN and evaporated (rotary evaporator, 20 min, 45°C; oven until dryness, max. 60°C for max. 18 h (recovery percentage, approx. 100%). Redissolved in 1000 ml 30:70 AcN:H₂O before analysis on HPLC-DAD (Agilent 1200 series DAD HPLC system; Phenomenex Hypersil 3 µm C8-DBS (150x4.60 mm, 3 µm); 35°C; Opti-SOLV-TM 0.5 µm guard column; 190-400 nm; injection volume: 20 µl (5-100 µl); LOD, 0.002 mg/l PtB; LOQ, 0.005 mg/l PtB). The chemical composition of water samples is a highly variable. Care must therefore be taken to ensure full

resolution during chromatography, which is accomplished here by using a DAD detector. The method was tested at a number of surface waters from bracken-infested areas in Denmark and showed highly variable contents of PTA in the range of previous findings.

P20: Degradation of the carcinogen ptaquiloside from bracken fern in rain and surface water

Janni Sandersen, D.N. Lindqvist and L.H. Rasmussen

Department of Technology, Metropolitan University College, Denmark. Corresponding author:

jasa@phmetropol.dk

Ptaquiloside (PTA) is a carcinogen present in *Pteridium* species (bracken ferns) as well as in a number of other ferns worldwide. The fact that *Pteridium* species can be found all around the world, and has been known for causing diseases in both humans and animals, has made the subject of big interest for researchers and veterinarians. PTA causes bladder cancer among bovines, blindness among sheep and acute bleedings among calves, hence the toxicology of bracken fern has been studied intensively for decades, especially cancer in mammals. PTA is under suspicion of causing human gastric cancer. The interaction between PTA and animals are most studied in the context of Bracken ferns as these are very common and contains high concentrations of PTA. Ptaquiloside can be absorbed by humans through food (fern/contaminated milk/meat), air (spores) or drinking water (fern leachates). As PTA is highly soluble in water, and poorly absorbed in soil, it is presumed to play a major role in contaminating ground and surface water bodies used for drinking water purposes in bracken infested areas. Degradation of PTA in ground and surface waters is a function of pH and microbiological activity (bacteria/fungi). PTA is most stable in water around pH 4.5-6.5. The abiotic route of degradation can be acid as well as base catalyzed. The main degradation product pterosin B is not presumed toxic. Degradation of PTA was studied by LC-MS in three types of water from Denmark: rainwater (Copenhagen region), acid brown water (lake Bøllemosen), and neutral clear water (pond Skodsborg Dam). The effect of the microbiological activity was studied by applying sterile filtration. PTA in sterile water showed the expected pH dependent degradation (acid hydrolysis). PTA in raw water showed a 100% degradation within 50-100 h most probably due to microbiological activity. Findings of PTA in surface water must therefore indicate recent contamination, e.g., rain leachates or intruding soil water.