# EURL mycotoxins & plant toxins
# EURLWS-MP07
# Agenda
# 1-2 October 2024
# Van der Valk, Breukelen

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| **Tuesday, 1st October 2024** | | |
| **08:30** | Coffee | |
| 09:00 | Welcome & agenda | Josipa Grzetic Martens (EURL) |
| 09:15 | Update on legislation and emerging issues | Frans Verstraete (DG SANTE) |
| **10:15** | Coffee | |
| 10:45 | Proficiency test on cyanogenic glucosides in almonds and linseed | Patrick Mulder (EURL) |
| 11:05 | Proficiency test on selected cannabinoids in hemp flour and oil | |
| 11:25 | Proficiency test on ochratoxin A and aflatoxins in cocoa and maize | Josipa Grzetic Martens (EURL) |
| 11:40 | Aflatoxins in tigernuts | Marta Sopel (EURL) |
| 12:00 | Method optimization – aflatoxins in cocoa<br>Reuse of IAC columns: guidelines for reliable performance | Alwin Kruijt (EURL) |
| 12:20 | Hydroxyanthracenes in food supplements and beverages | Nathalie Gillard (CER Groupe) |
| **12:40** | Lunch | |
| 13:40 | Food safety in Netherlands – Practical aspects from NVWA | Bob Duijnhouwer (NVWA) |
| 14:10 | Detection and Characterisation of Citrinin and Modified Forms of Citrinin in *Penicillium expansum* and Real Samples of Food and Feed by LC-HRMS | Christoph Hutzler (BfR) |
| 14:35 | Naturally occurring psychoactive compounds in food products on Czech market | Radim Stepan (CAFIA) |
| **15:00** | Coffee break | |
| 15:30 | AQC discussion | Hans Mol (EURL) |
| **18:30** | Dinner | |
| **Wednesday, 2nd October 2024** | | |
| **08:30** | Coffee | |
| 9:00 | Sample-to-Solution Approaches for Natural Toxin Analysis at the Point of Need | Gert Salentijn (WUR) |
| 9:40 | Homogeneity of samples – *Datura* in corn<br>Survey results on tropane alkaloids in teff | Sylvia Kalli (EURL) |
| 10.10 | Method development and survey on cucurbitacins in food | Ilaria Di Marco Pisciottano (EURL) |
| **10.30** | Photo and coffee break | |
| 11:10 | EURL work program and updates | Josipa Grzetic Martens (EURL) |
| 11:45 | AQC follow up | Hans Mol (EURL) |
| 12:05 | Extra time for discussion, Q&A and closure | Josipa Grzetic Martens (EURL) |
| **12:30** | Lunch | |

## Detection and Characterisation of Citrinin and Modified Forms of Citrinin in *Penicillium expansum* and Real Samples of Food and Feed by LC-HRMS

Dr. Christoph Hutzler
German Federal Institute for Risk Assessment (BfR), Department for Safety in the Food Chain
German National Reference Laboratory for Mycotoxins and Plant Toxins in Food and Feed, Berlin, Germany

Compared to the LC-MS/MS method with quadrupole devices frequently used in routine target analysis of mycotoxins, analysis with LC-HRMS devices offers the advantage of not only being limited to the target analytes, but also to its already known or unknown modifications and transformation products. Biomarkers for citrinin are often found in human biomonitoring [1] without exactly knowing the source for this exposure. There is only limited knowledge about the occurrence of citrinin in food, as citrinin analysis are rarely performed in routine. Citrinin has different functional groups which allow the molecule to undergo chemical und enzyme mediated reaction in multiple ways. We detected citrinin and modified forms of citrinin while screening analyses with HRMS in different food and feed samples. As the reason for the source of the citrinin exposure resulting in the citrinin findings in human biomonitoring is still unclear, it might also be possible that modified forms of citrinin may contribute to the overall exposure.
An extract of an inoculate of a citrinin building fungus (Penicillium expansum) was analysed by LC-HRMS for modified forms of citrinin. By comparison to literature data and the use of HRMS software tools numerous of modified forms of citrinin were detected.
Taking into account the knowledge of the modified forms of citrinin, all citrinin positive samples were analysed once again using modified analytical methods using inclusion lists for triggering the recording of HR-MS/MS spectra for already known modifications of citrinin. Additionally, by software tools based on a reaction database the identification of additional metabolites of citrinin was processed.
In summary, several modified forms of citrinin could be identified in food and feed real samples. E.g. the detected modifications based on the following chemical reactions and combinations of them: desaturation, methylation, decarboxylation, reduction by loss of oxygen. A semi-quantification of selected modified forms of citrinin in food and feed samples was performed using a commercially available algorithm for the prediction of ionisation efficiencies.

[1] Degen GH, et al., Toxins (Basel). 2022 Dec 30;15(1):26. doi: 10.3390/toxins15010026.

## Naturally occurring psychoactive compounds in food products on Czech market

Radim Stepan, Daniela Paldusova, Martin Kubik, Petr Cuhra
Czech Agriculture and Food Inspection Authority (CAFIA), Czech Republic

Food products containing naturally occurring psychoactive compounds have been continuously introduced to the Czech market in last years. Hexahydrocannabinol (HHC), Hexahydrocannabinol-O-acetate (HHC-O) and Tetrahydrocannabiphorol (THCP) were banned by CZ government regulation in the beginning of year 2024 however products containing other naturally occurring compounds were introduced to Czech market in the meantime. In the present time food products (e.g. gummies, jellies, powders) containing hallucinogenic/psychotropic compounds such as Muscimol originating from *Amanita muscaria* fungi and stimulating/euphoric alkaloids such as Mitragynine and 7-hydroxymitragynin (kratom) originating from *Mitragynia speciosa* tree are normally available. Optimized analytical method including sample preparation and LC-MS/MS conditions together with examples of our findings will be shown in the presentation.

**Hydroxyanthracenes in food supplement and beverages**

Nathalie Gillard, Mathieu Dubois
CER Groupe, Belgium

Plants containing hydroxyanthracene derivatives are numerous and belong to different botanical families and genera. They are widely used in food supplements.
While EFSA (2013 EFSA Scientific Opinion on the scientific substantiation of a health claim related to hydroxyanthracene derivatives and improvement of bowel function) recognized that HAD in food can improve bowel function, they advised against long-term use and consumption at high doses due to potential safety concerns. Based on this concern regarding possible harmful effects of the consumption of HAD, a new scientific opinion was published in 2017. In this scientific opinion, EFSA stated that hydroxyanthracene derivatives should be regarded as genotoxic and carcinogenic. As a consequence, regulation 2006/1925 was amended in 2021 and aloe-emodin, danthron and emodin were added in the Annex III as prohibited substances.
A method was developed, validated for food supplement (tablets or liquids) and set under accreditation at CER Groupe since 2023.
Since its validation, food supplement samples were received for analysis either from Food Business Operators or from the Food Agency. High levels of HAD were detected in several samples which has lead to contest from the FBO.
Additional tests were therefore realized to guarantee that HAD glycosides were not hydrolyzed in aglycon forms during analysis and to confirm the specificity of the method for additional food supplement materials.

**Sample-to-Solution Approaches for Natural Toxin Analysis at the Point of Need**

Dr. Gert Salentijn
Wageningen University and Research, Netherlands

Occurrence of natural toxins in food is not easy to predict, and yet vitally important in a changing world. By taking the analysis to the point-of-need, more ground can be covered. Compared to the typical lab-based approach, all steps from an analytical workflow need to be considered from an entirely different perspective when carried out outside the lab. Therefore, different research fields and analytical strategies are combined to achieve such goals, including ambient ionization (portable) mass spectrometry, lateral flow immunochemistry, chemical surface modification for (paper) microfluidics, and 3D-printing. In this presentation, Gert Salentijn will elaborate on some recent examples from his team in the field of on-site sample preparation, and portable detection strategies in the context of food safety analysis.