EURL mycotoxins & plant toxins
EURLWS-MP08
Agenda
7-8 October 2025
Van der Valk, Breukelen

Tuesday, 7th October 2025		
08:30	Grab a coffee	
09:00	In memoriam – Patrick Mulder	
09:10	Lecture in honour of Patrick – Grayanotoxins in honey	Sylvia Kalli (EURL)
09:40	Coffee break	
09:55	General Info	Josipa Grzetic Martens (EURL)
10:00	Update on legislation and emerging issues	Frans Verstraete (DG SANTE)
11:00	Coffee break	
11:30	Unravelling the mysteries of modified and bound mycotoxins	Franz Berthiller (BOKU)
12:00	Proficiency test for hydrocyanic acid in linseed and almond	Marta Sopel (EURL)
12:10	Proficiency test for ochratoxin A in cured ham and raw kidney	
12:20	Proficiency test for tropane alkaloids in teff and tea infusions	
12:30	Lunch	
13:30	Tremorgenic, ergot and tropane alkaloids in forage feed	Ilaria Di Marco Pisciottano (EURL)
13:50	Mycotoxins and plant toxins in plant-based drinks	Arnold Bahlmann (BfR)
14:10	Glycoalkaloids – there's more than just potatoes	Christoph Czerwenka (AGES)
14:30	Coffee break (photo?)	
15:00	AQC discussion 1. Guidance document discussion 2. Reporting the results (harmonizing the approaches) 3. Discussion on criteria for feed	Hans Mol (EURL)
18:00	Dinner	
Wednesday, 8th October 2025		
08:30	Grab a coffee	
08:55	General Info	
09:00	Development of a High-Resolution Tandem Mass Spectral Library for Pyrrolizidine Alkaloids (PASL)	Leonie Straub (EURL/WUR)
09:20	The EU complex	Bob Duijnhouwer (NVWA)
09:40	Accelerating ergot alkaloids analysis while revealing rye–sclerotia contamination patterns	Laura Righetti (WUR)
10:00	Effect of thermal processing on opium alkaloids in bakery products	Sylvia Kalli (EURL)
10.20	Coffee break (photo?)	
11:00	EURL work program and updates	Josipa Grzetic Martens (EURL)
12:00	Extra time for discussion, Q&A and closure	
12:30	Lunch	

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Unravelling the mysteries of modified and bound mycotoxins

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Ochratoxin A (OTA) is a nephrotoxic, immunosuppressive and neurotoxic mycotoxin, produced by various fungi of the genus *Aspergillus* and *Penicillium*. Its frequent occurrence in food products such as cereals, spices, dried fruits and coffee poses public health risks in particular to infants and children. The European Food Safety Agency panel on Contaminants in the Food Chain stated in their latest opinion that "more data on occurrence and toxicity of modified OTA are needed".[1]
Fumonisin B_1 (FB1) is the most important fumonisin regarding occurrence and toxicity and often occurring in maize and its products. Symptoms induced by fumonisins include neural tube defects in newborns, liver and kidney apoptosis, brain lesions in horses and lung edema in swine. The latest risk assessment by the Joint FAO/WHO Expert Committee on Food Additives states that "studies should be undertaken to better understand the occurrence of bound fumonisins in different cereals … and their bioavailability after consumption".[2]

Modified forms of mycotoxins can arise during food processing (e.g. 2'R-OTA during coffee roasting)[3] and due to plant metabolism (e.g. mycotoxins-glucosides)[4]. Studies with plants are currently limited to cell cultures and it is not yet fully understood which metabolites are formed in culture plants, such as maize, and to which extent. In addition, mycotoxins might be bound e.g. to proteins and little is known about the occurrence and bio-availability of bound forms.

We screened fungal strains to assess OTA or FB1 production in several semi-synthetic liquid media. We then used $^{13}C_6$-glucose to produce fully ^{13}C labelled yeast and subsequently fully ^{13}C labelled yeast extract. The labelled sugar and yeast extract was used to produce ^{13}C-OTA and ^{13}C-FB1 in milligram amounts. The compounds were purified using liquid-liquid extraction, solid-phase extraction and preparative HPLC. The resulting ^{13}C-toxins were mixed with natural ones to treat mini-maize plants during flowering. High resolution mass spectrometry and MetExtract II data processing[5] was employed to discover all formed soluble OTA and FB1 metabolites of maize.

Currently we are using ^{14}C-labelled precursors to from radio-labelled OTA and FB1. Those toxins will again be used to treat maize plants. After exhaustive extraction, the remaining radioactivity will determine the amounts of bound forms. Finally bioavailability of the plant metabolites and bound forms will be assessed using rat trials.

Acknowledgements:
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References:

1. EFSA CONTAM Panel, 2020. EFSA Journal 18(5), 6113.
2. JECFA, 2018. WHO Food Additives Series, No. 74.
3. Cramer, B. et al., 2008. Journal of Agricultural and Food Chemistry 56(14), 5673-5681.
4. Berthiller, F et al., 2013. Molecular Nutrition and Food Research 57, 165-186.
5. Bueschl, C. et al., 2017. Analytical Chemistry 89(17), 9518-9526.

Mycotoxins and plant toxins in plant-based drinks

Arnold Bahlmann

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Plant-based milk alternatives have gained relevance in recent years due to the growing popularity of a vegan lifestyle, rising health consciousness and environmental concerns. These drinks can contain mycotoxins and plant toxins, which may pose health risks to consumers. To address this, a highly sensitive LC-MS/MS method was developed to detect these toxins in oat, soy and almond-based drinks. A total of 91 analytes were validated in accordance with EU regulation 2023/2782 and the latest draft of the EURL guidance documents on performance criteria. Among the 67 validated mycotoxins were regulated mycotoxins such as aflatoxins and ergot alkaloids as well as emerging mycotoxins such as *Alternaria* toxins and enniatins. The 24 validated plant toxins included pyrrolizidine and tropane alkaloids, as well as the quinolizidine alkaloid marker lupanine.
The optimized analytical method including sample preparation and LC-MS/MS conditions along with findings from the analysis of samples from the German market will be presented.

Glycoalkaloids – there's more than just potatoes

Christoph Czerwenka

Austrian National Reference Laboratory for Plant Toxins

For potentially toxic glycoalkaloids the focus has so far been mainly placed on those compounds present in potatoes and potato products, including changes during processing. However, various other *Solanum* species may also contain glycoalkaloids. Therefore, we developed a method in our laboratory that covers the main glycoalkaloids of potatoes, aubergines and tomatoes including the respective aglycons (5 alkaloids plus 3 aglycons). The challenges during method development caused by the in-source fragmentation of the glycoalkaloids will be discussed and the final method, which was fully validated for matrices with high water content, high starch content and high fat content will be presented.
The analysis of a number of potato and aubergine samples from the Austrian market showed that substantial concentrations of the respective alkaloids can be present and that a focus on potatoes as the sole source of glycoalkaloids may miss significant contributions.
For more exotic Solanum species the question of other significant alkaloids which are not covered by common methods and for which no standards are available arises. Two approaches, one using triplequadrupole mass spectrometry and one utilizing high-resolution mass spectrometry will be presented for the sample of a melon pear (*Solanum muricatum*). Besides two analytes that are in the scope of our method a third glycoalkaloid could be observed and tentatively identified based on high-resolution MS and MS/MS data.

Accelerating ergot alkaloids analysis while revealing rye–sclerotia contamination patterns

Laura Righetti

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Ergot alkaloids (EAs), produced by *Claviceps purpurea*, are toxic compounds that contaminate cereal crops and pose significant health risks to humans and animals. *C. purpurea* forms hardened fungal structures known as sclerotia, which are the main source of EAs in infected rye. Despite increasing regulatory attention, limited information is available on the dynamics of EA transfer from sclerotia to grain, and robust analytical tools are required to address this challenge.

In this talk, I will address two complementary aspects. The first focuses on the analytical development. Ion mobility mass spectrometry (IM-MS) has recently emerged as a powerful approach to shorten chromatographic analysis times while maintaining or even enhancing separation performance. By adding an orthogonal gas-phase separation step, IM-MS enables higher throughput workflows without compromising data quality. In this study, we developed a high-throughput LC–cyclic IM-MS methodology capable of analyzing 28 EAs, including 9 isomeric pairs, within only 4 minutes. This advancement directly addresses the analytical complexity of EA isomers and the challenging composition of sclerotia and cereal extracts.

The second aspect relates to the field study. Specifically, we analyzed 40 wholegrain rye samples from three different varieties together with their corresponding sclerotia to assess EA contamination profiles and potential correlations between fungal structures and host cereal. Non-targeted screening was applied to detect novel EAs or biosynthetic intermediates using molecular networking strategies based on structural similarities.
Together, these two perspectives provide both methodological advances and new biological insights into EA occurrence and transfer.