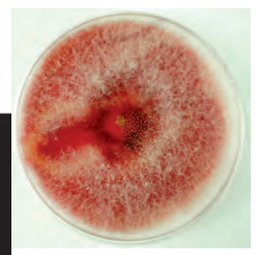
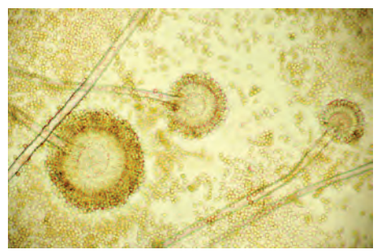
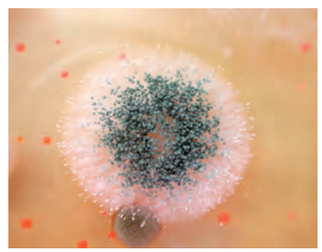
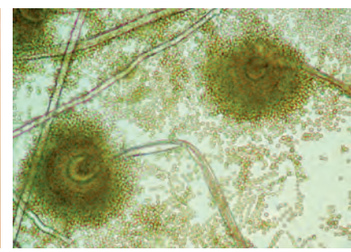
Society for Mycotoxin Research
Gesellschaft für Mykotoxinforschung e.V.

44th MYCOTOXIN WORKSHOP

Celle | HANNOVER – GERMANY

June 5th – 7th 2023

Conference Abstracts

44th

MYCOTOXIN WORKSHOP

is organized by Society for Mycotoxin Research
C/O Insitute of Food Quality and Food Safety University
of Veterinary Medicine Hannover, Foundation

Society for Mycotoxin Research
Gesellschaft für Mykotoxinforschung e.V.

cooperation with the Kazimierz Wielki University,
Faculty of Biological Sciences,
Department of Physiology and Toxicology

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Prof. Gisela Degen
Prof. Luca Dellafiora
Prof. Jan Grajewski
Prof. Hans Humpf
Prof. Doris Marko
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Program

MONDAY, 05 JUNE 2023

8:00 **Registration**

9:30–9:45 **Welcome**

OCCURENCE

9:45–10:00 Mycotoxin occurrence: a survey among major and minor winter cereals
L1 Marco Gozzi, Massimo Blandino, Chiara Dall'Asta, Renato Bruni, Laura Righetti

10:00–10:15 Putative tremorgenic mycotoxicosis in a dog – case report
L2 Leon Hart, Christina Rehagel, Michael Kuhn, Lukas Jozefowitz, Esther Haßdenteufe, Benedikt Cramer, Ewald Usleber

10:15–11:00 **Coffee break**

FOOD SAFETY I

11:00–11:15 Fusarium growth and production of toxins during malting
L3 Eva Maria Biehl, Sarah Schneidemann-Bostelmann, Ibrahim-Can Akpak, Michael Rychlik

11:15–11:30 Mycotoxins as agricultural & livestock biosecurity threats: from maize seeds to raw milk contamination
L4 Marta Sofia Carvalho Ferreira Malheiro Leite, Andreia Freitas, Jorge Barbosa, Fernando Ramos

11:30–11:45 Investigations into the mycotoxin-producing capacity of moulds isolated from cheese
L5 Daniela Schale, Bettina Seeger, Nadja Jeßberger, Sophie Kittler, Christina Rehagel, Leon Hart, Ewald Usleber, Madeleine Plötz

11:45–12:00 Thermal stability of the mycotoxin citrinin
L6 Lea Brückner, Benedikt Cramer, Hans-Ulrich Humpf

12:00–1:30 **Lunch**

1:30–1:45 Development and evaluation of sample preparation methods for mycotoxin analysis in plant-based milk alternatives by enzyme immunoassays
L7 Christina Rehagel, Ronald Maul, Kim Lara Gützkow, Ömer Akineden, Ewald Usleber

TOXICOLOGY I

1:45–2:00 Combined toxicity of aflatoxin B1 and deoxynivalenol: roles of ribotoxicity and cytochrome P450 enzymes

L8 Bernadette Willoquet, Gladys Mirey, Delphine Payros, Isabelle Oswald

2:00–2:15 Toxicity of enniatins A, A1, B, B1 and beauvericin on innovative *in vitro* Human cell models and their impacts on cellular gene expression

L9 France Coulet, Monika Coton, Emmanuel Coton, Nolwenn Hymery

2:15 – 2:30 Computer-empowered digging of big data to tackle toxicity assessment and bioremediation of emerging mycotoxins – a 4-year long romance with fusaric acid

L10 Lorenzo Pedroni, Chiara Dall'Asta, Gianni Galaverna, Luca Dellafiora

2:30–3:00 **Coffee break**

3:00–4:30 **Meeting of the Society for Mycotoxin Research**

5:00–6:00 Activities:

- Light art museum in Celle: modern art, light objects
- Guided tour of the residential palace
- Guided tour through the half-timbered houses with interesting facts from the city history
- Minigolf: have fun on 18 holes
- Explore Celle on your own: Ascent of the city tower: 283 steps to a view over Celle!

6:30 **Barbecue**

TUESDAY, 06 JUNE 2023

8:00 **Registration**

FOOD SAFETY II

9:00–9:15 Mycotoxin contamination in the Arab world: Highlighting the main knowledge gaps and discussing the current legislations

L11 Mohamed Fathi Abdallah, Muhammad Gado, Doha Abdelsadek, Fatma Zahran, Nada N. El-Salhey, Ohaila Mehrez, Sara Abdel-Hay, Sahar Mahrous, Karl De Ruyck, Elisabeth Varga

9:15–9:30 Mycotoxins in insects for food and feed

L12 Ronald Maul, Kim Lara Gützkow, Nina Kröncke, Johanna Ebmeyer, Nadja Kampschulte, Linda Böhmert, Cindy Schöne5, Nils Helge Schebb, Albert Braeuning, Rainer Benning

9:30–9:45 T-2 and HT-2 toxins in milling oats and oat kernels

L13 Inga Hennies, Jens Chr. Meyer

9:45–10:00 Cyclodextrins as potential mycotoxin binders: the summary of earlier and recent studies

L14 Miklós Poór, Eszter Fliszár-Nyúl

10:00–10:30 **Coffee break III**

HEALTH / EXPOSURE

10:30–10:45 Ochratoxin A and Parkinson disease: is there a link?

L15 Ariane Vettorazzi, Maria Serrano, Elba Beraza, Maria Izco, Amaya Azqueta, Elena Gonzalez-Peñas, Adela Lopez de Cerain, Lydia Alvarez-Erviti

10:45–11:00 The epigenetic interplay of mycotoxins & Epstein Barr virus towards childhood cancer

L16 Thanos Mouchtaris Michailidis, Sarah De Saeger, Rita Khoueiry, Grace Akinyi Odongo, Laura Corveleyn, Maarten Dhaenens, Zdenko Herceg, Marthe De Boevre

11:00–11:15 Short-time exposure to the *Fusarium* mycotoxins deoxynivalenol and zearalenone in cows fed a diet inducing rumen acidosis

L17 Lydia Kroon, Ant Koopmans, Antonio Gallo, Janine Saltzmann, Sven Dänicke, Ermino Trevisi, Regiane R Santos

11:15–11:30 New insights on citrinin exposure in German children and adults from a urine biomarker study

L18 Gisela H. Degen, Jörg Reinders, Martin Kraft, Yvonni Chovolou, Wolfgang Völkel, Felicia Gerull, Jan G. Hengstler, Hermann Fromme

11:30–11:45 Walls in camouflage: searching for molds and mycotoxins in military settings

L19 Wiebke Derz, Paul Elsinghorst

11:45–1:45 **Lunch**

TOXICOLOGY II

1:45–2:00 Analysing epigenetic toxicity caused by multi-mycotoxin exposure using an intestinal and hepatic cell culture model

L20 Yasmine Bader

2:00–2:15 New challenges in genotoxicity testing: the case of the *Alternaria* mycotoxins alternariol and altertoxin II

L21 Francesco Crudo, Chenyifan Hong, Vanessa Partsch, Giorgia Del Favero, Luca Dellafiora, Doris Marko

2:15–2:30 Does replication stress play a role in ochratoxin A genotoxicity?

L22 Christina Klotz, Julian Brode, Johannes Borchers, Angela Mally

2:30–2:45 Pitfalls and challenges when evaluating combinatory effects – a case study on zearalenone and isoflavones

L23 Dino Grgic, Barbara Novak, Elisabeth Varga, Doris Marko

2:45–3:00 Alternariol-sulfates as neglected conjugation products with toxicological potential

L24 Eszter Borsos, Luca Dellafiora, Chiara Dall'Asta, Giorgia Del Favero, Elisabeth Varga, Doris Marko

3:00–3:30 **Coffee break**

TOXICOLOGY III

3:30–3:45 New possibilities in mycotoxin research by functional intravital imaging

L25 Ahmed Ghallab, Reham Hassan, Maiju Myllys, Daniela González, Abdellatif Seddek, Andrea Gerdemann, Michael Kuhn, Benedikt Cramer, Hans Ulrich Humpf, Gisela H. Degen, Jan G. Hengstler

3:45–4:00 Insights into the metabolism, kinetics and related toxicity of ochratoxin A in mice models

L26 Michael Kuhn, Reham Hassan, Benedikt Cramer, Ahmed Ghallab, Gisela H. Degen, Jan G. Hengstler, Hans-Ulrich Humpf

4:00–4:15 Species differences of aflatoxin B1 metabolism in primary mouse, rat and human hepatocytes and in vivo in rodents

L27 Andrea Gerdemann, Benedikt Cramer, Matthias Behrens, Ahmed Ghallab, Reham Hassan, Gisela H. Degen, Jan G. Hengstler, Melanie Esselen, Hans-Ulrich Humpf

4:30–5:30 **Activities**

6:30 **Conference Dinner**

WEDNESDAY, 07 JUNE 2023

9:00 **Registration**

ANALYSIS / FUNGI I

9:15–9:30 LC-QToF-HRMS as an effective tool for detecting of co-contamination of mycotoxins and transformation products

L28 Marcus Trentzsch, Stefan Weigel, Christoph Hutzler

9:30–9:45 Acceleration of an HPLC-MS / MS multi-class method for the analysis of >1200 biotoxins, pesticides, and veterinary drugs

L29 Lidija Kenjeric, Michael Sulyok, Rudolf Krska, Kristyna Pavelicova, Alexandra Malachova

9:45–10:00 IR spectroscopy combined with LC-MS/MS analysis for rapid mycotoxin screening

L30 Stephan Freitag, Michael Sulyok, Rudolf Krska

10:00–10:15 Disarmed mycotoxins from *Stachybotrys chartarum*: insights into metabolism and biological activity of phenylspirodrimanes

L31 Alica Fischle, Katharina Steinert, Viola Haupt, Felix Schimang, Hans-Ulrich Humpf, Svetlana A. Kalinina

10:15–10:30 Effect of deoxynivalenol on soil nitrification

L32 Sven Korz, Katherine Munoz

10.30–11:00 **Coffee break**

FUNGI II

11:00–11:15 Influence of the velvet complex on development, secondary metabolism, aggressiveness and defense of *Penicillium expansum*

L33 Chrystian Zetina Serrano, Nadia Tahtah, Ophélie Rocher, Selma Snini, Yannick Lippi, Emilien L. Jamin, Isabelle P. Oswald, Olivier Puel, Sophie Lorber

11:15–11:30 Linking distinct extrolite profiles to the different genetic populations within *Penicillium roqueforti*

L34 Ewen Crequer, Jean-Luc Jany, Gwennina Cueff, Jeanne Ropars, Thibault Caron, Jens Frisvad, Johan Christiansen, Antoine Branca, Tatiana Giraud, Emmanuel Coton, Monika Coton

11:30–11:45 Environmental influences on the production of alternariol-sulfate by *Alternaria alternata*

L35 Adetoye Adeyemo, Markus Schmidt-Heydt

11:45–12:00 **Closing**

EXCURSION (free, limited, registration required)

12:30 pm

Bus departure for a visit of Bruker Daltonics in Bremen (free, limited, registration required).

Free bus tour to the factory of Bruker Daltonics in Bremen.
After the event no transfer back to Celle but to Bremen main station and airport.

2:30 pm–4:15 pm

Visit of the Bruker Daltonics factory of mass spectrometers (free, limited, registration required)

Guided tour through the production factory and visit of the demo labs showing the latest developments of Bruker Daltonics. For individual arrival: Meeting point in front of the main building, Fahrenheitstraße 4, 28359 Bremen

4:15 pm

Snacks, drinks and discussions / Shuttle transfer to Bremen station and airport

Free drinks and snacks. Opportunity to discuss with experts from Bruker.
Flights at 17:30 from Bremen to Munich or 19:10 to Frankfurt will be reached.

Tables of presentations

OCCURRENCE

FOOD SAFETY I

TOXICOLOGY I

FOOD SAFETY II

HEALTH / EXPOSURE

TOXICOLOGY II

TOXICOLOGY III

ANALYSIS / FUNGI I

FUNGI II

Tables of posters

OCCURRENCE AND ANALYSES OF MYCOTOXINS IN FOOD, FEED AND PHYSIOLOGICAL SAMPLES

TOXIGENIC FUNGI: GENETICS, BIOLOGY, NEW SECONDARY METABOLITES

MYCOTOXINS AND HUMAN/ANIMAL HEALTH

TOXICOLOGY AND RISK ASSESSMENT OF MYCOTOXINS

MYCOTOXIN AND FOOD/FEED SAFETY

LECTURES
AND SHORT COMMUNICATES

L1

MYCOTOXIN OCCURRENCE: A SURVEY AMONG MAJOR AND MINOR WINTER CEREALS

Marco Gozzi[1], Massimo Blandino[2], Chiara Dall'Asta[1], Renato Bruni[1], Laura Righetti[1,3,4]

[1]Department of Food and Drug, University of Parma, Parco Area delle Scienze 27/a, 43100 Parma, Italy
[2]Department of Agricultural Forest and Food Sciences, University of Turin, Largo Paolo Braccini 2, 10095 Grugliasco, Italy
[3]Laboratory of Organic Chemistry, Wageningen University, 6708 WE Wageningen, The Netherlands
[4]Wageningen Food Safety Research, Wageningen University & Research, P.O. Box 230, 6700 AE Wageningen, The Netherlands

Fusarium head blight (FHB) is a devastating disease of cereals caused by a complex of many fungal species that are known to infect many cereal crops [1-3]. Pathogenic fungi responsible for FHB also produce a wide range of mycotoxins that frequently co-occur in cereals [4]. Since co-occurrence poses a significant threat to public health, due to the additive or synergistic effects of these compounds, a multi-mycotoxin approach is of primary importance. In addition to cereal grains, it is well known that straw can be also contaminated with mycotoxins, posing serious threat to animal welfare due to its use as feed and also a potential risk for humans due to the persistence of these toxic compounds in the food chain [5]. In order to compare the susceptibility/resistance to mycotoxin accumulation of major and minor cereal crops, thirty-two varieties from different cereal crops, namely common wheat (*Triticum aestivum* L.), durum wheat (*T. turgidum* subs. *durum* Desf.), tritordeum (×*Tritordeum* Asch. & Graebn.) and barley (*Hordeum vulgare* L.), were grown over two consecutive years in open field and screened for multiple *Fusarium*-related mycotoxins in kernel and straw. Deoxynivalenol and its modified forms DON3Glc, 3Ac-DON, 15Ac-DON, T-2, HT-2, ZEN and Enniatin B were quantified by means of UHPLC-MS/MS. Based on the collected data, a susceptibility emerges for tritordeum to DON accumulation in kernels compared to the other crops tested, for the varieties considered. This is of particular relevance given its use as an alternative cereal to produce food products and require more studies. Finally, on the basis of the collected data, the straw proves to be at a high risk of contamination by mycotoxins, therefore representing a critical issue for food and feed safety.

[1] Audenaert, K.; van Broeck, R.; van Bekaert, B.; de Witte, F.; Heremans, B.; Messens, K.; Höfte, M.; Haesaert, G. Fusarium Head Blight (FHB) in Flanders: Population Diversity, Inter-Species Associations and DON Contamination in Commercial Winter Wheat Varieties. *Eur J Plant Pathol* 2009, *125*, 445-458, doi: 10.1007/s10658-009-9494-3.

[2] Karlsson, I.; Persson, P.; Friberg, H. Fusarium Head Blight From a Microbiome Perspective. *Front Microbiol* 2021, *12*, doi.org/10.3389/fmicb.2021.628373.

[3] Spaggiari, M.; Righetti, L.; Galaverna, G.; Giordano, D.; Scarpino, V.; Blandino, M.; Dall'Asta, C. HR-MS Profiling and Distribution of Native and Modified Fusarium Mycotoxins in Tritordeum, Wheat and Barley Whole Grains and Corresponding Pearled Fractions. *J Cereal Sci* 2019, *87*, 178-184, doi: 10.1016/j.jcs.2019.03.009.

[4] Scarpino, V.; Blandino, M. Effects of Durum Wheat Cultivars with Different Degrees of Fhb Susceptibility Grown under Different Meteorological Conditions on the Contamination of Regulated, Modified and Emerging Mycotoxins. *Microorganisms* 2021, *9*, 1-22, doi: 10.3390/microorganisms9020408.

[5] Křížová, L.; Dadáková, K.; Dvořáčková, M.; Kašparovský, T. Feedborne Mycotoxins Beauvericin and Enniatins and Livestock Animals. *Toxins (Basel)* 2021, *13*, doi.org/10.3390/toxins13010032.

L2

PUTATIVE TREMORGENIC MYCOTOXICOSIS IN A DOG – CASE REPORT

Leon Hart[1], Christina Rehagel[1], Michael Kuhn[2], Lukas Jozefowitz[3], Esther Haßdenteufel[3], Benedikt Cramer[2], Ewald Usleber[1]

[1]Chair of Dairy Sciences, Institute of Veterinary Food Science, Justus-Liebig-University, Giessen, Germany
[2]Institute of Food Chemistry, University of Münster, Germany
[3]Clinic for Small Animals, Department of Veterinary Clinical Sciences, Justus-Liebig-University, Giessen, Germany

Abstract

A seven-year-old female dog (Labrador mix), who had developed signs of generalized tremor shortly after ingestion of some unknown material, was presented by the owner at the Clinic for Small Animals. The owner reported that he had observed blue granules at the site where the suspect material was ingested. The physical examination revealed generalized muscle tremor, hypersalivation, tachypnea and tachycardia. Since intoxication with slug pellets (methiocarb or metaldehyde) was suspected, gastric lavage was carried out. The lavage liquid contained solids visibly resembling compost material, but no remains of slug pellets were found. Blood tests were conducted but showed no abnormalities. Following gastric lavage, non-specific detoxification therapy was carried out, which included lipid therapy, intravenous fluid therapy with buffered isotonic cristalloid solution, oral activated charcoal, and maropitant was given intravenously as an antiemetic. The symptoms disappeared within one day, and the dog was discharged.

The solid material recovered from the gastric lavage was analysed for neurotoxic mycotoxins by several enzyme immunoassays (EIA), namely ergoline alkaloids, penitrem A, paxillins, α-cyclopiazonic acid. The EIAs for penitrem A and paxilline were strongly positive, whereas no ergoline alkaloids or α- cyclopiazonic acid could be detected. Control analyses of sample extract by HPLC-MS/MS and HPLC-HRMS for penitrems A-E and paxilline qualitatively confirmed the EIA results. Mycological culture of lavage residues on malt extract agar, potato extract agar and Sabouraud dextrose agar yielded fungal mycelium (*Penicillium* spp., as determined by microscopy) which was extracted with methanol for EIA analyses. This extract was also positive for penitrem A. We conclude that penitrems and paxilline were the most likely causes of intoxication in this dog. Although neurotoxic food poisoning is not uncommon in dogs, specifically in food-motivated breeds like Labradors, well documented cases with confirmed analytical data for mycotoxins are very scarce. For Germany, this is the first reported case of penitrems and paxilline as putative causes for acute mycotoxicosis.

L3

TARGETED INOCULATION WITH FUSARIUM CULMORUM BEFORE GERMINATION: EFFECTS OF FUSARIUM TOXINS FROM MALTING THROUGH THE BREWING PROCESS

Eva M. Biehl, Ibrahim-Can Akpak, Michael Rychlik

Chair of Analytical Food Chemistry, Technical University of Munich (TUM),
Maximusvon Imhof-Forum 2, 85354 Freising, Germany

Fusarium species can infect almost all cereals, including barley, and can lead to reduced grain yield, loss of quality, and may be a risk to consumer safety due to the formation of mycotoxins. It is known that mycotoxins produced by *Fusarium* persist during malting and brewing processes and end up in the final beer product, posing a potential risk to consumer health. Given the global importance of beer as a beverage, it is crucial to develop cost-effective methods for mycotoxin analysis in beer. This would allow better monitoring of mycotoxin contamination and enable industry to take necessary measures to mitigate risks to consumer health. A model system for germinating barley was developed. Targeted inoculation with *Fusarium culmorum* before germination was used to produce defined, infected material for investigating *Fusarium* infection and mycotoxin production during the malting and the following beer brewing processes. The malting process was performed using a standard micro-malting procedure according to MEBAK guidelines and beer was brewed afterwards in a microbrewery scale [1]. Samples were taken at key steps during the malting and beer brewing processes and analyzed for 14 *Fusarium* toxins using different, validated LC-MS/MS methods that included the modified mycotoxin deoxynivalenol-3-glucoside (DON-3-Glc). Sample purification was achieved by a previous SPE clean-up step and quantification of mycotoxins was done via stable isotope dilution assay and matrixmatched calibration. Inoculation with *F. culmorum* did not result in clearly visible fungal growth within all approaches but resulted in toxin production in the germinating barley. This study found an increase in deoxynivalenol (DON) and DON-3-Glc during the malting process, with DON-3-Glc found in even higher concentrations than DON itself. The toxin concentrations were constantly measured during the processes. High concentrations of DON, 3-acetyl-DON and especially DON-3-Glc could also be determined in the beer brewed afterwards. These findings indicate that the modified form of the mycotoxin are also present and should be considered when assessing mycotoxin levels in cereals, malt and beer products.

[1] Jacob, F. (2016). MEBAK brautechnische Analysenmethoden-Rohstoffe: Rohfrucht, Gerste, Malz, Hopfen und Hopfenprodukte: Methodensammlung der Mitteleuropäischen Brautechnischen Analysenkommission. Selbstverlag der MEBAK, Freising-Weihenstephan. „Financial support in form of a PhD scholarship from the Cusanuswerk e.V. is acknowledged."

L4

MYCOTOXINS AS AGRICULTURAL & LIVESTOCK BIOSECURITY THREATS: FROM MAIZE SEEDS TO RAW MILK CONTAMINATION

Marta Leite[1,2,3]*, Andreia Freitas[2,3], Jorge Barbosa[3], Fernando Ramos[1,3]

[1]University of Coimbra, Faculty of Pharmacy, Health Science Campus, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal
[2]National Institute for Agricultural and Veterinary Research (INIAV), Rua dos Lágidos, Lugar da Madalena, 4485-655 Vila do Conde, Portugal
[3]REQUIMTE/LAQV, R. D. Manuel II, Apartado 55142, Oporto, Portugal

*corresponding author: marta.leite@iniav.pt

Maize value chain represents one of the most valuable and complex feed and food processes worldwide, with a global supply and consumption forecasted to expand in the coming years [1]. As an agricultural and industrial-based product, invasion, colonization, and production of mycotoxins by fungi can occur in pre-harvest and post-harvest stages of the maize food and feed chain, with a severe economic and health impact comprising direct losses in crops, production profitability, product safety and, ultimately, human and animal health [1,2]. Continuous monitoring of the occurrence and co-occurrence of mycotoxins throughout whole food chains presents a crucial process towards the implementation of accurate risk assessments and, consequently, the definition of risk management policies compliant with the application of correct mitigation and reduction strategies.

This work aimed at the determination of regulated, non-regulated and emerging mycotoxins in representative samples of the maize value chain, including agricultural field (seeds, flowering plants, and harvested maize, e.g. silage and maize grains), and dairy farm samples (animal feed and raw milk samples) from Portugal, through a validated QuEChERS based-analytical methodology comprising liquid chromatography coupled to sequential mass spectrometry detection (UHPLC-QTrap-MS/MS) [3,4]. Sampling plan design was performed as according to Commission Regulation (EC) nº 401/2006 [5]. Thirty-three agricultural fields from North, Center and Alentejo regions of Portugal were monitored in 2020; and eight dairy farms from the main dairy region of Portugal were analyzed for animal feed and respective feed ingredients. One-hundred raw milk samples were also collected from this region corresponding to 100 dairy farms, between 2020 and 2021. Solid samples were previously grinded to obtain fine powder, and all samples were ultimately stored at -20±2°C, until further analysis.

In relation to the overall occurrence data, regulated and emerging mycotoxins were identified in all samples. High contamination percentages were found in maize silage, which represented the major component contributing for the overall contamination in the final product (animal feed). Globally, the highest percentage of positives was found for the mycotoxins FB1 and FB2 in maize silage; DON in feed; ENNB in all analyzed samples; BEA and TTX in maize silage and feed. Concerning milk samples, at least one mycotoxin was found in 97% of the samples, with the highest prevalence for emerging mycotoxins. BEA and ENNB were found in 90% and 77% of samples, respectively, with FB1 and B2 being also found in 10 and 31 samples, respectively. No AFs, including AFM1, were found in the analyzed samples.

A first insight on a full screening of several regulated, non-regulated, and emerging mycotoxins in the maize value chain was achieved, allowing to perform a unique and innovative approach comprising a full assessment of a representative food chain. The high occurrence of mycotoxins, especially emerging mycotoxins, points out the need to perform further occurrence surveys in this matrix, towards a more sustainable and safer agriculture and industrial food processes, while protecting consumer's health.

[1] Leite, M., Freitas, A., Silva, A. S., Barbosa, J., & Ramos, F. (2021). Maize food chain and mycotoxins: A review on occurrence studies. Trends in Food Science and Technology, 115(2021), 307-331. https://doi.org/10.1016/j.tifs.2021.06.045

[2] Leite, M., Freitas, A., Silva, A. S., Barbosa, J., & Ramos, F. (2020). Maize (*Zea mays* L.) and mycotoxins: A review on optimization and validation of analytical methods by liquid chromatography coupled to mass spectrometry. Trends in Food Science and Technology, 99(2020), 542-565. https://doi.org/10.1016/j.tifs.2020.03.023

[3] Leite, M., Freitas, A., Barbosa, J., & Ramos, F. (2022). Comprehensive assessment of different extraction methodologies for optimization and validation of an analytical multi-method for determination of emerging and regulated mycotoxins in maize by UHPLC-MS/MS. Food Chemistry Advances, 2(2023), 1-12. https://doi.org/10.1016/j.focha.2022.100145

[4] Leite, M., Freitas, Barbosa, J., & Ramos, F. (2023). Mycotoxins in Raw Bovine Milk: UHPLC-QTrap-MS/MS Method as a Biosafety Control Tool. Toxins, 15(3), 1-15. https://doi.org/10.3390/toxins15030173

[5] European Commission (2006b). Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Official Journal of the European Union, 70(401), 12-34.

ACKNOWLEDGEMENTS This work was financially supported by the FCT – Portuguese Foundation for Science and Technology, I.P. [grant nº UIDB/50006/2020]. Marta Leite also wishes to acknowledge FCT, I.P., for supporting the present research, through the Ph.D. grant number SFRH/BD/141778/2018.

L5

INVESTIGATIONS INTO THE MYCOTOXIN-PRODUCING CAPACITY OF MOULDS ISOLATED FROM CHEESE

Daniela Schale[1], Bettina Seeger[1], Nadja Jeßberger[1], Sophie Kittler[1], Christina Rehagel[2], Leon Hart[2], Ewald Usleber[2], Madeleine Plötz[1]

[1]Institute of Food Quality and Food Safety, University of Veterinary Medicine Hannover, Germany
[2]Chair of Dairy Sciences, Institute of Veterinary Food Science, Justus-Liebig-University Giessen, Germany

The variety of mould-ripened cheese products on the market has increased significantly in recent years. This applies particulary the diversity of artisan cheeses, organic products and "farmer to consumer" products. Traditionally, defined mould cultures, such as *Penicillium camemberti* (white mould) and *P. roqueforti* (blue mould), are used in cheese-making dairies to produce mould-ripened cheeses. However, not all moulds that can be isolated from cheese are necessarily of defined origin. Depending on the production conditions, uncontrolled environmental contamination with moulds is also possible. While the potential for mycotoxin formation in classical white and blue mould cultures is relatively well known – at least qualitatively – environmental moulds contaminating the product during cheesemaking could cause a largely unexplored risk of mycotoxin production. The aim of this work is to obtain an overview of the identity and mycotoxin-forming capacity of moulds from commercial cheeses of the German market. The mycotoxin analyses include compounds known to be produced by *P. camemberti* and *P. roqueforti*. In addition, several other mycotoxin groups are also covered in this study. Fungal isolates were identified by Matrix Assisted Laser Desorption/ Ionisation (MALDI-TOF-MS) after culture and microscopic examinations, while mycotoxins are detected by a set of Enzyme-Linked Immunosorbent Assays (ELISA). Regarding fungal species, *P. roqueforti*, *P. camemberti*, *P. commune*, *P. aurantiogriseum*, *P. expansum*, *Geotrichum candidum*, *Alternaria alternata*, *Aspergillus niger*, *Scopulariopsis brevicaulis*, *Debaryomyces hansenii* and *Mucor sp.* have been detected so far. In culture on malt extract agar, some of these isolates were found to be strong producers of mycotoxins known to be produced by *P. camemberti* and *P. roqueforti*, e.g. α-cyclopiazonic acid, mycophenolic acid, and ergoline/clavine alkaloids. For some isolates, ELISA results also indicated the production of tremorgenic mycotoxins.

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L6

THERMAL STABILITY OF THE MYCOTOXIN CITRININ

Lea Brückner, Benedikt Cramer, Hans-Ulrich Humpf

Institute of Food Chemistry, University of Münster, Germany

Citrinin is a secondary microfungal metabolite and is attracting worldwide attention due to its toxic effects (nephrotoxic, teratogenic and embryotoxic). Typical citrinin producers are fungi of the genera *Penicillium*, *Aspergillus* and *Monascus*. Among the most affected foods are cereals, red mold rice and spices.

A study funded by the EFSA found that citrinin levels in processed cereals were significantly lower than those in unprocessed cereals. Overall, citrinin was detected in only 8% of the cereal samples tested [1]. In contrast, when human samples such as blood or urine are analyzed for citrinin, they usually show quite high levels of contamination [2,3].

The discrepancy of citrinin levels in processed and non-processed foods suggests that food processing and especially thermal processing has a decisive influence on the citrinin concentration in food. It is conceivable that citrinin reacts with components of the food matrix during food processing and is thus masked to conventional analytical techniques.

Therefore, in the course of this project model experiments were conducted to investigate which food components can react with citrinin upon heating. Important cereal components such as sugar, starch and functional groups in proteins were mimicked by model components. In comparison, citrinin was also heated under the same reaction conditions without reaction partners. The resulting reaction products were then analyzed using high-resolution UHPLC-DAD-ESI-QTOF methods and the data statistically evaluated using volcano-plot analyses, among other methods. Reaction products were isolated and further characterized with the aim to use them as standards for investigations in real food samples after precise structure elucidation by means of NMR spectroscopy.

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L7

DEVELOPMENT AND EVALUATION OF SAMPLE PREPARATION METHODS FOR MYCOTOXIN ANALYSIS IN PLANT-BASED MILK ALTERNATIVES BY ENZYME IMMUNOASSAYS

Christina Rehagel[1], Ronald Maul[2], Kim Lara Gützkow[2], Ömer Akineden[1], Ewald Usleber[1]

[1]Dairy Sciences, Institute of Veterinary Food Science, Justus-Liebig-University Giessen, Ludwigstrasse 21, 35390 Giessen, Germany
[2]Department Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Hermann-Weigmann-Strasse 1, 24103 Kiel, Germany

Plant-based milk alternatives (PBMAs) represent a complex matrix consisting of proteins, carbohydrates, lipids, dietary fibre, various additives (stabilisers, emulsifiers), and flavours, with the ingredients more or less stabilized as emulsions or colloidal dispersions. For analytical purposes, it is necessary to use appropriate sample preparation methods, to avoid matrix effects that may interfere with quantification. Published studies on the occurrence of mycotoxins in PBMAs exclusively involve liquid chromatography with tandem mass spectrometry or fluorescence detection, in combination with laborious or expensive sample pre-treatment methods, such as dispersive liquid-liquid microextraction or QuEChERS-like methods. While these methods are convenient in a laboratory environment, they are less suitable for rapid on-site quality control at the production site. This study, in a first approach, investigated the applicability of enzyme immunoassay (EIA) methods for direct testing of PBMAs without sample extraction. Mycotoxin analyses included aflatoxin B_1 (AFB_1), sterigmatocystin (STC), ochratoxin A (OTA), deoxynivalenol (DON), and T-2/HT-2-toxin (T-2/HT-2). Toxin standard curves prepared in diluted PBMA showed dilution-dependent deviations from the standard curves in plain buffer solution, indicating considerable matrix interference, affecting the reliability of the results. Initially, a dilution of PBMAs of at least 1:8 seemed to be sufficient to overcome matrix interference. This resulted in calculated detection limits of 0.4 µg/L (AFB_1), 2 µg/L (STC), 0.08 µg/L (OTA), 16 µg/L (DON), and 0.4 µg/L (T-2/HT-2). After analysis of 54 PBMA products from German retail stores, as many as 23 samples yielded positive results. However, most positive EIA results were close to the calculated detection limit. Control analyses of selected samples by LC-MS/MS for AFB_1, STC, and OTA qualitatively confirmed the presence of trace amounts of STC in some samples, but overall quantitative agreement was poor, including false-positive EIA results. It was assumed that the high diversity of ingredients used in PBMAs led to a highly variable degree of sample matrix interference, even in a 1:8 dilution. However, analysis of PBMA in higher dilutions would have conflicted with the need to achieve low detection limits. Consequently, we made further experiments aiming at 1) identifying possible causes of the matrix interference and 2) development of a convenient sample preparation method to overcome these effects. First results indicate that the more complex the composition of a PBMA product is the stronger are the observed non-specific effects on EIA detection. Experiments on sample preparation focus on eliminating interfering matrix compounds by filtration techniques, and by selective extraction/enrichment of mycotoxins by liquid-liquid-partitioning techniques.

L8

COMBINED TOXICITY OF AFLATOXIN B1 AND DEOXYNIVALENOL: ROLES OF RIBOTOXICITY AND CYTOCHROME P450 ENZYMES

Bernadette Willoquet, Gladys Mirey, Delphine Payros, Isabelle P. Oswald*

Toxalim, Research center in Food Toxicology, Université de Toulouse,
INRA, ENVT, INP-PURPAN, UPS, Toulouse, France

*corresponding author: isabelle.oswald@inrae.fr

Aflatoxin B1 (AFB1) and deoxynivalenol (DON) are two mycotoxins of particular concern because of their toxicity and occurrence in foodstuffs. AFB1, found mainly in cereals and nuts, is the most potent natural carcinogen. Primarily in the liver, it is bioactivated into AFB1-8,9-epoxide, a genotoxic compound, under the action of cytochrome P450 enzymes (CYP450), in particular CYP1A2 and CYP3A4 [1]. DON, the mycotoxin most present in foodstuffs, is found preferentially in cereals and cereal-based products. It mainly targets the intestine and the immune system but also the liver [2] [3].

In the context of the multiple exposure to mycotoxins, combined toxicity of AFB1 and DON has been investigated. First, genotoxicity has been evaluated in the hepatic HepG2 cell line, by In-Cell Western and immunostaining. As expected, AFB1 induced a dose-dependent genotoxicity while no effect of DON was observed. However, upon combined exposure to AFB1 and DON, a lower genotoxicity was observed as compared to AFB1 alone.

Since DON induces ribotoxic stress at the cellular level, the role of ribotoxicity in this phenotype has been studied. HepG2 cells were exposed to both AFB1 and model ribotoxins. As with DON, genotoxicity of AFB1 was inhibited, suggesting a role of its ribotoxicity in the phenotype.

The role of CYP450 enzymes has been investigated as well. The catalytic activities of CYP1A2 and CYP3A4 have been quantified using Promega P450-Glo™ assays. AFB1 alone had no effect; instead, exposure to DON, alone or in mixture, led to a decrease in both CYP450 activities. This result suggests that upon exposure to AFB1 and DON, the bioactivation of AFB1 is inhibited, explaining its weaker genotoxicity.

As a second approach, the genotoxicity and CYP450 expression upon exposure to AFB1 and DON have been studied in the HepaRP, a cell line that is surrogate for primary hepatocytes. The In-Cell Western and Western blot techniques allowed to observe both weaker genotoxicity and weaker expression of CYP1A2 and CYP3A4 upon exposure to the mixture, confirming the above-described phenotype in a more competent model.

In conclusion, this study demonstrates an interaction between AFB1 and DON that involves the ribotoxicity of DON and results in inhibited genotoxicity of AFB1, seemingly through its reduced bioactivation.

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L9

TOXICITY OF ENNIATINS A, A1, B, B1 AND BEAUVERICIN ON INNOVATIVE IN VITRO HUMAN CELL MODELS AND THEIR IMPACTS ON CELLULAR GENE EXPRESSION

France Coulet, Monika Coton, Emmanuel Coton, Nolwenn Hymery

Univ Brest, INRAE, Laboratoire Universitaire de Biodiversité et Écologie Microbienne, F-29280 Plouzané, France

Mycotoxins contaminate more than 70% of cereal crops worldwide and therefore constitute a public health problem [1]. Among mycotoxigenic molds, *Fusarium* spp. are the most recurring and are linked to enniatins (ENNs) contamination [2]. ENNs are considered «emerging» mycotoxins, a term referring to poorly characterized or only recently identified as secondary metabolites. However, their occurrence on cereals is particularly high. A few *in vitro* studies, mainly on ENNs B and B1, have shown various effects including DNA alteration, apoptosis, mitochondrial damage and production of reactive oxygen species. Yet, the lack of *in vitro* and *in vivo* ENN toxicity data prevents robust human health risk assessment [3-6]. It is therefore important to address these issues by mimicking human exposure conditions as closely as possible. In order to take into account the mycotoxin exposure process (i.e. through food consumption), we evaluated ENNs toxicity on liver cells as they are responsible for xenobiotic detoxification. Acute toxicity of enniatins A, A1, B, B1, and beauvericin was evaluated by cell viability tests on 2D and 3D hepatic cell line models (HepaRG). To further understand the impact of ENN B and ENN B1 (IC0, IC10 and IC30) toxicity, RNA-seq analyses on HepaRG spheroids, a more relevant hepatic model, were also performed and over- and under-expressed genes were identified. These key genes pinpointed the main pathways involved after acute exposure. The obtained data provides novel knowledge on ENNs toxicity after acute exposure and the impact on cell gene expression.

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L10

COMPUTER-EMPOWERED DIGGING OF BIG DATA TO TACKLE TOXICITY ASSESSMENT AND BIOREMEDIATION OF EMERGING MYCOTOXINS – A 4-YEAR LONG ROMANCE WITH FUSARIC ACID

Lorenzo Pedroni, Gianni Galaverna, Chiara Dall'Asta and Luca Dellafiora

Department of Food and Drug, University of Parma, Parma, Italy

Fusaric acid (FA) is a secondary metabolite produced by various *Fusarium* species, present with a relatively high incidence in a wide variety of food. FA was already described to be phytotoxic due to its capability to impair plant growth, and detoxification routes effective for plants have been already proposed (e.g. [1]). FA proved also to be cytotoxic to mammalian cells and toxic *in vivo* in several animal species, thus being listed among emerging mycotoxins [2]. However, the understanding of FA's toxicokinetics (TK) and toxicodynamics (TD) suffer of wide gaps of knowledge and the comprehension of its molecular mechanisms is still fragmentary. This scenario makes further data needed to ensure an informed assessment of the risk related to its presence in food. A computer-driven big data analysis was performed to tackle with such data gaps.

Specifically, an *in silico* target-fishing campaign was designed and applied to mine FA-interacting proteins out the available protein "structurome", which is referred to as the whole protein structural data made publicly available so far that includes more than 200 thousands crystallographic structures and 1 million computational structures (Protein Data Bank, last access 24th Feb 2023). The campaign was meant to identify proteins involved in the TK/TD of FA. The analysis identified the protein kinase A (PKA) as a novel biological target of FA [3], in addition to the dopamine beta hydroxylase (DBH) previously identified [4]. Of note, PKA was deemed a meaningful target from a food safety standpoint considering the potency observed and the level of contamination detected in food.

Also, the screening fished a bacterial cytochrome P450 (CYP) from *Rhodopseudomonas palustris* out of the CYP structures available at the time of analysis (roughly 600 structures) as capable of hydroxylate FA. This sequence was used to mine CYPs from human proteome, to shed light on the ADME-related actors in humans, though with no success due to the wide phylogenetic distance between human and bacterial CYPs. However, serendipitously, analogies with the CYPs of *Mucor* species were found. Interestingly, *Mucor ruoxii* was described as able to detoxify FA for plants via aliphatic hydroxylation, although the *Mucor's* enzyme(s) involved in such a process was unknown. Therefore, we used the CYP of *R. palustris* as a bait to grasp among the proteome of *Mucor* genus (more than 100 thousand sequences) CYPs theoretically able to hydroxylate FA. Although the characterization of *Mucor* genomes is still very fragmentary, three sequences were successfully identified deserving further investigations to verify their actual biotransforming activity over FA.

Finally, the effects of FA hydroxylation on the capability to interact with PKA and DBH was assessed via molecular modeling highlighting that those modifications seem not effective to prevent the interaction with the targets. This opens two hypothesis deserving further dedicated studies: i) unlike plants, hydroxylation of FA is not a detoxification route in humans; ii) if hydroxylated metabolites will test less toxic in humans, the underpinning mechanisms are likely to rely on TK-related reasons, such as an augmented excretion and/or phase II metabolism.

As a general remark, this 4-year long novel on FA extended the current knowledge on its toxicity, identifying a new biological target (PKA) and providing a preliminary toxicity evaluation of some FA phase I metabolites. Also, enzymes able to transform FA in derivatives less toxic for plants were identified and they could be considered for environmental bioremediation strategies in the future. Finally, computer driven studies over big data proved to be an effective analytical approach for a deep-analysis at a various level of complexity toward a thorough analysis of emerging mycotoxins.

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L11

MYCOTOXIN CONTAMINATION IN THE ARAB WORLD: HIGHLIGHTING THE MAIN KNOWLEDGE GAPS AND DISCUSSING THE CURRENT LEGISLATIONS

Mohamed F. Abdallah[1]*, Muhammad Gado[2], Doha Abdelsadek[3], Fatma Zahran[4], Nada N. El-Salhey[5], Ohaila Mehrez[6], Sara Abdel-Hay[7], Sahar Mahrous[8], Karl De Ruyck[9], Elisabeth Varga[10]

[1]Department of Veterinary Toxicology and Forensic Medicine, Assiut University, Assiut 71515, Egypt
[2]Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt
[3]Faculty of Pharmacy, Cairo University, Cairo 12613, Egypt
[4]Faculty of Pharmacy, Menoufia University, Menoufia 6131567, Egypt
[5]Faculty of Pharmacy, Kafrelsheikh University, Kafrelsheikh 33516, Egypt
[6]Faculty of Pharmacy, Ain Shams University 11566, Cairo, Egypt
[7]Faculty of Pharmacy, Tanta University, Tanta 31527, Egypt
[8]Department of Chemistry, Faculty of Science, Sohag University, Sohag 82524, Egypt
[9]Teagasc Food Research Centre, Ashtown, Dublin 15 D15 KN3K, Ireland
[10]Department of Food Chemistry and Toxicology, Faculty of Chemistry, University of Vienna, 1090 Vienna, Austria

Nowadays, mycotoxins are among the most alarming food and feed contaminants threatening human and animal health. Since the discovery of aflatoxins in the 1960s, our knowledge about mycotoxins increased dramatically: biological syntheses were unravelled; myriad analytical detection methods were developed; surveys in different matrices were conducted; investigations into toxicities took place; several methods for control have been tested; and legislative debates to protect the public from their toxic effects also continue. Based on that, hundreds of review articles were published to summarize different areas in the mycotoxin field, including their contamination per country or per region. However, mycotoxin contamination in the Arab world, which includes 22 countries in Africa and Asia, have not been specifically reviewed.

To this end, the present work has been conducted to review the contamination of mycotoxins in the Arab world to identify the main knowledge gaps needed to enhance the safety of food and feed. Our search, using several keywords, covered all the (non-)indexed publications written in English or Arabic or French. To the best of our knowledge, a total number of 306 papers were published between 1977 and 2021 focusing on the natural occurrence of mycotoxins in different matrices, which fall under one of 14 different categories (animal feed; animal products; baby food; cereals; cereal by-products; dairy products; legumes and pulses; nuts; spices; fruits and vegetables; juices and drinks; biomarkers; oils; and others). The relevant information (e.g., detected mycotoxins; number of samples; concentrations; method of detection) were extracted, processed and visualized using Microsoft Excel, R, GraphPad Prism and RAWGraphs.

The main results are presented as follow:

- Research on mycotoxins has increased over the years, however the accumulated data on their occurrences are scarce to non-existent in some countries and regions.
- State-of-the-art technologies on mycotoxin detection are not well implemented; neither are contemporary multi-mycotoxin detection strategies in the Arab world, evidencing needs for capacity building initiatives.
- Mycotoxin profiles differ among food and feed categories, as well as between human biofluids.

An overview will be presented relying on visualization for an easy take home message delivery. Furthermore, the current legislations in the Arab countries will be discussed in relation to the occurrence of mycotoxins in the Arab world.

L12

MYCOTOXINS IN INSECTS FOR FOOD AND FEED

Ronald Maul[1]*, Kim Lara Gützkow[1], Nina Kröncke[2], Johanna Ebmeyer[3], Nadja Kampschulte[4], Linda Böhmert[3], Cindy Schöne[5], Nils Helge Schebb[4], Albert Braeuning[3], Rainer Benning[2]

[1]Max Rubner-Institute, Federal Research Institute of Nutrition and Food,
Department Safety and Quality of Milk and Fish Products, 24103 Kiel, Germany
[2]University of Applied Sciences Bremerhaven, Germany
[3]German Federal Institute for Risk Assessment, Department Food Safety, 10589 Berlin, Germany
[4]University of Wuppertal, Faculty of Mathematics and Natural Sciences, Chair of Food Chemistry,
42119 Wuppertal, Germany
[5]German Federal Institute for Risk Assessment, Department Safety in the Food Chain, 10589 Berlin, Germany

*corresponding author: Ronald.maul@mri.bund.de

The search for alternative sources for animal protein required by a growing world population has been steadily intensified in agricultural and nutritional research for years. The use of insects, which have a small ecological footprint due to their very efficient nutrient and water balance, is a promising approach [1]. The acceptance of low processed insects as food is low in the European population. However, the use of insects in the animal feed sector in fish and poultry feeding seems to be generally accepted. At present, the same feed law requirements apply to the feeding of the permitted insect species as to all farm animals, i.e. there are minimum standards for the quality and safety of the initial feed. This is intended to ensure not only microbiological but also chemical safety along the process chain, since the possible accumulation of contaminants in the insect could pose a potential risk for their use in food and feed production. The presentation will show recent data on the effects of different mycotoxins on the insects as well as the transfer and metabolism in the insect with special focus on the impact of aflatoxin B_1 (AFB_1) in *Tenebrio molitor* larvae. In the in vivo trial it was found that the larvae tolerate up to 10 mg/kg AFB_1 in the feed with little effect on the growth rate. Analysis of the larvae and their excrements revealed the formation of phase I metabolites including a novel monohydroxylated AFB_1 derivative. Mass balance quantification of ingested AFB_1 revealed that 87% of the initial toxin remain undetected in larval body or residue. Analysis of histone H2Ax phosphorylation in human liver cells as a surrogate for genotoxicity showed that extracts from exposed larvae did not exhibit an elevated toxic potential [2]. The low recovery of the initial AFB_1 content leaves uncertainties about the fate of a big part of the toxin. Nevertheless, according to the mutagenicity assay results, the remaining toxic potential of the larvae appears to be low. Although present EU regulation still prohibits the further use as animal feed, it may be assumed that the resulting insects could be a safe source for e.g. poultry or fish feeding. The results of the present study are in line with prior studies on other mycotoxins also documenting the efficient excretion of e.g. trichothecenes or zearalenone from the larval body [3,4]. In consequence it should be discussed whether larvae of *T. molitor* or the black soldier fly (*Hermetia Illucens*) could be utilized for the remediation of mycotoxins from contaminated grains, helping to reduce food/feed loss due to fungal spoilage of various grain commodities.

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L13

T-2 AND HT-2 TOXINS IN MILLING OATS AND OAT MILLING PRODUCTS

Inga Hennies*, Jens Chr. Meyer

H. & J. Brüggen KG, Hafenstraße 5, D-23568 Lübeck, Germany

*corresponding author: jens.meyer@brueggen.com

The common oat (*Avena sativa* L.) is well known for its nutritional benefits, although it carries the risk of contamination with mycotoxins due to its susceptibility to the growth of various fungi.

Oats that can be processed in an oat mill are called milling oats in the following. In addition to legal requirements, there are also physical requirements for milling oats that are important for processing [1], [2].

Although oats are grown worldwide, hardly any oats from countries outside the European Union (EU) were processed in the EU due to an import duty of € 89 per tonne [3]. However, this is not valid for oats from the United Kingdom (UK) and will gradually change for Canadian oats due to the free trade agreement with the EU [4]. In the European Union, approximately 7.6 million tonnes of oat grains were produced in 2022. Of this, approximately 1 million tonnes (c. 13%), grown mainly in Finland, Sweden, Germany and Ireland, will be processed as milling oats, while the majority of over 5 million tonnes will be used as feed oats (EU-27 oats balance sheet – [5].

The toxins T-2 and HT-2 belong to the Group A Trichothecenes and are mainly produced by *Fusarium langsethiae* [6], [7], [8], [9], and on a lesser scale also by *F. poae* and *F. sporotrichioides* [7], [10].

In 2022, the European Commission commenced more formally reviewing the necessity for maximum limits for the sum of T-2 and HT-2 toxins, and a draft Commission Regulation was circulated to stakeholders [11]. A reduction rate of 96% was assumed for the sum of T-2 and HT-2 toxins starting from 1250 µg/kg for milling oats down to 50µg/kg for oat milling products

The reduction rate of the T-2 and HT-2 toxin content depends on 3 factors. These are the cleanliness of the milling oats upon mill intake, the proportion of husk and the growth of *Fusarium langsethiae* into the oat kernel [12], [13], [14]. Therefore, milling oat consignments were selected in order to understand the reduction rates on industrial scale. The average reduction rate of all examined production runs was 85% for larger kernels and 66% for thinner kernels. Only one sample was able to achieve the 96% reduction rate assumed by the European Commission. It was therefore investigated why de-hulling alone is not sufficient to sufficiently reduce the sum of T-2 and HT-2 toxins.

Recent studies by Divon et al. [14] showed that *Fusarium langsethiae* is able to also grow into the outer layers of oat kernels. The extent of growth into oat kernels was investigated on laboratory scale in order to achieve the most accurate possible identification [15]. For this purpose, samples from the harvest years 2011 to 2016 were examined. Samples were processed with a laboratory de-huller and thus oat kernels and husks were separated from each other. The oat grains and the corresponding oat kernels were analysed according to the DNA amount of *F. langsethiae* and the sum of T-2 and HT-2 toxins. The correlation of both parameters was significant ($R^2 = 0.85$; $p < 0.01$) in oat kernels (no husk). The reduction of the sum of T-2 and HT-2 toxins is thus clearly limited by nature.

Oat flakes analysed in Ireland, the UK and Germany showed for the sum of T-2 and HT-2 toxins mean levels of 34, 52 and 18 µg/kg respectively. 95 Percentile were at 100, 105 and 50 µg/kg respectively [16], [17], [18]. Interestingly highest values observed in Germany were coming mainly from Finland and the Baltics [18].

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L14

CYCLODEXTRINS AS POTENTIAL MYCOTOXIN BINDERS: THE SUMMARY OF EARLIER AND RECENT STUDIES

Miklós Poór, Eszter Fliszár-Nyúl

Department of Pharmacology, Faculty of Pharmacy, University of Pécs, Pécs, Hungary

Cyclodextrins (CDs) are ring-shaped cyclic oligosaccharides built up from glucose units. The internal cavity of CDs can accommodate apolar guest molecules, including certain mycotoxins. With the chemical modification of CDs, their complex formation abilities can be further improved. Insoluble (but water-swellable) CD polymers are also available, showing promising impacts regarding the removal of xenobiotics from aqueous matrices. Therefore, CD technology may be suitable for toxin removal and/or for analytical sample preparation.

The formation of mycotoxin-CD complexes can cause strong increase in the emission signal of fluorescent mycotoxins, as it has been demonstrated with zearalenone, zearalenols, zearalenone-14-sulfate, and alternariol. This impact of CDs makes possible the more sensitive fluorescent detection of the above-listed mycotoxins.

Mycotoxins typically form barely stable complexes with native CDs. However, certain chemically-modified derivatives showed strong interactions with mycotoxins: e.g., quaternary-ammonium-beta-CD with ochratoxin A, dimethyl-beta-CD with zearalenone and zearalenols, or sugammadex with alternariol.

Insoluble beta-CD bead polymer (BBP) successfully extracted some mycotoxins from aqueous solutions, including alternariol, ochratoxin A, and zearalenone. In addition, BBP can also bind masked/modified derivatives of alternariol and zearalenon, where the polymer was highly suitable for the removal of sulfate derivatives and induced the moderately strong extraction of glucosides. In addition, CD polymers considerably decreased the amounts of zearalenone in corn beer as well as alternariol and ochratoxin A in red wine samples.

Since the formation of highly stable ligand-CD complexes can entrap the mycotoxin in the CD cavity and consequently can limit its cellular uptake, we tested the protective effects of CDs against mycotoxin-induced toxicity using in vitro and in vivo models. In our experiments performed on HeLa cells and on zebrafish embryos, certain CDs showed strong protective action against alternariol- and zearalenone-induced toxicity.

Based on the above-listed observations, CD technology seems to be suitable for the development of new mycotoxin binders. Therefore, CDs may be applied for analytical mycotoxin extraction, for the removal of mycotoxins from certain beverages, and/or for the reduction of mycotoxin-induced toxicity.

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L15

OCHRATOXIN A AND PARKINSON DISEASE: IS THERE A LINK?

Ariane Vettorazzi[1,4]*, María Serrano[1], Elba Beraza[1], María Izco[2], Amaya Azqueta[1,4], Elena González-Peñas[3], Adela López de Cerain[1,4], Lydia Alvarez-Erviti[3]

[1]Department of Pharmacology and Toxicology, MITOX Research Group, School of Pharmacy and Nutrition, University of Navarra, Pamplona, Spain
[2]Laboratory of Molecular Neurobiology, Center for Biomedical Research of La Rioja (CIBIR), Logroño, Spain
[3]Department of Pharmaceutical Technology and Chemistry, MITOX Research Group, School of Pharmacy and Nutrition, University of Navarra, Pamplona Spain
[4]IdiSNA, Navarra Institute for Health Research, Pamplona, Spain

The main human health concern related to ochratoxin A (OTA) exposure is related to its nephrotoxicity. Regarding long term effects, OTA is considered genotoxic and carcinogenic. However, its mechanism of genotoxicity is still unclear.

Recently, our group published [1] preliminary data on long-term effects of OTA on Parkinson's Disease (PD) related molecular features (alpha-synuclein pathology and dopaminergic dysfunction) and motor dysfunction six months after the end of a 28 day oral treatment in mice (0.21 or 0.5 mg/kg b.w.). The mechanism was further validated *in vitro* in a human SH-SY5Y neuroblastoma cell line overexpressing alpha-synuclein.

New preliminary results obtained within a nationally-funded project "Mycotoxins and Parkinson: the missed link" (Gobierno de Navarra, 2019-project 43), as well as new insights linking DNA damage and PD will be discussed. More concretely, results of i) an *in vivo* study aiming at unraveling if the neurodegenerative process previously observed might start at the enteric nervous system and ii) an *in vitro* study (comet assay) evaluating if alpha-synuclein over-expression affects DNA damage, will be presented.

The current set of results provide preliminary data to understand the potential neurodegenerative effect of OTA. The *in vitro* and *in vivo* models used could be also interesting tools for the evaluation of long-term effects of other potential neurotoxins.

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Keywords: Ochratoxin, Parkinson, DNA damage, neurodegeneration, alpha-synuclein, mycotoxin

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L16

THE EPIGENETIC INTERPLAY OF MYCOTOXINS & EPSTEIN BARR VIRUS TOWARDS CHILDHOOD CANCER

Thanos M. Michailidis[1,2], Sarah De Saeger[1,2,3], Rita Khoueiry[4], Grace A. Odongo[4], Laura Corveleyn[2,5], Maarten Dhaenens[2,5], Zdenko Herceg[4], Marthe De Boevre[1,2]

[1]Centre of Excellence in Mycotoxicology and Public Health, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium
[2]CRIG, Cancer Research Institute Ghent, Ghent, Belgium
[3]Department of Biotechnology and Food Technology, Faculty of Science, University of Johannesburg, Doornfontein Campus, Gauteng, South Africa
[4]Epigenomics and Mechanisms Branch, International Agency for Research on Cancer/World Health Organization, Lyon, France
[5]Laboratory of Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences, Ghent University, Gent, Belgium

Vulnerable populations in low- and middle-income countries (LMICs) are daily exposed to dietary carcinogens such as mycotoxins. Infection by Epstein Barr virus (EBV) is linked to a childhood cancer called Burkitt lymphoma (BL) which is endemic in parts of sub-Saharan Africa where chronic mycotoxin exposure co-exists. It was recently demonstrated *in vitro* that aflatoxin B1 (AFB1) modifies the DNA methylome and promotes EBV infection in B-cells. In addition, both AFB1 and EBV may alter DNA methylation levels and deregulate the expression of cancer-related genes. The aim of this study is to assess the epigenetic interaction between mycotoxins and EBV (and other infections) in affected populations and validate the underlying mechanisms using *in vitro* and *in vivo* models. Accurate exposure assessments of mycotoxins and oncogenic viruses will be conducted in an established cohort of African infants and children, embedded in the MISAME-III cohort. DNA methylation status was assessed in the blood of the children and is associated with the levels of mycotoxin exposure and EBV infection status. Furthermore, the epigenetic toxicity following mycotoxin exposure and EBV infection will be integrated with the gene expression profile, in the cohort, as well as in cell lines and humanized mice. The outcome of this research will elucidate the mechanistic pathway(s) of environmentally-induced cancer. Understanding the mechanisms by which mycotoxins and viruses interact to deregulate the epigenome and induce tumors will provide insights to both the scientific community and governmental officials on how to overcome this public health challenge with focus on LMICs.

Keywords: Mycotoxins; Epstein Barr virus; Epigenetics; Burkitt lymphoma

L17

SHORT-TIME EXPOSURE TO THE *FUSARIUM* MYCOTOXINS DEOXYNIVALENOL AND ZEARALENONE IN COWS FED A DIET INDUCING RUMEN ACIDOSIS

Lydia Kroon[1], Ant Koopmans[1], Antonio Gallo[2], Janine Saltzmann[3], Sven Dänicke[3], Erminio Trevisi[2], Regiane R Santos[1]

[1]Schothorst Feed Research, P.O. Box 533, 8200 AM Lelystad, The Netherlands
[2]Department of Animal Science, Food and Nutrition (DIANA), Faculty of Agricultural, Food and Environmental Sciences, Università Cattolica del Sacro Cuore, 29122 Piacenza, Italy
[3]Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, D-38116 Brunswick, Germany

The rumen microflora of dairy cows can deactivate some important mycotoxins, but this capacity may decrease if the rumen microbial population is not functioning properly. For instance, sub-acute rumen acidosis (SARA) in involved in ruminal microbiota dysbiosis. Therefore, one may suggest that dietary contamination with *Fusarium* mycotoxins together with SARA may affect the ruminal capacity to degrade mycotoxins and increase the negative impact of these mycotoxins on production performance. In the present study was conducted with two treatment groups, each consisting of eight high productive dairy cows (~37 kg/day). In brief, the dietary treatments were split into four feeding periods, where in period 1 (d0-21) all experimental cows were fed a ration with negligible levels of mycotoxins (Control). In period 2 (d21-40; MYC), all cows were fed a ration naturally contaminated with deoxynivalenol (DON; 1.4 ppm) and zearalenone (ZEN; 0.6 ppm), followed by a period 3 (d40-42) where cows were divided into two groups, where one of the treatments (MYC) remained being fed a contaminated ration, and the MYC + SARA was fed the contaminated ration with increasing levels of fermentable carbohydrates (3.7 to 6.3% wheat on a dry matter basis) to induce acidification of rumen pH. Finally, in period 4 (d42-44), cows from MYC group were fed the control diet from period 1, and MYC + SARA cows were fed the control diet from period 1 with an increased level of fermentable carbohydrates (13.7% wheat). Contaminated diets were prepared with wheat and corn batches naturally contaminated with DON, and a sugar beet pulp batch naturally contaminated with ZEN to reach levels of about 1.4 ppm DON and 0.6 ppm ZEN in the total mixed ration. Dietary contamination with mycotoxins did not affect rumen pH but tended ($P = 0.09$) to increase the period where the rumen pH was lower than 5.8. Increase in the levels of fermentable carbohydrates during days 40 – 42 tended ($P = 0.10$) to decrease average rumen pH. When the levels of fermentable carbohydrates were increased to 13.6% on days 42-44, a decrease in the rumen average pH ($P = 0.01$) was observed. Dietary contamination with mycotoxins impaired ($P = 0.05$) feed efficiency together with a decrease ($P = 0.04$) in the fat- and protein corrected milk yield ($P < 0.04$). Cows fed the MYC diet presented a decrease in the milk fat percentage ($P = 0.02$), milk fat:protein ratio ($P < 0.001$), and milk urea ($P = 0.01$) when compared with the control diet. In the period of 40-42 days, a decrease in milk urea ($P = 0.01$) and serum glucose ($P = 0.02$) were detected when cows were fed the MYC + SARA diet. In the last period, the cows were fed the control diet and submitted to a rumen acidification presented a decrease in serum calcium ($P = 0.01$) and myeloperoxidase ($P = 0.03$), together with an increase in serum Mg ($P = 0.05$). The serum levels of ZEN and its metabolites were not affected by rumen pH. Serum levels of DON tended to decrease ($P = 0.07$) in cows fed the MYC diet and submitted to rumen acidification. However, when the cows were fed the control diet and submitted to rumen acidification, the DON serum level tended to increase ($P = 0.10$), and this effect was significant ($P = 0.045$) when the sum of DON and its metabolite de-epoxy-DON (DOM1) were considered. Induction of rumen acidification does not increase ZEN absorption in dairy cows but may prolong the exposure to DON+DOM1 in cows fed to this mycotoxin for a short period.

L18

NEW INSIGHTS ON CITRININ EXPOSURE IN GERMAN CHILDREN AND ADULTS FROM A URINE BIOMARKER STUDY

Gisela H. Degen[1], Jörg Reinders[1], Martin Kraft[2], Yvonni Chovolou[2], Wolfgang Völkel[3], Felicia Gerull[4], Jan G. Hengstler[1], Hermann Fromme[5]

[1]Leibniz Institut für Arbeitsforschung, Ardeystrasse 67, D-44139 Dortmund, Germany
[2]Landesamt für Natur, Umwelt und Verbraucherschutz NRW; D-45133 Essen, Germany
[3]Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, D-80538 München
[4]Landeslabor Berlin-Brandenburg, Fachbereich IV-4, D-12489 Berlin
[5]Institut und Poliklinik für Arbeits-, Sozial- und Umweltmedizin, D-80336 München

Citrinin (CIT), a mycotoxin produced by several fungal species is known to exert nephrotoxicity, and a contaminant in food and feed. So far, legal limits for CIT exist only for food supplements based on rice fermented with red yeast *Monascus purpureus* [1]. Since CIT contamination is not regularly analyzed in grains or spices, available data on its occurrence and levels in food commodities are insufficient for conducting a conventional exposure assessment. Yet, human biomonitoring, *i.e.* analysis of CIT and its metabolite dihydrocitrinone (DH-CIT) in biological samples such as spot urines allows to estimate an exposure [2]. To investigate exposure to CIT in the German population, a total of 138 urine samples from adults (24-61 years) and 175 from children (2-14 years) collected in three regions were analysed by a targeted LC-MS/MS based method for presence of CIT and DH-CIT [3].

At least one of the biomarkers was detected and quantified in all urine samples which indicate a widespread dietary exposure to the mycotoxin in Germany. Interestingly, biomarker concentrations of CITtotal (sum of CIT and DH-CIT) were higher in children urines (range 0.04–7.62 ng/mL; median of 0.54 ng/mL) than in urines from adults (range 0.05–3.50 ng/mL; median 0.30 ng/mL). The biomarker levels (CITtotal) measured in individual urines served to then calculate the probable daily CIT intake, and compare it to a value of 0.2 µg/kg b.w./day defined as 'level of no concern for nephrotoxicity' by the European Food Safety Authority. Median exposure of German adults was 0.013 µg/kg b.w., with only one urine donor exceeding the provisional tolerable daily intake (pTDI) for CIT. Median exposure of children was 0.05 µg/kg b.w. per day (*i.e.* 25% of the pTDI); however, CIT exposure in twelve individuals (6.3% of our study group) exceeded the limit value, with a maximum daily intake of 0.46 µg/kg b.w. (*i.e.* 231 % of the pTDI).

In conclusion: The new results reveal non-negligible exposure to CIT in some individuals in Germany, mainly in children. Therefore, further biomonitoring studies are required and also investigations aimed to identify the major sources of CIT intake with food commodities are encouraged.

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L19

WALLS IN CAMOUFLAGE: SEARCHING FOR MOLDS AND MYCOTOXINS IN MILITARY SETTINGS

Wiebke Derz[1], Paul W. Elsinghorst[1,2,*]

[1]Central Institute of the Bundeswehr Medical Service Munich, 85748 Garching, Germany
[2]Institute of Nutrition and Food Sciences, University of Bonn, 53121 Bonn, Germany

*corresponding author: paulelsinghorst@bundeswehr.org

Indoor mold is anchored in public awareness as a considerable health hazard as a mycosis from inhalation of spores is correlated with an increased prevalence of respiratory symptoms, allergies, asthma, and possibly an impaired immune response. However, in addition to such mycosis also mycotoxicosis can occur from inhaled mycotoxins as known from food contamination [1]. To assess the risk by mold-infested indoor environments cultural mycology of air samples is generally applied to identify the fungi, while analysis of mycotoxins relies on corresponding dust samples [1,2]. In a military context uncommon cases like moldy ammunition or aircraft interiors with hidden mold behind cover panels provide exotic samples. As sampling and methodology must also be applicable in deployed missions, different fungi and derived toxins must be considered depending on the particular climate conditions.

This presentation will illustrate the highlights from a current project of the German Armed Forces with real-life samples collected from several infested objects including buildings and equipment. Airborne fungi were identified by microscopy and MALDI-TOF analysis after incubation, while dust samples were screened by LC-MS/MS for 28 mycotoxins commonly observed in indoor environments. Comparing the identified mycotoxins from the dust samples to the ones expected from the fungi observed by air sampling will guide further development of a routine analysis setup by the German Armed Forces Medical Service.

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L20

ANALYSING EPIGENETIC TOXICITY CAUSED BY MULTI-MYCOTOXIN EXPOSURE USING AN INTESTINAL AND HEPATIC CELL CULTURE MODEL

Bader Yasmine[1*], Goessens Tess[1], Almey Ruben[2], M. Michailidis Thanos[1], Dhaenens Maarten[2], De Saeger Sarah[1,3], De Boevre Marthe[1]

[1]Centre of Excellence in Mycotoxicology and Public Health, University Ghent, Belgium
[2]ProGenTomics, Laboratory of Pharmaceutical Biotechnology, Ghent University, Ghent, Belgium
[3]Faculty of Science, University of Johannesburg, Johannesburg, South Africa

*corresponding author: Yasmine.bader@ugent.be

During the past decades, awareness of mycotoxin contamination in food and feed has increased. Previous European cohort-based research, the European Prospective Investigation into Cancer and Nutrition (EPIC), has shown chronic low-dose intake of multiple mycotoxins (i.e. deoxynivalenol (DON), patulin (PAT), sterigmatocystin (STC)) to be associated with an increased risk of colorectal carcinoma (CRC). Furthermore, a Malawian cohort investigated the association between multi-mycotoxin biomarker profiles and hepatic illness (cirrhosis and hepatocellular carcinoma (HCC)) and observed an association among ochratoxin (OTA), citrinin (CIT) and HCC. The paradigm of cohort-based association is causality which needs to be further resolved. Therefore this study aims to clarify the toxicological impact of DON, PAT, STC, CIT, OTA and *Alternaria* mycotoxins through heritable non-genetic histone post-translational modifications (hPTMs). This will allow us to gain a more in-depth understanding of crucial toxicological aspects as well as the potential mechanism behind carcinogenesis. As the genomic sequence does matter in cancer so do epigenetic alternations, which are reversible changes in gene expression without permanently changing the genome sequence.
Caco-2 (Cancer coli-2) and HepG2 (Human hepatocellular carcinoma) cells will be used as *in vitro* models for human intestine and hepatic, respectively. Prior to the hPTMs analysis, the cytotoxicity of these compounds will be determined using the cell viability MTS analysis. The hPTMs which are covalent enzymatic modifications of histones and important determinants of the epigenetic status (e.g. acetylation and methylation of lysine's and arginine's residues), will be analysed by liquid chromatography-tandem mass spectrometry, thoroughly validated for purpose. As a pioneer in the field, this presentation will highlight results regarding mycotoxin-induced cytotoxicity and hPTM induction, to investigate causal links between multiple mycotoxins and carcinogenesis. This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No 946192, HUMYCO).

L21

NEW CHALLENGES IN GENOTOXICITY TESTING: THE CASE OF THE *ALTERNARIA* MYCOTOXINS ALTERNARIOL AND ALTERTOXIN II

Francesco Crudo[1]*, Chenyifan Hong[1], Vanessa Partsch[1], Giorgia Del Favero[1], Luca Dellafiora[2], Doris Marko[1]

[1]Department of Food Chemistry and Toxicology, Faculty of Chemistry, University of Vienna, Vienna, Austria
[2]Department of Food and Drug, University of Parma, Parma, Italy

*corresponding author: francesco.crudo@univie.ac.at

Genotoxicity testing is a crucial step in the evaluation of the safety of chemicals and food contaminants. One of the most commonly used genotoxicity assays is the comet assay, which measures different types of DNA damage (e.g. single-strand breaks, double-strand breaks (DSBs)). However, this method is time-consuming and does not allow to assess a large number of samples simultaneously, which has led researchers to explore alternative assays such as the in-cell western assay of γH2AX. This assay assesses the induction and repair of DSBs by detecting the presence of the phosphorylated histone 2AX (γH2AX) in the cell nucleus. The key phosphorylation step, initiated in response to DSBs, is performed by members of the phosphoinositide 3-kinase-related kinases family (i.e. ATM, ATR, and DNA-PK) [1]. Since this assay only indirectly detects DSBs, other cellular events could potentially affect the assay results, which adds to the complexity of interpretation.

In the present study, we assessed the ability of two genotoxic *Alternaria* mycotoxins, namely alternariol (AOH) and altertoxin II (ATX-II), to induce γH2AX formation in HepG2 cells. In parallel, the extent of cytotoxicity was controlled by applying the CellTiter-Blue® assay.

Results of the study showed the ability of AOH to decrease cell viability and induce the formation of γH2AX. However, the increase in γH2AX fluorescence signal triggered by AOH was found to be moderate and rapidly reached a plateau. Based on these results and other available data, we hypothesized the possible inhibition by AOH of the kinases involved in the formation of γH2AX as a cause for the low level of γH2AX induction observed. In order to validate this hypothesis, an i*n vitro/in silico* approach was employed. In particular, the mycotoxin AOH (5 µM and 50 µM) was co-incubated with the known DSB-inducer chemotherapeutic drug doxorubicin (2.5 µM), and genotoxicity was evaluated by comparing the signals in the γH2AX assay with the level of DNA damage in the comet assay. Results obtained clearly demonstrated the ability of AOH to suppress the doxorubicin-induced formation of γH2AX at both concentrations tested. This result as such might suggest a suppression of DSBs, but the results from the comet assay clearly showed an increase in DNA damage in cells co-exposed to doxorubicin and AOH compared to the single treatments. To further support the formulated hypothesis, an *in silico* docking study was performed. Results obtained confirmed the ability of AOH to interact with the ATP pocket of the kinases involved in the phosphorylation of the histone. Similar experiments were also conducted by using the *Alternaria* mycotoxin ATX-II, which demonstrated also a weak capacity to trigger the formation of γH2AX.

In conclusion, our study emphasizes the challenges associated with using the γH2AX assay for genotoxicity testing and highlights the possibility of obtaining false negative results due to inhibition of the kinases responsible for γH2AX formation.

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L22

DOES REPLICATION STRESS PLAY A ROLE IN OCHRATOXIN A GENOTOXICITY?

Klotz Christina[1], Brode Julian[1], Borchers Johannes[1], Mally Angela[1]

[1]University of Würzburg, Department of Toxicology, Würzburg, Germany

The mycotoxin and renal carcinogen Ochratoxin A (OTA) causes weak genotoxic effects in mammalian cells by a mechanism that is still not resolved. In view of the specific spectrum of mutations, chromosomal damage and mitotic aberrations induced by OTA, which is neither compatible with DNA adducts nor with oxidative DNA damage, the European Food Safety Authority considered in its recent assessment that the genotoxic effects of OTA may derive from unresolved replication stress. Replication stress (i.e. impaired progression of replication forks and/or DNA synthesis) is increasingly recognized as a major source of genomic instability and hallmark of cancer. To test the role of replication stress in OTA genotoxicity, the aims of the present work were (1) to examine if OTA interferes with DNA replication, (2) to establish a causal link between replication stress, mitotic aberrations and genomic damage induced by OTA and (3) to characterize the cellular response to OTA mediated replication stress. Using the DNA fiber assay to study replication fork dynamics at monomolecular resolution, we observed a slight but statistically significant reduction in replication fork velocity in human kidney cells (HK-2) exposed to OTA after only 1 h of treatment. In contrast to the alkylating agent cisplatin which only affected replication forks proximal to DNA lesions, all replication forks were found to be equally affected in cells treated with OTA, indicating global slowing of replication fork progression in response to OTA. Immunofluorescence and western blot analysis demonstrated a significant concentration-related increase in yH2AX in cells exposed to OTA. Importantly, co-staining of newly replicating DNA with thymidine analogs such as 5-chloro-2´-deoxyuridine (CldU) and 5-ethynyl-2'-deoxyuridine (EdU) revealed yH2AX to be co-localized with nascent DNA, suggesting a causal link between DNA replication and DNA damage induced by OTA. Immunofluorescence analysis in cells arrested in G1/S or late G2 revealed that exposure to OTA during S phase, but not mitosis, induces a significant concentration-related increase in γH2AX, mitotic aberrations and cell death, providing further support that OTA may act primarily during S-phase of the cell cycle rather than interfere directly with the mitotic machinery. However, analysis of ATR-Chk1 and ATM-Chk2 signaling by immunofluorescence/immunoblotting suggests that OTA does not effectively activate DNA damage response pathways as observed in response to hydroxyurea. This may allow cells with under-replicated DNA or unrepaired DNA damage to escape checkpoint control and continue into mitosis, potentially leading to the mitotic aberrations and chromosome segregation defects observed in response to OTA. In support of this, 53BP1, a biomarker of DNA double-strand breaks that either arise or persist through mitosis, was significantly enriched in G1 cells treated with OTA during S phase. Taken together, these results provide first experimental evidence for perturbation of DNA replication as an early key event in OTA genotoxicity.

L23

PITFALLS AND CHALLENGES WHEN EVALUATING COMBINATORY EFFECTS – A CASE STUDY ON ZEARALENONE AND ISOFLAVONES

Dino Grgic[1,2], Barbara Novak[3], Elisabeth Varga[1], Doris Marko[1]

[1]Department of Food Chemistry and Toxicology, Faculty of Chemistry, University of Vienna, Vienna, Austria
[2]Doctoral School in Chemistry, University of Vienna, Vienna, Austria
[3]DSM – BIOMIN Research Center, Tulln, Austria

Daily, humans and animals are exposed to multiple known and unknown substances which might have a negative health impact alone or in combination. Zearalenone (ZEN) is a common contaminant of food and feed and is referred to being a mycoestrogen due to its endocrine disrupting potential caused by the structural similarity with the hormone 17ß-estradiol. Also, secondary plant metabolites might have estrogenic potential like the soy isoflavones (ISF) genistein (GEN) and daidzein (DAI) which are associated with both beneficial and negative effects. Analysis of different animal feed indicates that the co-occurrence of these estrogen mimicking compounds is up to 60% [1]. While in the past, toxicological evaluations were solely based on the effects of single substances, the trend is going towards the investigation of combinatory effects.

In the present study, we investigated *in vitro* the estrogenic potential of the mycoestrogen ZEN and some of its metabolites alone or in combination with GEN, DAI, and the DAI-metabolite equol (EQ). As previously shown, depending on the concentrations and selected ratios, the combination of myco- and phytoestrogen were found to potentiate the estrogenic stimuli compared to the respective single substances in Ishikawa cells [2]. This cell line expresses both estrogen receptors α (ERα) and β (ERβ). Therefore, following the OECD-guideline No 455 the luciferase reporter gene assay was performed for selected concentrations using the stably transfected hERα-HeLa-9903 cells expressing only ERα. While ZEN and its metabolites have a relative high binding affinity to both ERs, ISF preferably interact with ERβ and hence no enhanced estrogenic effects in the hERα-HeLa-9903 cell line were expected. Indeed, no synergistic effects were induced when compared to the effect of the single substances. However, at ISF concentrations above 1 µM a superinduction of the luciferase activity was observed in hERα-HeLa-9903 cells that surpassed by far the effect induced by the positive control (1 nM 17-ß-estradiol) and demonstrated a clear limitation of the actual OECD method 455 when isoflavones are involved.

In conclusion, these results indicate that synergistic estrogenic effects are especially to be considered in tissues where both isoforms of the estrogen receptor are expressed and the selected cell lines should be chosen with care.

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L24

ALTERNARIOL-SULFATES AS NEGLECTED CONJUGATION PRODUCTS WITH TOXICOLOGICAL POTENTIAL

Eszter Borsos[1,2], Luca Dellafiora[3], Chiara Dall´Asta[3], Giorgia Del Favero[1,4], Elisabeth Varga[1], Doris Marko[1]

[1]Department of Food Chemistry and Toxicology, Faculty of Chemistry, University of Vienna, Vienna, Austria
[2]Doctoral School in Chemistry, University of Vienna, Vienna, Austria
[3]Department of Food and Drug, University of Parma, Parma, Italy
[4]Core Facility Multimodal Imaging, Faculty of Chemistry, University of Vienna, Vienna, Austria

The ubiquitously occurring mycotoxin alternariol (AOH) has been associated with several adverse effects *in vitro*, including cytotoxicity, genotoxicity, mutagenicity, immunosuppression, as well as hormone-mimicking properties. Therefore, investigating the extent and path of its metabolism is of great interest and a prerequisite to unveil the impact of biotransformation on the expectable toxicological outcome.

Regarding the phase II metabolism of AOH, sulfation and glucuronidation constitute the most relevant pathways. In HT-29 cells, the DNA-strand-breaking potential of AOH was fully diminished through its glucuronidation, underpinning its detoxifying role [1]. In contrast, conjugation with a sulfate moiety in the third position of AOH does not abolish estrogenic properties of the parent compound [2]. This finding underlines the demand to re-evaluate the importance of sulfation as metabolic pathway. To this end, a selected panel of *in vitro* models have been exposed to AOH, and the produced metabolites were assessed with LC-MS. Indeed, two alternariol-*O*-sulfates could be identified for the first time as the major metabolization product of AOH in the human hepatocarcinoma cell line HepG2. Moreover, AOH was found to undergo sulfation not only in HepG2 and Caco-2 cells [3] but also in the human endometrial adenocarcinoma cell line Ishikawa – a well-established, estrogen-sensitive human cell model. As a result, it can be postulated that when assessing the estrogenic activity of AOH with an Ishikawa-based assay setup, in fact, a metabolic mixture is studied rather than exclusively the parent compound.

In conclusion, we identified metabolization products of AOH in mammalian *in vitro* models, determined the extent and partly even the time-dependency of their formation, and raised heretofore scarcely discussed aspects based on the acquired datasets.

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L25

NEW POSSIBILITIES IN MYCOTOXIN RESEARCH BY FUNCTIONAL INTRAVITAL IMAGING

Ahmed Ghallab[1,2]*, Reham Hassan[1,2], Maiju Myllys[1], Daniela González[1], Abdellatif Seddek[2], Andrea Gerdemann[3], Michael Kuhn[3], Benedikt Cramer[3], HansUlrich Humpf[3], Gisela H. Degen[1], Jan G. Hengstler[1]

[1]Leibniz Research Centre for Working Environment and Human Factors, Technical University Dortmund, Ardeystr. 67, 44139 Dortmund, Germany
[2]Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, South Valley University, Qena 83523, Egypt
[3]Institute of Food Chemistry, Westfälische Wilhelms-Universität Münster, Corrensstr. 45, 48149 Münster, Germany

*presenting author: ghallab@ifado.de

Abstract

Intravital two-photon-based imaging allows real-time observation of biological processes in living animals. Using this technology, we have established a toolbox to functionally analyze livers and kidneys of mice and rats in health and disease. Videos can be recorded at subcellular resolution (200 nm). Overview imaging over several liver lobules or kidney nephrons is also possible. A temporal resolution in the millisecond range can be achieved[1]. A disadvantage of this technology is that it requires labelling with fluorescent dyes. Luckily, many mycotoxins provide autofluorescence. For example, aflatoxin B_1 (AFB_1) and ochratoxin A (OTA) provide strong blue autofluorescence when excited at specific wavelengths, which allows label free imaging. This novel method allowed new insights about AFB_1 and OTA kinetics in health and disease, e.g.: (a) subcellular spatiotemporal intravital kinetics of AFB_1 and OTA in liver and kidney can be visualized anesthetized animals[2]; (b) the influence of hypoalbuminemia on the spatiotemporal tissue distribution of OTA and consequences for organ toxicity can be studied[3]; (c) spatio-temporal differences of AFB_1 kinetics in mice and rats can be recorded. In conclusion, the newly established intravital imaging technique may provide valuable additional information to conventional techniques.

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L26

INSIGHTS INTO THE METABOLISM, KINETICS AND RELATED TOXICITY OF OCHRATOXIN A IN MICE MODELS

Michael Kuhn[1], Reham Hassan[2], Benedikt Cramer[1], Ahmed Ghallab[2], Gisela H. Degen[2], Jan G. Hengstler[2], Hans-Ulrich Humpf[1]

[1]Institute of Food Chemistry, University of Muenster, 48149 Münster, Germany
[2]Leibniz Research Centre for Working Environment and Human Factors, Technical University Dortmund, 44139 Dortmund, Germany

Ochratoxin A (OTA), a secondary metabolite of *Aspergillus* and *Penicillium* fungi species is a widespread mycotoxin found in processed and unprocessed food like grains, nuts and coffee. OTA is known to have nephrotoxic and carcinogenic effects, but the underlying mechanisms of its toxicity have not been concluded. A crucial factor for OTA toxicity, metabolism and potential bioactivation is its affinity to albumin in blood. In order to understand the relevance of albumin, imaging experiments on tissue uptake and hepatotoxic effects in albumin knockout mice treated with OTA compared to wild type mice have been performed [1]. In these experiments, a strong increase of hepatotoxicity was observed in heterozygous albumin deficient mice.

Here we present the data of the analysis of blood, bile and urine samples from the mice in this study. For this purpose, a new sensitive targeted LC-MS/MS method was developed allowing the quantification of the analytes OTA, its hydroxylated derivates 4-hydroxy-ochratoxin A (4OHOTA) and recently identified 7'hydroxy-ochratoxin A (7'OH-OTA), ochratoxin α (OTα) formed by cleavage of the phenylalanine moiety, the dechlorinated product ochratoxin B (OTB), ochratoxin A – dihydroquinone (OTHQ), and the opened-lactone form (OP-OTA).

Analysis reveals that next to OTA, 4OHOTA, 7'OH-OTA, OTB, OTHQ and OP-OTA were found in all matrices in varying amounts. In urine and bile, especially the hydroxylated derivates as well as OP-OTA was formed in much higher amounts in the heterozygous albumin deficient mice compared to the wild type mice. As expected, clearance of OTA from blood of homozygous albumin deficient mice was faster than from blood of wild type mice. Here, we present and discuss the data from this study, comparing the quantified metabolites in the different matrices and mouse strains (wild type, heterozygous and homozygous albumin deficient mice). Furthermore, kinetics of OTA elimination from blood via urine and bile of the different mouse strains will be shown, demonstrating the relevance of albumin binding for the toxicity of OTA.

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L27

SPECIES DIFFERENCES OF AFLATOXIN B1 METABOLISM IN PRIMARY MOUSE, RAT AND HUMAN HEPATOCYTES AND *IN VIVO* IN RODENTS

Andrea Gerdemann[1], Benedikt Cramer[1], Matthias Behrens[1], Ahmed Ghallab[2], Reham Hassan[2], Gisela H. Degen[2], Jan G. Hengstler[2], Melanie Esselen[1], Hans-Ulrich Humpf[1]*

[1]Institute of Food Chemistry, University of Muenster, Corrensstraße 45, 48149 Münster, Germany
[2]Leibniz Research Centre for Working Environment and Human Factors (IfADo), Ardeystraße 67, 44139 Dortmund, Germany

*corresponding author: humpf@uni-muenster.de

Aflatoxin B_1 (AFB_1) is a mycotoxin contaminant of food and feed, and a risk for human and animal health due to its mutagenic, carcinogenic and hepatotoxic activity [1]. AFB_1 is known to exert species-dependent toxicity and carcinogenicity that may be related to its metabolism and formation of DNA adducts in the respective species. The present study focused on the kinetics of AFB_1 metabolism in primary hepatocytes of mouse, rat and human origin using chromatographic separation combined with mass spectrometric detection which allows a selective and sensitive quantification of AFB_1 and its metabolites. For quantification, commercially available metabolites such as aflatoxin P_1 (AFP_1) aflatoxin M_1 (AFM_1), aflatoxin Q_1 (AFQ_1) and aflatoxicol (AFL) were used, and known AFB_1 conjugates, i.e. AFB_1-*N*-acetylcysteine, AFB_1-glutathione (AFB_1-GSH), AFB_1-guanine, and AFB_1-lysine were synthesized. Upon treatment of cells with AFB_1, samples from the medium were collected up to 24 h. Additionally, the cellular DNA was analyzed at specific time-points to analyze AFB_1-DNA adducts which are linked to the carcinogenicity and mutagenicity of the compound.

Fast metabolization and comparably high levels of the far less toxic AFP_1 as main metabolite were observed for mice hepatocytes. Furthermore, AFB_1-GSH and AFM_1 were formed quickly, and only low amounts of AFB_1-DNA adducts were detected, which disappeared almost completely from the DNA up to 24 h. In contrast, rat hepatocytes metabolized AFB_1 significantly slower and formed mainly AFM_1 and AFB_1-GSH. Compared to mouse hepatocytes higher amounts of AFB_1-DNA adducts were detected in the lysates which stayed on a relatively high level up to the end of the experiment, explaining the higher carcinogenic potency of AFB_1 in rats in comparison to mice. The main human metabolites were AFM_1 as well as AFQ_1 and AFL, which had only a minor role in the other species.

AFB_1 was also applied to groups of mice and rats, and samples from plasma, urine and bile were collected for up to 24 h. The analysis of the metabolite pattern in samples of this *in vivo* time-course showed results which compare quite well to the *in vitro* experiments with primary rodent hepatocytes.

Overall, the results demonstrate a strong correlation between the sensitivity of certain species towards AFB_1 and their respective metabolism.

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L28

LC-QTOF-HRMS AS AN EFFECTIVE TOOL FOR DETECTING OF CO-CONTAMINATION OF MYCOTOXINS AND TRANSFORMATION PRODUCTS

Markus Trentzsch, Stefan Weigel, Christoph Hutzler

German Federal Institute for Risk Assessment (BfR), Department for Safety in the Food Chain,
Max-Dohrn-Str. 8-10, 10589 Berlin, Germany

Compared to the LC-MS/MS method with quadrupole devices frequently used in routine target analysis of mycotoxins, analysis with QToF-HRMS devices offers the advantage of not only being limited to the target analytes, but also to all other ionizable substances in the sample to be able to detect and mass spectrometrically characterize. In addition to the regulated mycotoxins and the "emerging mycotoxins" currently in focus, other secondary metabolites of mycogenic origin as well as known and previously undescribed transformation products of mycotoxins can be determined. For this purpose, selected flour samples (n = 10), in which deoxynivalenol (DON) had previously been detected by LC-MS/MS target analysis, were tested with LC-QToF-HRMS in full scan and "information dependent analysis" (IDA) mode with a positive result and negative ionization analyzed. The comparison of the MS and MS/MS spectra obtained with commercially available and self-created mass spectral libraries provided a hit list of 20 to over 50 mycotoxins per analyzed sample, which was classified according to criteria such as MS/MS spectrum match, agreement of the high-resolution masses and plausibility of the retention time must be checked. If the appropriate standard is available, the identity can be verified experimentally.

In addition to DON and its modified forms (DON-3-glucoside, 3- and 15-acetyl-DON), numerous other known or maximum-level-controlled mycotoxins such as ergot alkaloids, ochratoxin A, zearalenone and enniatines were detected in the real samples examined. In addition, other mycotoxins that are less of a focus, such as emodin, 15-hydroxyculmorone and trichodermin, were identified by database comparison of the mass spectra. In addition, the mass spectrometric characterization of six possible modifications of the potential Alternaria mycotoxin infectopyron [1], which could be identified in several real samples, was successful. Additionally, several feed samples tested positive for common Alternaria mycotoxins were analyzed with LC-QToF-HRMS and found to be positive for infectopyrone and some of its modifications besides other co-contaminating mycotoxins.

The LC-QToF-HRMS technique thus represents an effective tool to close knowledge gaps about the possible co-exposure to a variety of mycotoxins and other substances in different matrices by the possibility of simultaneous analysis for unknown mycotoxins, transformation products and substances from other regulatory areas in a sample.

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L29

ACCELERATION OF AN HPLC-MS / MS MULTI-CLASS METHOD FOR THE ANALYSIS OF >1200 BIOTOXINS, PESTICIDES, AND VETERINARY DRUGS

Lidija Kenjeric[1], Michael Sulyok[2], Rudolf Krska[2,3], Kristyna Pavelicova[4], Alexandra Malachova[1]

[1]FFoQSI GmbH, FFoQSI Austrian Competence Centre for Feed & Food Quality, Safety and Innovation, Tulln, Austria
[2]University of Natural Resources and Life Sciences Vienna (BOKU), Department of Agrobiotechnology (IFA-Tulln), Institute of Bioanalytics and Agro-Metabolomics, Konrad-Lorenz-Straße 20, 3430 Tulln an der Donau, Austria
[3]Institute for Global Food Security, School of Biological Sciences, Queens University Belfast, University Road, Belfast, BT7 1NN, Northern Ireland, United Kingdom
[4]Department of Chemistry and Biochemistry, Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemedelska 1, Brno, Czech Republic

Our laboratory has been utilizing a quantitative multi-method with an annual throughput of 7500 samples and 20,000 injections. This method is able to measure >1000 analytes in 40 minutes, within 2 injections in positive/negative ionization modes, 20 minutes each. There are 1194 MRM transitions in positive mode and 782 MRM transitions in negative mode, respectively. A flow rate of 1 ml/min requires a high amount of eluents which is not environmentally friendly. In order to contribute to the ongoing green transition, we decided to accelerate the current method via fast polarity switching (FPSW), and thus decrease the consumption of eluents and energy considerably. The aim of this study was to squeeze the method into 1 injection lasting 20 min avoiding impairment in its accuracy and precision. A limited factor in the scheduled MRM mode is the software automatically calculated dwell time based on the number of concurrent MRMs, the retention time window width, and the cycle time. The application of FPSW significantly reduced the dwell time from 25 ms to 10 ms for the analytes eluting from 8 to 16 min. Such a low dwell time may potentially affect the precision of the method. In order to assess the repeatability, repeated injections of a neat-solvent standard (n=15) were performed. The acquired data were compared with the original method. The repeatability of injections of FPSW with 5.8 % on average was not significantly worse than 4.7 % in the case of the original method comprising two separate injections. In order to decide whether the implementation of this accelerated method is feasible without significant deterioration of the data quality, the influence of FPSW on the absolute and relative matrix effects in food and feed matrices will be investigated as the next step.

L30

IR SPECTROSCOPY COMBINED WITH LC-MS/MS ANALYSIS FOR RAPID MYCOTOXIN SCREENING

Stephan Freitag, Michael Sulyok, Rudolf Krska

Institute of Bioanalytics and Agro-Metabolomics, Department of Agrobiotechnology, IFA Tulln, University of Natural Resources and Life Sciences, Vienna

Thousands of mycotoxin analyses are performed in Europe per year. Liquid chromatography linked to mass spectrometry (LC-MS/MS) as well as enzyme-linked immunosorbent assays (ELISAs) are among the predominantly used techniques for that purpose [1]. Their popularity is linked to the fact that LC-MS/MS allows to screen for several hundred analytes at once including all regulated mycotoxins besides providing unmatched data quality [2]. The technique, however, is costly as complex equipment and expert knowledge is needed. ELISAs on the other hand are easier to perform and cost-effective, but often suffer from cross-reactivities besides using a lot of consumables. In this context infrared (IR) spectroscopy has demonstrated high potential as alternative to established rapid mycotoxin screening platforms like ELISAs. IR spectrometer operation is straightforward as hardly any sample preparation is needed, enabling an analysis time of a few minutes. However, method development is challenging as due to the limited sensitivity of IR spectroscopy the direct measurement of mycotoxins is not feasible. In fact, IR spectroscopic approaches track fungal induced sample changes and correlate them with the mycotoxin value obtained by external reference analysis using chemometrics methods [3].

In the presented study we demonstrate the development and potential of an IR spectroscopic method for rapid mycotoxin screening. LC-MS/MS analysis was not only exploited to obtain the needed mycotoxin reference value for IR model development, but also to identify trends in the sample set by analyzing data of non-regulated secondary fungal metabolites. Thereby, it will be shown that the utilization of targeted multi-analyte LC-MS/MS data, during chemometric analysis, holds further potential beyond the usage as reference value for IR method development.

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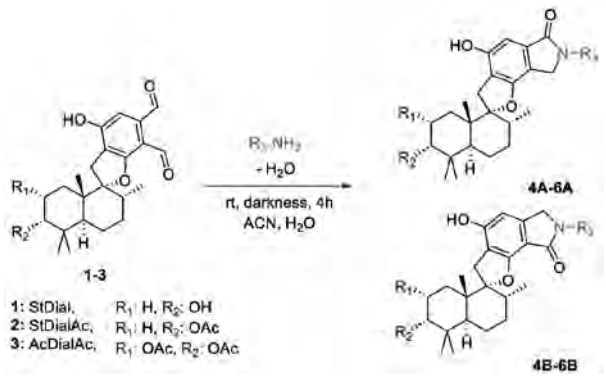
L31

DISARMED MYCOTOXINS FROM *STACHYBOTRYS CHARTARUM*: INSIGHTS INTO METABOLISM AND BIOLOGICAL ACTIVITY OF PHENYLSPIRODRIMANES

Alica Fischle[1], Katharina Steinert[1], Viola Haupt[1], Felix Schimang[1], Hans-Ulrich Humpf[1], Svetlana A. Kalinina[1]

[1]Institute of Food Chemistry, University of Münster, Münster, Germany

Phenylspirodrimanes (PSD) are produced solely across the *Stachybotrys* species, and knowledge about the biosynthetic formation of these highly toxic compounds is quite extensive. Chemically, PSDs present as structurally diverse triprenyl phenols, where derivatives carrying an *o*-dialdehyde group at their aryl residue have been shown to be especially susceptible to nucleophilic attacks from primary amines, resulting in the formation of isoindolinones. Multiple biological activities have been described for these compounds, including anticarcinogenic, antiviral, and plasminogen activation properties [1-3]. In our recent project, we focused on stachybotrydial (StDial, **1**), stachybotrydial acetate (StDialAc, **2**), and acetoxystachybotrydial acetate (AcDialAc, **3**) as starting materials in a semi-synthetic approach with e.g., agmatine, and arginine, to obtain new isoindolinone-derivatives (**4A-6A, 4B-6B**). Purification was performed *via* preparative high performance liquid chromatography (HPLC) coupled to an UV-detector, while structure elucidation was conducted *via* nuclear magnetic resonance (NMR) and high-resolution mass spectrometric screening (HRMS). Additional investigation of serine protease inhibition warranted further screening of the obtained products as potential thrombin inhibitors, some of which exhibited high specificity and await further investigation. Furthermore, cytotoxicity was determined on A549 (lung cancer cells) as well as HepG2 (liver cancer cells) which resulted in lower toxicity of the semi-synthetically derived products compared to the starting material [1]. Additionally, studies concerning metabolism (phase I and phase II) were conducted. Overall, we present extensive investigations regarding the most promising products derived *via* semi-synthesis of starting materials **1-3** with various primary amines which include analytical characterization as well as broad biological studies. This highlights *Stachybotrys chartarum*'s vast potential concerning medical treatments to come.



Reaction scheme of semi-synthesis: PSDs (**1-3**) reaction with a primary amine under mild conditions to isoindolinones with potential isomeric forms A and B (**4A-6A, 4B-6B**).

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L32

EFFECT OF DEOXYNIVALENOL ON SOIL NITRIFICATION

Sven Korz and Katherine Muñoz

Institute for Environmental Sciences, RPTU (University of Kaiserslautern-Landau), Landau, Germany

Deoxynivalenol (DON) has been detected in soils [1,2], for example as a consequence of an *in situ* production or when it is washed off from infested plants. Secondary metabolites serve important biological soil functions [3], thus there is growing interest in DON and its contribution in soil biogeochemical processes.

Soil nitrification is the processes of biological oxidation of ammonium ($N\text{-}NH_4$) to nitrite followed by the oxidation of the nitrite ($N\text{-}NO_2$) to nitrate ($N\text{-}NO_3$). This process allows for the balance in N-cycle in soils, that is relevant for soil functionality and productivity. Secondary metabolites of plants, such as polyphenolic compounds, i.e. Resveratrol and procyanidins, are known to affect this process in soil [4,5]. Therefore, the question arises whether secondary metabolites of fungi may also affect nitrification, since fungal growth and fitness in soil depend on N species and availability.

In this study we investigated DON for its effect on the potential nitrification rate (PNR). The PNR of soil samples was determined by measuring the microbial oxidation of excess ammonium to nitrate over 24 hours in an aqueous phosphate buffer suspension [6]. Soil samples were taken from a Riparian area in the east of the Landau in Rhineland-Palatinate, which are known for its strong contribution to N-cycle. A soil subsample was treated at a level of 3 µg DON $(g\ soil)^{-1}$ and the results were compared to a non-spiked control group. The test was carried out in quadruplicates.

Preliminary data shows that DON significantly increased the nitrification rate ($N\text{-}NO_3$ over $N\text{-}NH_4$) in the tested soil by averagely 28 ± 1 % compared to the control group (t-test, $t=6.8382$, $df=5.7913$, $p<0.001$, $n=4$). This observation provides evidence on the effect of DON on soil nitrifying bacteria or its enzymes and therefore potentially new understanding *Fusarium* phytopathogenicity. In further studies we aim to validate these results, including soils with different land use and DON treatment in different concentrations.

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Supplementary material:

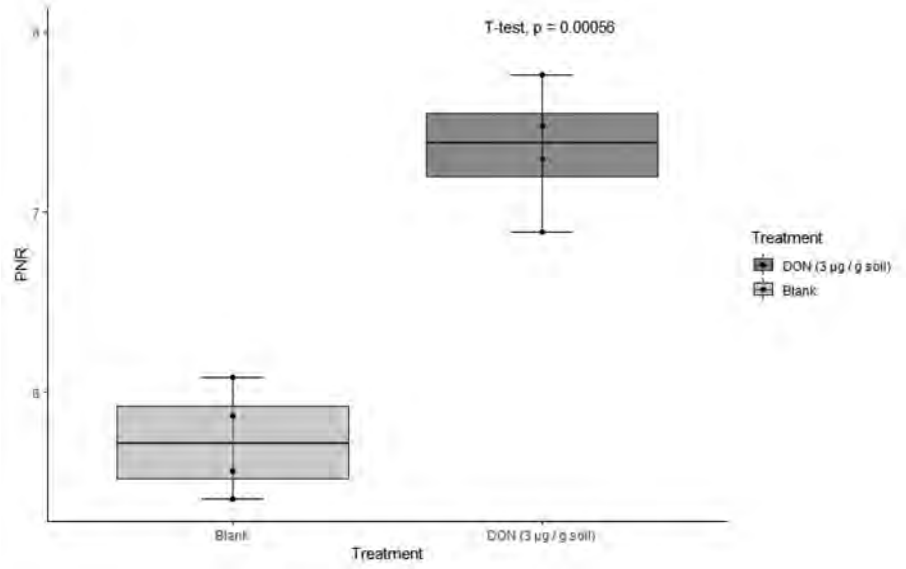

Fig. 1: Potential nitrification rate (PNR) of DON spiked soil compared to a non-spiked control group (Blank). The PNR was determined by individual linear models, that explain the microbial nitrate formation over time.

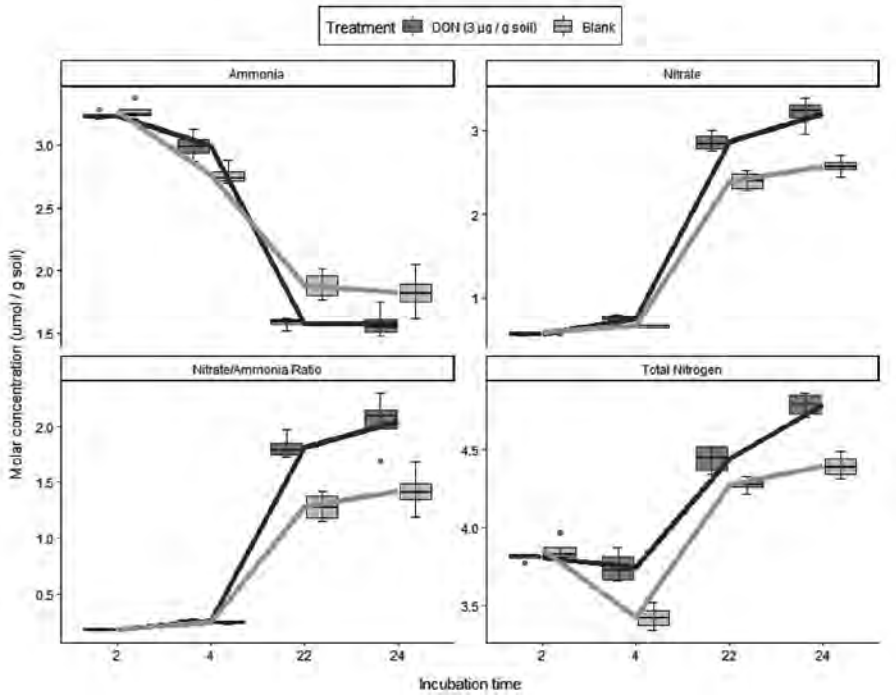

Fig. 2: Molar concentrations of ammonium and nitrate in the soil suspensions (DON spiked and non-spiked control group) over 24 h.

L33

INFLUENCE OF THE VELVET COMPLEX ON DEVELOPMENT, SECONDARY METABOLISM, AGGRESSIVENESS AND DEFENSE OF *PENICILLIUM EXPANSUM*

Chrystian Zetina-Serrano, Nadia Tahtah, Christelle El Hajj Assaf, Ophélie Rocher, Selma Snini, Yannick Lippi, Emilien L. Jamin, Isabelle P. Oswald, Olivier Puel*, Sophie Lorber

INRAE, Biosynthesis and Toxicity of Mycotoxins ToxAlim UMR1331 Toulouse, France

*corresponding author: olivier.puel@inra.fr

Penicillium expansum, the causal postharvest agent of blue mold disease in apples, is considered the main source of patulin in human dietary. In addition to the patulin, *P. expansum* has, as the great number of *Aspergillus* and *Penicillium* species, the capability to produce an extensive number of secondary metabolites (SM), which possess a multitude of functions such as fungal development, protection and defense, virulence factors and medical applications. They include citrinin, chaetoglobosins, roquefortines, expansolides. The production of these fungal secondary metabolites is controlled by specific (located inside clusters) and global transcription factor in response to various stimuli. Among the global transcription factors well characterized in the model organism *Aspergillus nidulans*, we find the velvet complex, a transcriptional complex conserved in filamentous fungi regulated by light in this latter. This complex, involving three global transcription factors: VelB, LaeA and VeA, plays also an important role in the regulation of development, sporulation, pathogenicity in many fungal species. Previous characterization of the role of velB and veA in the regulation of secondary metabolism as well as several physiological processes in *P. expansum* was partially achieved by our group. These three proteins can also act in concert with other proteins and form other protein complexes such as the VosA/VelB complex.

In order to determine what is really regulated by the complex velvet and what are the physiological functions and secondary metabolites regulated by VeA, LaeA and Velb independently to Velvet complex, a null PeΔ*veA*, PeΔ*velB* and PeΔ*laeA* mutant strains were generated in *P. expansum*.

The transcriptomic analysis showed that three mutants share 448 differently expressed genes (DEGs): 349 down expressed and 99 up-regulated. Among the down-regulated genes, there are all genes of patulin, citrinin and chaetoglobosin A genes clusters. This expression change was confirmed by HPLC-DAD and LC-MS analyses by a drastic decrease of the production of these mycotoxins, confirming an involvement of the velvet complex. Among down regulated gene, there are also many genes coding to carbohydrate enzymes involved in the plant cell wall degradation enabling a better colonizing of the fruit by the fungus.

This body of evidences showed that the velvet complex has a significant impact on the development of *P. expansum*. This was confirmed *in vivo* on Golden delicious cultivar. The involvement of Velvet in virulence towards the host organism has been previously demonstrated in other plant or animal pathogen species. But what about their defense against other microorganisms? A partial answer is given by the transcriptional data with a down-regulation of the three antifungal proteins.

L34

LINKING DISTINCT EXTROLITE PROFILES TO THE DIFFERENT GENETIC POPULATIONS WITHIN *PENICILLIUM ROQUEFORTI*

Ewen Crequer[1,2], Jean-Luc Jany[1], Gwennina Cueff[1], Thibault Caron[2], Jeanne Ropars[2], Jens Frisvad[3], Johan Christiansen[3], Antoine Branca[2], Tatiana Giraud[2], Emmanuel Coton[1] and Monika Coton[1]*

[1]Univ. Brest INRAE, Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, F-29280 Plouzané, France
[2]Université Paris-Saclay, CNRS, AgroParisTech, Laboratoire Ecologie Systématique et Evolution, UMR 8079, France
[3]Department of Biotechnology and Biomedecine, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark

*corresponding author: monika.coton@univ-brest.fr

Penicillium roqueforti is a well-known fungal species used for blue-veined cheese production but also for its potential to produce some extrolites, including mycotoxins, such as mycophenolic acid, roquefortin C or PR toxin [1,4]. Previous studies detected four differentiated genetic populations in *P. roqueforti*, associated with distinct environments: two cheese populations including a population specific to Roquefort PDO cheeses, and two other populations growing on silage and wood, respectively, but also considered as food contaminants. Adaptation to cheese has been found in both cheese populations, with contrasted phenotypic traits [2]. These cheese populations are the results of independent domestication events for cheese making without recent gene flow and with strong bottlenecks [3]. Based on sampling of non-inoculated Termignon cheeses and on population genomics, we found a fifth population in *P. roqueforti*. Here, we first addressed the question as to whether *P. roqueforti* populations have different mycotoxin-production profiles and then tried to decipher what genomic differences in biosynthetic gene clusters (BCG) explain these findings.

We determined extrolite production profiles of 48 strains from the five populations using miniaturised YES agar method for extrolite production and extraction and HR-LC-MS analyses. Three profiles were observed with higher extrolite productions for "non-cheese" population strains, a low production profile among "non-Roquefort" population strains and an intermediate profile with low PR-toxin production for "Roquefort", "Termignon" and other "non-cheese" strains. A first glimpse of the on-going *in silico* and metabolomics analyses show distinct genomic determinants explaining the observed differences including the deletion in a key gene (*mpaC*) involved in mycophenolic acid production in the non-producing population. This suggests that low mycotoxin production is a trait selected by human domestication of *Penicillium roqueforti*.

This study should provide new insights into this industrially important fungal species, and improve our understanding of its domestication mechanisms and the genetic basis explaining evolution of mycotoxin production.

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L35

ENVIRONMENTAL INFLUENCES ON THE PRODUCTION OF ALTERNARIOL-SULFATE BY *ALTERNARIA ALTERNATA*

Adetoye Adeyemo, Markus Schmidt-Heydt*

Max Rubner – Institut, Federal Research Institute of Nutrition and Food,
Department of Safety and Quality of Fruit and Vegetables, Karlsruhe, Germany

*corresponding author: markus.schmidt-heydt@mri.bund.de

The Fungus *Alternaria alternata* is known to infect a wide variety of fruits and vegetables which can lead to a contamination with mycotoxins of the aforementioned consumables. One of the most well-known and present mycotoxins produced by the genus *Alternaria* is Alternariol (AOH) with its plethora of derivates like Alternariolmonomethylether (AME). Beyond that, there are a number of so-called modified mycotoxins like the recently discovered Alternariol-Sulfate (Soukup, Kohn et al. 2016). Until now Alternariol-Sulfate (AOH-sulf) never played a major role in matters of research and safety, most likely due to the low amounts found in samples when compared to mycotoxins like Alternariol or Tenuazonic acid but it may be involved in processes such as virulence and colonization as it has been recently described for AOH (Wojciechowska, Weinert et al. 2014, Geisen, Graf et al. 2015, Wenderoth, Garganese et al. 2019).

In our current studies, we were able to evaluate the influence of environmental factors such as light, water activity, pH-value, and oxidative stress on the production of Alternariol-Sulfate. We performed stress experiments with multiple different *Alternaria alternata* isolates and measured the production of AOH-sulf by HPLC. Not only were we able to draw the conclusion, that the influence of environmental factors on the AOH-sulf production is extensive, but also that stress factors like white light or oxidative stress can lead to a tremendous increase up to up to about 104,3 µg/ml which is about 10 times the amount of AOH-sulf we measured in non-stress conditions produced. Those new findings might be worrying in the sense that neither function nor toxicity of AOH-sulf are known, while the severity of AOH-sulf contaminations might be underestimated at this point in time.

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ABSTRACTS OF POSTERS

P1

SHOULD MYCOTOXIN CONTAMINATION OF ITALIAN GRAINS BE A CONCERN?

Juliane Lima da Silva[1,2], Luana Izzo[2], Luigi Castaldo[2], Sonia Lombardi[2], Jaqueline Garda-Buffon[1], Alberto Ritieni[2]

[1]Chemistry and Food School, Rio Grande Federal University, Rio Grande, Brazil
[2]Pharmacy Department, Università Degli Studi di Napoli Federico II, Naples, Italy

Cereal grains are the major source of dietary energy and nutrients for human beings and livestock, which are exposed to different sources of fungal and bacterial contamination, such as fungus and consequently mycotoxins [1]. These toxins may cause different toxic effects in animals and humans, including carcinogenic, mutagenic, teratogenic, immunotoxic, and estrogenic damage [2]. It is estimated that between 25 and 50% of cereal products worldwide are contaminated with mycotoxins, and 5 to 10% are irreversibly contaminated, leading to exposure risks and economic losses [3]. In that regard, the aim of the present study was to evaluate the 24 mycotoxins occurrence in 200 samples of Italian wheat grain and estimate the exposure to these toxins.

The samples (n = 200) were provided by several farmers located in different fields in Campania, South Italy. The simultaneous extraction was using the QuEChERS approach, then representative homogenous samples (2.5 g) were weighed into a 50 mL falcon tube and 5 mL of HPLC grade water was added. After 2 min of vortexing, 5 mL of ACN acidified with 5% formic acid was added and vortexed for 2 min. After 5 min of centrifugation (5000 rpm), the supernatant was filtered and then analyzed by UHPLC-Q-Orbitrap HRMS. The method was validated according to European regulations [4, 5]. Linearity, matrix effect, specificity, trueness, precision, and sensitivity were evaluated.

As a result, 12 mycotoxins were detected in the analyzed Italian grains, and 116 samples showed contamination with at least one mycotoxin. ZEN metabolites, α-ZAL (19.58 to 147.20 µg/kg) and β-ZAL (31.48 to 176.88 µg/kg), were the most frequently detected compounds, being detected in 84 and 14 samples, respectively. The third most frequent mycotoxin was AOH, appearing in 13 samples with concentrations ranging from 0.66 to 2.08 µg/kg. HT-2 was detected in 9 samples (3.3 – 28.34 µg/kg), β-ZOL in 4 (6.52 – 12.44 µg/kg), DON in 3 (104.08 – 292.62 µg/kg), and ZAN in 2 (4.58 and 4.88 µg/kg) samples. α-ZOL, T2, ENN A1, ENN B1, and ENN A were detected in just one sample each.

Although more than 116 samples were contaminated with mycotoxins, none exceeded the limits recommended by regulatory agencies. DON, one of the mycotoxins of greatest concern among those studied, was the one with the highest level of contamination (292.62 µg/kg). However, below the maximum limit established by the European Union for unprocessed wheat (1750 µg/kg) [6]. This value is far below other studies made in Italian wheat grains that also found DON as the mycotoxin with the highest level of contamination [7, 8]. These results showed that, although contamination by mycotoxins is a reality in the Italian grains studied (58% of samples were contaminated), the levels found do not pose a risk to public health and respect the established regulatory limits.

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P2

CANNABIS INDICA LLAM. SOURCE OF MOLDS AND MYCOTOXINS?

Klaudia Rydzkowska, Iwona Ałtyn*, Jan Grajewski, Magdalena Twarużek

Kazimierz Wielki University, Faculty of Natural Sciences, Institute of Experimental Biology, Department of Physiology and Toxicology, Chodkiewicza 30, 85-064 Bydgoszcz, Poland

*corresponding author: iwonaalt@ukw.edu.pl

Keywords: CBD, molds, mycotoxin, contamination.

Cannabis indica Llam. belong to the order *Rosales*, family *Cannabaceae*, genus *Cannabis*, species – *C. sativa* L., both with industrial hemp (*C. sativa* L. var. sativa) and narcotic *C. sativa* L. indica. Hemp divide into several groups due to their genetic plasticity, which allows them to adapt to changing geographical and climatic conditions and to almost any substrate. For over 5,000 years, the pharmaceutical industry has used hemp for medicinal and ceremonial purposes. New bioactive compounds are still being discovered. The potential of this genre is therefore still inexhaustible and unfathomable.

The aim of this study was to evaluate molds and mycotoxins contamination of dietary supplements containing hemp in the form of dried material, available in Poland. The material consisted of 18 samples of dietary supplements containing hemp in the form of dried material. The mycological examination was performed on the YGC medium, and the results were expressed as the number of colony-forming units (CFU) per gram of sample. The obtained molds cultures were identified by genus. Mycotoxins determination was performed using HPLC-FLD method (immunoaffinity column AflaTest from Vicam-AF; immunoaffinity column OchraPrep from R-Biopharm Rhône Ltd–OTA).

Mycological analysis of dietary supplements in the form of dried plants showed a significant level of infection with molds, the average number of which was 1.4×10^5 cfu/g. The most frequently identified mold types were *Eurotium* spp. (45%) and *Cladosporium* spp. (34,9%).

AFs were detected in only one sample (AF G_1=2,3 ppb, AF B_1 <LOQ). The results showed that none of the analyzed samples contained OTA above the established limit of detection (LOD).

Studies on the presence of toxic metabolites in *Cannabis indica Lam.* show that only one of the analyzed samples contained AF. Nevertheless, it is very important that this products should be inspected by appropriate sanitary units for presence of molds and mycotoxin contamination.

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P3

FUNGAL AND MYCOTOXINS PRESENCE IN PORTUGUESE GROCERIES STORES – AN INTERVENTION IN THE SCOPE OF ONE HEALTH APPROACH

Carla Viegas[1,2], Bianca Gomes[1], Renata Cervantes[1,2], Marta Dias[1,2], Pedro Pena[1,2], Magdalena Twarużek[3], Robert Kosicki[3], Susana Viegas[1,2]

[1]H&TRC-Health & Technology Research Center, ESTeSL- Escola Superior de Tecnologia da Saúde, Instituto Politécnico de Lisboa
[2]NOVA National School of Public Health, Public Health Research Centre, Comprehensive Health Research Center, CHRC, NOVA University Lisbon, Lisbon, Portugal
[3]Faculty of Biological Sciences, Department of Physiology and Toxicology Kazimierz Wielki University Bydgoszcz, Poland

ABSTRACT

Although the use of preservatives and aseptic processing measures mitigate the food spoilage, certain fungi developed resistance to these treatments and can persist in storage and distribution environments and produce mycotoxins. In addition, the emergence of new pathogens and increased prevalence of fungicide resistant strains due to climate change and spread of fungal phytopathogens into warming climates, represent an additional challenge regarding food security. This study intends to characterize the fungal and mycotoxins presence in Portuguese Groceries Stores (GS).

Fifteen GS were assessed in three common sampling sites (checkout, fruits/vegetables area, and warehouse/dispenser). Electrostatic dust cloths (N=39) were used to assess fungal contamination, by culture-based methods and molecular tools (selected *Aspergillus* sections), and to detect mycotoxins. Mycotoxins were analyzed by an LC-MS/MS system. Separation and detection was carried out using high performance liquid chromatograph (HPLC) Nexera (Shimadzu) with a mass spectrometry detector API 4000 (Sciex).

Fruits/vegetables sampling location was the most contaminated (fungi) area in the GS. *Aspergillus* section *Fumigati* and *Fusarium* species were observed. Concerning mycotoxins, only fumonisin B2 was detected in 2 samples (5%), and both values were below the limit of quantification (LOQ).

Overall, *Aspergillus* section *Fumigati* observed in EDC samples should be highlighted, since in the recently published WHO fungal priority list to guide research, development and public health action, this section was listed in the critical priority group due to clinical relevance. Additionally, also *Fusarium* species, listed by the same WHO list in the high priority group, were observed corroborating the need to intervene in this specific indoor setting. Besides the fungal clinical relevance, highlighted by the WHO list, both species/sections toxicological potential should also be considered. Results obtained claim attention to a human health and food safety concerns and the need of applying a One Health approach.

Keywords: Groceries stores; Fungal contamination; Mycotoxins; One health approach.

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P4

INVESTIGATIONS OF OTA, AFLATOXIN B_1 AND ITS BIOSYNTHETIC PRECURSORS IN FEED

Kim Lara Gützkow[1]*, Kai Kristoffer Lie[2], Sascha Rohn[3], Ronald Maul[1]

[1]Max Rubner-Institut, Federal Research Institute of Nutrition and Food,
Department Safety and Quality of Milk and Fish Products, Kiel, Germany
[2]Institute of Marine Research, Bergen, Norway
[3]Technische Universität Berlin, Institute of Food Technology and Food Chemistry, Berlin, Germany

*corresponding author: kimlara.guetzkow@mri.bund.de

Food and feed are frequently contaminated with mycotoxins like aflatoxins, fumonisins and trichothecenes due to fungi infestation of crop plants in the field or during storage [1]. As crops can be infested with different fungal strain in parallel, feed may be at risk of co-contamination with different mycotoxins. Thus, synergistic effects of e.g. ochratoxin A and aflatoxins might play an important role for the overall toxic effect exerted by a food or feed item. In addition, rarely studied biosynthetic precursors of aflatoxin B_1 (AFB_1) like sterigmatocystin (STC) move into the scientific focus due to their toxicity as single compound but also with respect to additive or synergistic effects. In addition to exerting toxic effects in farm animals, some mycotoxins may be transferred into livestock products and therefore, may impact the safety of human consumers [2].

The first aim of the present study was to develop a sensitive and robust HPLC-MS/MS simultaneous quantification method based on a modified QuEChERS approach for the aflatoxin, their biosynthetic precursors and OTA in different feed.

Method validation demonstrated the challenging analysis of the aforementioned mycotoxins in complex matrix of feed. Due to the heterogenous composition of feed items, the recovery and sensitivity is decreased to varying extent in the different commodities, thus, requiring an internal standard for compensation. Moreover, by contrast oats as an apparently less complex matrix, even more difficulties were noticed resulting in even less sensitivity. For typical compound feed, analyte loss of 30 to 40% was observed when spiked toxins were allowed to settle on the matrix. This decrease in the recovery rate cannot be compensated by adding the internal standard during sample extraction.

Applying the optimised method to 97 conventional feed samples revealed mycotoxin contaminations in about 50% of the tested samples. However, the concentrations were mostly low in this particular set of samples (between < LOQ and maximum concentrations: AFB_1 0.5 µg/kg, STC 1.8 µg/kg, OTA 2.7 µg/kg). Contaminations with STC were detected more frequently and with higher concentrations than with AFB_1. Since a similar toxicity of STC and AFB_1 can be assumed further investigations are necessary to estimate the adverse effect on animal health. However, so far no data concerning the transfer rates of STC exist.

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P5

OCCURRENCE OF "EMERGING" MYCOTOXINS AND THEIR PRODUCERS IN SPRING BARLEY AND THEIR RELATION TO NUTRIENT COMPOSITION IN LITHUANIA

Eimantas Venslovas, Audronė Mankevičienė, Yuliia Kochiieru, Sigita Janavičienė

Lithuanian Research Centre for Agriculture and Forestry, Akademija, Lithuania

Spring barley is one of the main sources of food and feed therefore, it is very important to ensure the best grain quality [1]. However, barley grains can be affected by diseases and one of the most devastating diseases is *Fusarium* head blight (FHB) [2]. It is caused by *Fusarium* species which produce mycotoxins as their secondary metabolites [3]. The main species in Europe responsible for FHB are *F. graminearum*, *F. culmorum* and *F. poae*. However, species like *F. avenaceum* and *F. tricintum* are becoming increasingly important since they can produce "emerging" mycotoxins such as moniliformin (MON) and enniatins (ENNs) [4]. There is still a lack of information, however it is thought that these mycotoxins might act synergistically, therefore it is recommended to monitor and study their co-occurrence with other *Fusarium* mycotoxins [5]. Interest has recently increased in these mycotoxins as it is still unclear what the potentially negative health effects might be and there are still no regulations or recommendations that sets maximum levels of these mycotoxins in food or feed [6]. Therefore, the aim of this study was to evaluate the situation of barley grain in Lithuania, assessing its infection by *Fusarium* fungi and contamination with "emerging" mycotoxins that they produce, as well as the impact of these mycotoxins on nutrient composition.

In 2020–2021 two spring barley varieties, *Laureate* and *Luokė*, were studied. During the barley growing season, the temperature in 2020 was lower than in 2021, except at harvest time (August). June and July in 2020 had higher rainfall than 2021, but in 2021 more rainfall was recorded at the beginning and at the end of the barley growing season. For the detection of Fusarium fungal contamination of grains the classical morphological analysis (Agar plate method) was used. The method used for the detection of mycotoxins was the High-Performance Liquid Chromatography – Mass Spectrometry (HPLC-MS) method. The main nutrient components (dry matter, crude protein, crude fat, crude fiber, crude ash, starch, phosphorus, calcium, zinc, potassium, iron) were determined in the chemical research laboratory using reference chemical methods: Kjeldahl, Soxhlet extraction, gravimetric, polarimetric, atomic absorption spectrometry, spectrophotometry methods. Statistical analysis was conducted using SPSS Statistics, version 25 (IBM Inc.). Significant differences between mycotoxin concentrations were calculated using T-test. The Pearson correlation coefficient was used to detect relations between different mycotoxins, fungi, and nutrient components.

Our study showed that fungi producing MON and ENNs were the predominant fungi in 2020. In 2020, the infections with *F. tricintum* and *F. avenaceum* in the *Luokė* variety were 10 times and in *Laureate* variety almost 6 times higher than in 2021. Although, no differences were observed in the evaluation of fungi contamination between varieties. In 2020 the average concentration of MON was 27 µg/kg, while in 2021 it was not detected. It is also noted that in 2020, several times higher concentrations of ENNs B, B1 and A were found. ENNs B, B1 and A concentrations respectively were 19, 8 and 6 times higher than in 2021. Statistically significant positive correlations were observed between fungi and mycotoxins: *F. avenaceum* positively correlated with ENN B concentrations, *F. tricintum* with MON, ENNs B, B1 and A concentrations. Due to the low concentrations of mycotoxins detected, we did not observe many correlations between mycotoxins and nutrient components.

However, some negative correlations were found: MON and ENNs negatively correlated with crude ash, also ENNs negatively correlated with crude fibre.

It is noted that there is a risk of "emerging" mycotoxins production in Lithuania, which might have a negative impact on nutrient composition, however further research should be carried out in order to find out more about the potential synergistic effects on health and impact on food and feed products quality.

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P6

LC-MS/MS ANALYSIS OF ALTERNARIA AND FUSARIUM TOXINS IN CEREALS AND CEREAL-BASED FOODS

Fabian Dick[1]*, Alena Dietz[1], Michael Rychlik[1]

[1]Chair of Analytical Food Chemistry, Technical University Munich (TUM), Maximus-von-Imhof-Forum 2, 85354 Freising, Germany

*corresponding author: fabian.dick@tum.de

Cereals are often infected by fungi capable to produce mycotoxins. Of the latter, Fusarium species often infect the plants during growth while Alternaria species are known as saprophytic plant pathogens. Mycotoxins produced by the latter genera include regulated toxins like deoxynivalenol and zearalenone as well as emerging mycotoxins like alternariol, tenuazonic acid and enniatins.

In the study presented here we developed a multi-mycotoxin method to analyse 23 different Alternaria and Fusarium toxins in cereals in one LC-MS/MS run. After triple extraction the sample work-up involves a modified QuEChERS clean-up followed by dispersive solid phase extraction. The method was validated according to Hädrich and Vogelgesang (1998). The LODs range from 0.001 to 3.28 µg/kg while the LOQs are between 0.005 to 11.8 µg/kg.

The screening of over 80 different cereals and cereal products showed the prevalent co-occurrence of Alternaria and Fusarium toxins. The frequent contamination of rice and rice based products with emerging Alternaria toxins indicates the need to implement emerging toxins in routine methods.

P7

OCCURRENCE OF CYTOCHALASIN E IN MALTING BARLEY AND MALT IN THE CZECH REPUBLIC

Marek Pernica, Jan Martiník, Rastislav Boško, Zdeněk Svoboda, Sylvie Běláková, Karolína Benešová

Research Institute of Brewing and Malting, Mostecká 7, 614 00 Brno, Czech Republic

The microscopic fungus *Aspergillus clavatus* hardly occurs as a field contaminant of plants. It belongs to storage fungi, when it was found in a number of products stored in unsuitable conditions with high humidity. Its growth is mainly associated with increased temperatures during the malting of barley and other cereals [1]. Cytochalasin E is one of the toxic secondary metabolites produced by *Aspergillus clavatus* [2]. It is an inhibitor of actin polymerization in platelets. It also damages blood capillary cells, allows extravascular diffusion of plasma fluid, and prevents angiogenesis (regeneration of blood capillaries). It has mainly cytotoxic effects.

Cytochalasin E was analyzed in Czech malting barley and malt. For the extraction, the QuEChERS method was used and the mycotoxin was identified and quantified using ultra performance liquid chromatography with mass spectrometric detection. A total of 598 barley and malt samples were analyzed. In total, almost 1% of barley samples and 21% of malt samples were contaminated. The concentration of cytochalasin E in barley ranged from 13.5 µg/kg to 52.2 µg/kg with mean being 23.3 µg/kg and in malt ranged from 0.6 µg/kg to 135.2 µg/kg with mean being 25.1 µg/kg.

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P8

READY, SET, DETECT: SIMPLIFYING MYCOTOXIN ANALYSIS FOR THE FOOD INDUSTRY

Peter Carl[1,2], **Timm Schwaar**[1,2]

[1]SAFIA Technologies GmbH, Berlin, Germany
[2]Federal Institute for Material Research and Testing (BAM), Berlin, Germany

In the past year, Germany experienced 15 product recalls due to mycotoxin contamination in various foodstuffs, including figs, melon seeds, pistachios, rice, peanuts, and almonds [1]. These recalls not only pose a threat to public health but also result in substantial financial losses for the affected companies and insurers, amounting to an estimated 1.3 million Euros per recall [2]. Despite the severity of this issue, there is a lack of a comprehensive screening method for mycotoxins in foodstuffs, which leaves many food products susceptible to contamination and recall. To address this gap, we have developed the SAFIA Mycotoxin System, a suspension array fluorescence immunoassay (SAFIA) that enables rapid, cost-effective detection of multiple mycotoxins.

The SAFIA Mycotoxin System is a comprehensive solution for the detection of mycotoxins along the food supply chain. It comprises an analysis kit, a software for simplified data analysis, and a flow cytometer. The core technology is a particle-based competitive multiplexing immunoassay adapted from an assay system for detecting pollutants in environmental samples [3]. The utilized microparticles have a three-layered structure comprising a polymeric core with a fluorescent dye serving as an analyte code, an inorganic shell for protection of the core, and a surface modified with hapten-structures (binding sites for antibodies) and anti-fouling properties (to repel non-specific binding of matrix substances originating from foodstuffs). The assay utilizes high-selective antibodies that bind competitively to the hapten-structure on the particles or free analytes in solution. Both fluorescent signals (analyte code and secondary antibody) are read-out via a flow cytometer.

Using this system, we developed a 7-plex immunoassay for the parallel detection of aflatoxins (Total B1, B2, G1, G2), fumonisins (Total B1, B2, B3), ochratoxin A, deoxynivalenol, T-2 toxin, and zearalenone. The limits of detection achieved by SAFIA are well below the regulated maximum levels set by the EU commission regulation EU 1881/2006, demonstrating its high sensitivity and suitability for detecting mycotoxins in various foodstuffs. Furthermore, an internal control was introduced to detect false-positive errors, which is a persistent problem in classical immunoassays, such as ELISA or lateral flow assays. Flow cytometric read-out enables SAFIA to be executed in a wash-free mix-and-read manner, making the analysis fast and easy to perform. SAFIA is adapted to the 96-well plate format for high-throughput screening. On further, the assay can be executed in a tube-format that requires no additional material other than one pipette for both sample preparation and on-site detection.

We thoroughly validated SAFIA using reference materials from different foodstuffs, including samples with high protein content (grain, corn), high-fat content (nuts, oil), high-sugar content (figs, raisins), high-water content (fruits, juices), and special matrices (spices and herbs). We found that only a few matrices interfere with SAFIA. In most materials, good recovery rates (mostly 70-130%) and excellent precision (mean CV < 11% for total analysis and all analytes) were achieved.

In conclusion, the SAFIA Mycotoxin System provides a simple solution for rapid detection of mycotoxins in foodstuffs, with the potential to reduce the incidence of product recalls and protect public health.

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P9

DETERMINATION OF ERGOT ALKALOIDS IN RYE FLOUR BY ELISA AND LC-MS

Jan Martiník*, Rastislav Boško, Zdeněk Svoboda, Sylvie Běláková, Karolína Benešová, Marek Pernica

Research Institute of Brewing and Malting, Mostecká 971/7, 614 00 Brno, Czech Republic

*corresponding author: martinik@beerresearch.cz

The ergot fungus (*Claviceps purupurea*) is causative agent of ergot on cereals such as rye, wheat, barley and oats. Spores of this fungus infect cereals and grasses during flowering and produce characteristic black sclerotia (*Secale cornutum*) which contain varying amounts of ergot alkaloids [1].

From January 1st, 2022, Commission Regulation (EU) No 2021/1399 of 24th August 2021 amending Regulation (EC) No 1881/2006 as regards maximum levels of ergot sclerotia and ergot alkaloids in certain foodstuffs became active. Ground rye products must comply with the limit for ergot alkaloids of 500 µg/kg, which will be further reduced to 250 µg/kg in July 2024.

The aim of this study was to determine ergot alkaloids content in commercially available rye flours from Czech Republic by commercially available ELISA kit and by liquid chromatography method coupled with mass spectrometer. A total number of 27 rye flours were tested. The concentration of the sum of alkaloids detected by LC-MS ranged from 0 µg/kg to almost 10 000 µg/kg with mean being 783 µg/kg. The ELISA results were generally lower with range from < 29 µg/kg (< LOQ) to 236.9 µg/kg with two samples having concentration higher than detectable limit of 800 µg/kg.

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P10

DSM WORLD MYCOTOXIN SURVEY – FOCUS DACH REGION

Bettina Behler-Woechtl, Anneliese Mueller

CC Mycotoxin Risk Management, DSM Austria, Getzersdorf, Austria

The DSM World Mycotoxin Survey (previously known as BIOMIN World Mycotoxin Survey) has started in 2004. In 2022 the dataset contained 27.297 samples from 87 countries and 122.240 points of analyses were conducted. Most data are gained by LC-MS/MS, HPLC and ELISA. Currently, Aflatoxins (Afla), Zearlenone (ZEN), Deoxynivalenol (DON), T-2 toxin, Fumonisins (FUM) and Ochratoxins (OTA) are considered as the most relevant mycotoxins and thus are in the focus of the survey. However, analyzing only for these mycotoxins might lead to underestimation of the contamination of commodities, especially if co-contamination is not considered. The combination of mycotoxins in a diet might lead to enhancement of the negative effects [1]. State-of-the-art LC-MS/MS methods allow the detection of multiple mycotoxins in one run. With Spectrum Top® 50 DSM and Romer Labs® offer a commercially available multiple mycotoxin method covering >50 different mycotoxins and metabolites. For special cases and in research DSM cooperates with the Department of Agrobiotechnology (IFA-Tulln, University of Natural Resources and Life Sciences Vienna) who established a LC-MS/MS method covering >500 mycotoxins and other secondary metabolites [2]. The multiple data generated by these highly advanced methods are incorporated in the Survey.

In the DACH region (Germany, Austria, Switzerland) DON was the most prevalent mycotoxin with 54% (n=1586), followed by FUM (n=544, 43% positive) and ZEN (n=1396, 38% positive). The median contamination within the positive samples was 337, 124 and 39 ppb for DON, FUM and ZEN, respectively.

In corn kernels sampled in the DACH region, a prevalence of 86% and 60% for DON and ZEN was determined (n=235 and 224 for DON and ZEN, respectively). Median contamination was 618 ppb for DON and 453 ppb for ZEN. Prevalence of samples contaminated with at least two of the six major mycotoxins was 77%. Thus, corn needs to be considered as a major source of mycotoxins.

Finished feed as a mixture of different components is highly co-contaminated. In finished feed intended for poultry and swine nutrition sampled in DACH region, 94% of samples were contaminated with at least two of the six major mycotoxins. ZEN (91%), DON (87%) and FUM (79%) were the most prevalent mycotoxins in finished feeds samples, but also T-2 toxin could be found in 41% of samples. Maximum concentration in finished feed were high (760 ppb, 5700 ppb, 7682 for ZEN, DON and FUM, respectively).

The DSM World Mycotoxin Survey also covers unusual feed materials. Straw sampled in the DACH region was tested positive for DON in 87% and in ZEN in 14% of samples (n=23 and 21 for DON and ZEN, respectively). Thirteen samples (DE, AT) have been analyzed with the multi-mycotoxin method established by IFA Tulln [2]. On average 36 different mycotoxins and metabolites were detected per sample. Interestingly, the masked mycotoxin DON-3-glucoside is found in 62% of the samples (median of positives 103 ppb).

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P11

OCHRATOXIN A IN CHEESE: FIRST DATA ON THE OCCURRENCE IN SAMPLES FROM THE GERMAN MARKET AND ORGANISATION OF A PROFICIENCY TEST FOR QUALITY ASSURANCE FOR FURTHER MONITORING PROGRAMMES

Christoph Hutzler, Michael Weiß, Stefan Weigel

German Federal Institute for Risk Assessment (BfR), Department for Safety in the Food Chain, Max-Dohrn-Str. 8-10, 10589 Berlin, Germany

EFSA published a risk assessment on OTA in food in 2020 (1). Data on ochratoxin A (OTA) in cheese (n = 15) were also included in the exposure assessment, which indicate that cheese could be among the most relevant sources of exposure to OTA from food. The mean OTA levels in cheese were 2.24 µg/kg (lower bound) and 18.54 µg/kg (lower bound) in the 95th percentile. However, the database was very small with 15 samples, especially since the type of cheese could not be specified in all samples, so that the EFSA recommends generating further content data to reduce uncertainty. OTA has so far been detected mainly on traditionally produced cheeses with a long ripening period (principally on or near the rind) and most frequently on cheeses labeled 'Parmigiano Reggiano' and 'Grana Padano', so the investigations initially focused on these foods (grated and in one piece).

Since there is no standardized method for analyzing OTA in cheese, our lab developed, validated and used a method based on the standard DIN EN 17641:2022-12 for the determination of OTA and optional aflatoxin M1 in hard cheese and soft cheese. After QuEChERS extraction of milled hard cheese or an aqueous slurry of cheese, we established and validated three different variations based on LC-FLD analysis after clean up with immunoaffintiy columns (IAC) and LC-MS/MS analysis with and without IAC clean up.

We utilized the LC-FLD variation of the method to analyse 42 hard cheese samples labeled with 'Parmigiano Reggiano' or 'Grana Padano' from the German market purchased in 2022 from local retailers or from internet retailers. 5 samples were bought as flakes of hard cheese, 10 samples consisted of ground hard cheese and 24 samples were bought as packaged pieces of hard cheese. 70% of the grinded hard cheese samples resulted in quantifiable OTA contents above the LOQ of 0.2 µg/kg, whereas only 12.5% of the samples of packaged pieces and none of the flake samples resulted in OTA contents above the LOQ. Additionally Positive samples were confirmed by LC-MS/MS. A further evaluation and discussion of the results of this survey will be presented in this contribution.

Our OTA results from market samples clarify that there is a strong need for more data on the occurrence of OTA in cheese. As an important national approach to fill this data gap the German national monitoring programme includes a monitoring project on OTA in hard cheese and an additional routine topic on OTA in moulded soft cheese for 2023. In order to ensure the quality and comparability of the results of the individual labs participating in this monitoring programme our lab organised a proficiency test with 21 public labs from Germany and other European countries. The labs were able to analyse OTA in hard cheese and moulded soft cheese contaminated with OTA in the concentration range of 5-6 µg/kg with a Relative Standard Deviation for Reproducibility (RSDR, [%]) of 20% and 22% significantly below the target standard deviation of 25%. This demonstrates that the public labs involved in our PT are able to analyse OTA in cheese in high quality and consistency even there is no official standard method for this analyte matrix combination available now.

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P12

OCHRATOXIN A DETERMINATION IN MOUSE TISSUES AND PLASMA SAMPLES: A FAST AND SIMPLE HPLC-FLD METHOD

Elba Beraza[1*], María Serrano[1], Maria Izco[2], Lydia Alvarez-Erviti[2], Adela López de Cerain[1], Elena González-Peñas[3], Ariane Vettorazzi[1,4]

[1]Department of Pharmacology and Toxicology, MITOX Research Group, School of Pharmacy and Nutrition, University of Navarra, Pamplona, Spain
[2]Laboratory of Molecular Neurobiology, Center for Biomedical Research of La Rioja (CIBIR), Logroño, Spain
[3]Department of Pharmaceutical Technology and Chemistry, MITOX Research Group, School of Pharmacy and Nutrition, University of Navarra, Pamplona Spain
[4]IdiSNA, Navarra Institute for Health Research, Pamplona, Spain

A fast and simple high-performance liquid chromatography-fluorescence detection (HPLC-FLD) analytical method has been developed and validated for the determination of ochratoxin A (OTA) in mouse plasma, kidney, brain, intestine and liver. There are several methods published in the literature regarding OTA quantification. However, they either have been developed for other species and use big volume of samples or they do not present all the validation data. In the method described in this work, the calibration curves and chromatographic conditions are common for the five matrices, whereas the extraction method only varies for the liver. After extraction, the supernatants were dried and reconstituted with mobile phase before being injected into the HPLC system, analyzed on a 5µm (25 cm × 0.4 cm) Tracer Extrasil ODS2 column using FLD (excitation wavelength 225nm, emission wavelength 461nm). The mobile phase was 50:50 (v/v) acetonitrile–aqueous solution of formic acid (0.4%). The method was validated following the FDA and EMA guidelines according to the following parameters: selectivity, linearity, accuracy and precision (within and between-day), recovery, dilution integrity, carry-over effect, stability and robustness. All the validation criteria were met and recovery was very efficient in each case. The method has been successfully applied to the analysis of samples from mice treated for 28 days of OTA intraperitoneal administration (0.21 or 0.5 mg/kg b.w. dissolved in an aqueous NaHCO3 solution). Overall, the extraction procedure and the analytical conditions for the analysis of OTA in five different mouse matrices have been set. The developed method complies with the proposed objectives of being fast, robust and reproducible while allowing the quantification of OTA in tissues and plasma of individual mouse.

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P13

A STUDY ON THE AFLATOXIN M1 RATE AND SEASONAL VARIATION IN DAIRY PRODUCTS FROM WEST PART OF IRAN

Kousar Aghebatbinyeganeh[1], Mohamed F. Abdallah[2,3]*, Mohammad Hosein Movassagh[1]

[1]Department of Veterinary Medicine, Islamic Azad University, Shabestar Branch, Shabestar, Iran
[2]Department of Food Technology, Food Safety and Health, Ghent University, Ghent, Belgium
[3]Department of Veterinary Toxicology and Forensic Medicine, Assiut University, Assiut 71515, Egypt

Milk and milk products of livestock are the main source of human exposure to aflatoxin M1 (AFM1), the hydroxylated metabolite of aflatoxin B1 (AFB1). This toxin is resistant to different food preservation procedures such as pasteurization and sterilization. Although AFM1 is 10-fold less toxic than AFB1, it is still considered a genotoxic and carcinogenic food contaminant (EFSA, 2020).

The present study aimed at investigating the seasonality of AFM1 in milk, cheese, and yogurt products sold in Ilam city, located in the western part of Iran. A total of 120 samples consisting of raw milk (n=20), pasteurized milk (n=20), sterilized milk (n=20), traditional cheese (n=20), pasteurized cheese (n=20), and yogurt (n=20) were randomly collected during four consecutive seasons between January 2022 and November 2022, and the amount of AFM1 was measured by high-performance liquid chromatography with fluorescence detection (HPLC-FD). The results showed that 100% of the samples were contaminated with AFM1. Considering the seasonal variability, the contamination mean levels of AFM1 in samples obtained in summer were significantly higher ($P < 0.05$) than those obtained in winter. In addition, AFM1 concentrations in 100% of the yogurt samples and 70%, 45%, and 25% of the raw, sterilized, and pasteurized milk samples, respectively, were higher than the maximum tolerance limit accepted by European Union. The results of this research show the necessity of a monitoring program to control food safety for consumers.

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P14

THE PRODUCTION OF THE SATRATOXINS G AND H CORRELATES WITH THE LEVEL OF SPORULATION IN *STACHYBOTRYS CHARTARUM*

Katharina Tribelhorn[1], Magdalena Twarużek[2], Ewelina Soszczyńska[2], Christiane Baschien[3], Reinhard K. Straubinger[1], Frank Ebel[1] and Sebastian Ulrich[1]

[1]Chair of Bacteriology and Mycology, Institute for Infectious Medicine and Zoonoses, Department of Veterinary Science, Faculty of Veterinary Medicine, LMU Munich, Germany
[2]Department of Physiology and Toxicology, Faculty of Biological Sciences, Kazimierz Wielki University, Bydgoszcz, Poland
[3]Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

Stachybotrys chartarum is a toxic mold that can be isolated after infestation of moisture-damaged cellulosic materials (e.g., building material or animal feed). Macrocyclic trichothecenes (mainly satratoxins G and H) are the most cytotoxic mycotoxins produced by this species. Exposure of mammals to these metabolites can cause serious health problems. To evaluate the pathogenic potential of *S. chartarum* isolates, it is essential to cultivate them under conditions that reliably allow for toxin production.

In this study, three *S. chartarum* strains were cultivated on two Potato dextrose agar (PDA) from different manufacturers. Although nominally identical, the two media differed in one component: PDA from VWR Chemicals (PDA-V) comprises potato peptone (4.0 g/L), whereas PDA-S from Sigma-Aldrich contains potato infusion (4.0 g/L). We compared the resulting colonies, counted the number of spores and used a cytotoxicity assay to determine the toxicity of the samples. Furthermore, the satratoxins G and H were extracted from comparable cultures, purified and their concentrations were measured using an HPLC system.

Our data show that the use of PDAs from different manufacturers can lead to strikingly different results: Cultures on PDA-V grew and sporulated well and produced high satratoxin concentrations (SC), whereas cultures on PDA-S grew with sparse sporulation and lower SC. In addition, by counting the spores and analyzing the extracts we observed a strong correlation between SC and sporulation (Pearson correlation coefficient: $r \triangleq 0.95$). The cytotoxicity test showed that well-sporulating cultures were more cytotoxic than those with a weak sporulation, which correlated with the measured SC.

In summary, we provide evidence that the cultivation of *S. chartarum* on two almost identical PDAs leads to different growth patterns, sporulation rates and SC. For the first time, a clear positive correlation between sporulation and satratoxin production of *S. chartarum* was demonstrated. The level of sporulation depended on the nutrients contained in the medium. Even small differences in their composition can have a strong impact on the toxicity of the isolates, a finding that should be considered in future experiments and diagnostic procedures.

P15

COMPREHENSIVE INVESTIGATIONS CONCERNING APICIDIN-LIKE DERIVATIVES OBTAINED FROM PLANTPATHOGENIC FUNGUS *FUSARIUM FUJIKUROI*

Mika Lutsch[1], Alica Fischle[1], Hans-Ulrich Humpf[1], Svetlana A. Kalinina[1]

[1]Institute of Food Chemistry, University of Münster, Münster, Germany

Fusarium fujikuroi is a well described fungal plant pathogen that has been shown to produce a variety of secondary metabolites (SMs), i.e. pigments, mycotoxins and phytohormones, some of which induce disease in crops and lead to large economic losses in agriculture every year [1]. However, recent activation of an otherwise silent SM-producing gene cluster in *F. fujikuroi* reported the formation of a novel apicidin-like compound, apicidin F (APF) [2] (**Figure 1**). Genetic manipulation of this cluster lead to the production of two new apicidin-derivatives, namely apicidin J (APJ) and apicidin K (APK) [2] (**Figure 1**). Structurally, apicidins present cyclic tetrapeptides which have shown histone deacetylase (HDAC) inhibitory effects and are investigated as potential anti-tumorigenic agents [2,3,4]. Furthermore, antimalarial activities have been reported for apicidin and APF [2,5]. Screening of the genetically modified *F. fujikuroi* strain which is responsible for the APK production led to the investigations of a potentially novel APFderivative (**Figure 1**). The production of this compound is optimized by an amino acid supplementation approach accomplished at a microscale cultivation. The chemical structure is studied by application of liquid chromatographic coupled to high resolution mass spectrometric techniques, nuclear magnetic resonance as well as X-ray crystallography. Determination of *in silico* structure-activity relationship will be complemented by cytotoxicity and biological activity testing of APF derivatives.

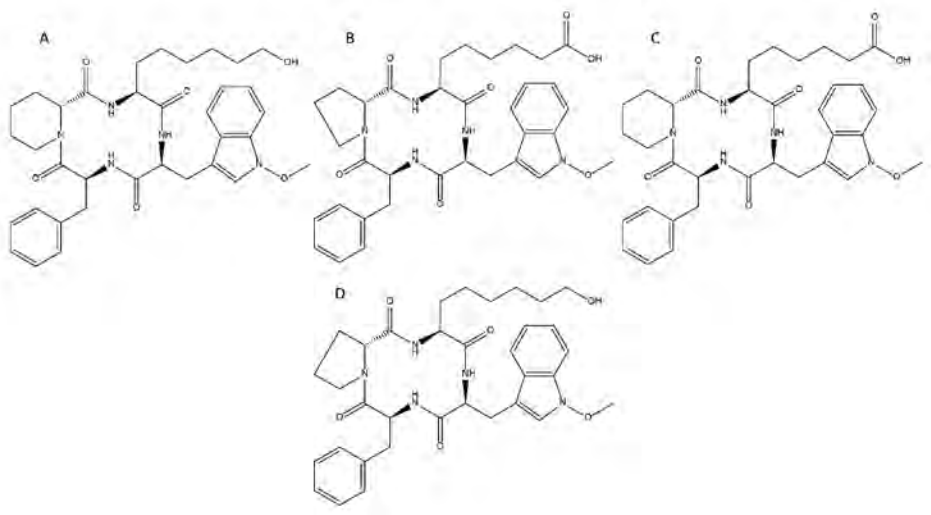

Figure 1: Chemical structures of apicidins K (**A**), J (**B**), F (**C**) and potential new feature (**D**).

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P16

INVESTIGATING THE EFFICACY OF INDIGENOUS ESSENTIAL OILS AS A BIOCONTROL AGENT FOR SAFE CONSUMPTION OF ALGERIAN DATE PALM (PHOENIX DACTYLIFERA) FRUITS

Imene Ghorbal[1], Sara Redouane Salah[1], Mohamed F. Abdallah[2,3], Ahmed Messai[4]

[1]Department of Life and Natural Sciences, University Mohamed khider Biskra, Algeria
[2]Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Ghent University, Belgium
[3]Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Assiut University, Egypt
[4]Department of Agricultural sciences, University Mohamed khider Biskra, Algeria

In many countries, the consumption of date palm (Phoenix dactylifera) fruits is intertwined with the societal, cultural, and religious customs and traditions of the people. Several countries, including Algeria, rely on the cultivation of date palms as a crucial component of their economy. In general, the fruits are marketed as fresh and/or dried fruits for direct consumption and they are also intensively incorporated into many food industries. Toxigenic fungi, particularly aflatoxigenic Aspergilli, have been associated with dates and date by-products [1, 2]. This contamination represents a non-negligible source of human (co-)exposure to mycotoxins [3]. Over the years, several strategies have been proposed to control the toxigenic fungi and their mycotoxins. However, biological control is often the preferred strategy as it is regarded as an environmentally-friendly approach.

The objective of the current project is to study the fungal contamination of date palm fruit samples collected from the southern region of Algeria. Additionally, the work aims to investigate the potential of some essential oils extracted from Algerian endemic plants such as Artemisia judaica, Artemisia herba alba, Artemisia absinthium, and Artemisia campestris for controlling the growth of toxigenic fungi and decreasing mycotoxin levels. In total, several samples of date fruits were randomly collected from date palm farms (n=55), and markets (n=55). Fungal isolation was carried out using two different plating methods (serial dilution and direct plating), while fungal identification was done at genus level using taxonomic keys followed by molecular and chemical identification approaches. The extraction of essential oils from the above mentioned plant species was performed by steam distillation method and their chemical compositions were revealed using gas chromatography-mass spectrometry. The potential antifungal activity against filamentous fungal strains isolated from date fruits were investigated using different in vitro tests. During the 44th Mycotoxin-Workshop, an overview of the research project, in combination with the obtained preliminary data, will be presented.

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P17

INFANT'S EXPOSURE TO MYCOTOXINS FROM THE CONSUMPTION OF MILK FORMULA AND BABY CEREAL IN CHILE

Claudia Foerster[1]*, Liliam Monsalve[1], Gisela Ríos-Gajardo[2]

[1]Universidad de O'Higgins/Instituto de Ciencias Agroalimentarias, Animales y Ambientales (ICA3), San Fernando, Chile
[2]Department of Food Science and Technology, Faculty of Pharmacy, University of Concepción, Concepción, Chile

*corresponding author: claudia.foerster@uoh.cl

Mycotoxins are metabolites of filamentous fungi that contaminate food, and their exposure is associated with various adverse health effects, including cancer induction and mutagenicity, as well as estrogenic, immunosuppressive, gastrointestinal, and renal disorders [1]. Infants (< 2 years) are the group where mycotoxins can show the worst effects, representing a unique combination of a restricted variety of food types, high-calorie intake based on body weight, and lower ability to eliminate toxins due to immaturity of excretory and metabolic processes. In addition, children have more future years to live than adults, so they have more time to accumulate toxins and develop chronic diseases [2,3].

The present study aims to assess the estimated daily intake (EDI) of aflatoxins (AFs), ochratoxin A (OTA), fumonisin (FUM), zearalenone (ZEN), deoxynivalenol (DON) because of baby cereal consumption, and aflatoxin M1 (AFM1), because of milk formula consumption in children less than 2 years old.

We sampled 4 types (wheat, rice, oats, 5 kinds of cereal) of the most consumed brand of baby cereal (n=30) and 2 batches of 12 brands of infant formulas (n=24) in national hypermarkets. We analyzed the samples with a commercial ELISA. Exposure was assessed by the EDI approach (mean and worst case, WCS) which considered: the levels found in a modified Lower Bound (mLB) and Upper Bound (UB); consumption in 3 groups < 6m, 7-12m, and 13-24m; adjusted by the weight of each group. The risk was estimated by the comparison of the EDI with a reference tolerable daily intake (TDI) or by the margin of exposure (MOE) in the case of carcinogenic mycotoxins.

The occurrence of AFM1 in milk formula was 63% (15/24), of which 29% could be quantified, with a range of 6–12 ng/L. The occurrence of mycotoxins in baby cereals was 3% for AF; 20% for OTA; 47% for ZEN; 30% for DON and 0% for FUM, with no levels > LOQ. The most frequent co-occurrences were ZEN-OTA and ZEN-DON, both with 17%. Wheat cereals were the most contaminated, mainly with ZEN (78%). According to exposure calculations, the MOE for AFM1 was less than 10,000 in all models. In the case of the exposure because of baby cereal consumption the EDI < TDI in the case of ZEN, DON, and FUM, and the MOE was > 10,000 in the case of Afs and OTA.

Although the concentrations of mycotoxins found in milk formula and cereals were within Chilean regulatory limits, aflatoxins were considered a problem for public health in children between 0 and 2 years of age. Special regulation for infants and young children is highly recommended in Chile, based on local risk assessments.

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P18

THE *IN VITRO* EFFICACY OF AN INNOVATIVE ANTI-MYCOTOXINS AGENT AGAINST EMERGING AND MASKED MYCOTOXINS

Insaf Riahi[1], Meritxell Sadurní[1], Raquel Codina[1], Laura Escrivá[2], Giuseppe Meca[2]

[1]Technical Department, BIŌNTE Nutrition, Reus, Spain
[2]Laboratory of Agrifood Biotechnology Department of Preventive Medicine Nutrition and Food Science Area Faculty of Pharmacy University of Valencia, Spain

Introduction:
Mycotoxins are low-molecular-weight secondary metabolites produced by fungi. Mycotoxins are considered a very important public health issue because of their adverse effects on animals and humans [1]. Emerging mycotoxins are mycotoxins that are not routinely determined and are not legislatively regulated, including beauvericin and enniatins. Masked mycotoxins formed by conjugation with polar compounds, as a plant defense mechanism, are not analyzed by conventional methods and may be more toxic than the original mycotoxin [2]. Therefore, the aim of the present study was to evaluate the *in vitro* efficacy of an anti-mycotoxins agent that contains selected binding material and natural extracts besides an exclusive combination of yeasts, against some emerging and masked mycotoxins.

Materials and Methods:
The adsorption test was carried out in buffer solutions at pH 3 and pH 7 for 90 min at room temperature, using a mycotoxin concentration of 5 mg/L and 10 mg/mL of the anti-mycotoxin product. Beauvericin, enniatin A, enniatin A1, enniatin B, enniatin B1 as emerging mycotoxins, besides ZEN-14-glucuronide as a masked mycotoxin were individually incubated in triplicate. After incubation, the samples were centrifugated and the supernatant was analyzed by HPLC- qTOF-MS/MS.

To evaluate the desorption capacity, the same mycotoxins at 5 mg/mL were incubated with the anti-mycotoxin product at 10 mg/ml under the aforementioned test conditions. Therefore, the samples were centrifugated and the resultant pellet was added to 1 mL of pH 7 PBS. The solution was centrifugated and the supernatant was analyzed by HPLC- qTOF-MS/MS. In addition, 1mL of methanol was added to the pellet resulting from the desorption performed at pH 7. The samples were then incubated for 30 min at room temperature and centrifugated to analyze the supernatant by HPLC- qTOF-MS/MS.

All results obtained were analyzed using the Mass Hunter (Agilent Technologies) software.

Results:
The *in vitro* efficacy of the anti-mycotoxin product against the emerging mycotoxins ranged from 99.5% to 100% (99.5% beauvericin, 100% enniatins). Concerning the efficacy against masked mycotoxins, the anti-mycotoxin product adsorbed 93% of ZEN-14-glucuronide. As for the desorption, percentages around 8.8 to 89.4% were only observed under forced methanol conditions, while null desorption was observed at pH 7.

Conclusions:
The innovative anti-mycotoxin product tested in the present study is a promising strategy with high *in vitro* efficacy against emerging (beauvericin and enniantins) and masked (ZEN-14-glucuronide) mycotoxins and low desorption.

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P19

TOXICO-PATHOLOGICAL EFFECTS OF OCHRATOXIN A AND ITS DIASTEREOISOMER UNDER IN OVO CONDITIONS AND IN VITRO EVALUATION OF THE TOXICITY OF THESE TOXINS AGAINST THE EMBRYO *GALLUS GALLUS* FIBROBLAST CELL LINE

Marcin Bryła[1], Krzysztof Damaziak[2], Magdalena Twarużek[3], Agnieszka Waśkiewicz[4], Łukasz Stępień[5], Marek Roszko[1], Adam Pierzgalski[1], Robert Kosicki[3], Ewelina Soszczyńska[3], Monika Łukasiewicz-Mierzejewska[2], Marta Chmiel[6], Wojciech Wójcik[2]

[1]Department of Food Safety and Chemical Analysis, Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology-State Research Institute, 02-532 Warsaw, Poland
[2]Department of Animal Breeding, Institute of Animal Science, Warsaw University of Life Sciences – SGGW, 02-786 Warsaw, Poland
[3]Department of Physiology and Toxicology, Faculty of Biological Sciences, Kazimierz Wielki University, 85-064 Bydgoszcz, Poland
[4]Department of Chemistry, Faculty of Forestry and Wood Technology, Poznan University of Life Sciences, 60-625 Poznan, Poland
[5]Department of Plant-Pathogen Interaction, Institute of Plant Genetics of the Polish Academy of Sciences, 60-479 Poznan, Poland
[6]Division of Meat Technology, Department of Food Technology and Assessment, Warsaw University of Life Sciences – SGGW, 02-787 Warsaw, Poland

Ochratoxin A (OTA) is produced by fungi from the *Aspergillus* and *Penicillium* genus, and may be present in many agricultural products from all over the world. OTA can be transferred from feed to animal tissues and bird's eggs. It is a potent nephrotoxin that causes renal toxicity and possesses carcinogenic, teratogenic, immunotoxic, and possibly neurotoxic properties. Ochratoxin A exhibits the highest embryotoxicity among all known mycotoxins. Poultry show a higher sensitivity to OTA toxicity, compared with mammals. Although OTA is described as a thermostable toxin, during heating at elevated temperature (common in feed production) it can be transformed into its diastereomer 2'R-ochratoxin A (2'R-OTA). In the presented study, the embryotoxicity of ochratoxin A (OTA) and its racemic derivative 2'R-ochratoxin A (2'R-OTA) under in ovo conditions was compared, as well as the in vitro embryotoxicity of these substances together with α-ochratoxin and ochratoxin B was evaluated, using chicken (*Gallus gallus domesticus*) embryo fibroblast cell lines. The in ovo tests included egg incubation of 8 different groups, 30 eggs each (2 control groups – eggs incubated without puncture or injection (standard incubation) and punctured eggs without injection; 6 experimental groups – eggs containing OTA or 2'R-OTA at 0.25, 0.50, and 0.75 μg/egg concentration). The results confirmed the impact of OTA on early and late embryo mortality, where chick hatchability decreased with increasing toxin dosage. Both OTA and 2'R-OTA showed embryotoxicity, however, in the case of the highest OTA diastereomer dose, nearly 11% higher hatchability of chicks was observed compared with the group that received OTA. The dosage of 2'R-OTA did not reduce parameters chick quality compared to chicks hatched from control group eggs. OTA concentrations were higher than 2'R-OTA detected in chicken organs such as liver and kidney, whereas 2'R-OTA concentrations were higher in blood serum and heart. The presented studies highlighted the differences in the ability to accumulate toxins in certain organs, which, to a certain extent, may affect the potential toxicity on individual organs. Additionally, during in vitro tests, when assessing the cytotoxic effects of OTA and its analogues toward the chicken embryonic cell line in an MTT assay, the cell metabolic activity was inhibited to a comparable extent at a 27-fold higher concentration of 2'R-OTA than OTA (0.24 μM). Also, comparably lower toxicity was attributed to the remaining OTA derivatives. The obtained results are the first to present comparative toxicological properties of OTA and its diastereomer and their impact on chicken embryogenesis.

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P20

CORRELATION BETWEEN THE EFFECTS OF INDIVIDUAL *FUSARIUM* MYCOTOXINS AND THEIR METABOLITES ON LIPID PEROXIDATION AND MEMBRANE LIPID COMPOSITION IN POULTRY

Szabina Kulcsár[1,2]*, Krisztián Balogh[1,2], Erika Zándoki[2], Omeralfaroug Ali[2], András Szabó[2], Miklós Mézes[1,2]

[1]Department of Feed Safety, Institute of Physiology and Nutrition, Hungarian University of Agriculture and Life Sciences, H-2100 Gödöllő, Hungary
[2]ELKH-MATE Mycotoxins in the Food Chain Research Group, Hungarian University of Agriculture and Life Sciences, H-7400 Kaposvár, Hungary

*corresponding author: szabina.kulcsar@gmail.com

Worldwide occurrence of mycotoxins is a very important issue in the aspect of both feed and food safety. Three Fusarium toxins, deoxynivalenol (DON), fumonisin B1 (FB1) and T-2/HT-2 toxin are of particular interest since they are frequent contaminants of cereal grains such as wheat and corn and co-occur frequently. Trichothecene mycotoxins are well known for oxygen-free radical formation which can cause oxidative damage, such as changing the lipid fatty acid composition of liver tissue and generating lipid peroxidation. The present study aimed to evaluate the combined effects of DON, T-2/HT-2 toxin, and FB1 in laying hens used mycotoxin doses once (low: T-2 toxin: 0.25mg/kg; DON: 5 mg/kg; FB1: 20 mg/kg) and twice as the EU recommended level of poultry feeds (high: T-2 toxin: 0.5mg/kg; DON: 10 mg/kg; FB1: 40 mg/kg) for 3-days. The liver samples were taken on days 1, 2 and 3 after beginning the feeding trial. In the intracellular biochemical studies, we focused on certain elements of the glutathione redox system. The hepatic membrane lipid alternations (the lipid profile of the phospholipids) was measured, and investigated the correlation with the rate of lipid peroxidation. The results showed that the multi-mycotoxin exposure affected the glutathione system, which was supported by the significantly higher reduced glutathione concentration in the low dose group on day 1 compared to the high dose group. By the way, the end-product of the lipid peroxidation, the malondialdehyde concentration was decreased than the control on day 3 also as an effect of the low dose. The effects of intoxication on the fatty acid composition of liver membranes were most pronounced after 3 days where ratio of saturated fatty acids to unsaturated ones increased, indicating that hepatocytes were undergoing oxidative stress. In summary, the well-known oxidative stress-inducing effect of the investigated mycotoxins occurred in multi-mycotoxin exposure, even at the low dose, therefore a synergistic effect among them would be suggested.

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P21

MYCOTOXINS IN WOMEN BODY FLUIDS

Gabriela Pilarska*, Magdalena Twarużek

Kazimierz Wielki University, Faculty of Biological Sciences, Department of Physiology and Toxicology, Chodkiewicza 30, 85-064 Bydgoszcz

*corresponding author: gpilarska@ukw.edu.pl

Keys: mycotoxin, zearalenone, ochratoxin A, human body fluids.

Mycotoxins are secondary metabolites of the metabolism of molds. They are mainly produced by molds such as *Aspergillus* spp., *Fusarium* spp. or *Penicillium* spp. These mycotoxins are detected in many agricultural products – cereals, feed and food of plant or animal origin for human consumption. Currently, according to FAO estimates, about 25% of the world's cereal grains, and according to some works up to 40%, are contaminated with at least one mycotoxin.

The problem of the occurrence of zearalenone (ZEN) and ochratoxin A (OTA) in food is very typical. Mycotoxins are characterized by their ability to move through the gastrointestinal tract into secretions, tissues and the circulatory system. Increasingly, biological material is being analysed for mycotoxin accumulation in blood, urine or breast milk. EFSA [1] report from 2020 noted that the mean of OTA concentration in blood was 0.12-2.76 ng/ml in urine was 0.21 ng/ml and in breast milk was 0.40-0.26 ng/g. Valitutti [2] et all in (2018) showed that mean of ZEN occurred in breast milk at concentration of 2.7 ng/ml. In contrast, the EFSA [3] report from 2011 showed that the incidence of ZEN occurred in the range between 0.0002-1.8 ng/ml in blood and 3.0-3.7 ng/ml in urine. It was noted that excessive accumulation of OTA in the human body can lead to kidney problems, while ZEN can cause hematotoxic effects through blood clotting disorders or modification of blood parameters. It was also noted that it is not weight, gender or BMI that affects the accumulation of these toxins, but geography, seasonal changes, and climate, which is a very important variable.

The health risks associated with the presence of mycotoxins in our environment is a global problem. Therefore, it is important to monitor their concentrations in both food and their transmission to humans and animals.

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P22

PREVALENCE OF ISOFLAVONES IN PORCINE FEED SAMPLES AND THEIR COMBINATORY EFFECT WITH ZEARALENONE ON PIGLETS

Barbara Novak[1], Dino Grgic[2,3], Elisabeth Varga[2], Doris Marko[2], Barbara Doupovec[1], Dian Schatzmayr[1]

[1]DSM – BIOMIN Research Center, Tulln, Austria
[2]Department of Food Chemistry and Toxicology, Faculty of Chemistry, University of Vienna, Vienna, Austria
[3]Doctoral School in Chemistry, University of Vienna, Vienna, Austria

Isoflavones (ISF) occur frequently and in high concentrations in soy-based animal feed products. They are present either as aglycones (e.g., daidzein (DAI) and genistein (GEN)) or as glycosides and are described to possess endocrine activity due to their structural similarity with 17ß-estradiol [1]. ISF commonly co-occur with the mycotoxin zearalenone (ZEN) in feed, which interferes with both estrogen receptors (ERα and Erβ) [2]. We investigated the prevalence and concentration of these compounds in 206 pig feed samples from the year 2022. Additionally, a feeding trial was conducted in which in total 40 female weaning piglets were fed either a control diet (without soy), a diet including ISF or ZEN (870-1100 µg/kg) or both compounds for 14 days. The ZEN-degrading enzyme zearalenone hydrolase ZenA (ZEN*zyme*®) was included in an additional fifth group together with ZEN and ISF. Vulva size (width and length) and uterus weight were determined at day 15 after euthanasia of the animals.

Aglycones and their glycosides as well as ZEN occurred in almost half of all analysed pig feed samples (42-50%); DAI and GEN were found in median concentrations of ~2000 µg/kg and their glycosides in concentrations of ~7000 and 3000 µg/kg, respectively. ZEN, on the other hand, was found only in a median concentration of 18 µg/kg, however individual samples reached concentrations up to 899 µg/kg. Dietary ZEN led to a significant increase of vulva area ($p = 0.0167$) and uterus weight ($p = 0.0165$) compared to the control group after 14 days. Also, the combination of ZEN+ISF significantly increased the vulva area ($p = 0.0101$), but elicited an even higher uterus weight than ZEN alone ($p = 0.0042$). If ZEN*zyme*® (40 U/kg) was included to the ZEN+ISF diet, no clinical effects were determined anymore.

As the co-occurrence of these substances in feed is common [3], the investigation of their synergistic effects on the reproductive system of animals as well as counteracting strategies are of great interest.

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P23

MECHANISMS OF TOXICITY OF THE FUSARIOTOXIN ZEARALENONE AT THE PORCINE INTESTINAL LEVEL

Valeria Cristina Bulgaru, Daniela Eliza Marin, Gina Cecilia Pistol, Ionelia Țăranu

National Research-Development Institute for Animal Biology and Nutrition (IBNA), 1 Calea Bucuresti, Balotesti, 077015, Ilfov, Romania

Zearalenone (ZEA) is a mycotoxin produced by fungi belonging to the genus *Fusarium*, that contaminates food and feed afecting both human and animal health. Among farm animals the pig is not only a great consumer of grains, but also has a native sensitivity to mycotoxins. As the main route of contamination with mycotoxins is oral, the intestine is among the first organs affected by the action of mycotoxins. The intestine plays a very important role as a physical barrier, moreover it is also involved in the immune response through the secretion of effector molecules such as cytokines.

There are studies showing that ZEA has a dual character, being either pro- or anti-inflammatory depending on the concentration, triggering different pathway mechanism which require further studies. The aim of the present *in vivo* study was to investigate the effect of two contrasting dietary concentration of ZEA (75ppb and 290ppb) on several signaling key molecules involved in important signaling pathways in porcine colon, one of the vulnerable tissues to inflammation and oxidative stress in pigs after weaning. The experiment was carried out on eighteen piglets for 30 days at the end of which colon samples were collected and the gene expression of certain signaling molecules involved in different pathways especially related to inflammation and oxidative stress such as Toll-like receptors signaling pathway (*TGFβ2*, *TLR4 MYD88*, *IRAK1*, *TRAF6*, *TAK1*, *AKT)*, MAPKs (*p38α*, *JNK 1/2*, *ERK1/2)* and nuclear receptors signaling pathway (*NF-kB*, *AP-1*). Pro-inflammatory markers (*IL-1β*, *TNFα*, *IL-8*, *IL-6*, *IFN-γ*) gene expression was also analyzed.

The results showed that the higher level of ZEA (290ppb) significantly increased the gene expression of TLR4/MYD88 molecules as well as that of MAPKs, NF-kB and AP-1, therefore an increase of the pro-inflammatory cytokines *IL-1β*, *TNFα*, *IL-8*, *IL-6*, *IFN-γ* was observed, showing a pro-inflammatory character of ZEA. By contrast, the data obtained for the lower concentration of ZEA (75ppb) have no effect on these markers. Some of them registered an insignificant decrease which might suggest an anti-inflammatory effect of ZEA.

P24

ARE THE RULES PROTECTING OUR FISH? FUMONISINS BELOW EU GUIDANCE LEVEL HAVE NEGATIVE EFFECTS ON SPHINGOLIPID METABOLISM IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

Roy Rosen[1], Pablo Cabo-Valcarce[2], Bozidar Raskovic[3], Michaela Thamhesl[1], Dian Schatzmayr[1]

[1]DSM – BIOMIN Research Center, 3430 Tulln, Austria
[2]Research Center for Animal Nutrition and Health, DSM Nutritional Products, F-68128, Village-Neuf, France
[3]Institute of Animal Sciences, Faculty of Agriculture, University of Belgrade, 11080 Belgrade, Serbia

The potential of the aquaculture sector to provide healthy and sustainable protein for the growing human population led to its intense growth and development in recent years. As is the case for terrestrial animals, feed used in aquaculture farming has a critical bioeconomic influence. Aquaculture feeds in particular are subject to constant development that is often driven by economic and environmental considerations. Among other changes, the reduction of these feeds' dependency on raw materials from marine origin led to the increasing use of globally sourced plant-based raw ingredients and consequently to an increased risk of various mycotoxin exposure. Globally, fumonisins are one of the most prevalent groups of mycotoxins detected [1]. They are produced by *Fusarium* species and are known for their negative effects, often caused and manifested by their influence on sphingolipid metabolism. European legislation specifies recommended guidance values for fumonisins in fish feeds of 10 mg/kg with the intention to ensure food and feed safety and the maintenance of animal welfare [2].

In contrast to other species, only a handful of studies describe the effects of fumonisins on salmonids. A single study from nearly two and a half decades ago described the difficulties of fumonisin recoveries from a salmonid diet and the alteration of *in vivo* sphingolipid metabolism caused by pure fumonisin incorporated into the diet [3].

The aim of this study was to close this knowledge gap. We assessed the influence of oral exposure of salmonids to fumonisins under EU guidance levels in two separate trials with different age groups of rainbow trout. Gut content metabolite analysis and the sphinganine-to-sphingosine (Sa:So) ratio were used as endpoints to assess the toxic effect as well as the detoxification efficacy of FumD, a commercial detoxification recombinant enzyme.

The results of our study suggest that short-term oral exposure to sub-regulatory levels of fumonisins negatively affects rainbow trout. It is possible that commercially available fish feeds that are in compliance with the European regulatory levels still put fish health and farmers' operation at risk. Hence these guidance levels should be revised and further studies should be aimed to reproduce and further investigate these findings. Nevertheless, detoxification of the feed by FumD proved to be effective and significantly reduce the effects observed in both endpoints.

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P25

DATABASE FOR PRIORITIZING EMERGING MYCOTOXINS BASED ON GENOTOXICITY AND CARCINOGENICITY POTENTIAL

Myriam Benito[1], Uxue Altuna[1], Elena Lizarraga[2], Elena González-Peñas[2], Adela López de Cerain[1], Ariane Vettorazzi[1]

[1]Department of Pharmacology and Toxicology, MITOX Research Group, School of Pharmacy and Nutrition, University of Navarra, Pamplona, Spain
[2]Department of Pharmaceutical Technology and Chemistry, MITOX Research Group, School of Pharmacy and Nutrition, University of Navarra, Pamplona Spain

The exact number of mycotoxins is unknown, however a significant fraction of the ~170,000 currently known natural products are of fungal origin [1]. Still, only a few have received attention as threats to human and animal health due to their toxic potential and worldwide contamination. Indeed, at the EU level, only aflatoxins, ochratoxin A, patulin, deoxynivalenol, zearalenone, fumonisins, T-2 toxin and HT-2 toxin, citrinin and ergot alkaloids are regulated in different foodstuffs [2]. The term "emerging mycotoxin" has been defined as "mycotoxins which are neither routinely determined, nor legislatively regulated, however the evidence of their presence is rapidly increasing" [1]. Advances in analytical methodologies as well the current context of climate change might increase the number of mycotoxins to be further evaluated from a toxicological point of view. In this regard, genotoxicity and carcinogenicity are main endpoints related to long term exposure.

We present the creation of a database of genotoxic and carcinogenic endpoints of emerging mycotoxins. For that purpose a systematic review has been carried out in Pubmed, in EFSA and WHO/FAO databases. The review was carried out following the PRISMA methodology.

Data extraction will be carried out in two consecutives phases: i) identification of emerging mycotoxins based on occurrence and human biomonitoring studies and ii) search of toxicological information (genotoxicity and carcinogenicity).

The database will allow to prioritize the mycotoxins that should be first evaluated for genotoxicity. Selected mycotoxins will be screened with the SOS/umu in absence and presence of metabolic liver activation.

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SUSCEPTIBILITY OF WEANLING AND ADULT MICE TO DEOXYNIVALENOL ON OVARIAN REPRODUCTIVE TOXICITY

Gisele A.A. de Lemos[1], Juliana R. Gerez[1], Julia B. da Costa[1], Marielen de Souza[1], Emerson Venâncio[1], Camila R. Ferraz[1], Waldiceu A. Verri[1], Eduardo Micotti da Gloria[2], Ana Paula F.L. Bracarense[1]

[1]Universidade Estadual de Londrina, Laboratory of Animal Pathology, Londrina, Brazil
[2]Universidade de São Paulo, Laboratory of Mycology, São Paulo, Brazil

Deoxynivalenol (DON) is associated with reproductive toxicity in various animal species. The frequent presence of DON in cereal-based foods and the high intake of these foods by children raises particular concern about the relative susceptibility of this subpopulation to adverse effects evoked by this mycotoxin. However, the question of whether age-related differences exist in the *in vivo* reproductive toxicity of DON has not been evaluated. Thus, the effects of DON on serum follicle stimulating hormone (FSH) levels and on the ovary (histology and oxidative stress response) of weanling and adult mice were investigated. For this, 20 weanling Swiss mice (21 days old) and 20 young adult mice (65 days old) were fed a control mycotoxin-free diet or diet containing 10 mg of DON/kg of feed for a period of 15 (weanling mice) and 28 days (adult mice), respectively. Ingestion of DON decreased serum FSH levels in weanling females, whereas an increase was observed in adult mice at these times. In ovaries, DON induced vacuolization of oocytes and disorganization of granulosa cells at both ages. DON altered the ovarian oxidative stress response in an age-specific manner; in weanling mice increased levels of superoxide were accompanied by a reduction of the antioxidant potential and an increase of lipoperoxidation, whereas in adults the increase of superoxide levels was associated with an increase of the antioxidant potential. In addition, in weanling mice, a reduction in the number of macrophages and increased levels of TNF-α were observed in the ovaries of the DON group, while in adult animals an increase in the number of macrophages and a trend to higher levels of TNF-α and IL-1 were observed when compared to the control group. When the effect of DON was evaluated on the follicular atresia, an increase in the atretic follicles was observed only in weanling animals fed the DON diet. Age-related differences in the reproductive toxicity of DON can be assigned to the fact that weaned animals show higher levels of intestinal DON absorption and a decreased ability to clear the toxin via urine when compared to adults. Our results may contribute to refining the current risk assessment concerning DON in both animals and humans with emphasis on the reproductive parameters of young age. Additional studies on the reproductive toxicity of DON in weanling animals and its effects on fertility later in life should be encouraged.

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AN *IN VITRO* APPROACH TO ASSESSING THE GENOTOXIC AND OXIDATIVE STRESS RESPONSE POTENTIALS OF DEOXYNIVALENOL AND ZEARALENONE

Maria Yu, Agneta Oskarsson and Johan Lundqvist

Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, Box 7028, SE-750 07 Uppsala, Sweden

Deoxynivalenol (DON) and zearalenone (ZEN) are two common mycotoxins primarily produced by *Fusarium* fungi and are well-recognized food contaminant compounds. According to the scientific opinions of EFSA's Panel on Contaminants in the Food Chain (CONTAM Panel), both mycotoxins are reported to be genotoxic *in vitro*; and clastogenic and aneugenic *in vitro* in the case of ZEN. Further, the genotoxicity of DON and ZEN may be related to oxidative stress-mediated mechanisms [1,2].

The genotoxic effects of DON and ZEN have been assessed in various experimental models [1-3]. The *in vitro* micronucleus (MN) test is one such genotoxicity test that detects micronuclei in the cytoplasm of interphase cells. Very few MN studies, however, have been conducted for DON and ZEN specifically in human cell types. As such, in our study we assessed the genotoxicity of DON and ZEN in the human lymphoblastoid TK6 cell line using the MN test. Micronucleus formations (%MN) and cytotoxicity were analyzed via flow cytometry. In addition, the oxidative stress response in the human mammary cell line AREc32 derived from MCF7 cells was evaluated using the NF-E2-related factor 2 (Nrf2) luciferase reporter gene assay.

For DON, the %MN in the highest non-cytotoxic concentration (0.78 µM) was not statistically different compared to the control. For ZEN, the %MN increase in the highest non-cytotoxic concentration (12.5 µM) was statistically significant compared to the control (p-value of 0.0007, $\alpha = 0.05$). For the oxidative response endpoint, no induction of Nrf2 activity was detected after treatment of cells with either DON or ZEN up to the highest concentration of 50 µM.

To the best of our knowledge, our study is the first to assess the genotoxic effects of DON and ZEN in the TK6 cell line using the sensitive method with MN detection by flow cytometry. For ZEN, genotoxicity was demonstrated as increased MN formations at non-cytotoxic concentrations. DON did not induce any significant MN formations in TK6 cells at non-cytotoxic concentrations. Contrary to what has been suggested elsewhere regarding the role of oxidative stress in the genotoxicity of DON and ZEN, we detected no oxidative stress response in the Nrf2 assay for either of these two compounds at concentrations higher than what was tested for genotoxicity. This study is ongoing and will also include a further evaluation of DON and ZEN in the MN test in the presence of an exogenous metabolic activation system.

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P28

REALISTIC CONCENTRATIONS OF *FUSARIUM* TOXINS IN FOLLICULAR FLUID TRIGGER APOPTOSIS AND ACTIVATE NLRP3 INFLAMMASOME IN BOVINE PRIMARY THECA CELLS

Guodong Cai[1,4], Hilda M Guerrero-Netro[2], Jianchun Bian[1], Isabelle P Oswald[3], Christopher Price[4], Imourana Alassane-Kpembi[4]

[1]College of Veterinary Medicine, Yangzhou University, Yangzhou 225009, Jiangsu, China
[2]Depto. de Reproducción, Facultad de Medicina Veterinaria Zootecnia, UNAM, Ciudad de México, México
[3]Toxalim (Research Centre in Food Toxicology), Université de Toulouse, INRAE, ENVT, INP-Purpan, UPS, Toulouse, France
[4]Centre de Recherche en Reproduction et Fertilité, Faculté de Médecine vétérinaire, Université de Montréal, St-Hyacinthe, Québec, Canada

Cattle are deemed less susceptible to mycotoxins due to the limited internal exposure resulting from rumen microbiota activity. However, the significant amounts of *Fusarium* mycotoxins deoxynivalenol (DON) and zearalenone (ZEA) frequently detected in bovine follicular fluid samples suggest that they could affect the ovarian function [1].

Both mycotoxins trigger several patterns of cell death and activate the NLRP3 inflammasome in the intestine [2,3]. *In vitro* studies have reported a number of their adverse effects on bovine oocytes. However, the biological relevance of such findings with regard to realistic concentrations of DON and ZEA in bovine follicular fluid is still not clear. Hence, it is important to better characterize the effects of dietary exposure to DON and ZEA on the bovine ovary.

Using bovine primary theca cells, this study investigated the effects of realistic patterns for bovine ovary exposure to DON and ZEA, but also DON metabolite DOM-1, on cell death and NLRP3 inflammasome activation.

Exposure to DON starting from 0.1 µM, significantly decreased theca cell viability. The kinetics of phosphatidylserine translocation and loss of membrane integrity showed that ZEA and DON, but not DOM-1, induce an apoptotic phenotype. At anticipated concentrations in follicular fluid of dairy cows based on current EU guidance values, the qPCR analysis of the expression of *NLRP3*, *PYCARD*, *IL-1β*, *IL-18*, and *GSDMD* indicated that DON and DOM-1 individually and in mixture, but not ZEA, activate NLRP3 inflammasome in theca cells.

Altogether, these results suggest that current UE guidance values for DON may not protect the bovine ovary from inflammatory disorders.

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EFFECT OF *ALTERNARIA* MYCOTOXIN – ALTERNARIOL MONOMETHYL-ETHER – ON CELL PROLIFERATION, APOPTOSIS AND DEATH OF SWINE IPEC 1 CELL LINE

Daniela Eliza Marin, Gina Cecilia Pistol, Cristina Valeria Procudin, Ionelia Taranu

National Research and Development Institute for Biology and Animal Nutrition, Balotesti, Ilfov, Romania

Alternariol monomethyl-ether (AME) is one of the major mycotoxins produced by fungi of the genus *Alternaria* and are common contaminant of food products. *Alternaria* mycotoxins are known to cause relevant economic losses and to have a negative impact on human and animal health. According to EFSA the data on the toxicity of Alternaria mycotoxins in livestock and in particularly in pig are generally lacking. The present study aimed to fill some knowledge gaps by studying the *in vitro* effect of AME on cell proliferation and cell death, cell cycle and apoptosis signaling pathways at the intestinal levels using porcine intestinal epithelial cells (IPEC-1). IPEC-1 cells were exposed to different AME concentrations (1-100µg/mL) after mycotoxin exposure for 24h versus solvent control. Treatment of cells with doses of 50 and 100 µg/mL AME, resulted in a dose-dependent loss of cell viability with 39% and 67% and an increase of late apotototic/cell death by 17 and 35 times as reported to untreated cells. Concentrations of AME higher than 5 µg/mL significantly increase the percentage of cells in G2/M phase of cell cycle, while decreasing the percentage of cells in G0/G1 and S phases. Exposure of IPEC1 cells to 10 µg/mL significantly decrease the number of Bcl2 activated cells, while no effect was observed for lower concentrations. Taken together our results have shown that AME interfere with cell proliferation, apoptosis and cell death through Bcl2 signaling pathway.

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ASSESSMENT OF THE EFFECT OF ALTERNARIOL MYCOTOXIN ON SWINE INTESTINAL PARAMETERS USING AN EXPLANT MODEL

Daniela Eliza Marin, Gina Cecilia Pistol, Cristina Valeria Procudin, Ionelia Taranu

National Research and Development Institute for Biology and Animal Nutrition, Balotesti, Ilfov, Romania

Alternariol (AOH) is produced by fungi of the genus *Alternaria* and can be found in fruits, vegetables, and grains. *Alternaria* mycotoxins are known to cause relevant economic losses and to have a negative impact on human and animal health (Solhaug et al., 2016; Kowalska et al., 2021). The present study aimed to investigate the effect of AOH in pigs, at the intestinal level using an explant model. Explants were obtained from the jejunum of three healthy piglets as described by Pinton et al (2021) and exposed or not to different concentration of AOH (5-10µg/mL). After 4h of incubations, explants were used for histological analyses and for the assessment of the expression of junction proteins (occludin and zonula 1) by western blot. The histological analysis of the intestinal mucosa in of the control group revealed a normal aspect of the mucosa, respectively the intestinal villi, the crypts and the chorion. Exposure to AOH 5µg and AOH 10 µg revealed a thinner mucosa and an alteration of mucosal structure. The intestinal epithelium is characterized by the apoptosis of enterocytes and the disintegration of the epithelium on certain areas, especially in the AOH 10 µg/mL group. These changes were not associated with an alteration of the expression of the expression of occluding and zonula 1 proteins. Our results have shown that the exposure to alternariol can interfere with the normal structure and function of the intestine of growing pig.

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VERSICOLORIN A IS A MITOCHONDRIAL TOXICANT FOR HUMAN COLON CELLS

Thierry Gauthier, Sylvie Puel, Isabelle P. Oswald, Olivier Puel

Toxalim (Research Centre in Food Toxicology), Université de Toulouse,
INRAE, ENVT, INP-Purpan, UPS, Toulouse, France;

Among aflatoxins, AFB1 is the most potent natural liver carcinogen known until so far [1]. Versicolorin A (VerA) is one of the intermediate metabolites in the AFB1 biosynthetic pathway. However, VerA may be present at rates exceeding the maximum level of AFB1 established by the European Union in many foodstuffs (corn, nuts, almond and pistachio) [2]. Its cytotoxicity and genotoxicity have been demonstrated in different types of human cells [3,4]. VerA displayed even a higher toxic potency for intestinal cells than AFB1 [2]. Our previous results relating to transcriptomic analysis indicated that VerA could affect the mitochondrial function of human colon cells [2]. Forty years ago, a former paper [5] reported that VerA was a potent uncoupler of oxidative phosphorylation in isolated rat liver mitochondria. The purpose of our present work was to highlight the capacity of VerA to alter oxidative phosphorylation (OXPHOS) in the human colon Caco-2 cells using the Seahorse real-time analyser (Agilent). We have shown that, as soon as 30 minutes exposure, VerA decreased the coupling efficiency of the OXPHOS at low concentration (5 µM) and inhibited the electron transfer chain (ETC) at high concentration (30 µM). Furthermore, the uncoupling effect was correlated to the decrease of the mitochondrial membrane potential measured by flow cytometry. On the contrary, after 24 hours exposure, VerA inhibited the ETC from 0.5 µM in a dose dependent manner and decreased the coupling efficiency at high concentration (10 µM). The qPCR analysis revealed that the 13 proteins encoded in the mitochondrial DNA and involved in the ETC were downregulated in the same way than the mitochondrial RNA Polymerase POLRMT.

Taken together, these results suggest that VerA is able to interact rapidly within the inner mitochondrial membrane to impair the proton motive force. Longer exposure induce a decreased expression of proteins of the ETC resulting in a lower respiration capacity. Both of these effects lead to a drop of the rate of ATP synthesis, contributing to the cell toxicity of VerA.

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INTERACTIONS OF MYCOTOXINS WITH SERUM ALBUMIN: TOXICOKINETIC IMPORTANCE AND OTHER CONSIDERATIONS

Ágnes Dombi, Miklós Poór

Department of Pharmacology, University of Pécs, Pécs, Hungary

Serum albumin is one of the most abundant plasma proteins in the circulation. Albumin binding is an important issue in the toxicokinetics of xenobiotics: the formation of stable ligand-albumin complexes can influence the tissue distribution and/or the elimination half-lives of the compounds bound. Besides its toxicokinetic role, albumin can also be considered as an affinity protein which may be a cheap alternative of antibodies when the formed mycotoxin-albumin complex is properly strong.

Rarely, certain reactive metabolites of mycotoxins (e.g., the epoxide derivative of aflatoxin B1) can form covalent adducts with albumin, which is a biomarker of exposure. However, the reversible interactions of mycotoxins with serum albumin are the most typical, leading to the formation of weak, moderately strong, strong, or highly stable mycotoxin-albumin complexes. For example, ochratoxins bind to albumin with extremely high affinity, resulting in their very long plasma elimination half-lives. Nevertheless, other mycotoxins, including alternariol, citrinin, and zearalenone also show strong interactions with the protein. Besides the parent mycotoxins, the complexation of certain mycotoxin metabolites with albumin seems to be relevant as well.

Usually, ligand-albumin interactions do not show significant species-dependent variations. However, some mycotoxins form highly stable complexes with certain specific albumins, such as ochratoxin A prefers human albumin, while zearalenols favor rat albumin. Based on the data available from animal experiments and human studies, these differences in albumin binding can affect the toxicokinetics (e.g., elimination half-lives) of the above-mentioned mycotoxins.

Recent studies underline that albumin can be utilized as an affinity protein in analytical sample preparation/enrichment, as it has been demonstrated with ochratoxin A and alternariol. This kind of application can be very useful in the extraction of emerging mycotoxins and/or masked/modified metabolites, where antibody-based products are not commercially available.

In this presentation, we aimed to summarize the major issues in regard to mycotoxin-albumin interactions, including the complex stability, albumin binding of metabolites vs. the parent compounds, species-dependent differences, and the possible application of albumin as affinity protein for mycotoxin binding.

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AN *IN SILICO* METHOD TO MINE BIOLOGICAL TARGETS AND BIOTRANSFORMING ENZYMES FOR EMERGING MYCOTOXINS – A CASE STUDY ON FUSARIC ACID

Lorenzo Pedroni, Gianni Galaverna, Chiara Dall'Asta and Luca Dellafiora

Department of Food and Drug, University of Parma, Parma 43124, Italy

This study established a methodology consisting of a computer-driven big data analysis, based on *in silico* target fishing, to address knowledge gaps related to the toxicity and mitigation strategies of emerging mycotoxins. The case study used to illustrate this approach is the mycotoxin fusaric acid (FA). FA is a phytotoxic and cytotoxic secondary metabolite produced by several *Fusarium* fungi, which can be found in a variety of food and feed [1]. Like most emerging mycotoxins, its mechanisms of action and effective mitigation strategies are still shady and should be deeply investigated.

The method presented here is based on the benchmark database for 3D biological macromolecules structures, namely Protein Data Bank (PDB; https://www.rcsb.org/) with more than 200,000 experimentally obtained structures publicly available. We focused on the *co-crystallome*, i.e. the whole structural data of ligands co-crystallized with biological macromolecules found in the PDB. This information was extracted and converted into a fit-for-purpose 3D structural database containing the 3D structures for more than 30,000 ligands (last database access 10th February 2023). Specifically, each ligand has at least one cross-link directed towards the structural information of the macromolecule it was co-crystallized with, which can be used to further model the activity of mycotoxin(s) under analysis. Specifically, a molecular modelling approach involving molecular docking and dynamics simulations allowed studying the geometrical stability of mycotoxin-macromolecule complex over time to predict the biological/toxicological actions of the query mycotoxin. Concerning FA, its 3D structure was used as a bait to fish ligands with a similar structure and consequently identify novel possible biological targets [2]. In fact, the whole procedure relies on the principle that similar compounds may interact with the same biological targets thanks to their chemical similarities. These were quantified using LiSiCA (Ligand Similarity using Clique Algorithm) [3] and the *co-crystallome* hierarchized according to its similarity to FA. The low-molecular weight PDB ligands 46L and 8ZU were identified as the most FA alike.

46L is an inhibitor of the protein kinase-A (PKA), suggesting an activity of FA over PKA as likely. The inhibitory potential was investigated via a molecular modeling approach based on docking simulations and molecular dynamics to study the complex stability over time to estimate the inhibitory activity of query mycotoxins. Regarding 8ZU, it was found as a substrate of a cytochrome p450 (CYP) from *Rhodopseudomonas palustris*, a CYP able to hydroxylate 4-allyl benzoic acids. A proteome-wide search was designed using the *R. palustris* CYP as a bait to mine the proteomes of all the available species to identify CYPs possibly capable of biotransforming FA. The primary sequence of more than 1,000,000 CYPs (https://www.ncbi.nlm.nih.gov/protein/, last database access 10th February 2023) was collected into a database useful for a BLAST analysis [4]. As a result, analogies were found with CYPs belonging to the *Mucor* genus, a species of which (i.e. *M. ruoxii*) is reported as capable of detoxifying FA for plants via hydroxylation [5]. Since the responsible enzyme is unknown, we built a local database containing all the 101,144 available *Mucor* protein sequences

and using the *R. palustris* CYP as query we successfully identified three sequences for further dedicated analysis. To that end, their 3D model structure was obtained via the AlphaFold2 ColabFold v1.5.2 [6] and molecular docking and dynamics analysis were performed to probe their FA hydroxylation capability.

In conclusion, this contribution describes how to set up a computer-driven methodology, easily applicable to other emerging mycotoxins, allowing the identification of a novel targets and enzymes possibly useful for their mitigation.

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INTERACTION BETWEEN FUMONISIN B1 AND DIETARY CONSTITUENTS PALMITIC ACID AND CHOLESTEROL IN HUMAN INTESTINAL CELLS *IN VITRO*

Janice Bergen[1,2], Endre Kiss[2], Doris Marko[2], Franz Berthiller[3], Giorgia Del Favero[1,2]

[1]Department of Food Chemistry and Toxicology, Faculty of Chemistry, University of Vienna, Vienna, Austria
[2]Core Facility Multimodal Imaging, Faculty of Chemistry, University of Vienna, Vienna, Austria
[3]Department of Agrobiotechnology, IFA-Tulln,
University of Natural Resources and Life Sciences, Vienna (BOKU), Austria

Fumonisin B1 (FB1), a mycotoxin mainly produced by *Fusarium verticillioides* and *F. proliferatum*, is found mainly in corn and a variety of grains [1]. On account of feed contamination, hepatotoxic, nephrotoxic, and neurotoxic effects have been observed in animals such as leukoencephalomalacia in horses and pulmonary edema in swine [1]. In humans, consumption of FB1 containing food my cause neural tube defects of newborns and its role to oesophageal cancer is still unclear [1]. Mechanistically, it has been demonstrated that FB1 disturbs the metabolism of sphingolipids due to its structural similarity [1], making it likely that the toxin could affect intestinal cells biomechanical compliance and barrier function related to altered integrity of the cell membrane. Additionally, many questions remain open regarding the toxicity of fumonisins, especially when it comes to potential interactions with matrix macroconstituents derived from dietary intake. Due to the chemical structure, fumonisins may interact through non-covalent binding with foodborne compounds, leading to hidden forms of fumonisins which may be disrupted upon digestion, ultimately releasing the unchanged parent forms of fumonisins in the gastrointestinal tract [2]. Additionally, based on mechanistic considerations, mycotoxins can potentially interact and or compete with dietary lipids, thus contributing to shape cell functions. This was already described for the food contaminant mycotoxin alternariol (AOH), which can antagonize the uptake of water-soluble cholesterol (MßCD-CHOL) in intestinal HT-29 cells [3]. Building on this, we aim evaluating possible interactions between the dietary lipid constituents palmitic acid (PA, 25 µM, 100 µM) and cholesterol (MßCD-CHOL 1 µM, 5 µM) in combination with FB1 (10 µM – 100 µM) comparing the human colon cancer cell line HCT116 to the non-transformed human epithelial intestinal cell line HCEC-1CT. In non-cytotoxic concentrations, first experiments indicated a modulation of cell membrane fluidity in the HCEC-1CT cells upon treatment with the combination of FB1 and MßCD-CHOL, as well as with the combination of FB1 and PA. This infers for potential increase of FB1 biological activity in presence of dietary lipids and opens new perspectives in the evaluation of the risk associated with dietary exposure to this class of compounds.

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INVESTIGATING THE COMBINED TOXICITY OF AFLATOXIN B1 AND FUMONISIN B1 ON HEPG2 USING RESPIROMETRY AND TRANSCRIPTOMIC ANALYSES

Xiangrong Chen, Mohamed F. Abdallah, Charlotte Grootaert, Andreja Rajkovic

Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

Aflatoxin B1 (AFB1) and fumonisin B1 (FB1) are widely (co-)detected in food and known for their hepatotoxicity in humans. Still, their combined toxicity needs to be investigated, especially the impact on mitochondria. In our previous work, we examined the effect of short-term exposure to different doses of AFB1, FB1, and their binary mixture (MIX) on the bioenergetic status of the HepG2 cells, a well-recognized in vitro model system to study liver cell function (Chen et al., 2022). The current work further investigated the (combined) effect of AFB1 and FB1 on the mitochondrial and glycolytic activity of the HepG2 cells using seahorse respirometry analysis and RNA transcriptome sequencing. The results showed that the co-exposure, especially at high doses, is more toxic due to a more inhibition of all parameters of mitochondrial respiration. However, FB1 contributes more to the MIX effects than AFB1. RNA transcriptome sequencing showed that the p53 signaling pathway, a major orchestrator of mitochondrial apoptosis, was differentially expressed. Moreover, the co-exposure has significantly downregulated Cx I, Cx II, Cx III, and Cx IV genes, representing the onset of the suppressed mitochondrial respiration in HepG2 cells.

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P36

EFFECT OF OCHRATOXIN A TREATMENT ON ALPHA-SYNUCLEIN AND CHAPERONE-MEDIATED AUTOPHAGY PROTEINS LEVELS IN NON-DIFFERENTIATED AND DIFFERENTIATED SH-SY5Y CELL LINES

Maria Serrano[1], Elba Beraza[1], Maria Izco[2], Lydia Albarez-Erviti[2], Adela Lopez de Cerain[1,3], Ariane Vettorazzi[1,3]

[1]Department of Pharmacology and Toxicology, Research Grupo MITOX, School of Pharmacy and Nutrition, Universidad de Navarra, Pamplona, Spain
[2]Laboratory of Molecular Neurobiology, Center for Biomedical Research of La Rioja (CIBIR), Logroño, Spain
[3]IdiSNA Institute for Health Research, Pamplona, Spain

Ochratoxin A (OTA) is a mycotoxin that contaminates a great variety of crops. The main concern is its genotoxicity/carcinogenicity. Although poorly explored, OTA has also showed some neurotoxic effects. On the other hand, Parkinson's Disease (PD) is characterized by the presence of Lewy bodies which contain aggregates of alpha-synuclein (α-syn). Some investigation lines have focused their attention on the degradation pathway of this protein the chaperone-mediated autophagy (CMA) pathway, in which two proteins and a miRNA stand out for their key role: the heat shock cognate protein 70 (hsc70), the lysosome associated membrane protein 2 (LAMP-2A) and the miRNA hsa-miR-193a-3p.

Previously, our group demonstrated [1], through *in vitro* studies with a human neuroblastoma cell line overexpressing α-syn (WT-α-syn SH-SY5Y), that exposure to subtoxic doses of OTA (100 and 200 nM) for 72h increases the levels of intracellular α-syn and its half-life, while upregulating the expression of hsa-miR-193a-3p and decreasing the levels of LAMP-2A.

The aim of this study was to achieve a better understanding of the kinetics of the dysregulation of α-syn, LAMP-2A and hsa-miR-193a-3p. For this purpose, both undifferentiated and differentiated WT-α-syn SH-SY5Y cells were treated with OTA 100 and 200 nM for 24, 48 and 72h. Then, the effects of OTA over α-syn, LAMP-2A and heat shock cognate protein 70 (hsc70) gene and protein expression were evaluated via RT-qPCR and Western Blot techniques, respectively. Effects of OTA treatment over miRNA hsa-miR-193a-3p were also studied.

No alterations in hsc70 mRNA nor protein levels were detected in neither of the two cell lines. On the other hand, both LAMP-2A mRNA and protein levels were downregulated over time, while hsa-miR-193a-3p expression was increased over time. For both LAMP-2A and hsa-miR-193a-3p, the effects were more pronounced in the differentiated SH-SY5Y cells compared to the non-differentiated SH-SY5Y cells.

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P37

CYCLOPIAZONIC ACID: A DANGEROUS CHIMERA IN OUR WHITE MOLD CHEESE. SCREENING OF PRODUCTS FROM THE FOOD MARKET WITH LC-MS/MS USING INTERNAL STANDARDS

Georg Häubl

Romer Labs Division Holding GmbH, Tulln, Austria

Chemically, cyclopiazonic acid (CPA) is a chimera between the ergot alkaloids and the *Alternaria* mycotoxin tenuazonic acid [1]. It contains the indole structure as the alkaloid part and the tetramic acid structure, responsible for its acid properties [2]. Its danger lies in its tremorgenic, neurochemical and mutagenic toxicity [3]. It is produced by certain *Penicillium* and *Aspergillus spp.* [4], including two important industrial molds for the production of fermented foods (*Penicillium camemberti* and *Aspergillus oryzae*) [5]. CPA is important as both single mycotoxin and co-contaminant, since many fungi can produce it simultaneous with other mycotoxins [6]; for example, many strains of *A. flavus* produce additionally to the very toxic aflatoxins, CPA as major metabolite also [4]. By consuming contaminated feed, animals can accumulate CPA in their muscles, milk and eggs and humans are exposed to CPA by ingesting these products, as well as by direct consumption of contaminated agricultural products [6]. Especially white mold cheese, like Camenbert, Brie, Coulommiers, which are produced by using *P. camenberti* cultures, can contain CPA [7]. Therefore, it is important to have accurate analytical methods for the detection and quantification of CPA in food and feed.

With an optimized HPLC-MS/MS method, CPA can be detected and quantified in in food and feed samples. To compensate the matrix effect in complex products and guarantee accurate quantification, fully carbon-13-labelled CPA was used as internal standard (IS). Cheese samples were extracted with 0.1% formic acid in acetonitrile. After centrifugation, the supernatant was spiked with the IS and directly injected into the HPLC-MS/MS, without any further clean-up or dilution step.

A validation of the developed method showed for the matrix white mold cheese an LOD of 0.02 ng/mL (0.2 µg/kg) and an LOQ of about 0.05 ng/mL (0.5 µg/kg). The recoveries of spiked cheese samples were close to 90%. In some commercially available white mold cheeses, high amounts of CPA (up to 3.8 mg/kg) could be found.

The ^{13}C-labelled CPA as IS for an HPLC-MS method compensates matrix effects and other fluctuations and is a good tool to get more reliable results. The presented method is applicable for detection of CPA in difficult matrices like white mold cheese and does not need sophisticated clean-up [7].

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PHOTOLYSIS AND PHOTOCATALYSIS OF ZEARALENONE UNDER DIFFERENT TYPES OF IRRADIATIONS IN THE AQUATIC ENVIRONMENT

Sandra Jakšić[1]*, Vesna Despotović[2], Szabolcs Bognár[2], Dušica Jovanović[2], Nenad Popov[1], Tamara Ivetić[3], Daniela Šojić Merkulov[2]

[1]Scientific Veterinary Institute "Novi Sad", Rumenački put 20, 21000 Novi Sad, Serbia
[2]University of Novi Sad Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia
[3]University of Novi Sad Faculty of Sciences, Department of Physics, Trg Dositeja Obradovića 4, 21000 Novi Sad, Serbia

*corresponding author: sandra@niv.ns.ac.rs

With frequent and constant contamination of cereals with mycotoxins, the problem of their presence in the environment becomes of great importance. Due to the leaching of zearalenone from contaminated fields and its toxic effects on human and animal health, it is important to examine the possibility of its removal from water. Advanced oxidation processes initiated by UV or solar irradiation have a high potential for degradation and transformation of hazardous substances from different types of water.

In this study, the degradation efficiency of zearalenone in deionized water was investigated in photolysis and photocatalysis under different irradiation sources. Two different lamps for photolysis were used in this study, and two inexpensive and eco-friendly catalysts were tested in the photodegradation of zearalenone. Namely, ZnO nanoparticles as catalysts were synthesized according to the principles of green chemistry using cheap and non-toxic banana peel extract and tartaric acid. The degradation of zearalenone was monitored by liquid chromatography with fluorescence detection method.

The quality of irradiation type had a significant effect on the kinetics and efficiency of zearalenone degradation in the case of photolysis. Higher efficacy with total degradation was observed by using photoreactor system (xenon lamp, Toption-V, China) during the 60 min, compared to the manually assembled photoreactor (halogen lamp), where only 24% zearalenone degraded after 120 min of irradiation. On the other hand, in the Toption-V photoreactor system using banana peel-based catalyst, as well as tartaric acid photocatalyst, acceptable removal efficiency, i.e. almost 100% of zearalenone degradation was achieved under simulated solar irradiation.

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OCCURRENCE OF DEOXYNIVALENOL (DON) AND ITS MODIFIED FORMS IN CEREALS AND CEREAL PRODUCTS IN GERMANY 2000–2021

Arnold Bahlmann, Stefan Weigel

German Federal Institute for Risk Assessment (BfR), Department for Safety in the Food Chain, Max-Dohrn-Str. 8-10, 10589 Berlin, Germany

Deoxynivalenol (DON) is one of the most frequently detected mycotoxins in cereals and cereal products. Several modified forms have been detected as well, among which 3-acetyl-DON (3-ac-DON), 15-acetyl-DON (15-ac-DON) and DON-3-glucoside (DON-3-G) are considered the most relevant ones. While DON is regulated in the European Union in food and feed, the modified forms are not addressed in current legislation.

In 2017, the European Food Safety Authority (EFSA) assessed the risk to animal and human health related to DON and these three modified forms in food and feed. Since the modified forms can be transformed to the parent compound after ingestion, the same toxicity was assumed. Accordingly, EFSA extended the tolerable daily intake (TDI) value for DON and established a group-TDI for DON, 3-ac-DON, 15-ac-DON and DON-3-G.

In the present study, data gathered by German official control laboratories within the German National Monitoring Programme from 2000-2021 were evaluated. Data on the occurrence of DON was available for more than 27000 cereals and cereal products intended for human consumption. For more than 7300 of these samples, the level of one or both of the acetylated forms was reported, while only 194 results were available for DON-3-G. A quantified level of DON was reported for 45% of all samples. For more than 95% of the samples, all three forms were below the limit of detection (LOD) or quantification (LOQ).

To assess the co-occurrence of DON and its three modified forms, the ratios of the modified forms to the parent compound was calculated for all samples. A modified medium bound approach was conceived to take into account the high percentage of left censored data: If the modified form was reported to be below LOD or LOQ, the level of the modified form was assumed as 50% of LOQ (for results below LOQ) or 50% of LOD (for results below LOD). Furthermore, samples were only considered, if the level of DON in the same sample was quantified to be at least 10-20 times as high as the LOQ of the corresponding modified form. This approach was found to yield sufficient numbers for statistical analysis, while maintaining quality of data allowing to draw meaningful conclusions.

In summary, the study shows that the acetylated forms of DON are present at low to negligible levels relative to DON in most types of cereals and cereal products. Exceptions are 3-ac-DON in oats and 15-ac-DON in maize which were found at 5-10 fold higher median relative levels compared to all other types of cereals. The median ratio of DON-3-G to DON was the highest median ratio for all modified forms investigated. No conclusions regarding the distribution of DON-3-G in different types of cereals could be drawn.

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PLANT EXTRACTS AS BIOCONTROL AGENTS AGAINST THE GROWTH OF *ASPERGILLUS CARBONARIUS* AND OCHRATOXIN A PRODUCTION IN GRAPES

Wiem Chtioui[1,2,3], Sandrina Heleno[2,3], Quirico Migheli[1,4], Paula Rodrigues[2,3]*

[1]Dipartimento di Agraria, Università degli Studi di Sassari, Sassari, Italy
[2]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal
[3]Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Bragança, Portugal
[4]Nucleo di Ricerca sulla Desertificazione, Università degli Studi di Sassari, Sassari, Italy

Natural plant extracts can provide an alternative to synthetic fungicides to prevent infection of grapes by *Aspergillus carbonarius* (Bainier) Thom. and reduce ochratoxin A (OTA) contamination. The present study investigates the antifungal and anti-mycotoxigenic properties of aqueous extracts of chestnut flower, cistus, eucalyptus, and orange peel at 10 mg/mL. Cistus and orange peel extracts appeared promising for inhibiting *Aspergillus* vegetative growth. All extracts were effective against OTA after ten days of grape storage, with inhibition ratios from 7.6% to 82.3%. Eucalyptus extract was particularly efficient in inhibiting OTA production by up to 80% but had no effect on fungal growth. Thus, it maintained the natural microbial balance in grapes. This study suggests that plant extracts may be useful sources of bioactive chemicals for preventing grape contamination and OTA production. However, further work is required to evaluate their organoleptic effects on grape before these extracts can be used in practical applications.

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SURVEY ON OCHRATOXIN A OCCURRENCE IN FERMENTED MEAT PRODUCTS

Marta Sopel, Hester van den Top, Monique de Nijs

Wageningen Food Safety Research, Akkermaalsbos 2, 6708WB Wageningen, The Netherlands

The mycotoxin ochratoxin A (OTA) is produced during the field period of cereals by the fungi Aspergillus and Penicillium. It can also be produced during the transport and storage, for example if the product is kept in too moist conditions, so the moulds producing ochratoxin A can grow. OTA is soluble in fat and has a high bioavailability and long half-life. When OTA is present in animal feed it can be transferred and accumulate in organs, meat, muscle tissue and blood of farm animals [1]. Fermented products such as dry meat can therefore contain OTA. Additionally, fermented dry meat products can be contaminated with fungi during ripening and storage, leading to more OTA production. The major OTA producers from the production and storage environment are A. alliaceus, A. carbonarius, A. ochraceus, A. steynii, A. westerdijkiae, P. nordicum and P. verrucosum. The fungus P. nordicum is a high OTA producer and is isolated mainly from particular meats such as ham and salami [2]. The European Food Safety Authority (EFSA) published an opinion in 2020 in which it is concluded that OTA can be carcinogenic to kidney in humans and the exposure of the European population to OTA is higher than previously assumed, whereby a significant contribution of OTA from processed meats cannot be ignored [3]. EFSA, however, highlighted the unequal data contribution from individual Member States, thus the exposure derived from cured meat could be overestimated. More data would enable to make better decisions on possible new legislation regarding the presence of OTA in meat products.

To estimate the occurrence of OTA in fermented meats, a straightforward method was developed and validated. Ochratoxin A is extracted from the sample with methanol and the extracts are cleaned on IAC columns dedicated to OTA and analyzed by LC-MS/MS. Quantification is carried out by external calibration of standards in solvent after normalization of the response to the isotopically labelled internal standard 13C20 OTA. The LOQ of the method is 0.2 ug/kg.

During validation, dry ham was found to be regularly contaminated with OTA. In 2023, the method will be used for a survey into the OTA contamination of 40 dry meat products available form Netherlands supermarkets. The results will be presented at the 44th Mycotoxin Workshop.

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INVESTIGATING ON STRESSOR'S EFFECT: POTENTIAL GLYPHOSATE APPLICATION AND SOIL PROCESSING INFLUENCE ON DEOXYNIVALENOL MYCOTOXIN PRESENCE AND ITS BIOTRANSFORMATION IN WHEAT KERNELS

Federico Ferrari[1], Laura Righetti[1,2,3], Massimo Blandino[4], Chiara Dall'Asta[1]

[1]Food and Drug Department, University of Parma, Viale delle Scienze 17/A, Parma 43124, Italy
[2]Laboratory of Organic Chemistry, Wageningen University, 6708 WE Wageningen, The Netherlands
[3]Wageningen Food Safety Research, Wageningen University & Research, P.O. Box 230, 6700 AE Wageningen, The Netherlands
[4]Department of Agricultural Forest and Food Sciences, University of Turin, Largo Paolo Braccini 2, 10095 Grugliasco, Italy

From FAO's latest forecast for world cereals production from December 2022 [1], wheat supply has undergone an increase over the last three years, conditioned by pandemic and political events that occurred, insisting on its enormous importance and interest in the human diet as an easily available commodity. Meantime, environmental and climatic changes are inducing a decrease in the global mean yield of wheat reaching 2030 [2], from -3% to 10% per degree of warming; thus, guaranteeing a safe and secure offer in the next years becomes a duty to cover. Knowledge and improved awareness of the xenobiotic effects on mycotoxins, which presence, strictly associated with climate change, threatens our next future food commodities, is a huge step to further understanding how they can be controlled and mitigated.

In this study deoxynivalenol (DON) mycotoxin has been evaluated in kernels according to a manmade stressor, glyphosate, and different soil conditions [3]. Open field experiments were conducted on wheat plants belonging to Aubusson (*T. aestivum* spp. *aestivum*) cultivar, grown in Carmagnola (IT), applying glyphosate in pre-sowing and during the caryopsis dough stage [4]; this design aimed to simulate opposite treatment between, respectively, European and northern American farmers. Moreover, the conversion of DON to its first metabolite deoxynivalenol-3-glucoside (D3G) was assessed to estimate the degree of biotransformation and plant reaction to abiotic stressors according to minimally processed soil and plowed ones.

Our introductory results, pondering the great variability that a real experimental field has, disclose a significant difference in glyphosate residues between the above-mentioned approaches meanwhile target mycotoxin presence and its conversion ratio to D3G appear to be mostly driven by soil processing where plants were cultivated.

Therefore, further investigation should be carried out in a wider view of crops as organisms subjected to multiple chemicals which can have synergistic effects or whose effect will benefit plant defenses against fungal infections and proliferation.

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FUNGAL FLORA AND MYCOTOXINS IN DRY CAT FOOD

Jurgita Jovaišienė, Ligija Andrijauskaitė, Gediminas Gerulis, Gintarė Vaičiulienė, Rimvydas Falkauskas, Violeta Baliukonienė

Lithuanian University of Health Sciences, Kaunas, Lithuania

Dry cat or other pet foods are the main types of pet food on the markets. One of the most common ingredients of dry food are cereals – vectors of harmful fungi (molds) and mycotoxins posing the risk to pet health [1]. The aim of the study was investigate dry cat food and determinate hygienic quality and contamination by mycotoxins. In the current study, 25 cat dry food samples randomly purches from the markets were investigated. Purchased cat food contain food materials of plant origin (cereals, rice, maize, peas, potatoes, beet pulp) in food plant origin varied from 20% – till 80% in total composition of food. As an indicator of poor hygiene and a potential microbiological contamination were calculated Total Yeast and Mold count (The method specified in ISO 21527-2:2008). Morphological method using light microscopy was applied to indentify molds genera in cat dry food. AFB1, ZEN, DON, OTA, T-2 were performed using TLC.

Total Yeast and Mold count in the analyzed dry cat food ranged from 1,9 to above 2,7 logCFU/kg. The highest Total Yeast and Mold count were determined in cat food with contain 30% food plant origin.

Seven genera of fungi were identified: *Penicillium* spp. (42%), *Aspergillus* spp. (28%), *Rhizopus* spp. (10%), *Cladosporium* spp. (8%), *Rhizopus* spp. (10%), *Fusarium* spp. (5%), *Verticillium* spp. (3%).

The following ranges concentration of mycotoxins were detected: AFB1 (1-1,8 µg/kg), ZEN (30-166 µg/kg), DON (50-500 µg/kg), OTA (1-6 µg/kg), T-2 (50-90 µg/kg). In two samples mycotoxins were not detected. In 2016, the European Commission issued a recommendation regarding the permitted levels of mycotoxins in animal feed products. The guidance values of mycotoxins ZEN, OTA and T-2 in cat feed with a moisture content of 12% were respectively: 200 µg/kg; 10 µg/kg and 50 µg/kg. AFB1 and ZEN values are not specified for cat food.

Conclusion. Despite the plant-based content and various plants materials in dry cat food, there is a risk of mold and toxin contamination that should be tested.

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POTENTIAL ROLE OF ALTERNATIVE HOST PLANTS TO MYCOTOXINS CONTAMINATION IN SPRING WHEAT

Sigita Janavičienė, Skaidrė Supronienė

Lithuanian Research Centre for Agriculture and Forestry, Akademija, Lithuania

*corresponding author: sigita.janaviciene@lammc.lt

Fusarium graminearum is an important pathogen that causes Fusarium head blight (FHB) in several cereal crops worldwide. The ability of this pathogen to contaminate cereal grain with trichothecene mycotoxins poses a risk to human and animal health. This study aimed to evaluate the potential role of *F. graminearum* isolated from an alternative host plant in the production of mycotoxins under different incubation conditions of spring wheat grain. Twelve *F. graminearum* strains were isolated from six alternative host plants (*Fallopia convolvulus* (L.) Löve, *Viola arvensis* Murray, *Brassica napus* L., *Euphorbia helioscopia* L., *Tripleurospermum inodorum* (L.) Sch., *Poa annua* L.) and two from the primary host plant spring wheat (*Triticum aestivum*). These strains were cultivated on spring wheat grains at 25 °C and 29 °C for 5 weeks. The mycotoxins produced were analysed with a high-performance liquid chromatograph (HPLC) coupled to a Thermo Scientific TSQ Quantiva MS/MS detector.

The obtained results showed that all strains of *F. graminearum* were found to grow mycelium on spring wheat grains and to produce particularly high levels of mycotoxins in the grains. The spring wheat grains were inoculated with *F. graminearum* strains that produced nivalenol (NIV), deoxynivalenol (DON), fusarenon X (FUS-X), 3-acetyl-deoxynivalenol (3-ADON), deoxynivalenol-3-ß-D-glucoside (D3G), 15-acetyl-deoxynivalenol (15-ADON), and zearalenone (ZEA). Regarding the strain-dependent potential for mycotoxin production, we found that all strains were able to produce DON and ZEA in spring wheat grains incubated at 25 °C. Slightly different results were obtained when spring wheat grains were incubated at 29 °C. This temperature was less favourable for producing particularly high concentrations of mycotoxins in spring wheat. *F. graminearum* strains produced DON and ZEA under both temperatures, with the mean concentrations varying from 363 to 112,379 µg kg^{-1} and from 1452 to 44,816 µg kg^{-1}, respectively. 15-ADON was detected in between 57% and 95% of the samples, and 3-ADON was detected in between 67% and 86% of the samples, depending on the incubation temperature. 15-ADON and 3-ADON were produced in lower amounts, and the mean concentrations varied from < 50 to 22,246 µg kg^{-1} and from <50 to 28,800 µg kg^{-1}, respectively. NIV was detected from 14 % to 43 %, D3G from 0 % to 43 % of samples depending on storage temperature. NIV and D3G were detected at very low concentrations or not detected at all. It is important, that FUS-X was detected from 28 % to 100 % of samples, and the concentrations varied from <10 to 220 µg kg^{-1}.

Our results indicated the possible role of dicotyledonous plants, including weeds, as a reservoir of inoculum sources of *F. graminearum*-induced Fusarium head blight, associated with the risk of mycotoxin contamination in spring wheat.

P45

IN VITRO DETOXIFICATION OF MYCOTOXINS CONCENTRATIONS

Violeta Baliukoniene[1]*, Gintare Vaiciuliene[1], Rimante Kacerauskaite[1], Jurgita Jovaisiene[1], Algimantas Paskevicius[2], Rimvydas Falkauskas[1], Gediminas Gerulis[1]

[1]Lithuanian University of Health Sciences, Lithuania
[2]Nature Research Centre, Vilnius University, Lithuania

Mycotoxins are a class of low molecule secondary metabolites which is mostly produced under specific environmental conditions by filamentous fungi within the genera *Aspergillus*, *Fusarium* and *Penicillium*. They are the most toxic mycotoxins and can cause the various toxigenic effects to dairy cows' organism, thereby causing acute or chronic poisoning of the body. Ruminants are generally considered to be less susceptible to the effects of mycotoxins than monogastric animals as the rumen microbiota are capable of detoxifying some of these toxins. Despite this potential degradation, mycotoxin-associated subclinical health problems are seen in dairy cows [1]. However, in high-productive dairy cows, mycotoxin-associated subclinical health problems may occur, reflected by vague and non-specific symptoms and periodic decrease in milk production [2]. Various approaches have been identified to reduce or prevent the adverse effects of mycotoxins on animal health and production. The most promising approach to decontaminate feed is the biological detoxification, the so-called biotransformation. The use of biological method to detoxify mycotoxins is based on elimination of the toxin (adsorption), elimination of the toxicity (biotransformation) and elimination of toxin-related effects.

The aim of the present study was ti determine the detoxification of mycotoxins (aflatoxin B1 (AFB1), zearalenone (ZEA), deoxynivalenol (DON), T-2 toxin) by feed additive with live yeast of *Saccharomyces cerevisiae* and yeast *Rhodotorula rubra* in a static gastrointestinal model *in vitro*.

In vitro a static gastrointestinal model with the presence of feed was established as screening tool to test the efficacy of a feed additive with live yeast of *Saccharomyces cerevisiae* (1.5×10^{10} CFU/g) and yeast *Rhodotorula rubra* (4×10^{7} cells/mL) claiming to reduce mycotoxins: AFB1 – 8.5 ± 0.85 µg/kg, ZEA – 500 ± 73.2 µg/kg, DON 791.5 ± 80.36 µg/kg and T-2 toxin 207.6 ± 31.98 µg/kg in samples of total mixed ration.

Feed samples with feed additive (1 kg / 0.15 g) or with yeast *Rhodotorula rubra* suspension (1 kg / 50 µL) were mixed with gastric simulation solution (pH 3) in an incubation flask. All samples were incubated in triplicate at 39 °C in a shaking incubator (150 rpm). After 0 h, 3 h, 6 h, 12 h samples were used for AFB1, ZEA, DON and T-2 toxin determination by HPLC.

After 12 h AFB1, DON and T-2 toxin were most effectively reduced by feed additive, respectively 47.38%, 41.17% and 75.91% ($p>0.05$). The lowest effect was determined to ZEA (20%) ($p>0.05$). *Rhodotorula rubra* were able to reduce mycotoxins (AFB1, ZEA, DON, T-2 toxin) concentrations in total mixed ration after 12 h, respectively 76.47%, 6%, 47.38%, 51.83% ($p>0.05$).

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P46

DETERMINATION OF MYCOBIOME ON ONIONS OF DIFFERENT CULTIVARS AT HARVEST TIME AND SPECIES ABUNDANCE SHIFT IN A STORAGE TRIAL WITH DIFFERENT DRYING METHODS

Karla Hell, Heidi Brandel, Nicole Mischke, Christoph Weinert, Markus Schmidt-Heydt*

Max Rubner – Institut, Federal Research Institute of Nutrition and Food,
Department of Safety and Quality of Fruit and Vegetables, Karlsruhe, Germany

*corresponding author: markus.schmidt-heydt@mri.bund.de

Filamentous fungi occur worldwide as food contaminants and can have serious health effects on humans and animals, since they can spoil food not only by decomposition but pose a serious risk through the formation of toxic secondary metabolites and their pathogenic and allergenic potential. The production of secondary metabolites such as mycotoxins depends on the fungal species and on environmental conditions such as temperature, pH, relative humidity, light, etc., but also the exposure to stress conditions. Poor storage conditions can therefore promote the growth of fungi and reduce the self-life of the product. Hence, it is important to monitor and optimize storage conditions.

Different onion varieties (old land varieties and hybrid varieties) have been analyzed for the development of fungal infestation in a storage trial lasting for up to 9 months under different storage conditions and for different drying methods. For this, organically grown onions harvested from a field trial have been separated into “outer skin”, “leaf base”, “root area” and “inner tissue” and samples of the individual tissues have been prepared for dilution series, to evaluate the extent of fungal infestation (colony forming units/CFU).

Detailed investigations by cultivation and subsequently identification of pure fungal cultures by ITS-/ housekeeping gene-sequencing revealed, that the foliage base, the root area and the outer skin of the onions in particular exhibit a high level of mycological diversity. In particular, fungi of the genera Fusarium, Aspergillus and Penicillium, and occasionally Alternaria, all described as species that are able to produce mycotoxins, and Rhizopus, occur here with great abundance. From a food safety point of view, it should be mentioned that a different “susceptibility” of the different onion varieties to fungal infestation, e.g. due to differences in sugar content and other factors, is clearly evident. As expected, the type of drying method (natural drying vs. condensation drying) also has an influence on shelf-life during storage. F. oxysporum (mycotoxin producer), A. niger (mycotoxin producer) and P. brasilianum, as well as P. glabrum are described in the literature as typical pathogens on onions and showed a change in abundance during storage. The significance of these findings for cultivation and storage, as well as the relevance for the consumer with regard to food safety, are important aspects discussed in the further course of the project (Chang, Ho et al. 2002, Merget, Sander et al. 2008, Irma, Shades et al. 2020). In some cases, mite infestation of the onions could also be documented, with an additional influence on, e.g. the migration of fungal spores into the interior of the onion, tissue damage due to feeding marks and the like.

The work described here is part of the ZwiebÖL project, which is being funded by the Federal Ministry of Food and Agriculture (BLE) under the reference/grant number 2819OE019.

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P47

TRICHODERMA AFROHARZIANUM ISOLATES SHOW HIGH INTRASPECIFIC VARIABILITY WITH RESPECT TO THEIR EFFICIENCY AS BIOCONTROL ORGANISMS

Christian Roder, Markus Schmidt-Heydt

Max Rubner – Institut, Federal Research Institute of Nutrition and Food,
Department of Safety and Quality of Fruit and Vegetables, Karlsruhe, Germany

corresponding author: markus.schmidt-heydt@mri.bund.de

Filamentous fungi are important food contaminants and can have serious effects on human and animal health through the formation of harmful mycotoxins and their pathogenic and allergenic potential. As part of the multidisciplinary project "AflaZ" with German and Kenyan research institutions, the potential use of Trichoderma afroharzianum MRI349 (TA349) (Braun, Woitsch et al. 2018, Landeis and Schmidt-Heydt 2021) and other isolates of the same species have been investigated on laboratory level as a natural biocontrol agent against Aspergillus flavus (AF) and Fusarium verticilloides (FV), two mycotoxigenic fungi relevant as contaminants on maize in Kenya.

TA349 was tested in different concentration mixtures in competition experiments and is able to effectively inhibit the growth of both fungi at a ratio of 10:90 for TA349 vs. AF and 60:40 for TA349 vs. FV, respectively. This was demonstrated phenotypically for TA349 vs. AF and by absolute DNA quantification using a targeted droplet digital PCR (ddPCR) approach. Furthermore, mycotoxin formation decreased significantly in both mycotoxigenic strains when grown in competition with TA349.

T. afroharzianum isolates investigated comparatively to TA349 in competition experiments showed variable efficacies in suppressing the growth of AF and FV. Interestingly, TLC fingerprint analyses of the different isolates showed a drastic change in secondary metabolite profiles when the fungi grew in competition. In particular, strain TA349 produced an unknown metabolite in excess under these conditions. Identity of this metabolite and its possible involvement in the competition mechanism are currently under investigation.

The present results confirm that TA349 is a promising biocontrol species for reducing the growth and mycotoxin production of A. flavus and F. verticilloides.

The work described here is part of the project AflaZ, which is being funded by the Federal Ministry of Food and Agriculture (BLE) under the reference AflaZ 2816PROC11.

P48

ALTERNARIA TOXINS IN WHEAT SAMPLES FROM EXPERIMENTAL FIELDS

Giuseppina Scialò[1], Merima Jasarevich[2], Barbara De Santis[1], Gabriele Chilosi[2], Arianna Palchetti[3], Luca D'Ambrosio[3], Francesca Debegnach[1]

[1]Department of Food Safety, Nutrition and Veterinary Public Health, Italian National Institute of Health, Rome, Italy
[2]Department of Innovation in Biology, Agri-food and Forest systems (DIBAF), University of Tuscia, Viterbo, Italy
[3]Food Analysis and Product Safety Laboratory, Environmental and Climate Protection Agency of the Autonomous Province of Bolzano, Bolzano, Italy

Alternaria toxins are secondary metabolites produced by fungi that can contaminate cereals, oilseeds, fruits, and vegetables. This research aimed to investigate the occurrence of Alternaria toxins alternariol (AOH), alternariol monomethyl (AME), and tenuazonic acid (TeA) in wheat samples from experimental fields trial followed along three harvesting years 2020, 2021, and 2022. Three different tillage techniques (*plowing*, *spading* and *ripping)* were combined with two different fertilizing procedures (mineral and organic by compost). Each year, the six experimental fields were conducted in triplicate, and a grand total of 54 wheat samples were collected and analysed. The analyses were performed by SPE clean-up and LC-MS/MS determination.

Over the 3 years considered, the obtained results confirm the contamination trend of AOH<AME<TeA. No difference was highlighted comparing mineral and organic fertilization procedures. No general trends were highlighted when the different treatments applied to the 3 soils are considered. However, high variability among the 3 replicates was observed, suggesting a heterogeneous distribution for the investigated mycotoxins. The average contamination levels were comparable in 2020 and 2021 for the 3 toxins while, during 2022 a significant decrease of contamination levels was observed. No indicative levels are reported for wheat in the Commission Recommendation (EU) 2022/553, but values are suggested for AOH, AME and TeA in cereal-based foods for infants and young children (2, 2 and 500 µg/kg for AOH, AME and TeA respectively). Mean values calculated for years 2020 and 2021 (4, 3 and 112 µg/kg for AOH, AME and TeA, respectively) are comparable with the indicative levels, however, taking into account the low level usually set for baby food category, this seems to raise a controlled contamination issue. However, due to scarce toxicity data available for Alternaria toxins and to suspected genotoxicity of AOH and AME, particular attention should be paid to the presence of these toxins in wheat as it is an essential source for cereal-based baby food products.

P49

EVALUATION OF STATISTICAL TREATMENT OF LEFT-CENSORED CONTAMINATION DATA: EXAMPLE ON DEOXYNIVALENOL OCCURRENCE IN PASTA AND PASTA SUBSTITUTE PRODUCTS

Alessandro Feraldi[1], Barbara De Santis[2], Marco Finocchietti[1], Francesca Debegnach[2], Antonio Mandile[1,2] and Marco Alfò[1]

[1]Department of Statistical Science, University of Rome "La Sapienza", Rome, Italy
[2]Department of Food Safety, Nutrition and Veterinary Public Health, Italian National Institute of Health, Rome, Italy

The handling of data on food contamination frequently represents a challenge because they are often left censored, i.e., they are composed by both positive and non-detected values; the latter observations are not quantified and provide only the information that they are below a lab-specific threshold value.

Besides deterministic approaches, that simplify the treatment by substituting non-detected values by fixed threshold or null values, there has been a growing interest in the application of stochastic approaches to the treatment of not quantified values. In this study, a Multiple Imputation method is applied to analyse contamination data on deoxynivalenol (DON), a mycotoxin that may be present in pasta and pasta substitute products.

An application of the proposed method to left-censored DON occurrence data is provided and the results are compared to those attained by using deterministic (substitution methods) methodologies. In this context, the stochastic approach seems to provide a more accurate, unbiased and realistic solution to the problem of left-censored occurrence data, with DON contamination mean values of the food considered equal to 139.4 µg/kg with a 95% confidence interval ranging from 137.0 to 141.9 µg/kg, in which lower and upper bound values of the substitution methods are included (*128.1 µg/kg and 160.8 µg/kg r*espectively).

The obtained sample of occurrence values may represent a suitable dataset to be used in the assessment of dietary exposure scenarios of the general population.

P50

COMPARISON OF DETECTION METHODS FOR DETERMINATION OF MYCOTOXINS IN CANNABIS AND CANNABIS DERIVED PRODUCTS

Juliane Kramer, Giorgia Greco, Diana Rohrbeck

KNAUER Wissenschaftliche Geräte GmbH, Hegauer Weg 38, 14163 Berlin (Germany)

Due to its potential healthcare benefits of cannabis and hemp, the market for this plant and its products have increased dramatically in the last years. In several states cannabis is already legal for medicinal and/or recreational use. Quality control before human consumption e.g., the determination of pesticide residues and mycotoxins in cannabis biomass and its derived products, is therefore mandatory. The number of regulated pesticides varies dependent on state/country/region. The U.S. Food & Drug Administration (FDA) sets a limit value of 20 ppb for mycotoxins in human food and animal feed [1]. The same values are set by Commission Regulation (EC) No 1881/2006 [2]. Organizations like AOAC are developing method requirements for mycotoxin determination in cannabis matrices. AOAC SMPR® 2021.010 defines aflatoxins B1/B2, aflatoxins G1/G2 and ochratoxin A as analytes of interests and specifies limits of quantification and qualification for cannabis biomass and cannabis derived products [3].

The most chosen detector for mycotoxin determination is the mass spectrometer (MS). The regulations are met easily but due to the complexity of an LC-MS system the operation can be challenging. Therefore, a systematic evaluation of other, less common detection methods is carried out. The same analytical method is applied with fluorescence detection (FLD), electrochemical detection (ECD), evaporative light scattering detection (ELSD) and MS. The results are compared related to the achievement of valid limit values and regarding to consumption of solvents, energy and handling/user friendliness. Cannabis analysis also includes sample preparation. Cannabis flowers and cannabis derived products (e.g. cannabis oil) are processed with different sample preparation protocols. Results are investigated and evaluated in terms of time, costs per sample and suitability for the used detectors.

[1] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-action-levels-poisonous-or-deleterious-substances-human-food-and-animal-feed#afla, 13/01/2023.

[2] Commission Regulation (EC) No 1881/2006 (consolidated text 01-07-2022), Setting maximum levels for certain contaminants in foodstuffs.

[3] AOAC SMPR® 2021.010, Standard Method Performance Requirements (SMPRs®) for Quantitative Analysis of Mycotoxins in Cannabis Biomass and Cannabis-Derived Products.

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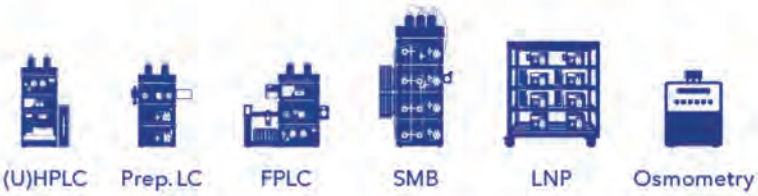

Comparison of detection methods for determination of mycotoxins in cannabis and cannabis derived products

Juliane Kramer, Diana Rohrbeck, Giorgia Greco
KNAUER Wissenschaftliche Geräte GmbH, Hegauer Weg 38, 14163 Berlin (Germany), www.knauer.net

Due to its potential healthcare benefits of cannabis and hemp, the market for this plant and its products have increased dramatically in the last years. In several states cannabis is already legal for medicinal and/or recreational use. Quality control before human consumption e.g., the determination of pesticide residues and mycotoxins in cannabis biomass and its derived products, is therefore mandatory. The number of regulated pesticides varies dependent on state/country/region. The U.S. Food & Drug Administration (FDA) sets a limit value of 20 ppb for mycotoxins in human food and animal feed [1]. The same values are set by Commission Regulation (EC) No 1881/2006 [2]. Organizations like AOAC are developing method requirements for mycotoxin determination in cannabis matrices. AOAC SMPR® 2021.010 defines aflatoxins B1/B2, aflatoxins G1/G2 and ochratoxin A as analytes of interests and specifies limits of quantification and qualification for cannabis biomass and cannabis derived products [3].

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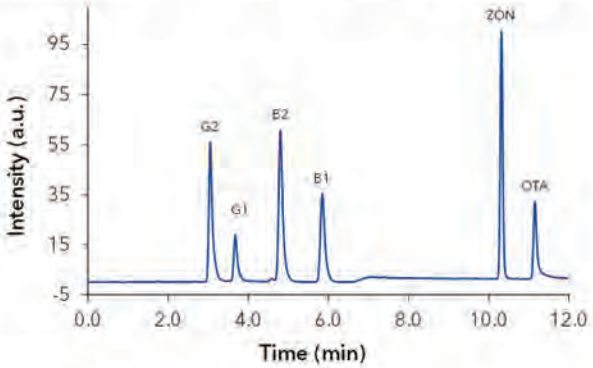

[1] Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed; https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-action-levels-poisonous-or-deleterious-substances-human-food-and-animal-feed#afla, 13/01/2023
[2] Commission Regulation (EC) No 1881/2006 (consolidated text 01-07-2022), Setting maximum levels for certain contaminants in foodstuffs
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