38th Mycotoxin Workshop
Berlin, 02 - 04 May 2016

Conference Abstracts

© visitBerlin, Foto: Wolfgang Scholvien

Your competent partner and consultant for food and feed testing

Fast and Reliable Detection of Residues & Contaminants

Our Service

- Accurate results on time
- Labelling control and advice
- Risk monitoring & sampling plans
- Sampling and on-site analysis with rapidust®
- Sample registration, tracking and test reports online (EOL)
- Accreditation according to DIN EN ISO/IEC 17025:2005

Our Competence Centres

- Mycotoxins
- Biotoxins
- Pesticides
- Metals & Elements
- Organic contaminants
- Dioxins & PCBs
- Veterinary drug residues

service@eurofins.de
www.eurofins.de

eurofins

MANAGING FOOD SAFETY TOGETHER.

38th Mycotoxin Workshop

Conference Abstracts

May 2 – 4, 2016

Berlin, Germany

Gesellschaft
für
Mykotoxinforschung e. V.
Society
for
Mycotoxin Research

Scientific Committee

The Society for Mycotoxin Research (Board of Directors)

Prof. Dr. Dr. habil. Manfred Gareis (President)
Chair of Food Safety, Ludwig-Maximilians-Universität, München

Prof. Dr. Petr Karlovsky (Vice President)
Molecular Phytopathology and Mycotoxin Research, Georg-August-University, Göttingen

Dr. Karsten Meyer (Secretary)
Institute of Animal Hygiene, TU München, Freising

Dr. Christoph Gottschalk (Treasurer)
Chair of Food Safety, Ludwig-Maximilians-Universität, München

Organizing Committee

Barbara Biermaier
Christoph Gottschalk
Florian Kaltner
Michaela Scheer
Sebastian Ulrich

Web support

Sebastian Ulrich
Helmut Wöhrle

Very special thanks to our sponsors:

Table of contents

Program..... 5

Sunday, May 1st 2016..... 5

Monday, May 2nd 2016..... 5

Tuesday, May 3rd 2016..... 7

Wednesday, May 4th 2016..... 8

Tables of presentations

Table of lectures..... 13

Table of posters..... 16

Abstracts of lectures..... 25

Abstracts of posters..... 65

List of authors..... 159

List of participants..... 169

Program

Conference venue: Federal Institute for Risk Assessment (BfR)

Location Marienfelde, Diedersdorfer Weg 1, 12277 Berlin

Sunday, May 1st 2016

19:00 Get together

Seminaris Hotel Berlin, Takusstr. 39, 14195 Berlin

Monday, May 2nd 2016

08:00 Registration

09:00 Welcome

Session 1: **Analytics (Martin-Lerche lecture hall)**

09:15
L1
Practical evaluation of screening test kits according to Regulation (EU) No 519/2014
Elena Cubero Leon, Katrien Bouten, Carsten Mischke, Jörg Stroka

09:30
L2
Development of a multiplex lateral flow immunoassay with quantum dots and gold nanoparticles as label for the detection of four mycotoxins
Astrid Foubert, Natalia Beloglazova, Andreja Rajkovic, Benedikt Sas, Annemieke Madder, Sarah de Saeger

09:45
L3
Electrochemistry coupled with mass spectrometry - a versatile tool to investigate metabolic processes of mycotoxins
Julia Keller, Hajo Haase, Matthias Koch

10:00
L4
MALDI Imaging mass spectrometry of mycotoxins in food
Sebastian Hickert, Benedikt Cramer, Matthias Letzel, Hans-Ulrich Humpf

10:15
L5
Identification of *Stachybotrys* spp. by MALDI-TOF MS
Sebastian Ulrich, Barbara Biermaier, Karin Schwaiger, Oliver Bader, Georg Wolf, Reinhard Straubinger, Manfred Gareis, Christoph Gottschalk

10:30 – 11:15 **Coffee break/Exhibition (Foyer)**

Session 2: **Co-occurrence and Co-exposure (Martin-Lerche lecture hall)**

11:15
L6
Co-occurrence and co-exposure in the risk assessment of mycotoxins – approaches discussed in EFSA
Lutz Edler

11:45
L7
Synergistic estrogenic effects of *Fusarium* and *Alternaria* mycotoxins
Katharina Vejdovszky, Kathrin Hahn, Benedikt Warth, Doris Marko

12:15 **L8**	Co-occurrence of aflatoxins, ochratoxin A and citrinin in "egusi" melon (*Colocynthis citrullus* L.) seeds consumed in Ireland and the United Kingdom Yinka Somorin, Adeyemi Akinyemi, Terenzio Bertuzzi, Amedeo Pietri
12:30 – 13:30	**Lunch (Foyer)**
13:00 – 14:00	**Poster Session/Exhibition (Foyer/Room D146)**
Session 3:	**Occurrence/Degradation/Detoxification (Martin-Lerche lecture hall)**
14:00 **L9**	Occurrence of mycotoxins (DON, ZEA) in feeding stuff from farms in the German state of Brandenburg Ulrike Korn
14:15 **L10**	Mycotoxin production of moulds on bread and cheese under household conditions Sandra Wolf, Madeleine Gross, Ewald Usleber
14:30 **L11**	Distribution of *Fusarium* mycotoxins and their respective modified analogues in naturally contaminated oats: effect of primary processing Lada Ivanova, Stefan Sahlstrøm, Ida Rud, Silvio Uhlig, Christiane Fæste, Anastasia Hole, Hege Divon
14:45 **L12**	*Alternaria* toxin production – from laboratory study to field and market analyses Theresa Zwickel, Horst Klaffke, Marina Müller, Michael Rychlik
15:00 **L13**	Deoxynivalenol sulfates: detoxification metabolites? Giorgia Del Favero, Lydia Wölflingseder, Gudrun Pahlke, Benedikt Warth, Philipp Fruhmann, Rudolf Krska, Rainer Schuhmacher, Gerlinde Wiesenberger, Gerhard Adam, Doris Marko
15:15 **L14**	Degradation of enniatins by *Clonostachys rosea*, *Acremonium strictum* and *Bacillus licheniformis* Rosine Ghislaine Suchfort, Petr Karlovsky
15:30 – 16:30	**Coffee break/Exhibition (Foyer)**
16:00 – 17:00	**Meeting of the members of the Society for Mycotoxin Research (Martin-Lerche lecture hall)**
16:00 – 17:00	**Poster Session/Exhibition (Foyer/Room D146)**
17:00	**Draught beer, wine and soft drinks (Foyer/Garden)**
18:00 – 23:00	**Barbecue**

Tuesday, May 3rd 2016

8:00	Registration (Foyer)
Session 4:	**Inhalation Exposure (Martin-Lerche lecture hall)**
9:00 **L15**	A review of 25 years of indoor air research in the context of dampness and toxigenic mold – a clinical perspective Eckardt Johanning
9:30 **L16**	Indoor inhalation exposure to mycotoxins and health effects Harriet Ammann
10:00 **L17**	How toxic is the air? – Methods for sampling and detection of mycotoxins Christoph Gottschalk, Barbara Biermaier, Eckardt Johanning, Wolfgang Völkel, Hermann Fromme, Manfred Gareis
10:15 **L18**	Transfer of mycotoxins from mouldy wallpaper to indoor air Brankica Aleksic, Marjorie Draghi, Sébastien Ritoux, Sylviane Bailly, Marlene Lacroix, Isabelle Oswald, Enric Robine, Jean-Denis Bailly
10:30 – 11:00	**Coffee break/Exhibition (Foyer)**
Session 5:	**Human Health/Toxicology I (Martin-Lerche lecture hall)**
11:00 **L19**	Occupational exposure to aflatoxin B1 – the case of a poultry slaughterhouse Susana Viegas, Luísa Veiga, Ana Almeida, Mateus dos Santos, Elisabete Carolino, Carla Viegas
11:15 **L20**	*In vitro* and *in vivo* toxicity of indoor *Stachybotrys chartarum* metabolites Elena Pieckova, Maria Majorosova, Marta Hurbankova, Aurelia Liskova, Sona Wimmerova
11:30 **L21**	Portuguese children exposure to multiple mycotoxins through food consumption: toward a holistic approach for multiple mycotoxins risk assessment Ricardo Assunção, Carla Martins, Elsa Vasco, Mariana Pinhão, Susana Loureiro, Maria João Silva, Paula Alvito
11:45 **L22**	Human biomonitoring to assess mycotoxin exposures in Bangladesh Nurshad Ali, Gisela H. Degen
12:00 **L23**	Influence of amino acid and halogen moiety on cytotoxicity of Ochratoxin A: Structure-activity studies with synthesized derivatives in comparison to natural OTA Ulrike Rottkord, Gerald Schwerdt, Michael Gekle, Hans-Ulrich Humpf

12:15 – 13:00	**Lunch (Foyer)**
13:00 – 13:45	**Poster Session/Exhibition (Foyer/Room D146)**
Session 6:	**Human Health/Toxicology II (Martin-Lerche lecture hall)**
13:45 **L24**	Sex-dependent gene expression of kidney transporters after ochratoxin A exposure in F344 rats Ariane Vettorazzi, Laura Pastor, Adela López de Cerain
14:00 **L25**	Impact of dietary deoxynivalenol and its decontamination with sodium sulfite (SoS) on peripheral blood mononuclear cells (PBMC) ex vivo in a porcine LPS-challenge model Anh Tuan Tran, Gina Pistol, Marleen Paulick, Jeannette Kluess, Jana Frahm, Sven Dänicke
14:15 **L26**	Zearalenone-14-glucoside: a fleeting phase-II plant metabolites for mammals? Luca Dellafiora, Gianni Galaverna, Chiara Dall'Asta
14:45	**Departure of the bus shuttle to Berlin "Hansabrücke"**
15:45 **18:15**	**Sightseeing tour on the Spree river boat "Alexander von Humboldt"** **Coffee and cake** **Arrival at "Fernsehwerft"** **10 min walk along the Spree**
18:30	**Welcome at "Spreespeicher" event location**
19:00 – 22:00	**Buffet & Brigitte Gedek Award for Mycotoxin Research**
22:00 – 02:00	**Dancing**

Wednesday, May 4th 2016

Session 7:	**Animal Health I (Martin-Lerche lecture hall)**
9:00 **L27**	Physiological costs of zearalenone (ZEN) exposure in carp (*Cyprinus carpio* L.) Constanze Pietsch
9:15 **L28**	Effects of a lipopolysaccharide (LPS) stimulus on the kinetics of deoxynivalenol (DON) in pigs chronically exposed to dietary DON Erik Bannert, Tanja Tesch, Jeanette Kluess, Jana Frahm, Susanne Kersten, Stefan Kahlert, Lydia Renner, Hermann-Josef Rothkötter, Sven Dänicke
9:30 **L29**	Deoxynivalenol-3-β-D-glucoside: In vitro cytotoxicity and in vivo oral bioavailability and hydrolysis in broiler chicken and pig Nathan Broekaert, Mathias Devreese, Thomas van Bergen, Stijn Schauvliege, Marthe De Boevre, Sarah De Saeger, Lynn Vanhaecke, Franz Berthiller, Herbert Michlmayr, Alexandra Malachová, Gerhard Adam, Kristel Demeyere, Evelyn Meyer, An Vermeulen, Siska Croubels

9:45 **L30**	Ingestion of feed contaminated with ergot alkaloids induces morphological and transcriptomic changes in the intestine of piglets Viviane Mayumi Maruo, Philippe Pinton, Ana Paula Bracarense, Jean-Paul Métayer, Isabelle P. Oswald
10:00 **L31**	The pig intestinal explants: an original model for the analysis of the effects of mycotoxins Philippe Pinton, Alix Pierron, Delphine Payros, Joelle Laffitte, Anne Marie Cossalter, Yannick Lippi, Pascal Gourbeyre, Isabelle P. Oswald
10:15 – 11:00	**Coffee break/Exhibition/Poster Session (Foyer/Room D146)**
Session 8:	**Animal Health II/Miscellaneous (Martin-Lerche lecture hall)**
11:00 **L32**	Influence of mycotoxin binders on the oral bioavailability of doxycycline in pigs Thomas De Mil, Mathias Devreese, Sarah De Saeger, Mia Eeckhout, Siska Croubels
11:15 **L33**	Disposition of enniatin B and B1 in broiler chickens: oral bioavailability and toxicokinetics Sophie Fraeyman, Mathias Devreese, Gunther Antonissen, Siegrid De Baere, Michael Rychlik, Siska Croubels
11:30 **L34**	Use of a large-scale qPCR approach to understand the anti-aflatoxigenic effect of Eugenol Isaura Caceres, Rhoda El Khoury, Isabelle P. Oswald, Olivier Puel, Jean-Denis Bailly
11:45 **L35**	Oosporein - mycotoxin or colonization-facilitating antioxidant? Wuttiwat Jitjak, Franz Hadacek, Vladimir Chobot, Julian Dopstadt, Hans-Ulrich Humpf, Petr Karlovsky, Niwat Sanoamuang
12:00 **L36**	Investigations on the influence of mycotoxins on biogas production Katrin Harms, Johannes Ostertag, Michael Lebuhn, Bernhard Munk, Mathias Hartel, Fabian Lichti, Karsten Meyer
12:15	**Closing Session**

MYCOTOXIN STANDARD SOLUTIONS

- Aflatoxin B1
- Aflatoxin B2
- Aflatoxin G1
- Aflatoxin G2
- Deoxynivalenol
- Fumonisin B1
- Fumonisin B2
- Fumonisin B3
- Zearalenone

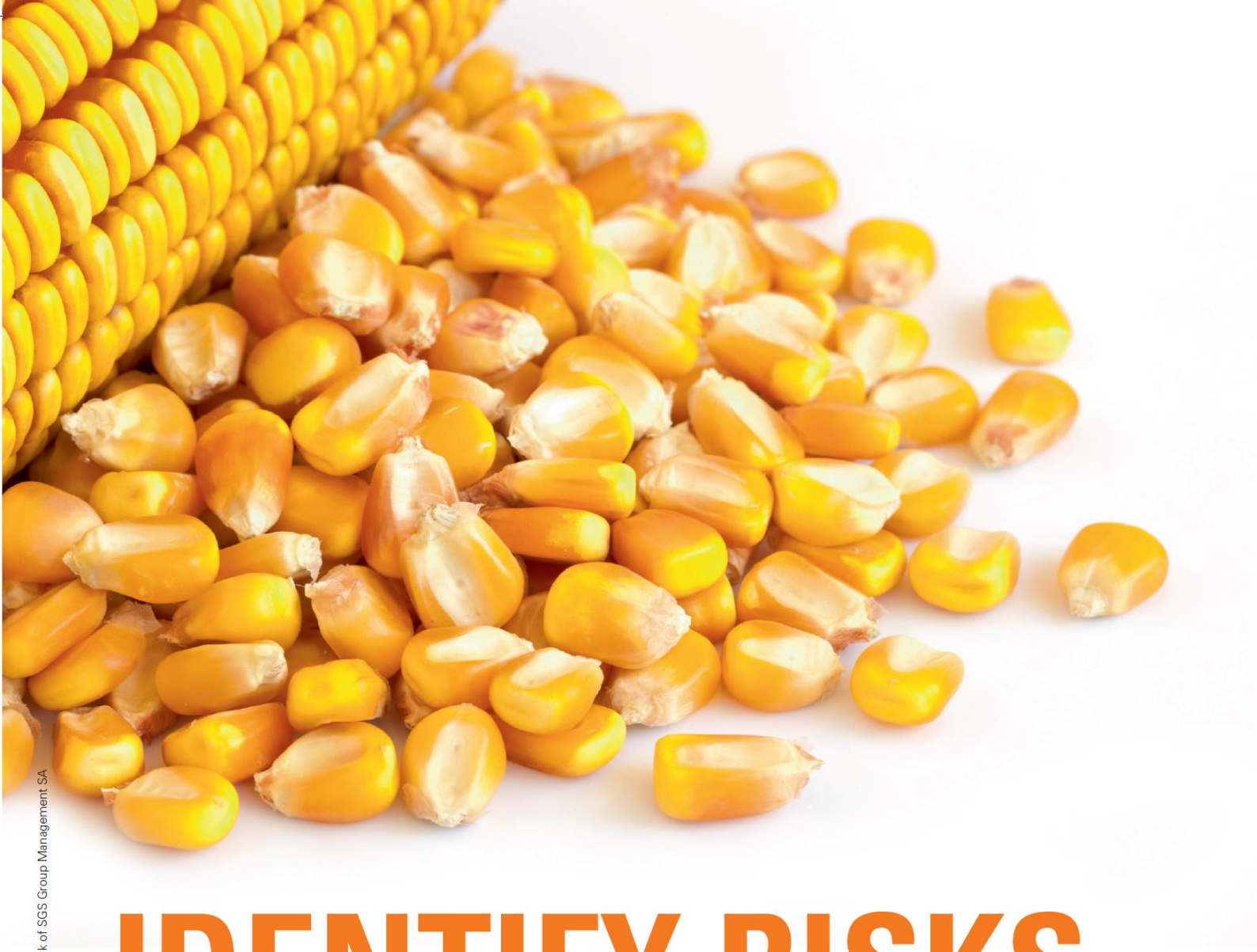
Food Analysis – Animal Feed Analysis – Pharmaceutical Raw Material Analysis

www.cfmot.com

TEL +49 (0) 9231-9619-0 / FAX +49 (0) 9231-9619-60

Oskar Tropitzsch

WEB WWW. CFMOT.DE / MAIL INFO@CFMOT.DE



IDENTIFY RISKS

MYCOTOXIN ANALYSES PERFORMED BY SGS

WE CAN PROVIDE YOU WITH

- sampling on site as well as in the country of origin
- combination packages for different mycotoxins
- low limits of determination for dietetic foods
- rush analyses within 8 or 24 hours

At the same time, you can make use of SGS's long-standing expertise in analyses and assessment as well as our global network of laboratories and regional offices.

WE ANALYSE

- aflatoxins (B1, B2, G1, G2, aflatoxin M1)
- ochratoxin A
- fusarium toxins such as deoxynivalenol, nivalenol, T2 toxin, HT2 toxin and zearalenone
- fumonisins B1, B2, B3
- patulin
- alkaloids of rye ergot (ergot alkaloids)

CONTACT

SGS Germany GmbH
Rödingsmarkt 16
D-20459 Hamburg
t +49 40 30101 - 687
f +49 40 30101 - 963
de.food.bergedorf@sgs.com
www.sgsgroup.de

SGS IS THE WORLD'S LEADING INSPECTION, VERIFICATION, TESTING AND CERTIFICATION COMPANY.

WHEN YOU NEED TO BE SURE

SGS

Table of presentations

Table of lectures

Analytics (Session 1)

L1 **Practical evaluation of screening test kits according to Regulation (EU) No 519/2014**
Elena Cubero Leon, Katrien Bouten, Carsten Mischke, Jörg Stroka

L2 **Development of a multiplex lateral flow immunoassay with quantum dots and gold nanoparticles as label for the detection of four mycotoxins**
Astrid Foubert, Natalia Beloglazova, Andreja Rajkovic, Benedikt Sas, Annemieke Madder, Sarah de Saeger

L3 **Electrochemistry coupled with mass spectrometry - a versatile tool to investigate metabolic processes of mycotoxins**
Julia Keller, Hajo Haase, Matthias Koch

L4 **MALDI Imaging mass spectrometry of mycotoxins in food**
Sebastian Hickert, Benedikt Cramer, Matthias Letzel, Hans-Ulrich Humpf

L5 **Identification of *Stachybotrys* spp. by MALDI-TOF MS**
Sebastian Ulrich, Barbara Biermaier, Karin Schwaiger, Oliver Bader, Georg Wolf, Reinhard Straubinger, Manfred Gareis, Christoph Gottschalk

Co-occurrence and Co-exposure (Session 2)

L6 **Co-occurrence and co-exposure in the risk assessment of mycotoxins – approaches discussed in EFSA**
Lutz Edler

L7 **Synergistic estrogenic effects of *Fusarium* and *Alternaria* mycotoxins**
Katharina Vejdovszky, Kathrin Hahn, Benedikt Warth, Doris Marko

L8 **Co-occurrence of aflatoxins, ochratoxin A and citrinin in "egusi" melon (*Colocynthis citrullus* L.) seeds consumed in Ireland and the United Kingdom**
Yinka Somorin, Adeyemi Akinyemi, Terenzio Bertuzzi, Amedeo Pietri

Occurrence/Degradation/Detoxification (Session 3)

L9 **Occurrence of mycotoxins (DON, ZEA) in feeding stuff from farms in the German state of Brandenburg**
Ulrike Korn

L10 **Mycotoxin production of moulds on bread and cheese under household conditions**
Sandra Wolf, Madeleine Gross, Ewald Usleber

L11 **Distribution of *Fusarium* mycotoxins and their respective modified analogues in naturally contaminated oats: effect of primary processing**
Lada Ivanova, Stefan Sahlstrøm, Ida Rud, Silvio Uhlig, Christiane Fæste, Anastasia Hole, Hege Divon

L12 ***Alternaria*** **toxin production – from laboratory study to field and market analyses**
Theresa Zwickel, Horst Klaffke, Marina Müller, Michael Rychlik

L13 **Deoxynivalenol sulfates: detoxification metabolites?**
Giorgia Del Favero, Lydia Wölflingseder, Gudrun Pahlke, Benedikt Warth, Philipp Fruhmann, Rudolf Krska, Rainer Schuhmacher, Gerlinde Wiesenberger, Gerhard Adam, Doris Marko

L14 **Degradation of enniatins by *Clonostachys rosea*, *Acremonium strictum* and *Bacillus licheniformis***
Rosine Ghislaine Suchfort, Petr Karlovsky

Inhalation Exposure (Session 4)

L15 **A review of 25 years of indoor air research in the context of dampness and toxigenic mold – a clinical perspective**
Eckardt Johanning

L16 **Indoor inhalation exposure to mycotoxins and health effects**
Harriet Ammann

L17 **How toxic is the air? – Methods for sampling and detection of mycotoxins**
Christoph Gottschalk, Barbara Biermaier, Eckardt Johanning, Wolfgang Völkel, Hermann Fromme, Manfred Gareis

L18 **Transfer of mycotoxins from mouldy wallpaper to indoor air**
Brankica Aleksic, Marjorie Draghi, Sébastien Ritoux, Sylviane Bailly, Marlene Lacroix, Isabelle Oswald, Enric Robine, Jean-Denis Bailly

Human Health/Toxicology (Sessions 5 and 6)

L19 **Occupational exposure to aflatoxin B1 – the case of a poultry slaughterhouse**
Susana Viegas, Luísa Veiga, Ana Almeida, Mateus dos Santos, Elisabete Carolino, Carla Viegas

L20 ***In vitro*** **and *in vivo*** **toxicity of indoor *Stachybotrys chartarum*** **metabolites**
Elena Pieckova, Maria Majorosova, Marta Hurbankova, Aurelia Liskova, Sona Wimmerova

L21 **Portuguese children exposure to multiple mycotoxins through food consumption: toward a holistic approach for multiple mycotoxins risk assessment**
Ricardo Assunção, Carla Martins, Elsa Vasco, Mariana Pinhão, Susana Loureiro, Maria João Silva, Paula Alvito

L22 **Human biomonitoring to assess mycotoxin exposures in Bangladesh**
Nurshad Ali, Gisela H. Degen

L23 **Influence of amino acid and halogen moiety on cytotoxicity of Ochratoxin A: Structure-activity studies with synthesized derivatives in comparison to natural OTA**
Ulrike Rottkord, Gerald Schwerdt, Michael Gekle, Hans-Ulrich Humpf

L24 **Sex-dependent gene expression of kidney transporters after ochratoxin A exposure in F344 rats**
Ariane Vettorazzi, Laura Pastor, Adela López de Cerain

L25 Impact of dietary deoxynivalenol and its decontamination with sodium sulfite (SoS) on peripheral blood mononuclear cells (PBMC) ex vivo in a porcine LPS-challenge model
Anh Tuan Tran, Gina Pistol, Marleen Paulick, Jeannette Kluess, Jana Frahm, Sven Dänicke

L26 Zearalenone-14-glucoside: a fleeting phase-II plant metabolites for mammals?
Luca Dellafiora, Gianni Galaverna, Chiara Dall'Asta

Animal Health (Sessions 7 and 8)

L27 Physiological costs of zearalenone (ZEN) exposure in carp (*Cyprinus carpio* L.)
Constanze Pietsch

L28 Effects of a lipopolysaccharide (LPS) stimulus on the kinetics of deoxynivalenol (DON) in pigs chronically exposed to dietary DON
Erik Bannert, Tanja Tesch, Jeanette Kluess, Jana Frahm, Susanne Kersten, Stefan Kahlert, Lydia Renner, Hermann-Josef Rothkötter, Sven Dänicke

L29 Deoxynivalenol-3-β-D-glucoside: In vitro cytotoxicity and in vivo oral bioavailability and hydrolysis in broiler chicken and pig
Nathan Broekaert, Mathias Devreese, Thomas van Bergen, Stijn Schauvliege, Marthe De Boevre, Sarah De Saeger, Lynn Vanhaecke, Franz Berthiller, Herbert Michlmayr, Alexandra Malachová, Gerhard Adam, Kristel Demeyere, Evelyn Meyer, An Vermeulen, Siska Croubels

L30 Ingestion of feed contaminated with ergot alkaloids induces morphological and transcriptomic changes in the intestine of piglets
Viviane Mayumi Maruo, Philippe Pinton, Ana Paula Bracarense, Jean-Paul Métayer, Isabelle P. Oswald

L31 The pig intestinal explants: an original model for the analysis of the effects of mycotoxins
Philippe Pinton, Alix Pierron, Delphine Payros, Joelle Laffitte, Anne Marie Cossalter, Yannick Lippi, Pascal Gourbeyre, Isabelle P. Oswald

L32 Influence of mycotoxin binders on the oral bioavailability of doxycycline in pigs
Thomas De Mil, Mathias Devreese, Sarah De Saeger, Mia Eeckhout, Siska Croubels

L33 Disposition of enniatin B and B1 in broiler chickens: oral bioavailability and toxicokinetics
Sophie Fraeyman, Mathias Devreese, Gunther Antonissen, Siegrid De Baere, Michael Rychlik, Siska Croubels

Miscellaneous (Session 8)

L34 Use of a large-scale qPCR approach to understand the anti-aflatoxigenic effect of Eugenol
Isaura Caceres, Rhoda El Khoury, Isabelle P. Oswald, Olivier Puel, Jean-Denis Bailly

L35 Oosporein - mycotoxin or colonization-facilitating antioxidant?
Wuttiwat Jitjak, Franz Hadacek, Vladimir Chobot, Julian Dopstadt, Hans-Ulrich Humpf, Petr Karlovsky, Niwat Sanoamuang

L36 Investigations on the influence of mycotoxins on biogas production
Katrin Harms, Johannes Ostertag, Michael Lebuhn, Bernhard Munk, Mathias Hartel, Fabian Lichti, Karsten Meyer

Table of posters

P1 **Determination of aflatoxin M1 in raw milk samples from Assiut city, Egypt**
Mohamed Fathi Abdallah, Bilge Kilicarslan, Gözde Girgin, Terken Baydar

P2 **Mini-survey of fungal and bacterial metabolites in dried dates palm from Egypt using LC-MS/MS**
Mohamed Fathi Abdallah, Rudolf Krska, Michael Sulyok

P3 **Innovative approaches for risk-oriented global supply chain analysis**
Elisabeth Achten, Husam Ibrahem-Aroud, Milena Zupaniec, Janet Riedl, Helmut Schafft, Monika Lahrssen-Wiederholt

P4 **Determination of aflatoxin M1 by a sensitive HPLC-FD method in urines from two Bangladeshi cohorts reveals frequent dietary exposure to aflatoxin B1**
Nurshad Ali, Meinolf Blaszkewicz, Wolfram Föllmann, Gisela H. Degen

P5 **Natural occurrence of Deoxynivalenol in feedstuffs and its risk assessment for livestock health**
Taekyung An, SeungRan Hong, Han Sub Jang, Chan Lee

P6 **New successes in the elucidation of the *Fusarium fujikuroi* metabolome**
Birgit Arndt, Slavica Janevska, Isabel Krug, Bettina Tudzynski, Hans-Ulrich Humpf

P7 **The role of water activity in mycotoxin production: are results obtained with solutions of sodium chloride and glycerol reliable?**
Vincent Ayugi, Katharina Pfohl, Petr Karlovsky

P8 **Effects of a lipopolysaccharide (LPS) stimulus on the kinetics of zearalenone (ZEN) and its metabolites in pigs chronically exposed to dietary ZEN**
Erik Bannert, Tanja Tesch, Jeannette Kluess, Jana Frahm, Susanne Kersten, Stefan Kahlert, Lydia Renner, Hermann-Josef Rothkötter, Sven Dänicke

P9 **Application of an enzyme immunoassay for the detection of Paxilline in beer**
Julia I. Bauer, Madeleine Gross, Ewald Usleber

P10 **Dietary supplements: occurrence of ochratoxin A in brewer`s yeast**
Barbara Biermaier, Christoph Gottschalk, Madeleine Gross, Karin Schwaiger, Manfred Gareis

P11 **Impact of Deoxynivalenol (DON) and DON-sulfates on oxidative stress and the redox-sensitive Nrf2/ARE signalling pathway**
Dominik Braun, Lydia Wölflingseder, Giorgia Del Favero, Gudrun Pahlke, Benedikt Warth, Philipp Fruhmann, Rudolf Krska, Rainer Schuhmacher, Gerhard Adam, Doris Marko

P12 **Incidence of *Fusarium* (modified) mycotoxins in Nigerian maize**
Cynthia Adaku Chilaka, Marthe De Boevre, Olusegun Atanda, Sarah De Saeger

P13 **Nano-immuno detection of Ochratoxin with gold nanoparticles as labels**
Priyanka Choudhari, Jyutika Rajwade, Dhananjay Bodas, Vandana Ghormade

P14 **Comparison of blood sampling methods for investigating the concentrations and ratios of sphingoid bases and their phosphates as fumonisin biomarkers in pigs**
Sonia Colicchia, Franz Berthiller, Heidi Elisabeth Schwartz-Zimmermann

P15 **The evaluation of DON and D3G content of native contaminated pig feed before and after in vitro digestibility test with HPLC–ESI–MS/MS method**
Krisztián Czompó, Orsolya Vida, Edit Deák

P16 **Role of fumonisins in the colonization of maize, sorghum, rice and beet seedlings with Fusarium verticillioides**
Raana Dastjerdi, Petr Karlovsky

P17 **An *in vitro* model to assess the adsorption of drugs to mycotoxin binders in a buffered, feed matrix**
Thomas De Mil, Mathias Devreese, Patrick De Backer, Siska Croubels

P18 **Development of an LC-MS/MS method for the simultaneous determination of beauvericin, enniatins (A, A1, B, B1) and cereulid in cereal and cereal-based food matrices**
Marlies Decleer, Andreja Rajkovic, Benedikt Sas, Annemieke Madder, Sarah De Saeger

P19 **Dilute and shoot – enough to pass a PT?**
Kay Dietrichkeit*, Lilli Reinhold

P20 **Biomarkers of effect prove the in vivo efficacy of a fumonisin degrading enzyme (FUM*zyme*®) in swine**
Barbara Doupovec, Ilse Dohnal, Doris Hartinger, Corinna Kern, Roger Berrios, Dian Schatzmayr

P21 **Mycotoxin Screening of Brewing Barley by Optical Spectroscopy and Ion Mobility Spectrometry (IMS)**
Alexander Erler, Dennis Klier, Toralf Beitz, Michael Kumke, Hans-Gerd Löhmannsröben

P22 **Determination of Fumonisin B1 and B2 in different temperature conditions using autosampler in needle derivatization by HPLC/FLD**
Hunor Farkas*, Biljana Marosanovic, Milena Jaksic, Svetlana Cujic, Dragana Marinkovic

P23 **Ochratoxin A and aflatoxins in selected herbal medical supplements**
Anna Filipiak-Szok, Magdalena Twarużek, Marzanna Kurzawa, Katarzyna Kuźmińska, Anna Błajet-Kosicka, Edward Szłyk, Jan Grajewski

P24 **Intestinal metabolism of plant metabolites of mycotoxins ("masked mycotoxins") in the pig cecum model**
Christine Focke, Hans-Ulrich Humpf

P25 **Zearalenone and enzymatic hydrolysis products – correlation of the estrogenic potential *in vitro* and *in vivo***
Sebastian Fruhauf, Andreas Höbartner, Bertrand Grenier, Elisavet Kunz-Vekiru, Gerhard Adam, Gerd Schatzmayr, Wulf-Dieter Moll, Michaela Thamhesl

P26 **A study on *Fusarium* mycotoxins in donkey (*Equus asinus*) feed and milk**
Madeleine Gross, Julia Bauer, Christian Puck Ploetz

P27 **Optimized synthesis of butenolide: a journey towards isotope labelled butenolide**
Maximilian Haider, Philipp Fruhmann, Julia Weber, Philipp Skrinjar, Christian Hametner, Hannes Mikula, Johannes Fröhlich, Gerhard Adam

P28 **Deoxynivalenol, deoxynivalenol-3-glucoside and 3-acetyldeoxynivalenol in processed oat: effect of steaming, flaking and cooking on mycotoxin level**
Anastasia Hole, Ida Rud, Stefan Sahlstrøm, Lada Ivanova, Gunnar Sundstøl Eriksen, Hege Hvattum Divon

P29 **Mycotoxin co-occurence in subsistence farmed maize from Zimbabwe and related risk assessment**
Melody Hove, Marthe De Boevre, Carl Lachat, Liesbeth Jacxsens, Loveness Nyanga, Sarah De Saeger

P30 **Comparison of liquid chromatography coupled with fluorescence and mass spectrometry detection in the determination of aflatoxin M1 in dairy products**
Piotr Jedziniak, Marta Piątkowska, Olga Burek, Katarzyna Pietruszka

P31 **Development and validation of HPLC-MS/MS method for determination of deoxynivalenol, de-epoxy-deoxynivalenol and their 3α-sulfates in freeze-dried eggs**
Nada Jurisic, Heidi Elisabeth Schwartz-Zimmermann, Elisavet Kunz-Vekiru, Dian Schatzmayr, Franz Berthiller

P32 **Influence of mycotoxin DON on LPS distribution and clearance in pigs**
Stefan Kahlert, Lydia Renner, Erik Bannert, Tanja Tesch, Jeannette Kluess, Constanze Nossol, Anikó Barta-Böszörményi, Hermann-Josef Rothkötter, Sven Dänicke

P33 **Indole-based alkaloids from *Penicillium expansum* and *Penicillium crustosum***
Svetlana Kalinina, Annika Jagels, Sebastian Hickert, Benedikt Cramer, Hans-Ulrich Humpf

P34 **Determination of fumonisin B1 and B2 in corn and corn products by HPLC-FLD with automatic pre-column derivatization after strong anion exchange clean-up**
Florian Kaltner, Corina Rampl, Michael Rychlik, Thomas Zimmermann, Alexander Rohe

P35 **Phase II-Metabolism of Alternariol and Alternariol Monomethyl Ether in Carrot and Tomato Explant Cultures**
Beate N. Kohn, Hannah Renner, Andreas A. Hildebrand, Erika Pfeiffer, Manfred Metzler, Mirko Bunzel

P36 **Mycotoxins influence on yeast growth and productivity in alcoholic fermentation**
Daniela Kotz, Stephan Rose, Dian Schatzmayr, Gerd Schatzmayr

P37 **^{13}C labeled standards – a reliable tool to overcome matrix effects in LC-MS/MS analysis**
Lilian Kuster

P38 **Effects of graded levels of deoxynivalenol in an artificially contaminated diet on body weight, feed and water intake in broiler chickens**
Annegret Lucke, Barbara Doupovec, Qendrim Zebeli, Josef Böhm

P39 **Effects of citric and lactic acid treatment on common mycotoxins in artificially contaminated feed samples**
Annegret Lucke, Hauke Harder, Elke Humer, Josef Böhm, Barbara Metzler-Zebeli, Qendrim Zebeli

P40 **Respiratory toxicity of indoor aspergilli and penicillia**
Maria Majorosova, Elena Pieckova, Marta Hurbankova, Sona Wimmerova

P41 **Phomopsins in legumes – a multidimensional approach**
Ronald Maul, Svenja Schloß, Corinna Herz, Evelyn Lamy, Matthias Koch, Sascha Rohn

P42 **Glucosylation of trichothecene toxins by plant UDP-glucosyltransferases**
Herbert Michlmayr, Elisabeth Varga, Alexandra Malachova, Wolfgang Schweiger, Philipp Fruhmann, Christian Hametner, Rudolf Krska, Franz Berthiller, Gerhard Adam

P43 **Heterokaryon incompatibility/compatibility and phenotypic characterisation of *Aspergillus flavus* isolates in low and high risk zones in Kenya**
Alfred Mitema, Rafudeen Suhail, Okoth Sheila, Iyer Revel

P44 **Feed from Austria and Germany tested for more than 380 mycotoxins and secondary metabolites**
Michele Muccio, Christina Schwab-Andics, Karin Naehrer, Paula Kovalsky, Rudolf Krska, Michael Sulyok

P45 **Phenotypic and phylogenetic characterization of *Alternaria infectoria* isolated from wheat in Germany and Russia**
Müller Marina E.H., Kahl Sandra M., Ulrich Andreas, Kirichenko Albina A.

P46 **Is plastic mulching a risk for Deoxynivalenol/Nivalenol soil contamination in strawberry cultivation?**
Katherine A. Muñoz, Markus Schmidt-Heydt, Dörte Diehl, Rolf Geisen, Gabriele E. Schaumann

P47 **Analysis of fourteen mycotoxins in corn and corn derived-products during wet-milling by LC-MS/MS**
Hee-Min Noh, Woori Kim, Chanvorleak Phat, Dong-Ho Kim, Chan Lee

P48 **Initial response to low deoxynivalenol (DON) doses in combination with low lipopolysaccharide (LPS) concentration and their consequence on interleukin production**
Constanze Nossol, Anikò Barta-Böszörményi, Michael Oster, Klaus Wimmers, Lydia Renner, Stefan Kahlert, Sven Dänicke, Hermann-Josef Rothkötter

P49 **Optimization of Total Aflatoxin Recovery Levels in Overparticular Matrices Using a Twenty-Minute ELISA by Dilution Normalization and Two Incubation Steps**
Antonios Ntantasios, Sotirios Athanasiou, Christos Chatzoglou, Nikolaos Natsaridis

P50 **Variability in accumulation of *Fusarium* mycotoxins in grain of winter wheat breeding lines artificially inoculated with *F.culmorum* in 2015**
Piotr Ochodzki, T. Góral, H. Wiśniewska, I. Grzeszczak, M. Kwiatek

P51 ***Fusarium graminearum* infection via wounding promotes mycotoxin contamination of maize ears**
Elisabeth Oldenburg, Frank Ellner

P52 **Recent information about mycotoxins and the IARC classification of human carcinogens**
Vladimir Ostry, Frantisek Malir, Yann Grosse, Jakub Toman

P53 **Application of an aqueous, time-saving, simple, extraction method for multiple mycotoxins.**
Elise Palmer, John Davenport, Xinwen Wang

P54 **Risk assessment of fumonisin B1 and B2 in feed stuff for livestock**
JuHee Park, JooWon Lim, Hansub Jang, Chan Lee

P55 **Complex formation of cyclodextrins with zearalenone as a tool to decrease toxin exposure**
Miklós Poór, Beáta Lemli, Sándor Kunsági-Máté, Nikolett Sali, Tamás Kőszegi, Lajos Szente

P56 **Development and first applications of an LC-ESI-MS/MS multi-method for the quantification of *Alternaria* toxins**
Hannes Puntscher, Benedikt Warth, Philipp Skrinjar, Doris Ellmer, Hannes Mikula, Doris Marko

P57 **Determination of total fumonisins and aflatoxins levels in some grain sorghum lines in Iran**
Vahid Rahjoo, Mohammad Taghi Feizbakhsh

P58 **Reduction of Ochratoxin A in beer by using genetically improved lager brewing yeasts**
Sandra Rainieri

P59 **Development of *in vitro* model plant system to study the detoxification ability of different wheat varieties**
Laura Righetti, Enrico Rolli, Chiara Dall'Asta

P60 **Variety specific control of fusarium head blight and mycotoxin reduction in winter wheat by the use of fungicides**
Bernd Rodemann, Tim Baumgarten

P61 **Deoxynivalenol and its modified forms in Croatian wheat harvested in 2015**
Bojan Šarkanj, Lidija Brodar, Michael Sulyok, Rudolf Krska, Tomislav Klapec

P62 **Reduction of T-2 and HT-2 toxins in oats by combined mechanical and thermal energies**
Henning S. Schmidt, Stefanie Becker, Benedikt Cramer, Hans-Ulrich Humpf

P63 **Necessity of different moieties of the ochratoxin A molecule to influence collagen formation or gene expression: structure-activity-studies with synthesized derivatives in comparison to natural OTA**
Gerald Schwerdt, Ulrike Rottkord, Marie-Christin Schulz, Hans-Ulrich Humpf, Michael Gekle

P64 **How does the *Fhb1* Locus of Wheat Affect the Ability to Detoxify DON – a Hypothesis**
Gerald Siegwart, Wolfgang Schweiger, Benedikt Warth, Herbert Michlmayr, Sonia Vautrin, Barbara Steiner, Rudolf Krska, Rainer Schuhmacher, Helene Berges, Hermann Buerstmayr, Gerhard Adam

P65 **Deoxynivalenol and its metabolite deepoxy-deoxynivalenol: multi-endpoint analysis for the evaluation of cellular effects**
Alexandra Springler, Sabine Hessenberger, Nicole Reisinger, Corinna Kern, Veronika Nagl, Gerd Schatzmayr, Elisabeth Mayer

P66 **Rapid dust screening by means of LC-MS/MS to obtain occurrence patterns of *Fusarium*, *Aspergillus*, *Penicillium* and *Alternaria* toxins in corn**
Simone Staiger, Mareike Reichel, and Scarlett Biselli

P67 **Co-occurrence of Fusarium, Aspergillus, Penicillium, Alternaria toxins and Ergot alkaloids in European wheat and rye samples screened by means of dust analyses**
Simone Staiger, Mareike Reichel, and Scarlett Biselli

P68 ***Fusarium* resistance evaluation in the set of winter wheat varieties from Common EU catalogue**
Lenka Štěrbová, Jana Chrpová

P69 **WGS Sequencing of the citrinin producer Penicillium citrinum and subsequent characterization of the citrinin gene cluster**
Dominic Stoll, Najim Touhami, Rolf Geisen, Markus Schmidt-Heydt

P70 **Improving the determination of aflatoxins – a never ending story for the analyst?**
Joerg Stroka*, Stefanka Bratinova

P71 **Purification of enniatins from *F. tricinctum* cultures on solid white bean medium**
Rosine Ghislaine Suchfort, Petr Karlovsky

P72 **Functional characterization of ethylene biosynthesis candidate genes of *Fusarium graminearum***
Thomas Svoboda, Alexandra Parich, Alexandra Malachova, Maria Doppler, Clemens Schmeitzl, Gerlinde Wiesenberger, Rainer Schuhmacher, Gerhard Adam

P73 **Relationship between body core temperature and inflammatory markers in pigs exposed to oral deoxynivalenol (DON) and systemic lipopolysaccharide (LPS)**
Tesch Tanja, Bannert Erik, Kluess Jeannette, Frahm Jana, Hüther Liane, Kersten Susanne, Renner Lydia, Kahlert Stefan, Rothkötter Hermann-Josef, Dänicke Sven

P74 **Prevalence of ochratoxin A and ochratoxigenic microfungi in medicinal herbs and teas**
Jakub Toman, Frantisek Malir, Vladimir Ostry, Vladimir Dvorak, Katerina Kotalova, Tomas Roubal

P75 **Ochratoxin A and aflatoxin B1 in breakfast cereals from Serbian market**
Ljilja Torovic, Gorica Vukovic

P76 **New plasmids allowing positive-negative selection for fungal transformation and efficient Cre-loxP mediated marker recycling**
Krisztian Twaruschek, Pia Spörhase, Gerlinde Wiesenberger, Gerhard Adam

P77 **Evaluation of cytotoxicity and mould contamination of selected meadow plants**
Magdalena Twarużek, Romuald Dembek, Ewelina Soszczyńska, Jan Grajewski

P78 **Mycological contamination and cytotoxic evaluation of used cars' dust filters**
Magdalena Twarużek, Ewelina Soszczyńska, Justyna Kwiatkowska, Jan Grajewski

P79 **Green young barley as potential source of moulds and mycotoxins**
Magdalena Twarużek, Robert Kosicki, Anna Błajet-Kosicka, Iwona Ałtyn, Josef Böhm, Jan Grajewski

P80 **Prevalence of *Aspergillus* section *fumigati* in Portuguese slaughterhouses: a fungal and mycotoxin concern**
Carla Viegas, Tiago Faria, Raquel Sabino, Anita Quintal Gomes, Susana Viegas

P81 **Co-exposure to mycotoxins – the case of waste management workers**
Susana Viegas, Bernd Osteresch, Susana Almeida, Benedikt Cramer, Hans-Ulrich Humpf, Carla Viegas

P82 **Toxigenic fungi in coffee samples: a menace to public health**
Carla Viegas, Cátia Pacifico, Ana Cebola de Oliveira, Ana Pintão, Anita Quintal Gomes, Susana Viegas

P83 **Assessment of toxigenic fungi in poultry feed**
Carla Viegas, Cátia Pacifico, Tiago Faria, Ana Cebola de Oliveira, Anita Quintal Gomes, Susana Viegas

P84 **Aflatoxins and ochratoxin A in different types of flours marketed in Serbia**
Gorica Vukovic, Ljilja Torovic

P85 **DATEX.CZ – Natural occurrence of mycotoxins in foodstuffs in year 2011-2014**
Jiri Vyslouzil, Vladimir Ostry, Marcela Dofkova, Irena Rehurkova, Jiri Ruprich

P86 **Optimized Synthesis of Deoxynivalenol-15-β-D-glycosides**
Julia Weber, Philipp Fruhmann, Christian Hametner, Alois Schiessl, Georg Häubl, Johannes Fröhlich, Hannes Mikula

P87 **Mechanism of the genotoxic potential of *Alternaria* mycotoxins**
Nadin Wedler, Stefanie Fleck, Ann Liza Piberger, Andrea Hartwig

P88 ***In Situ* Analysis of Trichodiene as a Volatile Biomarker for the Fast and Non-Invasive Gas-Phase Analysis of Cereal Mycotoxins**
Martina Werneburg, Julian Gebauer, Matthias Koch

P89 **Non-toxic degradation products of NX-2 and NX-3**
Gerlinde Wiesenberger, Elisabeth Varga, Christian Hametner, Gerhard Adam, Franz Berthiller

P90 **Resistance of winter triticale breeding lines to accumulation of *Fusarium* metabolites in grain**
Halina Wiśniewska, Tomasz Góral, Piotr Ochodzki, Michał Kwiatek, Maciej Majka, Dorota Walentyn-Góral, Iga Grzeszczak, Zofia Banaszak, Mirosław Pojmaj, Danuta Kurleto, Marcin Konieczny, Grzegosz Budzianowski, Alicja Cicha, Kazimierz Paizert, Janina Woś

P91 **Aurofusarin, a secondary metabolite of *Fusarium* fungi, induces oxidative stress and DNA damage in HT29 colon carcinoma cells**
Konstantin Wolters, Benedikt Warth, Gudrun Palke, Michael Sulyok, Doris Marko

P92 **Interaction between fungivorous Collembola and mycotoxin-producting *Fusarium* species**
Yang Xu, Katharina Pfohl, Petr Karlovsky

MYCOTOXIN SOLUTIONS

Neogen's mycotoxin test range includes both rapid on-site lateral flow solutions and ELISA test kits for laboratory use.

OUR TESTS INCLUDE ASSAYS FOR:

AFLATOXIN
FUMONISIN

DON
OCHRATOXIN

ZEARALENONE
T-2/HT-2

NOW AVAILABLE

NEW WATER BASED MYCOTOXIN TESTS

Neogen now offers an ELISA and lateral flow test for aflatoxin which use an aqueous extraction method, eliminating the need for harsh solvents in the lab.

Veratox® for Total Aflatoxin and **Reveal® Q+** MAX for Aflatoxin detect aflatoxin B_1, B_2, G_1, and G_2 with superior cross-reactivity.

Why not contact us to find out more?

COMING SOON **Reveal Q+ MAX** lateral flow tests for: DON • Zearalenone • Fumonisin • Ochratoxin • T-2/HT-2

NEOGEN®

Tel: +44 (0) 1292 526 097 • +44 (0) 1292 525 603
Email: mykotoxine_de@neogeneurope.com • Web: www.neogeneurope.com

Abstracts of lectures

L1

Practical evaluation of screening test kits according to regulation (EU) No 519/2014

Elena Cubero Leon, Katrien Bouten, Carsten Mischke, Joerg Stroka*

European Commission, Joint Research Centre, IRMM, Geel, Belgium

* Corresponding author: joerg.stroka@ec.europa.eu

Deoxynivalenol (DON) is a mycotoxin produced by various Fusarium species, which contaminates grains and cereal-based food and feed. In order to actively decrease the presence of DON in food and feed, maximum values have been set at European level with Regulation 1881/2006 (1).

DON is found in almost half the samples taken into account in the European Food Safety Authority's Opinion on DON (2). While the highest levels were observed in wheat, maize and oat grains and derived products, higher levels of DON were found in feed compared with food. DON levels exceeded the maximum limits for food or guidance values for feed in less than 2 % of the cases (2). This means that more than 98% of the tested samples were found compliant, which makes this contamination scenario interesting for early testing by rapid screening methods.

In 2014 the European Union has put in place the necessary legal framework for the validation of rapid screening methods with Regulation 519/2014 (3). The here presented work aims at applying this validation scheme to three commercial lateral flow immunoassays for DON.

Test kits were evaluated for their reliability and usefulness to verify compliance with EC permitted maximum levels. The performance of the test kits was assessed through a single-laboratory validation following the provision of Regulation 519/2014 which provided information about the cut-off value allowing samples to be classified as compliant or as "suspects". Occurrence data coming from monitoring programmes was used to demonstrate the usefulness of this approach on the basis of the calculated cut-off values.

References:

1. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union, L364/5.

2. Scientific report of EFSA (2013). Deoxynivalenol in food and feed: occurrence and exposure. EFSA Journal, 11(10):3379.

3. Commission Regulation (EU) No 519/2014 of 16 May 2014 amending Regulation (EC) No 401/2006 as regards methods of sampling of large lots, spices and food supplements, performance criteria for T-2, HT-2 toxin and citrinin and screening methods of analysis. Official Journal of the European Union, L147/29.

L2

Development of a multiplex lateral flow immunoassay with quantum dots and gold nanoparticles as label for the detection of four mycotoxins

Astrid Foubert[1]*, Natalia Beloglazova[1], Andreja Rajkovic[2], Benedikt Sas[3], Annemieke Madder[4], Sarah De Saeger[1]

[1]Faculty of Pharmaceutical Sciences, Laboratory of Food Analysis, Ghent University, Ghent, Belgium; [2]Faculty of Bioscience Engineering, Department of Food Safety and Food Quality, , Laboratory of Food Microbiology and Food Preservation, Ghent University, Ghent, Belgium; [3]Faculty of Bioscience Engineering, Department of Food Safety and Food Quality, Food2Know, Ghent University, Ghent, Belgium; [4]Faculty of Sciences, Department of Organic Chemistry, Laboratory for Organic and Biomimetic Chemistry, Ghent University, Ghent, Belgium

* Corresponding author: astrid.foubert@ugent.be

Today, with the increased regulatory requirements in food safety the demand for rapid, sensitive and accurate methods to detect biological and chemical contaminants has increased. In particular, tests that can be completed within minutes would enable processors to take quick corrective actions when contaminants are detected, which is also the case for mycotoxins. Hence, rapid methods like the lateral flow immunoassay (LFIA) are rapid, user-friendly and sensitive on-site tests suitable for this purpose.

Here, we present the development of two multi-mycotoxin LFIA systems by using different kind of labels (quantum dots (QDs) and colloidal gold nanoparticles (CG). By comparing the two labels conclusions can be made about their sensitivity. For the QD-LFIA and CG-LFIA green, red, orange-emitted QDs and gold nanoparticles were used, respectively. Both of them were able to detect four mycotoxins i.e. deoxynivalenol (DON), zearalenone (ZEN) and T2/HT2 in different matrices (barley and wheat). The test is based on an indirect competitive approach. First, the QDs were solubilized by coating them with polymer or silica, which also made covalent bioconjugation with antibodies (Abs) possible. Next, the specific Abs were immobilized on the QDs by carbodiimide chemistry. In case of the gold nanoparticles the bioconjugation was based on electrostatic interaction. The mycotoxin ovalbumin (OVA) conjugates (DON-OVA, ZEN-OVA and T2-OVA) were synthesized and immobilized as three test lines on the membrane. The test is completed within 15 minutes and there is no need for any mathematical or statistical processing of the obtained results. This detection method is a user-friendly and sensitive detection method with cut-offs (DON:1 000 µg/kg, ZEN: 80 µg/kg, T2/HT2: 80 µg/kg) according to EU legislation. Taking everything together, the development of a LFIA with QDs as label required 20 times less labelled Abs and 4 times less mycotoxin conjugates to receive an assay with the same cut-off levels as the CG-LFIA. Furthermore, the signals of the QD-LFIA were much brighter and easier to interpret than the ones from the CG-LFIA.

Acknowledgement: This work is financial supported by the BOF Special Research Fund from Ghent University, GOA project no. 01G02213.

L3

Electrochemistry coupled with mass spectrometry – a versatile tool to investigate metabolic processes of mycotoxins

Julia Keller[1]*, Hajo Haase[2], Matthias Koch[1]

[1]Bundesanstalt für Materialforschung und -prüfung, Berlin, Germany; [2]Technische Universität Berlin, Berlin, Germany

* Corresponding author: julia.keller@bam.de

To understand the metabolic fate of food relevant mycotoxins in vitro systems were mainly used as the method of choice, so far. Yet, in recent years coupling of electrochemistry mass spectrometry (EC-MS) gained increasing importance as promising technique for fast simulation of metabolic processes and was successfully applied in particular for drug metabolism [1].

The aim of our work was to investigate the potential of EC-MS to predict phase I metabolites of priority mycotoxins and to compare the results with in vitro experiments. Hence, the EU-regulated Fusarium mycotoxins zearalenone (ZEN) and patulin as well as dihydroergocristine (DHEC) as model compound of ergot alkaloids were electrochemically oxidized and analyzed by EC-MS for the first time.

Electrochemical conditions were set-up individually for each of the three mycotoxins. By using a coulometric flow through cell with a diamond working electrode oxidation of the chosen mycotoxins was observed after applying potentials between 1.7 and 2.0 V vs. Pd/H_2.The electrochemically generated reaction products were analyzed online by mass-spectrometric detection.

All of the three chosen mycotoxins were electrochemically converted to mono- and/or dihydroxylated products confirming the results of ZEN related metabolism studies [2,3] and in case of DHEC own results from in vitro assays. Due to a lack of metabolism studies concerning the oxidative fate of patulin, interpretation of EC-MS data and performing microsomal studies is of particular relevance.

Beside the identified products from electrochemical oxidation of ZEN, patulin and DHEC there is still a number of yet unknown compounds. Additional structural characterization of detected compounds by NMR and X-ray analysis will be facilitated by their large-scale production using preparative EC cells.

1. Karst, U., Electrochemistry/mass spectrometry (EC/MS) - A new tool to study drug metabolism and reaction mechanisms. Angewandte Chemie-International Edition, 2004. 43(19): p. 2476-2478.

2. Metzler, M., E. Pfeiffer, and A.A. Hildebrand, Zearalenone and its metabolites as endocrine disrupting chemicals. World Mycotoxin Journal, 2010. 3(4): p. 385-401.

3. Drzymala, S.S., et al., Estrogenicity of novel phase I and phase II metabolites of zearalenone and cis-zearalenone. Toxicon, 2015. 105: p. 10-12.

L4

MALDI Imaging Mass Spectrometry of Mycotoxins in Food

Sebastian Hickert[1,2], Benedikt Cramer[1], Matthias Letzel[3], Hans-Ulrich Humpf[1,2]*

[1]Institute of Food Chemistry, WWU Münster, Münster, Germany; [2]NRW Graduate School of Chemistry, Münster, Germany; [3]Institute of Organic Chemistry, WWU Münster, Münster, Germany

* Corresponding author: Humpf@wwu.de

Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS) generates ions by irradiation of co-crystallized analytes and a matrix substance with a laser beam. The specific matrix substance is ionized by absorbing the energy at the emission wavelength of the laser and this process leads to the ionization of the analytes. The laser beam can be used to raster surfaces generating mass spectra at different positions, e.g. a tissue section. This information allows the visualization of distribution patterns of analytes within a tissue. Until now most applications of MALDI-Imaging-MS deal with physiological tissues such as brain or liver sections of mammals.

This work describes the development of a workflow for tissue preparation and application of MALDI-Imaging-MS (MALDI-IMS) to food samples infected with fungi of the genera Fusarium and Aspergillus and the spatially resolved analysis of ochratoxin A in grapes, fumonisins of the B- and C-series in maize and aflatoxins in Brazil nuts.

The obtained results show differences in the distribution of the analysed toxins. While some substances are only detectable at the location of fungal spoilage, other toxins are distributed homogenously within the food sample. These insights can help to identify possibilities and limitations for mechanical reduction of the mycotoxins contamination e.g. by removal of visible mould or certain parts of infected foodstuff. Furthermore this technique provides new insights into the infection mechanisms and mycotoxin excretion of moulds on plant material.

L5

Identification of *Stachybotrys* spp. by MALDI-TOF MS

Sebastian Ulrich[1]*, Barbara Biermaier[1], Karin Schwaiger[1], Oliver Bader[2], Georg Wolf[3], Reinhard K. Straubinger[3], Manfred Gareis[1], Christoph Gottschalk[1]

[1]Chair of Food Safety, Ludwig-Maximilians-University, Munich, Germany; [2]Medical Microbiology, University Medical Center, Goettingen, Germany; [3]Chair of Bacteriology and Mycology, Ludwig-Maximilians-University, Munich, Germany

*Corresponding author: ulrich@ls.vetmed.uni-muenchen.de

Stachybotrys (*S*.) spp. are omnipresent moulds and able to produce highly cytotoxic secondary metabolites (JARVIS, 2003). *S. chartarum* chemotype S and *S. dichroa* are known as producers of macrocyclic trichothecenes. They grow on cellulose rich materials such as wilting plants e.g. hay, straw (HARRACH et al., 1987) and culinary herbs (BIERMAIER et al., 2015), but also on wallpapers and other indoor items (JOHANNING et al., 1996). Thus, *Stachybotrys* spp. pose a risk for human and animal health alike.

Currently applied methods for the identification of toxigenic strains comprise phenotypic and genotypic description in combination with determination of mycotoxins, e.g. by LC-MS/MS. In recent years, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has proven to be a fast and easy method for the identification of microorganisms after minimal sample pretreatment. However, for filamentous fungi the routine methods of sample preparation are often not suited for the generation of high quality mass spectra for their reliable identification due to their rigid cell walls.

Here we report on an optimized protocol for the extraction of mainly ribosomal proteins from *Stachybotrys* spp. for subsequent MALDI-TOF MS analysis. The optimized protein extraction protocol enabled the generation of MALDI-TOF MS reference mass spectra for eleven Stachybotrys species (*S. chartarum* chemotype A and S, *S. chlorohalonata*, *S. theobromae*, *S. parvispora*, *S. kampalensis*, *S. cylindrospora*, *S. complementi*, *S. longispora*, *Memnoniella echinata*, *Melanopsamma pomiformis*). A total of 45 isolates originating from different habitats (hay, straw, culinary herbs and indoor materials) were analyzed by the MALDI-TOF MS technique. Twenty-seven and 18 isolates out of 45 were identified as *S. chartarum* and *S. chlorohalonata*, respectively. The results were confirmed by sequencing the ITS region (*internal transcribed spacer*) and the *tri*5-gene (trichodiensynthase).

HARRACH, B., BATA, A., SÁNDOR, G., VÁNYI, A., 1987. Isolation of macrocyclic and non macrocyclic trichothecenes (*stachybotrys* and *fusarium* toxins) from the Environment of 200 III Sport Horses. Mycotoxin Research 3, 65-68.
JARVIS, B.B., 2003. *Stachybotrys chartarum*: a fungus for our time. Phytochemistry 64, 53-60.
JOHANNING, E., BIAGINI, R., HULL, D., MOREY, P., JARVIS, B., LANDSBERGIS, P., 1996. Health and immunology study following exposure to toxigenic fungi (*Stachybotrys chartarum*) in a water-damaged office environment. International Archives of Occupational and Environmental Health 68, 207-218.
BIERMAIER, B., GOTTSCHALK, C., SCHWAIGER, K. & GAREIS, M. (2015) Occurrence of *Stachybotrys chartarum* chemotype S in dried culinary herbs. Mycotoxin Res 31, 23-32

This work is financially supported by the Brigitte and Wolfram Gedek-Stiftung (Ismaning, Germany).

L6

Co-occurrence and co-exposure in the risk assessment of mycotoxins - approaches discussed in EFSA

Lutz Edler*

Division on Biostatistics, German Cancer Research, Heidelberg, Germany

* Corresponding author: edler@dkfz.de

Most previous risk assessments of mycotoxins performed by the European Food Safety Authority (EFSA) since 2004 focused on one toxin and co-exposure was rarely considered except at few instances where a group TDI was established. Co-exposure to mycotoxins has become an issue, when analytical sensitivity increased and multi-mycotoxin methods allowed joint analyses. Co-occurrence of different mycotoxins produced by the same fungi in one sample shifts the paradigm of assessing the risk of a single mycotoxin to the assessment of a key mycotoxin (also called parent mycotoxin) joint with its alterations recently denoted modified forms. Two examples where the EFSA has been asked by the European Commission for a joint assessment will be presented: DON and its acetylated and modified forms, specifically DON, 3-Ac-DON, 15-AC-DON and DON-3-glucoside, and ZEN and its modified forms (identified as phase I and phase II metabolites).

One of the biggest challenges for the evaluation of these multi-dimensional occurrence data is data incompleteness and missing data: the analytical result of a sample cannot be fully quantified since the level of contamination is below the limit-of-quantification and /or below the limit-of-detection, or, in the extreme, no measurement exists, but zero concentrations cannot be assured. Therefore, adequate tools for transparent and correct reporting in tables and graphics are needed that account the complexity of left-censored data. Screening for possible sources of bias is highly relevant such that the risk assessment informs adequately on inherent uncertainty.

Another challenge concerns the establishment of a group health-based guidance value comprising both the parent and the modified mycotoxins. Therefore, the toxicokinetic (TK) availability of co-occurring mycotoxins in the critical target organs, their joint toxicodynamic (TD) effects and possible differences in their mode-of-action need to be considered. In the presence of enough TK and TD data a group of mycotoxins might be combined using relative efficiency/potency factors relating the modified forms to the parent mycotoxin; otherwise one might consider a conservative approach assuming same critical toxicities for parent and modified forms.

Further issues such as the role of biomarkers, hazard characterization of farm animal when fed naturally contaminated feed, dose-dependency of the quality of interactions, adjustment for different molecular weights, and role of non-oral exposure, and also issues related to the origin of samples should be noted.

The work is supported by the author's membership in the EFSA Panel on Contaminants in the food chain (CONTAM) and his function as chair of the Working Group on Fusarium toxins and his membership in the BFR Kommission für Kontaminaten und andere unerwünschte Stoffe in der Lebensmittelkette and its Ausschuss Mykotoxine.

L7

Synergistic estrogenic effects of *Fusarium* and *Alternaria* mycotoxins

Katharina Vejdovszky, Kathrin Hahn, Benedikt Warth, Doris Marko

University of Vienna, Department of Food Chemistry and Toxicology,
Waehringer Str. 38, A-1090 Vienna, Austria

Corresponding author: doris.marko@univie.ac.at

Current risk assessment of mycotoxins, as for most chemical substances, is based on the effects of single compounds. However, concern on a potential enhancement of risks by interactions of single substances in naturally occurring mixtures has greatly increased recently. Toxic secondary metabolites formed by various fungal species are found as natural contaminants in food. This very heterogeneous group of compounds triggers multiple toxic mechanisms, including endocrine disruptive potential. In this study the combinatory effects of three mycoestrogens were investigated in detail. This includes the endocrine disruptors zearalenone and α-zearalenol produced by *Fusarium* fungi and alternariol, a cytotoxic and estrogenic mycotoxin formed by *Alternaria* species. For evaluation of effects, estrogen-dependent activation of alkaline phosphatase and cell proliferation were tested in the adenocarcinoma cell line Ishikawa. The estrogenic potential varied amongst the single substances. The majority of combinations, even at very low concentrations in case of α-zearalenol, showed strong synergism with respect to alkaline phosphatase activation. These toxicity-potentiating phenomena of mycotoxin mixtures highlight the urgent need to incorporate combinatory effects into future risk assessment, especially for endocrine disruptors. To the best of our knowledge, this study presents the first investigation on synergistic effects of mycoestrogens.

L8

Co-occurrence of aflatoxins, ochratoxin A and citrinin in "egusi" melon (Colocynthis citrullus L.) seeds consumed in Ireland and the United Kingdom

Yinka Somorin[1]*, Adeyemi Akinyemi[2], Terenzio Bertuzzi[3], Amedeo Pietri[3]

[1]Microbiology, National University of Ireland, Galway, Ireland; [2]Agronomy, Osun State University, Ejigbo Campus, Ejigbo, Nigeria; [3]Feed & Food Science and Nutrition Institute, UCSC, Via Emilia Parmense, 84, 29122 Piacenza, , Italy

* Corresponding author: yinkasomorin@yahoo.com

The natural co-occurrence of aflatoxins (AFs), ochratoxin A (OTA) and citrinin (CIT) in melon seed samples obtained from retailers and households in Ireland and the United Kingdom (UK) was evaluated. AFs and OTA were determined by HPLC with fluorescence detection while CIT was analysed by HPLC-MS/MS. AFB_1 and AFB_2 were detected in all samples (100%) collected whereas AFG_2 had the lowest incidence (27.3%) of the aflatoxins. Total AFs were detected with concentrations ranging from 0.3 to 82 µg kg^{-1} (Mean = 12.0 µg kg^{-1}) while AFB_1 ranged from 0.2 to 66.5 µg kg^{-1} (Mean = 9.7 µg kg^{-1}). Commercially retailed samples showed a significantly higher AFB_1 contamination ($p<0.05$) than the household samples. OTA occurred in 3 (13.6%) samples while 4 (18.2%) were contaminated with CIT at very low levels. To our knowledge, this is the first report of co-occurrence of AFs, OTA and CIT in "egusi". From this study, 68% of the melon seed samples were contaminated above the 2 µg kg^{-1} EU limits fixed for AFB_1. These results highlight the need for the development of strategies to reduce AF contamination in "egusi", so as to improve the acceptability of Nigerian melon seeds exports to the EU markets.

Whole "egusi" seeds Milled "egusi"

L9

Occurrence of mycotoxins (DON, ZEA) in feeding stuff from farms in Brandenburg

Ulrike Korn*

Landeskontrollverband Berlin-Brandenburg eV, Waldsieversdorf, Germany

* Corresponding author: korn@lkvbb.de

The Landeskontrollverband Berlin-Brandenburg eV (LKV BB) analyzes feeding stuff for farmers from Brandenburg. The analysis of mycotoxins like deoxynivalenole (DON), zearalenone (ZON), ochratoxin A, aflatoxins and T2/HT2 are part of the examination spectrum of the LKV BB. These examinations are more and more requested in the last years.

In contrast to research studies, in which feeding stuff is analyzed with a defined target, the samples analyzed in the LKV BB are samples from farmers whose livestock have health problems. Type of feeding stuff and animal species define the mycotoxins which were supposed to be analyzed.

Against this background many different types of feeding stuff were analyzed, for example maize products, cereals or mixed feed (Figure 1). The most requested analyses were DON and ZEA.

In the speech, results of the above mentioned analyses will be presented focusing on DON and ZEA. Additionally, a short overview will show how farmers deal with such contaminated feeding stuff.

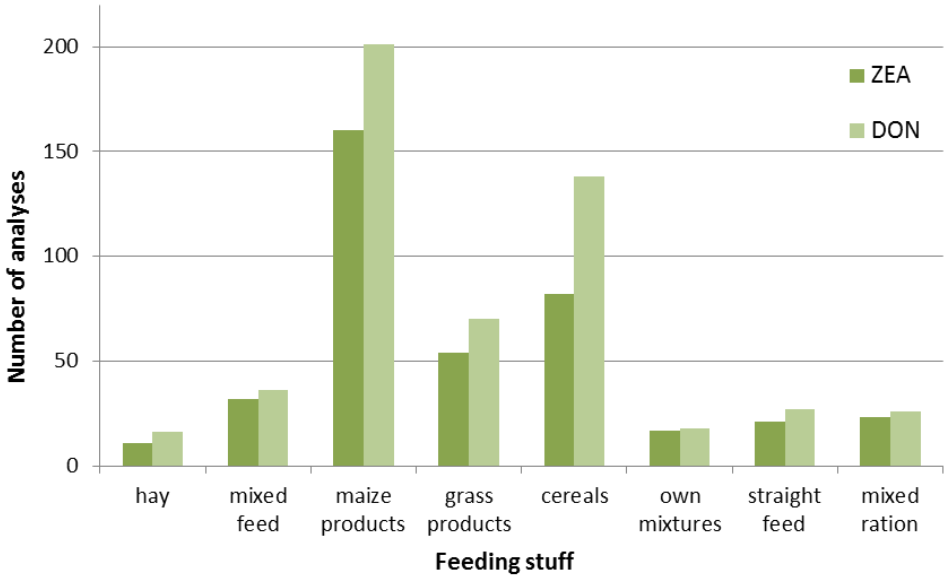


Figure 1: Numbers of analyses of DON and ZEA in the different types of feed

L10

Mycotoxin production of moulds on bread and cheese under household conditions

Sandra M. Wolf, Madeleine Gross, Ewald Usleber*

Justus-Liebig-University, Veterinary Faculty, Institute of Veterinary Food Science, Chair of Dairy

* Corresponding author: Ewald.Usleber@vetmed.uni-giessen.de

Bread and cheese getting mouldy during storage in the home environment is a common, everyday event. It is also trivial that fungal species involved in this process may produce mycotoxins. However, analytical data on toxin production during fungal spoilage under household conditions seem to be scarce. In this study, cheese and bread was purchased from local retailers and stored for up to 3 months, refrigerated (bread, cheese, 7 °C) or at 21/24 °C (bread only). Additionally, samples of mouldy bread and cheese were obtained from private households. Spontaneous fungal spoilage was allowed to proceed into extreme forms and to the worst possible visible result. Beginning several days (1-2 weeks latest) after storage, all samples developed visible mould infestation. Moulds were roughly grouped according to the predominant color, but mixtures of colonies also were quite common. Black Aspergilli and blue-grey Penicillia were the predominant moulds. Fumonisin B2 (FB2) and Isofumigaclavine A (IsoFuA) were used as marker toxins to identify toxin production. Samples were analysed by enzyme immunoassays, some samples were additionally analysed for FBs by HPLC-FLD. On bread, spoilage with black moulds at 21/24 °C yielded high contamination with FB2 in a range of 3-81 mg/kg (mean 30 mg/kg). Bread with blue-green fungal spoilage yielded IsoFuA at levels of 8-108 µg/kg (mean 60 µg/kg). At 7 °C, little or no growth of black moulds occurred. FB2 and IsoFuA levels increased with storage time, but visibly non-mouldy parts were always essentially free of toxin. In cheese, blue-grey moulds producing IsoFuA (4-1100 µg/kg) grew on most samples after several days and were the predominant colony type, again toxin levels increased with storage time. Visibly non-infested areas were essentially free of IsoFuA, even if massive mould spoilage was only a few centimeters away. In conclusion, spoilage of bread under household conditions with black moulds yields high levels of FB2, blue-grey moulds yield IsoFuA. In cheese, spoilage results in IsoFuA contamination. Rework of such materials, either as food or as feed, would result in a mycotoxin recycling at toxicologically relevant levels.

L11

Distribution of Fusarium mycotoxins and their respective modified analogues in naturally contaminated oats: effect of primary processing

Lada Ivanova[1]*, Stefan Sahlstrøm[2], Ida Rud[2], Silvio Uhlig[1], Christiane Fæste[1], Anastasia Hole[2], Hege Divon[1]

[1]Norwegian Veterinary Institute, Oslo, Norway; [2]Nofima, Ås, Norway

* Corresponding author: lada.ivanova@vetinst.no

Oats (Avena slativa L.) ranks seventh in world cereal production and are considered to be an important source of many valuable components of nutritional and biological importance, i.e., proteins, fats, carbohydrates, fiber, minerals and vitamins. Because of these properties, the amount of oats used for human consumption has increased progressively during the last years. Unfortunately, the quality of this grain crop is often compromised by mycotoxin contamination, which is considered to be relatively ubiquitous despite efforts to control the problem. The concern regarding the public health grew with the discovery of masked toxins, which own their name to the ability to escape detection by existing routine analytical methods. Therefore, development of rapid, sensitive, and specific methods for determination of mycotoxins and their conjugated forms in cereals and cereal-based products was highly desirable.

In this study we investigated the fate of selected trichothecene mycotoxins as well as their conjugated derivatives during oats processing. Furthermore, we applied a state-of-the-art method based on high-resolution mass spectrometry for the chemical analysis of the samples. Quantitative mapping of selected Fusarium mycotoxins in the fractions collected during the processing trials consisting of dehulling, pearling, and sequential milling was performed. In this context, the mycotoxins DON, HT-2, and T-2 as well as their conjugated derivatives were the main focus of the present pilot-scale study.

Dehulling and sequential pearling had a significant effect on the mycotoxin contamination in the oats (> 83%). However, we noticed accumulation of conjugated mycotoxins, especially DON-3-glucoside and HT-2-3-glucoside in the inner fractions of kernels. In addition, the distribution pattern of parent and conjugated toxins in collected fractions was inhomogeneous, reflecting differences in the metabolization observed in plants.

L12

Alternaria toxin production – from laboratory study to field and market analyses

Theresa Zwickel[1], Horst Klaffke[1], Marina E.H. Müller[2], Michael Rychlik[3]

[1]Federal Institute for Risk Assessment (BfR), Berlin, German, [2]Leibniz-Centre for Agricultural Landscape Research (ZALF), Institute of Landscape Biogeochemistry, Müncheberg, Germany, [3]Technische Universität München, Chair of Analytical Food Chemistry, Freising, Germany

* Corresponding author: theresa.zwickel@bfr.bund.de

Alternaria ssp. are worldwide occurring fungi, which can infect cultivated plants and lead to several plant diseases resulting in harvest losses and reduction in quality. They are able to produce a broad spectrum of possibly human or animal health endangering mycotoxins, which can occur as natural contaminants within the food and feed chain [1]. The formation of the toxins depends on the *Alternaria* species and is influenced by various environmental factors.

To gain a better understanding of the *Alternaria* toxin production a laboratory study regarding the influence of temperature, substrate and incubation time on the formation of these toxins by *Alternaria infectoria* and *A. tenuissima* was carried out. Under the optimized conditions (25 °C, 14 days, wheat kernels) 100 different *Alternaria* isolates from four species groups (*A. alternata, A. infectoria, A. arborescenc, A. tenuissima*) were examined for the presence of the before mentioned as well as modified (e.g. AOH3S, AME3S, ALT3S) *Alternaria* toxins and infectopyrone, a potential mycotoxin produced by *A. infectoria* using HPLC-MS/MS and HRMS. Comparison of the resulting mycotoxin patterns will be discussed.

An insight into the contamination situation of food and feed with *Alternaria* toxins is a high priority in order to assess human and animal exposure and possible health risks [2]. Therefore, two different multi-mycotoxin methods for the determination of tenuazonic acid (TeA), alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (TEN), altenuene (ALT), iso-altenuene (iso-ALT), altertoxin I (ATX-I), altertoxin II (ATX-II), stemphyltoxin III (STTX-III), altenuisol (ATL), altenuic acid III (AA-III) and AAL-Toxin TB1 and TB2 were developed and validated. A QuEChERS [3] based extraction procedure was applied for solid and pasty food and feed samples like tomato products, several cereal products and forage maize. For liquids such as wine and various types of fruit and vegetable juice a solid phase extraction procedure by using diatomaceous earth was used. The quantification of the mycotoxins was carried out by means of the matrix-matched calibration curve or the standard addition method using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) in a single run time of less than 10 minutes. Over 300 commercially available food samples (tomato based products, cereal products, fruit juices and wine) from the German market and 100 maize feed sampled obtained from German agricultural fields were analysed between the years 2013 and 2015. TeA was the predominately occurring mycotoxin followed by AOH, TEN and AME. Data obtained from this market study will be presented and discussed.

1. EFSA, Scientific Opinion on the risks for animal and public health related to the presence of Alternaria toxins in feed and food. EFSA Journal 2011;9(10):2407 2011b.

2. Lorenz, N.; Klaffke, H.-S.; Kemmlein, S.; Itter, H.; Lahrssen-Wiederholt, M., Report: Aktueller Sachstand zum Thema „Alternaria-Toxine“ aus Sicht des gesundheitlichen Verbraucherschutzes. J. Verbr. Lebensm. 2012, 7, 359-365.

3. Payá, P.; Anastassiades, M.; Mack, D.; Sigalova, I.; Tasdelen, B.; Oliva, J.; Barba, A., Analysis of pesticide residues using the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) pesticide multiresidue method in combination with gas and liquid chromatography and tandem mass spectrometric detection. Analytical and bioanalytical chemistry 2007, 389, 1697-1714.

L13

Deoxynivalenol sulfates: detoxification metabolites?

Giorgia Del Favero[1]*, Lydia Wölflingseder[1], Gudrun Pahlke[1], Benedikt Warth[1], Philipp Fruhmann[2], Rudolf Krska[3], Rainer Schuhmacher[3], Gerlinde Wiesenberger[4], Gerhard Adam[4], Doris Marko[1]

[1]Dept. of Food Chemistry and Toxicology, Univie, Vienna, Austria; [2]Institute of Applied Synthetic Chemistry, TU Wien, Vienna, Austria; [3]Department IFA-Tulln, BOKU, Tulln, Austria; [4]Dept. of Applied Genetics and Cell Biology, BOKU, Tulln, Austria

* Corresponding author: giorgia.del.favero@univie.ac.at

The trichothecene mycotoxin deoxynivalenol (DON) is a well-known mycotoxin and one of the most relevant food contaminants in Europe. Recently, two sulfo-metabolites of DON have been discovered in artificially inoculated wheat, namely the DON-3-sulfate (DON3S) and DON-15-sulfate (DON15S; Warth et al., 2015 Anal. Bioanal. Chem. 407(4):1033-9). In addition, DON3S was also identified as a urinary metabolite in humans (unpublished results). Since the primary mechanism of action of DON is the inhibition of the protein synthesis at ribosomal level, the first step for the comparison of the DON sulfates with the parent compound was the performance of an in vitro translation assay. In agreement with literature, DON induced a concentration dependent inhibition of the ribosomal activity, whereas, interestingly, DON3S did not inhibit protein translation at concentrations of up to 100 µM. DON15S was shown to be a moderate inhibitor of mammalian ribosomes (IC50 of about 47 µM). In order to characterize the toxicological relevance of DON3S and DON15S in comparison to the parent compound, initial experiments were performed on the human colorectal adenocarcinoma cell line HT-29. After 24 h of incubation, DON triggered a concentration-dependent decrease of the cellular viability (sulforhodamine B assay). On the contrary, the DON sulfates did not show any cytotoxic effect. Thus, at a first glance, the DON3S and DON15S could be considered as detoxification products of the parent compound DON. However, intriguingly, both compounds induced significantly cellular proliferation of colorectal carcinoma cells starting from the concentration of 0.1 µM. In conclusion, even though the DON-sulfates do not seem to share, in our experimental systems, the same biological effects of the parent compound DON, their activity on the cellular proliferation of intestinal cells opens new perspectives in the evaluation of the risk associated to the entrance of trichothecene mycotoxin and their metabolites into the food chain.

The authors thank the Austrian Science Fund (project SFB Fusarium F3701, F3702, F3706, and F3718) and the City of Vienna Jubilee Funds for the University of Natural Resources and Life Sciences, Vienna (project MycoMarker) for the financial support.

L14

Degradation of enniatins by *Clonostachys rosea*, *Acremonium strictum* and *Bacillus licheniformis*

Rosine Ghislaine Suchfort, Petr Karlovsky*

Molecular Phytopathology & Mycotoxin Research, Georg-August University Göttingen, Göttingen, Germany

* Corresponding author: pkarlov@gwdg.de

This study was conducted in order to isolate enniatin-degrading microorganisms (fungi, yeast or bacteria) from sources including soil, water, grains, cereal- based food, and fruits. Wash fluid from these materials was amended with enniatins (final concentration 1 mg/ml) and incubated for 7 d at room temperature. The cultures were diluted 25-times in minimal medium with enniatins and incubated for further 21 d. The cultures were plated on full agar media. A total of 114 isolates of bacteria and/or yeast-like microorganisms and 23 fungal isolates were obtained. These strains were inoculated into the minimal media with enniatins as sole carbon source described above. After 3 d (bacteria) or 10 d (fungi) culture supernatants were analyzed for enniatin content by HPLC-UV (RP chromatography, detection at 210 nm). One bacterial strain and two fungal strains were able to transform enniatins into new products. Although enniatin content was reduced in most other cultures, no new products were identified in these cultures by HPLC. Several enniatin transformation products were identified using liquid chromatography coupled to mass spectrometry. Morphological, biochemical and molecular characterization of the enniatin-transforming isolates results in taxonomical assignments to bacterium Bacillus licheniformis and fungi Clonostachys rosea (Gliocladium roseum) and Acremonium strictum. The first two strains originated from soil and the third from Hazel nut.

L15

A review of 25 years of indoor air research in the context of dampness and toxigenic mold – a clinical perspective

Eckardt Johanning *

Columbia University, New York, N.Y. & Occupational and Environmental Life Science, Albany, N.Y.

* Corresponding author: johanningeckardt@verizon.net

Buildings can impact the health of occupants and workers. Some of the known factors that can adversely impact the health after chronic water infiltration have described over the years, although there is an ongoing discussion about the environmental factors, pathology, methodology of investigation and causation analysis. It has been shown that within 24-48 hours water intrusion can lead to excessive growth and amplification of mold and other microorganisms, including mycotoxin producing fungi. There is a consensus among experts that a variety of microbes, microbial fragments and by-products are associated with adverse health effects which include respiratory problems and effects on other organs that may be explained by allergic or toxic-irritant reactions [1, 2]. Mycotoxicosis had been described as a "condition looking for a disease" [3]. Clinical experience has shown that the health reaction may vary greatly depending on exposure characteristics and individual factors (gender, age, atopy, route of exposure, etc.). In this presentation sentinel health investigations will be reviewed that have lead over the years to international cooperation and research efforts, inter-disciplinary conferences, and public health, professional- or technical guidelines.

In sentinel investigation unusual presentations of building related diseases or conditions are explored to develop prevention strategies and determine further research priorities. Starting with representative case histories that involved workers, home owners and children who presented in a specialty clinic with seemingly non-specific health complaint and symptoms the complex variety of pathologies (i.e., immune system abnormalities, respiratory organs, neurological and neuro-cognitive disorders), the complexity of medical tests and interpretations are reviewed on the clinical level. Such investigations often lead to further group studies, using health questionnaires and collection of epidemiologic data or health assessments after interventions. However, epidemiological data may not be sufficient evidence in a causation analysis and specific laboratory studies or experimental animal studies are necessary to postulate a cause-and-effect relationship. In a cluster situation of unusual indoor air complaints of museum employees the first large scale investigation in the USA of exposure to toxigenic Stachybotrys atra will be appraised, which lead to the now widely accepted "New York City DOH Guidelines on the assessment and remediation of mold in buildings" [4, 5]. Followed by an investigation of a child with a rare genetic abnormality, who was misdiagnosed and insufficiently treated by her hospitalists, until an environmental home study showed the connection of the respiratory disease with excessive dampness and growth of mold in their New York Spanish Harlem apartment [6]. Further cases of neurological and neuro-cognitive abnormalities of patients with a history of indoor exposure to toxigenic molds (including trichothecene producers) are reviewed and relevant studies and publications will be discussed [7, 8, 9]. Research in the last decades has shown that in most cases no single agents are the culprit, but in-vivo interactions between toxigenic fungi and bacteria and complex immunological-organ reactions of by the host(s) may lead to the spectrum of allergic and non-allergic (toxicological) health disorder that one may encounter in the medical office. Nevertheless, based on these experiences and knowledge guidelines for the remediation and controls have been recently reviewed [10]. This study was conducted in

order to isolate enniatin-degrading microorganisms (fungi, yeast or bacteria) from sources including soil, water, grains, cereal- based food, and fruits. Wash fluid from these materials was amended with enniatins (final concentration 1 mg/ml) and incubated for 7 d at room temperature. The cultures were diluted 25-times in minimal medium with enniatins and incubated for further 21 d. The cultures were plated on full agar media. A total of 114 isolates of bacteria and/or yeast-like microorganisms and 23 fungal isolates were obtained. These strains were inoculated i

1. Husman, T., *Health effects of indoor-air microorganisms.* Scand. J. Work Environ. Health, 1996. **22**(1): p. 5-13.
2. Panel of experts (Harriet Ammann, S.A., Tony Colantonio, Jean Cox-Ganser, Anne Hyvarinen, Eckardt Johanning, Philip Morey). *Proceedings of the international audit of the methods used and recommendations made by the Direction de santé publique de l'Agence de la santé et des services sociaux de Montréal regarding the health impacts of chronic water damage in buildings.* 2014. Montreal, Quebec, Canada: Direction de santé publique; Agence de la santé et des services sociaux de Montréal (2015).
3. Schiefer, H.B., *Mycotoxins in Indoor Air: A critical toxicological viewpoint*, in *Indoor Air '90, Proceedings of the 5th International Conference on Indoor Air Quality and Climate*. 1990, Indoor Air '90: Toronto, Canada. p. 167-172.
4. Johanning, E., et al., *Health and immunology study following exposure to toxigenic fungi (Stachybotrys chartarum) in a water-damaged office environment.* Int Arch Occup Environ Health, 1996. **68**(4): p. 207-18.
5. Klitzman, S., et al., *Guidelines on Assessment and Remediation of Fungi in Indoor Environments*. 2002.
6. Johanning, E., et al., *Clinical experience and results of a Sentinel Health Investigation related to indoor fungal exposure.* Environ Health Perspect, 1999. **107 Suppl 3**: p. 489-94.
7. Auger, P.L., *Mycotoxins and neurotoxicity*, in *Fungi and Bacteria in Indoor Air Environments - Health effects, detection and remediaton*, E. Johanning and C.S. Yang, Editors. 1994, Eastern New York Occupational Health Program: Albany, NY. p. 161-167.
8. Gordon, W.A., E. Johanning, and L. Haddad, *Cognitive impairment associated with exposure to toxigenic fungi*, in *Bioaeorosols, Fungi and Mycotoxins: Health effects, assessment, prevention and control*, E. Johanning, Editor. 1999, Eastern New York Occupational and Environmental Health Center: Albany. p. 94-98.
9. Gordon, W.A., et al., *Cognitive impairment associated with toxigenic fungal exposure: a response to two critiques.* Appl Neuropsychol, 2006. **13**(4): p. 251-7.
10. Johanning, E., et al., *Review of health hazards and prevention measures for response and recovery workers and volunteers after natural disasters, flooding, and water damage: mold and dampness.* Environ Health Prev Med, 2014. **19**(2): p. 93-9.

L16

Indoor Inhalation Exposure to Mycotoxins and Health Effects

Harriet Ammann*

University of Washington, School of Public Health and Community Medicine, Department of Environmental and Occupational Health Sciences, Seattle, Washington, USA

* Corresponding author: h.ammann@comcast.net

Understanding of agents of mycotoxin exposure via inhalation has evolved from assumptions that mold spores are the primary vehicle. It has become clear that mycotoxin-containing fragments of spores and hyphal fragments are produced during germination and growth. Since mycotoxins are exotoxins, they are secreted by the fungus on to surfaces where they grow. Mycotoxin-bearing dust particles, as well as fungal fragments can be re-entrained into the air through building and human activity, and breathed by occupants. Depending on size, the particle to spore ratio can be in the hundreds of thousands to millions. Particles in aggregate have a much larger surface to volume ratio than spores per unit mass, and the large aggregate surface area can adsorb and carry greater amounts of adsorbed toxins than spores can. Inhalation of small particles provides a direct route to the bloodstream for systemic distribution, in contrast to ingestion in which the enterohepatic circulation routes the absorbed particles/toxins to the liver for detoxification, before being returned to the general circulation. Systemic effects from inhalation of mycotoxins, especially to immune tissues, have been shown in rats and pigs. The nose also provides direct access to the central nervous system via axonal transport along the olfactory, trigeminal and vagus nerves. Inhalation experiments with rodents, pigs and rhesus monkeys show that such transport of mycotoxins and particles to the brain where apoptosis of neural cells and oxidative damage has been demonstrated.

L17

How toxic is the air? – Methods for sampling and detection of mycotoxins

Christoph Gottschalk[1]*, Barbara Biermaier[1], Sebastian Ulrich[1], Eckardt Johanning[2], Wolfgang Völkel[3], Hermann Fromme[3], Manfred Gareis[1]

[1]Chair of Food Safety, Ludwig-Maximilians-University, Munich, Germany; [2]Columbia University, New York, N.Y. & Occupational and Environmental Life Science, Albany, N.Y., U.S.A.; [3]Bavarian Health and Food Safety Authority, Department of Chemical Safety and Toxicology, Munich, Germany

*Corresponding author: christoph.gottschalk@lmu.de

Inhalation exposure to mycotoxins can occur in residential and occupational settings such as in mouldy indoor environments or buildings equipped with HVAC-systems and at workplaces like farms, waste sorting units or cereal storages (Fromme et al. 2016). Depending on the environmental conditions the spectrum of moulds and possibly occurring mycotoxins could be very broad and there are various ways of toxins becoming airborne: Mycotoxins can be bound to fungal spores or microdust particles while the toxins themselves probably are released from the fungal organism by active segregation into guttation droplets (Gareis and Gottschalk 2014).

Collecting of samples for the assessment of indoor air toxicity comprises many kinds of samples such as bulk samples, dust, settled dust, and air filters. However, only air sampling could provide quantitative results for a most reliable assessment of an inhalation exposure to mycotoxins.

Mycotoxins can be measured by HPLC, LC-MS/MS, immunoassays, and effect-based bioassays. The latter are limited to mycotoxins of the respective mode of action/endpoint, e.g. cytotoxicity, while mass spectrometry and ELISA are subject to partly strong matrix effects hampering reliable results. LC-MS/MS would be the method of choice for toxins where ^{13}C-stable isotope standards are available for quantification. Due to the variety of different sampling methods and methods for quantification of mycotoxins available data are hardly comparable and not easy to assess. In this lecture we will give an overview on current techniques and propose a standard approach for sampling and quantification of mycotoxins in order to harmonize and simplify air toxicity assessments.

References

Fromme H, Völkel W, Gareis M, Gottschalk C. Overall internal exposure to mycotoxins and their occurrence in occupational and residential settings – An overview. Int J Hyg Environ Health 219 (2016) 143–165

Gareis M and Gottschalk C: *Stachybotrys* spp. and the guttation phenomenon. Mycotox Res 30 (2014) 151-159

L18

Transfer of mycotoxins from mouldy wallpaper to indoor air

Brankica Aleksic[1], Marjorie Draghi[2], Sébastien Ritoux[2], Sylviane Bailly[1], Marlene Lacroix[1], Isabelle Oswald[1], Enric Robine[2], Jean-Denis Bailly[1]*

[1]INRA, UMR 1331 Toxalim - Research center in food technology, F - 31027, Toulouse, FRANCE;
[2]CSTB, Scientific and Technical Center for Construction, Laboratory for Microbiology of Environments Interiors and Sustainable Development, Marne-la-Vallée, FRANCE

* Corresponding author: jd.bailly@envt.fr

In industrialized countries, people spend 80-95% of their time inside the buildings. At least 20% of the buildings in North America and Europe have one or more signs of dampness; indoor dampness and molds are common worldwide. The development of these fungi in habitats can cause immuno-allergic pathologies. Some of the species commonly observed in indoor environments are also able to produce mycotoxins. The danger associated with exposure to these contaminants by air (inhalation of toxin or contaminated particles) or by contact with contaminated materials are very poorly documented.

We first studied levels of mycotoxins that could be produced by fungal contaminants during their development on wallpaper, one of the most often used material in indoor furnishing. The molds/mycotoxins that were studied: Aspergillus versicolor/sterigmatocystin, Penicillium brevicompactum /mycophenolic acid and Stachybotrys chartarum/ macrocyclic trichothecenes (Roridin L2, verrucarin J and satratoxins G and H). These species-toxins were chosen due to their high prevalence in mouldy indoor environment and especially in case of water damage, possible toxicity of corresponding mycotoxins but also the differences in mycelial structure and conidial organization that could let think of different behaviours towards aerolic solicitations.

To evaluate the possible aerosolization of fungal particles and associated mycotoxins in indoor air, analysis were performed in specific experimental system built to reproduce in the laboratory the mechanical action of air flows that correspond to air circulation due to heating, opening a window, etc.

Results indicate that aerosolization from contaminated materials is possible for several toxins, collecting hundreds (thousands) nanograms of toxins in air. As suspected, three species of interest had different behaviour during aerosolization. Air flow sufficient to significantly aerosolize particles from contaminated material strongly differed from one specie to another.

It was noted that toxins are mostly found in particles whose size corresponds to the size of spore, but also in smaller ones, which can reach bronchi but also bronchiole and alveoli. These data are interesting for risk assessment related to mouldy environments.

This work was supported by the French Environment and Energy Management Agency (ADEME) and Scientific and Technical Center for Construction (Centre Scientifique et Technique du Bâtiment, CSTB). This work is done in the framework of the PRIMEQUAL project.

L19

Occupational exposure to Aflatoxin B1 – the case of a poultry slaughterhouse

Susana Viegas[1,2], Luísa Veiga[3], Ana Almeida[1], Mateus dos Santos[1], Elisabete Carolino[1], Carla Viegas[1,2]

[1]Environment and Health RG, Lisbon School of Health Technology, Polytechnic Institute of Lisbon, Lisbon, Portugal; [2]Centro de Investigação em Saúde Pública (CISP/ENSP/UNL), Lisboa, Portugal; [3]Lisbon School of Health Technology, Polytechnic Institute of Lisbon, Lisbon, Portugal

* Corresponding author: susana.viegas@estesl.ipl.pt

Aflatoxin B1 (AFB_1) is a secondary metabolite produced by the fungi *Aspergillus flavus* and is the most potent hepatocarcinogen known in mammals and has been classified by the International Agency of Research on Cancer as Group 1 carcinogen. Although dietary exposure to AFB_1 has been extensively documented, there are still few studies dedicated to the occupational exposure topic. In view of the recent findings regarding AFB_1 occupational exposure in poultry production and other occupational settings related with animal production, it was considered relevant to clarify if there is also exposure in poultry slaughterhouses.

Occupational exposure assessment to AFB_1 was done with a biomarker of internal dose that measures AFB_1 in the serum by enzyme-linked immunosorbent assay. Thirty workers from a slaughterhouse were enrolled in this study. A control group (n = 30) was also considered in order to know AFB_1 exposure resulting from food consumption in Portuguese population. Fourteen workers (47.0%) showed detectable levels of AFB_1 with values from 1.06 ng mL^{-1} to 4.03 ng mL^{-1}, with a mean value of 1.73 ng mL^{-1}. No AFB_1 was detected in serum of individuals used as controls. Despite uncertainties regarding the exposure route that is contributing more to exposure (inhalation or dermal) is possible to state that exposure to AFB_1 is occurring in the studied slaughterhouse unit. It seems that reducing AFB_1 contamination in poultry production can have a positive result in reducing occupational exposure to this setting.

L20

In vitro and in vivo toxicity of indoor Stachybotrys chartarum metabolites

Elena Pieckova*, Maria Majorosova, Marta Hurbankova, Aurelia Liskova, Sona Wimmerova

Slovak Medical University, Bratislava, Slovakia

* Corresponding author: elena.pieckova@szu.sk

A mould Stachybotrys chartarum (Ehrenb. ex Link) Hughes colonizing indoor environments is supposed to belong to the causative agents of ill health of their occupants, esp. babies. The cellulolytic fungus is considered to be a tertiary colonizer of surfaces in affected buildings. Adverse health effects of S. chartarum known in the occupational hygiene, result from its toxins. There is only limited knowledge on them in dwellings. It is able to produce trichothecene mycotoxins or atranones as well as spirolactams. S. chartarum isolates originated from mouldy walls in Slovakia were studied for their ability to produce toxicants with certain toxicity in vitro and in vivo. Chloroform extracts of endo- and exometabolites of its growth on a liquid medium with yeast extract and sucrose at 25 °C for 14 d were used in the experiments. In vitro toxicity was studied on chick tracheal organ cultures and the rat lung epithelial type II cells. In vivo toxicity of the uncharacterized mixtures of metabolites was evaluated after the intratracheal instillation (4 microg in 0.2 % dimethylsulphoxide; diacetoxyscirpenol as the positive control) in Wistar male rats. After 3 d, haematological parameters were measured in peripheral blood and inflammatory response biomarkers in bronchoalveolar lavage fluid (BALF) and statistically analysed. Based on the results of HPTLC of fungal metabolites, the S. chartarum isolates represented an atranone-type of the mould. Its metabolites showed no ciliostatic activity at 20 microg. mL^{-1}. There was no difference in the cytotoxicity of exo- and endometabolites of the strain expressed as the decreasing alkaline activity in rat lung type II cells at the same concentration. Cell wall coarsing and fragmentation were apparent in the lung epithelium. The exometabolites proved to be erythrocyte (Ery) suppressors (decreasing of total Ery count, haemoglobin and haematocrite) and cytotoxic substances (decreased viability of alveolar macrophages, proportion of living cells and increased lysosomal cathepsin D activity in BAL cells) for the lung tissue. Inflammation indicated by significantly higher total BALF cell and lower alveolar macrophages counts, with increased number of granulocytes related to the BALF cells, was detected as well. S. chartarum toxic metabolites can contribute to damage of upper/lower airways and the haematopoetic cells in exposed occupants of mouldy buildings. The results were even more pronounced when the effect of fungal co-cultures and additional environmental stressors had been analyzed (Piecková et al, 2015).

The publication resulted from the project realization

L21

Portuguese children exposure to multiple mycotoxins through food consumption: toward a holistic approach for multiple mycotoxins risk assessment

Ricardo Assunção[1]*, Carla Martins[2], Elsa Vasco[3], Mariana Pinhão[3], Susana Loureiro[4], Maria João Silva[5], Paula Alvito[6]

[1]INSA, IP; IIFA, Universidade Évora; CESAM, Lisbon/Évora/Lisbon, Portugal; [2]INSA, IP; NOVA University of Lisbon; CESAM, Lisbon, Portugal; [3]INSA, IP, Lisbon, Portugal; [4]Department of Biology & CESAM, Aveiro, Portugal; [5]INSA, IP; ToxOmics, New University of Lisbon, Lisbon, Portugal; [6]INSA, IP; CESAM, Lisbon, Portugal

* Corresponding author: ric.assuncao@gmail.com

Humans can be exposed to multiple chemicals at once from a variety of sources, and human risk assessment of multiple chemicals poses several challenges to scientists, risk assessors and risk managers. Ingestion of food is considered a major route of exposure to many contaminants, namely mycotoxins, especially for vulnerable population groups, as children. A lack of sufficient data regarding mycotoxins children risk assessment, could contribute to an inaccuracy of the estimated risk. Efforts must be undertaken to develop initiatives that promote a broad overview of multiple mycotoxins risk assessment. The present work, developed within the MYCOMIX project, aims to assess the risk associated to the exposure of Portuguese children (< 3 years old) to multiple mycotoxins through consumption of foods primarily marketed for this age group. A holistic approach was developed applying deterministic and probabilistic tools to the calculation of mycotoxin daily intake values, integrating children food consumption (3-days food diary), mycotoxins occurrence (HPLC-UV, HPLC-FD, LC-MS/MS and GC-MS), bioaccessibility (standardized in vitro digestion model) and toxicological data (in vitro evaluation of cytotoxicity, genotoxicity and intestinal impact). A case study concerning Portuguese children exposure to patulin (PAT) and ochratoxin A (OTA), two mycotoxins co-occurring in processed cereal-based foods (PCBF) marketed in Portugal, was developed. Main results showed that there is low concern from a public health point of view relatively to PAT and OTA Portuguese children exposure through consumption of PCBF, considering the estimated daily intakes of these two mycotoxins (worst case scenarios, 22.930 ng/kg bw/day and 0.402 ng/kg bw/day, for PAT and OTA, respectively), their bioaccessibility and toxicology results. However, the present case study only concerns the risk associated with the consumption of PCBF and child diet include several other foods. The present work underlines the need to adopt a holistic approach for multiple mycotoxins risk assessment integrating data from exposure, bioacessibility and toxicity domains in order to contribute to a more accurate risk assessment.

This research was performed under the MycoMix project “Exploring the toxic effects of mixtures of mycotoxins in infant food and potential health impact” (PTDC/DTP-FTO/0417/2012) and CESAM Lisboa UID/AMB/50017/2013, both funded by the Fundação para a Ciência e Tecnologia (FCT), Portugal.

L22

Human biomonitoring to assess mycotoxin exposures in Bangladesh

Nurshad Ali, Gisela H. Degen*

IfADo-Leibniz Institut für Arbeitsforschung, Dortmund, Germany

* Corresponding author: degen@ifado.de

In Bangladesh surveillance of mycotoxin contamination in food is insufficient. As biomonitoring covers intake from all sources, analysis of urine biomarkers was used to study mycotoxin exposure in inhabitants of Bangladesh. An LC-MS/MS based multi-method first served to select main mycotoxins of interest, i.e. aflatoxin B1 (AFB1), ochratoxin A (OTA), citrinin (CIT), deoxynivalenol (DON). Then analysis by sensitive specific methods for AFM1 (metabolite of AFB1), OTA, CIT, dihydrocitrinon (HO-CIT) and DON, served to determine concentration ranges in urines from Bangladeshi cohorts a German reference cohort. Findings of this first systematic biomonitoring study in the Bangladeshi population are presented with comments on possible risks associated with mycotoxin exposure.

AFB1: AFM1 was not detected by HPLC-FD analysis in urines of German adults. But, AFM1 is found in >40% of Bangladeshi urines. AFM1 biomarker levels (range 1.7-190 pg/mL) vary with regions and sampling season (see Poster by Ali et al.), yet overall the new data show frequent exposure of the Bangladeshi population to the hepato-toxin AFB1, and at levels which raise concerns. As AFB1 is a potent mutagenic carcinogen, no “safe” intake values can be defined, and exposure should be kept as low as possible (ALARA principle).

OTA and CIT: Biomarkers of exposure to these nephrotoxins are present in most samples. Total CIT (CIT+HO-CIT) in Bangladeshi urines is much higher in winter (mean 3.77, max 48.12 ng/mL) than in summer (mean 0.51, max 5.70 ng/mL). Also OTA levels in winter (mean 0.19, max 1.75 ng/mL) are higher than in summer (mean 0.06, max 0.55 ng/mL). Analysis of Bangladeshi pregnant women urines confirms frequent concomitant exposure to OTA and CIT. In German adults, the OTA urine level (mean 0.21, max 1.82 ng/mL) is similar to that in Bangladeshi cohorts in winter. CIT levels in German urines (total mean 0.14, max 0.58 ng/mL) are clearly lower than those found in all Bangladeshi samples. Estimates of OTA exposure from urine data are hampered by its low excretion rate (<3%). Biomarker data for CIT and its urine excretion rate of 35% per day served to calculate probable daily intake and compare it to a preliminary tolerable daily intake (TDI) of 0.2 µg/kg bw set by EFSA. The TDI for CIT was not exceeded in the German cohort, but in up to 24% of Bangladeshi adults. CIT and OTA exposure should be further monitored, including also other groups (e.g. children), and in light of possible combined effects.

DON: Analysis for all Bangladeshi cohorts reveal low DON exposure, but higher urine DON levels (mean 9.02, max 38.44 ng/mL) in German adults. Probable daily DON intake calculated for persons in both countries is below the TDI of 1 µg/kg/d set by WHO, indicating that present DON exposures are not of concern.

L23

Influence of amino acid and halogen moiety on cytotoxicity of Ochratoxin A: Structure-activity studies with synthesized derivatives in comparison to natural OTA

Ulrike Rottkord[1], Gerald Schwerdt[2], Michael Gekle[2], Hans-Ulrich Humpf[1]*

[1]Institute of Food Chemistry, Westfälische Wilhelms-Universität, Münster, Germany; [2]Julius-Bernstein-Institute of Physiology, Martin-Luther-Universität, Halle-Wittenberg, Germany

* Corresponding author: humpf@uni-muenster.de

The mechanism why the mycotoxin ochratoxin A (OTA) impairs cell and organ function is still not solved in detail. However all indicates that an interaction with target molecules is indispensable for any observed adverse effect. This interaction depends on characteristics of the target molecule as well as on the OTA molecule itself. OTA has different structural moieties which may be relevant for these interrelations including a halogen (chlorine) and an amino acid group (phenylalanine). To test their importance for the impact of OTA detailed structure-activity studies with various OTA derivatives were performed. For this 23 OTA derivatives were available, which were modified by either an exchange of the halogen moiety against another halogen (fluorine, iodine or bromine) or by the amino acid moiety against another one (tyrosine or alanine) or a combination of both. Additionally, the configuration of the 3R carbon atom was changed to 3S. These derivatives were tested in human renal cells for their ability to induce cell death (cytotoxicity, apoptosis, necrosis), their impact on collagen protein secretion and for their influence on gene expression. It turned out that the substitution of the amino acid moiety against tyrosine or alanine almost completely prevented the adverse effects of OTA. The exchange of the halogen moiety had minor effects and the inversion of the stereochemistry at C3 did not prevent the OTA effects. Therefore, we conclude that the amino acid moiety of OTA is a prerequisite for the interaction of OTA with its target molecules.

Supported by the Deutsche Forschungsgemeinschaft (DFG) HU 730/12-1 and SCHW1515/2-1)

L24

Sex-dependent gene expression of kidney transporters after ochratoxin A exposure in F344 rats

Ariane Vettorazzi*, Laura Pastor, Adela López de Cerain

Department of Pharmacology and Toxicology. University of Navarra, Pamplona, Spain

* Corresponding author: avettora@unav.es

Ochratoxin A (OTA) is a mycotoxin that contaminates several food commodities such as cereals, nuts and spices. It is considered a potent renal carcinogen in rats but its mechanism of action is still not understood. Moreover, a high male susceptibility to tumor formation has been demonstrated in rats.

Different susceptibilities to OTA toxicity might be due to variations in transport mechanisms in kidney cells. Therefore, the aim of this project was to analyze, by RT-qPCR, the sex-dependent gene expression response of the main renal proteins (Oat, Abc, Oatp and Pept families) that might be involved in OTA transport in F344 rats. Firstly, sex differences were studied in control animals (NaHCO3 0.1M pH 7.4). Then, temporal gene expression profiles (24h, 48h, 72h, 96h, 1 and 2 months) were studied after single (0.5 mg/kg bw) and repeated oral administrations (0.5 mg/kg bw/day for 7 and 21 days). OTA concentrations in plasma and kidneys were measured in all experiments.

At basal level, Oat2 and Oat5 showed female-predominant expression while Oat1, Bcrp, Oatp1 demonstrated a higher expression in male F344 rats.

After single OTA administration, females showed a general decrease in all transporters studied, mainly after 48 hours. In males, early expression changes were observed; with an increase in Oat2 expression and a decrease of Mrp2 and Bcrp. The highest sex differences involved Oat transporters: Oat2, Oat3 and Oat5 significantly increased in males while Oat1, Oat2 and Oat5 decreased in females.

After OTA repeated administrations, both sexes presented a gene expression downregulation of Oats and Pept2. This response appeared earlier in females than in males. In females, Oat3 did not show any change at any time-point and all the transporters of the Abc family tended to be upregulated, especially Mrp2. Males also showed an upregulation of Mrp2 but Bcrp and Oatp1 were downregulated.

The results will be discussed in relation to OTA plasmatic and kidney concentrations and the transport direction of each kidney transporter. These molecular changes might be implicated in the highest male susceptibility to OTA renal carcinogenesis.

This work was supported by the University of Navarra through the PIUNA Project ¨Efecto cancerígeno de la ocratoxina A: influencia del sexo en el mecanismo de acción". Laura Pastor thanks the "Asociación de Amigos" (University of Navarra) for the pre-doctoral grant received.

L25

Impact of dietary deoxynivalenol and its decontamination with sodium sulfite (SoS) on peripheral blood mononuclear cells (PBMC) ex vivo in a porcine LPS-challenge model

Anh Tuan Tran[1]*, Gina Pistol[2], Marleen Paulick[1], Jeannette Kluess[1], Jana Frahm[1], Sven Dänicke[1]

[1]Friedrich Loeffler Institut, Braunschweig, Germany; [2]Institute for Animal Biology and Nutrition, Ilfov, Romania

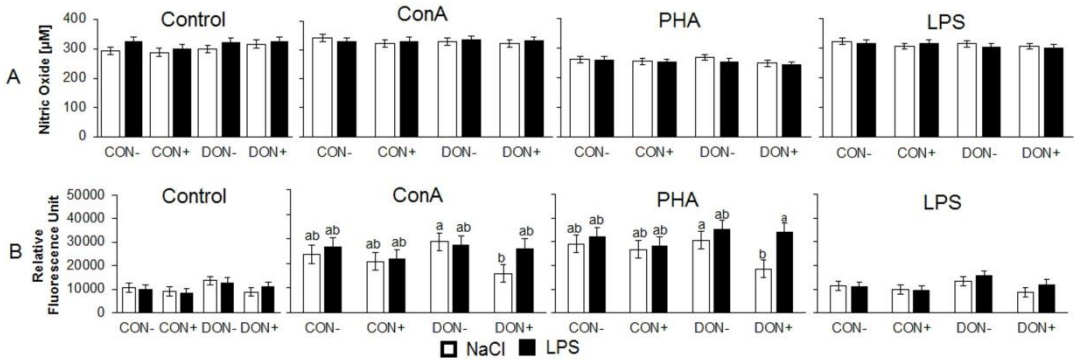
* Corresponding author: AnhTuan.Tran@fli.bund.de

We investigated the effects of feeding a DON contaminated diet, either untreated or treated with SoS on ex vivo PBMC in lipopolysaccharide (LPS) challenged and non-challenged pigs.

80 piglets (7.59±0.92kg) were equally assigned to one of four diets (35% barley, 27% wheat, 10% maize) for 37d: CON- (control diet), CON+ (maize + 5g SoS/kg), DON- (contaminated maize; 6mg DON/kg feed), and DON+ (contaminated maize + 5g SoS/kg; 0.8mg DON/kg feed). Ten pigs of each group were injected i.p. with either 7.5µg LPS/kg BW or placebo (0.9% NaCl). After 2h blood was collected and PBMC isolated on a Ficoll gradient. PBMC were cultivated (37°C, 5% CO2) and treated for 72h: control (medium RPMI 1640), ConA (12.5µg/ml concanavalin A), PHA (10µg/ml phytohaemagglutinin), and E.coli-LPS (O111:B4, 1µg/ml LPS). Supernatants were collected, proliferative capacity (Alamar Blue assay) and nitric oxide (NO) release (Griess assay) were analysed. Data were analysed with a 3-factorial ANOVA (diet, SoS, LPS) and differences (Student's t-test) were significant at $p \leq 0.05$.

Release of NO from PBMC ex vivo was neither affected by diet, SoS treatment or LPS challenge. PBMC proliferative capacity ex vivo was strongly increased by ConA and PHA, whereas LPS stimulation ex vivo hardly elicited any proliferative effect compared to control. PBMC from non-challenged pigs fed SoS-treated diets showed a significantly decreased ex vivo proliferation for ConA and PHA stimulation (DON- vs. DON+: $p<0.05$), but only a trend for control and LPS-treatment ex vivo. However, this effect was absent in PBMC of their LPS-challenged counterparts.

In vivo DON exposure alone had no impact on ex vivo PBMCs, but SoS treatment of DON-diets depressed the proliferative capacity of PBMC (ConA and PHA stimulation) from non-challenged pigs. However, this SoS-effect was annulled in their LPS-stimulated counterparts and was also absent in PBMCs from both control-fed pigs. Further analyses elucidating the observed effects are in progress.



Experimental results obtained from Griess (panel A) and Alamar Blue assays (panel B). Differences (Lsmeans+SEM) were considered significant at p≤0.05 (letters)

L26

Zearalenone-14-glucoside: a fleeting phase-II plant metabolites for mammals?

Luca Dellafiora, Gianni Galaverna, Chiara Dall'Asta*

Department of Food Science, University of Parma, Parma, Italy

* Corresponding author: chiara.dallasta@unipr.it

Zearalenone (ZEN) is a well-known foodborne mycotoxin produced by several species of Fusarium fungi that can be found as contaminant primarily in small-grain cereals, maize and derived products thereof. ZEN raises concern for mammals due to the xenoestrogenic activity via the binding and activation of the estrogen receptors (ERs). In plant it can be converted to the glycoconjugate ZEN-14-glucoside (ZEN14Glc) upon phase-II metabolism. Even though it is not still regulated, it can be compartmented in the edible parts, thereby entering the food and feed production chains. Whilst the hazard for health is under debate, the mainstream tends to consider the glycosylation an inactivating modification as the binding to the ERs is prevented in vitro. Nevertheless, our results clearly showed that ZEN14Glc had relevant xenoestrogenic activity – and not citotoxic effects – in terms of activation of ERs in estrogen-sensitive cancer cells, while the binding in vitro was drastically reduced. The hydrolysis to ZEN was proposed as underling mechanism. In addition, we demonstrated that ZEN14Glc can be converted to ZEN not only by cell cultures but also by plasma proteins (e.g. serum albumin) via (pseudo)enzymatic mechanism. An integrated in silico/in vitro approach provided structural basis of both xenoestrogenic behavior and hydrolysis phenomenon by proteins. Overall, our results strongly suggests the inclusion of ZEN14Glc in further and more detailed risk assessment study, also on the account of the possible (pseudo)enzymatic reversion to ZEN in plasma and the consequent interaction with plasma proteins. Accordingly, implications for human health might be questioned.

L27

Physiological costs of zearalenone (ZEN) exposure in carp (Cyprinus carpio L.)

Pietsch Constanze*

Zürcher Hochschule für Angewandte Wissenschaften, Wädenswil, Schweiz

* Corresponding author: pies@zhaw.ch

The occurrence of mycotoxins in fish nutrition increasingly gains attention. One of the most important contaminants in fish feeds is zearalenone (ZEN). However, systemic effects on fish and possible metabolic costs have not yet been investigated. In order to fill this gap a feeding trial with juvenile carp was conducted. The fish were fed ZEN-contaminated diets at three concentrations for four weeks. Possible reversible effects of ZEN were evaluated by feeding an additional group with the mycotoxin for four weeks period and the uncontaminated diet for further two weeks. Possible ZEN effects on kidney, spleen, liver and muscle tissue were investigated to get an organism-wide aspect of ZEN effects. Most organs appeared to (over)compensate ZEN effects during the exposure to this mycotoxin, which caused metabolic costs. Oxygen consumption increased in fish treated with the two higher ZEN concentrations via the diet. The differences between the treatments persisted also after the recovery phase of two weeks. Thus, the present study provided evidence of effects of ZEN on enzyme activities and lipid peroxidation in organs, immune parameters and metabolic oxygen demand. This is the first evidence for increased metabolic costs in a fish species due to exposure to the mycotoxin ZEN. Consequently, these results raise concern about welfare of fish raised in aquaculture.

L28

Effects of a lipopolysaccharide (LPS) stimulus on the kinetics of deoxynivalenol (DON) in pigs chronically exposed to dietary DON.

Erik Bannert[1], Tanja Tesch[1], Jeannette Kluess[1]*, Jana Frahm[1], Susanne Kersten[1], Stefan Kahlert[2], Lydia Renner[2], Hermann-Josef Rothkötter[2], Sven Dänicke[1]

[1]Friedrich-Loeffler Institute, Brunswick, Germany; [2]Otto-von-Guericke University, Magdeburg, Germany

* Corresponding author: Jeannette.Kluess@fli.bund.de

We investigated the kinetics of DON in endotoxemic pigs differing in site of LPS entry, either pre- or post-hepatic.

Fifteen barrows were exposed to a mycotoxin-contaminated diet (4.59 mg DON/kg) for 28d and equipped with jugular and portal catheters. Infusion of 7.5 µg LPS/kg BW or 0.9% NaCl resulted in three groups: CONjug-CONpor, CONjug-LPSpor, LPSjug-CONpor. Blood samples were taken at −30, +15, +30, +45, +60, +75, +90, +120, +150 and +180 min relative to infusion start, pigs were sacrificed at 195 min and liver, bile, urine and liquor cerebrospinalis collected. Samples were analyzed for DON and its metabolite de-epoxy-DON (de-DON) simultaneously by in-house-validated LC-MS/MS (Agilent Technologies, Böblingen, Germany). Samples were either treated or non-treated with β-glucuronidase in order to establish free, total and conjugated DON/de-DON in matrices. Plasma data were evaluated using PROC MIXED (SAS 6.1, Cary, USA) with group, catheter and time as main factors and their interactions. Data of bile, urine, liquor and liver were evaluated using non-parametric procedures (StatSoft Inc., Tulsa, USA).

DON and de-DON were detected in all matrices (except liquor, no de-DON) in descending order: urine > bile > liver > plasma > liquor. Post prandial rise of DON (+15 to +60 min) was higher in portal than in jugular blood ($p \leq 0.05$) and decreased from +60min significantly in both LPS-infused groups ($p \leq 0.05$). This LPS-impact was also detected in liver tissue ($p \leq 0.05$) and in particular the hepatic DON conjugation was dramatically lower in LPS treated animals ($p \leq 0.05$). Although the effect of LPS-infusion site was not always clear, DON levels in liquor tended to be lower in LPSjug-CONpor ($p = 0.09$) compared to the other two groups. Conjugated DON in bile tended to be higher in jugular LPS-groups compared to portal-infused pigs ($p = 0.06$). Overall, DON kinetics was altered in endotoxaemic pigs while site of LPS-infusion only marginally influenced liquor and bile toxin residue levels.

L29

Deoxynivalenol-3-β-D-glucoside: In Vitro Cytotoxicity and In Vivo Oral Bioavailability and Hydrolysis in Broiler Chicken and Pig

Nathan Broekaert[1]*, Mathias Devreese[1], Thomas van Bergen[1], Stijn Schauvliege[1], Marthe De Boevre[1], Sarah De Saeger[1], Lynn Vanhaecke[1], Franz Berthiller[2], Herbert Michlmayr[2], Alexandra Malachová[2], Gerhard Adam[2], Kristel Demeyere[1], Evelyn Meyer[1], An Vermeulen[1], Siska Croubels[1]

[1]Ghent University, Ghent, Belgium; [2]University of Natural Resources and Life Sciences, Vienna, Austria

* Corresponding author: nathan.broekaert@ugent.be

In addition to DON, cereals are often co-contaminated with modified forms of DON, such as deoxynivalenol-3-β-D-glucoside (DON3G), 3-acetyl-deoxynivalenol (3ADON) or 15-acetyl-deoxynivalenol (15ADON). Due to the lack of information on toxicity and bioavailability of modified mycotoxins, current risk assessment assumes that these modified forms are equally toxic to their respective unmodified counterparts.

The goal of the in vitro study was to determine the intrinsic cytotoxicity of modified DON forms towards porcine intestinal epithelial cells by means of a flow cytometric technique. Quantification of viable cells, apoptotic and necrotic cells indicate following toxicity ranking: DON3G << 3ADON < DON ≈ 15ADON.

For the in vivo part, cross-over animal trials were performed with intravenous and oral administration of DON3G and DON to broiler chickens and pigs. Systemic and portal plasma concentrations of DON and DON3G were quantified using liquid chromatography-tandem mass spectrometry. Liquid chromatography coupled to high-resolution mass spectrometry was used to unravel phase II metabolism of DON. Data were processed via tailor-made compartmental toxicokinetic models.

The results in broiler chickens indicate that DON3G is not hydrolysed to DON in vivo. Furthermore, the absolute oral bioavailability of DON3G in broiler chickens was low (3.79±2.68%) and comparable to that of DON (5.56±2.05%). After oral DON3G administration to pigs, only DON was detected in plasma, indicating a complete presystemic hydrolysis. However, the absorbed fraction of DON3G, recovered as DON, was approximately 5 times lower than after oral DON administration, 16.1±5.4% compared to 81.3±17.4%. Additionally, analysis of phase II metabolites revealed that biotransformation of DON in pigs mainly consists of limited glucuronidation, whereas in chickens extensive conjugation with sulfate occurs, these observations might explain the differences in sensitivity of these species to DON.

Although in vitro studies demonstrate a decreased toxicity of DON3G compared to DON, the species dependent toxicokinetic data and in vivo hydrolysis to DON illustrate the toxicological relevance of DON3G.

This research was funded by a Ghent University Special Research Fund grant (No. I/00105/01), the Vienna Science and Technology Fund (WWTF LS12-012), the Austrian Science Fund (FWF) (SFB F3708), the Austrian Federal Ministry of Science, Research and Economy and the Austrian FTE foundation.

L30

Ingestion of feed contaminated with ergot alkaloids induces morphological and transcriptomic changes in the intestine of piglets

Viviane Mayumi Maruo[1]*, Philippe Pinton[2], Ana Paula Bracarense[3], Jean-Paul Métayer[4], Isabelle P. Oswald[2]

[1]Universidade Federal do Tocantins, Araguaína, Brazil; [2]INRA, Toxalim UMR 1331, Toulouse, France; [3]Universidade Estadual de Londrina, Londrina, Brazil; [4]ARVALIS - Institut du végétal, Villerable, France

* Corresponding author: vivimaruo@uft.edu.br

Ergot alkaloids (EAs) are mycotoxins mainly produced by Claviceps fungi. They caused numerous poisonings characterized by gangrene and convulsions, a condition known as ergotism. Acute poisoning is rare but EAs are still of concern due to the increase of their occurrence in the last years. Few studies have investigated the toxicity of EAs in pigs. Swine, apart from its importance as livestock, is also a choice species as biomedical model for human health and disease. Most important findings in pigs exposed to EAs include reduction in feed intake and body gain, damage on liver function, agalactia and reduced weight gain of litters. As for other food contaminants, the intestine is of particular importance since it is the first site of absorption of toxins and a protection barrier preventing the entry of antigens.

The present study investigated in pigs, the effects of EA-contaminated feed on intestine morphology, histology and cytokines and junction proteins gene expression. Sclerots from naturally contaminated wheat were mixed to regular diets at different levels: 0, 1.2 g/kg and 2.5 g/kg corresponding to 0, 2.1 and 5 mg of EAs/kg, respectively. Thirty six castrated male crossbreed pigs 34 days old were assigned to the 3 groups and received experimental diets during 28 days. Weight and feed consumption were measured at day 0, 14 and 28. At the end of the experiment, 6 males of each group were euthanized and the jejunum was sampled for histology or gene expression analysis. Daily feed intake of animals consuming the high ergot diet was reduced by 20 % and their weight gain was lower in the first 14 days of treatment, compared to control group. In both ergot diets groups, morphometrical analysis revealed a significant decrease of jejunal villi height (-22% and -19%) and jejunal Peyer's patches (-29% and -29%) compared to control. The jejunal lesion score from high ergot group was 240% higher than in control group and the analysis revealed edema of lamina propria and cytoplasmic vacuolization of enterocytes. The number of goblet cells was reduced by 34% in both ergot diets groups, compared to control. The expression of junction proteins mRNA in the jejunum showed a tendency towards increase that might reflect an attempt of the tissue to reestablish its function. The jejunum profile of TLR 4, NFkB, IL-6, IL-8 and TNFa mRNA showed a dose-response downregulation. In conclusion, sub-chronic ingestion of EAs induced tissue lesions and altered different functional capacities of the intestine and may thus increase the susceptibility of animals to enteric infectious diseases.

V.M. Maruo was supported by a fellowship from Conselho Nacional de Pesquisa e Desenvolvimento, CNPq,Brazil.
The authors thank the technicians who carried out the animal trial at the experimental station of ARVALIS – Institut du végétal (France)

L31

The pig intestinal explants: an original model for the analysis of the effects of mycotoxins

Philippe Pinton, Alix Pierron, Delphine Payros, Joelle Laffitte, Anne Marie Cossalter, Yannick Lippi, Pascal Gourbeyre, Isabellle.P Oswald*

INRA, Toxalim, Toulouse, France

* Corresponding author: isabelle.oswald@toulouse.inra.fr

The intestine, the major site for nutrient's absorption is the first barrier following the ingestion of food contaminants. Mycotoxins are secondary metabolites produced by fungi and contaminating cereals. Pig is exposed to contaminated feed and is very sensitive to deoxynivalenol (DON), a toxin produced by Fusarium. To study the effects of DON on the pig intestine, we developed a model of intestinal explants.

From a segment of pig intestine, explants of 6 mm diameter were prepared and exposed between 1 to 12 h to the toxin in cell culture plates. Stored in fixative solution or frozen, the explants were then used for histological study and for studying the genes or proteins expression. We demonstrated that DON (i) alters the morphology of the intestinal epithelium, ii) modulates, depending on time and along the proximo-distal axis of the intestine, the expression of genes coding for pro-inflammatory cytokines (iii) activates the Mitogen-Activated Protein Kinases signaling pathway. These data are correlated with in vivo and in vitro experiments.

The intestinal explants allow testing simultaneously dozens of experimental conditions with histological, biochemical and molecular approaches. They are a complementary approach to the in vivo experiments and to cell culture models and an original tool for studying the effects on the intestine of food contaminants, additives or food components. At the same time, they represent an alternative in the requirements of reduction, refinement and replacement for laboratory animal based research.

L32

Influence of mycotoxin binders on the oral bioavailability of doxycycline in pigs

Thomas De Mil[1], Mathias Devreese[1]*, Sarah De Saeger[2], Mia Eeckhout[3], Siska Croubels[1]

[1]Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium; [2]Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium; [3]Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

* Corresponding author: mathias.devreese@ugent.be

Mycotoxin binders are feed additives that aim to adsorb mycotoxins in the gastro-intestinal tract (GIT) of animals, making them unavailable for systemic absorption. The antimicrobial drug doxycycline (DOX) is often used in pigs and is administered through feed or drinking water, hence DOX can come in contact with mycotoxin binders in the GIT. This paper describes the effect of four mycotoxin binders on the absorption of DOX in pigs.

In the first experiment, thirty healthy pigs were randomly allocated to one control group and four test groups (Clay 1 to 3 and Yeast 1 group, n=6/group) and a two-way cross-over design was applied. The animals received a gastric bolus administration of one of the four mycotoxin binders (Clay 1 – 3, Yeast 1), dispersed in tap water. The control group received only tap water. Immediately after the mycotoxin binder (or water), a single dose of Doxylin® was administered to all groups. The dose of mycotoxin binders corresponded with the daily intake and was estimated using a 2 g/kg inclusion rate (low exposure) or 10 g/kg inclusion rate (high exposure). The dose of Doxylin® was 10 mg DOX/kg BW. Blood samples were collected and doxycycline was quantified by LC-MS/MS.

In the second experiment, the field conditions were simulated. Thirty other healthy pigs were allocated to the same control and test groups as described above. For the test groups, feed was supplemented with one of the four mycotoxin binders at an inclusion rate of 2 g/kg feed. The feed of the control group contained no mycotoxin binder. This feed regime was maintained for two weeks, and after this period all animals received feed medicated with Doxylin® (270 mg DOX/kg feed). Blood samples were taken daily for 5 consecutive days and analyzed by LC-MS/MS.

Results indicated that interactions are possible between some of the mycotoxin binders dosed at 10 g/kg feed but not at 2 g/kg feed. When applying field conditions, no influences were seen on the plasma concentrations of DOX.

L33

Disposition of enniatin B and B1 in broiler chickens: oral bioavailability and toxicokinetics

Sophie Fraeyman[1]*, Mathias Devreese[1], Gunther Antonissen[1], Siegrid De Baere[1], Michael Rychlik[2], Siska Croubels[1]

[1]Ghent University, Merelbeke, Belgium; [2]Technische Universität München, Freising, Germany

* Corresponding author: sophie.fraeyman@ugent.be

The emerging Fusarium toxins enniatin B (ENN B) and enniatin B1 (ENN B1) are frequently encountered feed contaminants. Seventy-eight and 67% of the poultry feed samples analysed by Mendes de Souza et al. (2013) were contaminated with ENN B and B1 respectively, with maximum levels up to 4.6 µg/kg ENN B and 12 µg/kg ENN B1[1].

Notwithstanding their high prevalence, little is known about the possible effects of ENNs on poultry health. To elucidate the absorption, distribution, metabolism and excretion (ADME) processes of these mycotoxins in broiler chickens, a toxicokinetic study was performed. Each animal received 0.2 mg ENN B or B1/kg body weight via an intracrop bolus and a single intravenous injection in a two-way cross-over design. Plasma concentrations were determined with an in-house developed and validated LC-MS/MS method. Moreover, plasma was screened for main phase I and II metabolites using UHPLC-HR-MS. The absolute oral bioavailability (F) and main toxicokinetic characteristics were calculated using non-compartmental analysis.

Preliminary results demonstrated that ENN B and ENN B1 are poorly absorbed after oral administration in broiler chickens, with an F < 15 and 10%, respectively. The preliminary volume of distribution (Vd) was approximately 30 and 20 L/kg for ENN B and ENN B1, respectively. Furthermore, results showed a similar clearance for both ENN B and B1, i.e. approximately 7 L/h/kg.

1. Mendes de Souza et al. Sci World J. 2013, DOI: 10.1155/2013/427369.

L34

Use of a large-scale qPCR approach to understand the anti-aflatoxigenic effect of Eugenol

Isaura Caceres *, Rhoda El Khoury , Isabelle Oswald , Olivier Puel , Jean Denis Bailly

INRA, UMR 1331 Toxalim, Research Center in Food Toxicology, Toulouse , France

* Corresponding author: isauracaceres@hotmail.com

Produced by several species of Aspergillus, Aflatoxin B1 (AFB1) is considered as one of the most dangerous mycotoxins as being carcinogenic for humans and animals. Therefore, it is essential to avoid its presence in food and for that, several methods have been developed. Utilization of fungicides is nowadays one of the most common methods; nevertheless, their use is not environmental or economically sound. Thus, as an alternative strategy, the use of natural compounds capable of inhibiting fungal toxin production is investigated. Indeed, several natural extracts such as plant extracts, essential oils and spices have shown interesting anti-aflatoxinogenic effects. Among them, eugenol was identified as able to inhibit the AFB1 production in some Aspergillii. However, its mechanism of action is yet to be elucidated. Production of AFB1 is associated with the expression of a 70 kB cluster and not less than 21 enzymatic reactions are necessary for its production. Additionally, several external factors interfere with the synthesis process of this toxin. Latter's can be classified in different families such as: Velvet complex, RAS family, genes involved in oxidative stress response, transcription and environmental factors, G-protein receptors, etc. According to this, a molecular tool composed of 62 genes, including the entire gene cluster involved in the production of AFB1 was developed in order to understand the mechanism of action of eugenol. We demonstrated that 0.5 mM of Eugenol in MEA culture medium totally inhibits the production of AFB1 by Aspergillus flavus without a significant fungal growth modulation. We have also confirmed that the presence of eugenol induces a transcriptional inhibition of the cluster and modulates the expression of certain global regulation factors as VeA, MtfA and MsnA among others.

The authors would like to thank the Consejo Nacional de Ciencia y Tecnología (CONACYT) Mexico, funding Isaura Caceres. This work was financed by the projects Aflafree (ANR-11-ALID-0003), Aflared (joint project, 001-2012 STDF-AIRD), and ToxinFree (Campus France PHC cèdre 32763).

L35

Oosporein - mycotoxin or colonization-facilitating antioxidant?

Wuttiwat Jitjak[1], Franz Hadacek[2]*, Vladimir Chobot[3], Julian Dopstadt[4], Hans-Ulrich Humpf[4], Petr Karlovsky[2], Niwat Sanoamuang[5]

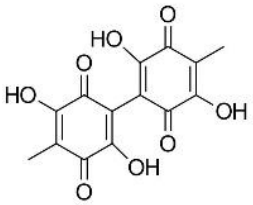
[1]International College, Khon Kaen University, Khon Kaen, Thailand; [2]Molecular Phytopathology and Mycotoxon Research, Göttingen, Germany; [3]Molecular Systems Biology, University of Vienna, Vienna, Austria; [4]Institut für Lebensmittelchemie, Münster, Germany; [5]Plant Sciences and Agricultural Resources, Khon Kaen, Thailand

* Corresponding author: fhadace@uni-goettingen.de

Oosporein is a red symmetrical 1, 4-bibenzoquinone derivative and has been identified as metabolite of various ascomycete and basidiomycete fungi, most notably of the ascomycete genus Beauveria, especially in the species B. bassiana and B. brongniartii. Various toxic properties have been ascribed to this mycotoxin, the insecticidal being the most notable; a special focus was on cockchafer control. Various studies, however, support the notion that oosporein facilitates more the fungal colonization of the insect than its killing. A potential target protein of oosporein is still unknown.

Gelatinomyces siamensis is an ascomycete fungus that produces notable, dark red pigmented fruit bodies on bamboo twigs and occurs in the Nam Nao national park in Petchaboon, Thailand. When cultured in vitro, it produces a red pigment that turned out to be oosporein. This was confirmed by comparing the preparatively isolated compound with published UV, MS and NMR data.

A pre-screening of the unidentified compound especially pointed out strong antioxidant (DPPH assay), but less toxic activity. A more detailed assessment of redox activity revealed that oosporein, albeit possessing redox cycling properties (square wave voltammetry), lacked the pro-oxidative activity that characterizes many toxic quinones. Contrary, it showed strong antioxidant activity (deoxyribose degradation assay) that compares to and even excels that of many well-known antioxidants occurring in plant fruits. These results suggest that potentially pathogenic fungi not only may profit from metabolites with toxic activity but also from such with antioxidant activity, the latter of which can help by assisting the infection process. In congruence with its observed occurrence in fruiting bodies of a bamboo endophyte, no pronounced phytotoxic activities could be demonstrated for oosporein so far. Health-harming and protecting activities can often be caused by the the identical compound, as it is known for many plant toxins and drugs, their actual activity depending on their concentration and the quality of their chemical environment.



Oosporein, a bibenzoquinone mycotoxin

Hussam Ibrahim Aroud (Göttingen) helped with preparative chromatography, Katharina Pfohl (Göttingen) with LC-MS, and Lenka Kubicova (Vienna) with antioxidant assays. W.J. acknowledges financial support from Erasmus Mundus Action 2.

L36

Investigations on the influence of mycotoxins on biogas production

Katrin Harms[1]*, Johannes Ostertag[2], Michael Lebuhn[2], Bernhard Munk[2], Mathias Hartel[2], Fabian Lichti[2], Karsten Meyer[1]

[1]Lehrstuhl für Tierhygiene, TUM, Freising, Germany; [2]Bavarian State Research Center for Agriculture LfL, Freising, Germany

* Corresponding author: Katrin.Harms@wzw.tum.de

Maize and grass silages are often used as substrate in agricultural biogas production. A contamination of silages with toxinogenic moulds such as Penicillium, Monascus or Aspergillus species is a common problem. In addition to the loss of energy of the substrate caused by microbial respiration, the occurrence of mycotoxins may influence biogas production.

Preliminary investigations on adverse effects and the stability of single mycotoxins in the biogas fermentation process have been conducted in batch trials. The fermentation substrate was donated with solutions of common feed contaminants like Fusarium toxins and aflatoxin B1, but also typical silage toxins like mycophenolic acid, roquefortine C or monacolin K. The biogas yield was measured continuously. After 25 days the digestates were screened for mycotoxin residues by LC-MS/MS. In these batch trials, only the addition of mycophenolic acid (50 µg/g) resulted in reduced gas yields. However, a remarkable reduction of the measurable amounts of the added toxins in the digestates (up to 100%) was observed.

In further investigations with larger scaled continuous flow digesters artificially moulded silages were used. These silages were inoculated with either Monascus ruber or Pencillium roqueforti strains as dominant fungal species. Toxin analysis of the silages contaminated with Monascus ruber revealed the presence of large amounts of monacolin K, but also fumigaclavin C, gliotoxin, trypacidin, fumagillin and fumonisin were detected. The silage inoculated with Penicillium roqueforti contained predominantly roquefortine C.

Both biogas processes with substrates moulded by Monascus ruber broke down after 70 days, the Penicillium-contaminated digesters were disturbed a few days later, while the gas yield of the control fermenter fed with silage of good quality remained stable. The reduced methane quota obtained in all experiments using mouldy silages may be caused by increased respiration during storage or inhibitory activities of mycotoxins. The digestates showed a remarkable accumulation of monacolin K and roquefortine C, respectively.

The study is performed in cooperation with the Bavarian State Research Center for Agriculture (LfL), Department for Quality Assurance and Analytics, the Institute for Animal Nutrition and Feed Management and the Institute for Agricultural Engineering and Animal Husbandry; and is financially supported by the grant of the Bavarian Ministry of Economic Affairs and Media, Energy and Technology.

SAFE SOLUTIONS
TOTAL NUTRITION

MYCOTOXINS STICK TO TOXFIN™

Kemin's TOXFIN is the duct tape of toxin binders: it sticks to even the trickiest of mycotoxins throughout the gastrointestinal tract, preventing mycotoxins from entering the blood stream of the animal, while leaving behind the beneficial nutrients.

TOXFIN offers the most complete protection for your animals, using super-efficient, innovative and carefully selected adsorbents. Stick to TOXFIN and get optimal health and performance of your animals.

Remove unwanted toxins with TOXFIN.

www.kemin.com

Certain statements may not be applicable in all geographical regions. Product labeling and associated claims may differ based upon government requirements.

KEMIN® INSPIRED MOLECULAR SOLUTIONS™

BIOMIN Research & Development

Turning science into sustainable solutions

Our strong in-house R&D and global cooperation with leading institutions form the basis by which innovative solutions are developed for our customers.

research.biomin.net

Naturally ahead

Biomin®

Abstracts of posters

P1

Determination of aflatoxin M1 in raw milk samples from Assiut city, Egypt

Mohamed Fathi Abdallah[1,2]*, Bilge Kilicarslan[1], Gözde Girgin[1], Terken Baydar[1]

[1]Department of Toxicology, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey;

[2]Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt

* Corresponding author: mohamed.fathi@hacettepe.edu.tr

Aflatoxins are one of the most significant food contaminants produced mainly by Aspergillus flavus and Aspergillus parasiticus. Aflatoxin M1 (AMF1), the hydrolyzed form and the main metabolite of Aflatoxin B1, is secreted into milk of dairy animals which are consuming aflatoxins contaminated feedstuffs.

Searching through the internet database, only general screening methods such as ELISA have been used for detection of the mycotoxin in that area of Upper Egypt. Therefore, the aim of the study was to evaluate the occurrence of AFM1 in raw milk samples marketed in Assiut city.

A total of 20 raw milk samples were purchased from local shops in Assiut, Upper Egypt. Skimmed milk samples, obtained from raw milk by centrifugation, were passed through Afla M1 immunoaffinity column for clean-up. AFM1 separation and detection was performed by using HPLC-FLD system. The limits of detection (LOD) and quantitation (LOQ) for AFM1 were 0.008 µg/kg and 0.02 µg/kg, respectively.

The results showed that AFM1 is detected in all examined milk samples (100%) and 14 samples (70%) were above the maximum permissible level in the European Union (0.05 µg/kg). The concentrations ranged from 0.02µg/kg to 0.19 µg/kg except only one sample was under limit of quantification. In conclusion, more attention and strict regulations are highly recommended in order to control dairy milk contamination with mycotoxins.

This work is financially supported by Hacettepe University Scientific Research Projects Coordination Unit (#014 D06 301 002-620).

P2

Mini-survey of fungal and bacterial metabolites in dried dates palm from Egypt using LC-MS/MS

Mohamed Fathi Abdallah[1,2]*, Rudolf Krska[1], Michael Sulyok [1]

[1]Center for Analytical Chemistry, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences Vienna (BOKU), Tulln, Austria; [2]Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt;

*Corresponding author: mohamed.fathi@vet.au.edu.eg

Date palm is a seasonal plant growing mainly in the Arabian Gulf and Middle East countries. Dates are an integral part of Arabian diet and known as a good source of energy. Egypt is the major producer of dates with 1470000 MT followed by Iran and Saudi Arabia (1). Investigations on the occurrence of mycotoxins in dried fruits including dates have been mostly on aflatoxins and ochratoxin A so far. Furthermore, data concerning these threating contaminants in dates from Egyptian markets are so limited and not up to date. However, modern multi-toxin LC-MS/MS methods aim at the quantification of a wide range of mycotoxins in a single extract. The performed survey aimed to determine the co-occurrence of a wide range of mycotoxins in dried dates distributed in different markets at Assiut Governorate, Egypt using LC-MS/MS. The samples were analysed using dilute and shoot approach and LC-MS/MS (5500 QTrap) as described before in the literature (2).

Overall, 30 different metabolites have been quantified in 28 dates samples collected during the last three months of 2015. Aflatoxin and ochratoxin A were detected in only one and three samples, respectively. The overall metabolite pattern indicates the prevalence of Aspergillus niger, as malformins were found in 71% of the samples. In addition, several unspecific metabolites such as physcion, asperglaucide, asperphenamate, emodin and kojic acid were frequently detected.

In conclusion, large scale surveys in dates and dates products are required in order to further broaden the knowledge on the natural occurrence and co-occurrence of mycotoxins. Also, good management and proper storage conditions should be applied to minimize the chance for the storage fungi growth and mycotoxin production.

References:

(1) FAO. (2012). http://faostat.fao.org/site/339/default.aspx

(2) Malachová A, Sulyok M, Beltrán E, Berthiller F, Krska R. Optimization and validation of a quantitative liquid chromatography-tandem mass spectrometric method covering 295 bacterial and fungal metabolites including all regulated mycotoxins in four model food matrices. J Chromatogr A. 1362, 145-156.

P3

Innovative approaches for risk-oriented global supply chain analysis

Elisabeth Achten, Husam Ibrahem-Aroud, Milena Zupaniec, Janet Riedl, Helmut Schafft, Monika Lahrssen-Wiederholt

Federal Institute for Risk Assessment, Berlin, Germany

Corresponding authors: Elisabeth.Achten@bfr.bund.de, Husam.Ibrahem-Aroud@bfr.bund.de, Milena.Zupaniec@bfr.bund.de

The increasing complexity and globalization of supply chains create challenges with respect to food safety and the related risk assessment. International foodborne disease outbreaks in the recent past have demonstrated the lack or the limited availability of data and information required for precise risk assessment. Within this respect three projects, comprising software models as well as analytical strategies, have recently started at the BfR that aim to increase supply chain integrity.

One project aims to develop interactive models/software systems, which may serve as risk assessment tools for complex issues and improve the predictability of emerging or potential food risks. For demonstration purposes we have chosen maize and its contamination with mycotoxins to develop the approach “Global supply chain analysis” consisting of the visualization of the global maize supply chains and a hazard analysis in terms of probability calculations of the occurrence of mycotoxins in each step of the maize supply chain.

The second project aims to provide reliable data to the software system by developing an analytical method for the determination of mycotoxins in foodstuff. In this project, a fast, reliable liquid chromatography/tandem mass spectrometric multi-mycotoxin method will be developed for the determination of mycotoxins in fruit juices, especially in apple and orange juices due to their high consumption. We aim to optimize this method for different juice matrixes and apply it to detect the occurrence or the degradation of mycotoxins in each step of the juice production. These data could also be used for the improvement of the risk assessment and could subsequently help to minimize the amount of these undesired substances in the final product.

The third project deals with the geographical origin authentication of agricultural commodities, in particular with the “authentication of maize as feed and food material”. The geographical origin is typically mentioned in the declaration and shipping documents of a product. In terms of proactive hazard identification it is preferable to verify the authenticity on the product itself. Therefore, several chemical methods will be investigated to characterize samples of grain maize, such as infrared and nuclear magnetic resonance spectroscopy as well as isotope ratio mass spectrometry and elemental analysis. Statistical interpretation of the resulting data will lead to classification models which can distinguish samples according to their geographical origin. To create models for different spatial levels (global, European, national) authentic samples with confirmed geographical origin are needed.

P4

Determination of aflatoxin M1 by a sensitive HPLC-FD method in urines from two Bangladeshi cohorts reveals frequent dietary exposure to aflatoxin B1

Nurshad Ali, Meinolf Blaszkewicz, Wolfram Föllmann, Gisela H. Degen*

Leibniz-Institut für Arbeitsforschung (IfADo), Dortmund, Germany

* Corresponding author: degen@ifado.de

Aflatoxin B1 (AFB1), a potent hepatocarcinogen and the most toxic mycotoxin known, can contaminate various food commodities, especially in hot and humid climates that favour the growth of aflatoxin-producing fungi. As data on the presence of AFB1 in food and feed in Bangladesh are insufficient to assess human exposure, biomonitoring was used in a preliminary study where the occurrence of metabolite aflatoxin M1 (AFM1) in urines as suitable biomarker of AFB1 exposure has been screened by ELISA (Ali N et al. Arch Toxicol, in press, DOI 10.1007/s00204-015-1601y). These initial data led us to conduct a follow-up study where we applied a more sensitive method (IAC clean-up of urines and HPLC-FD) to measure the AFM1 concentration in urines of adult Bangladeshi cohorts. To account for possible seasonal and regional differences in mycotoxin exposure, in total 164 urines were collected (n=69 in summer, n=95 in winter) from residents of a rural and an urban area, among which there were 62 participants enrolled in both sampling periods. AFM1 was detected in 40% of all urine samples at a range of 1.7–104 pg/mL in summer and in 42% of samples at a range of 1.8–190 pg/mL in winter season. The mean level of urinary AFM1 was higher in winter (27.7±42.6 pg/mL) than in summer (13.6±21.2 pg/mL) season, and a significant difference was observed at the mean AFM1 level between the rural (40.4±48.3 pg/mL) and the urban (3.6±1.2 pg/mL) cohort. Urinary AFM1 levels did not show significant associations between consumption of certain types of food by the participants and their biomarker levels, except a positive trend for higher rice consumption in winter season.

The frequent detection of AFM1 in urines indicates widespread dietary AFB1 exposure of the cohorts that varies by season and region. Further biomarker-based studies in children and cohorts from other parts of the country are indicated to gain more insight into AFB1 exposure in the population of Bangladesh. This is warranted in light of rather high urinary AFM1 concentrations when compared to such data from other countries.

Acknowledgement: Nurshad Ali is grateful for a PhD stipend from the Deutsche Akademische Auslandsdienst (DAAD).

P5

Natural occurrence of Deoxynivalenol in feedstuffs and its risk assessment for livestock health

Taekyung An[1], SeungRan Hong[1], Han Sub Jang[2], Chan Lee[1]*

[1]Brain Korea 21 PLUS Project for Department of Food Science and Technology, Chung-Ang University, Anseong, Republic of Korea; [2]National Agricultural Products Quality Management Service, Gimcheon, Republic of Korea

* Corresponding author: chanlee@cau.ac.kr

Deoxynivalenol (DON) is one of mycotoxins contaminated in cereal-based foods such as barley, rice, corn, wheat, oats and other grains in the field or during storage. Especially, DON affects livestock health by causing acute vomiting, diarrhea, feeds intake refusal, fever, reduced body weight gain and even death. So far, the permitted level of the DON in livestock has not been established in South Korea. For this reason, the contamination level of DON was monitored in 754 animal feeds samples including 578 compound feeds and 176 feeds ingredients produced in KOREA during 2009-2014. In addition, daily dietary exposure for cows, pigs, and chickens were estimated for further documentation to regulate the level of DON in feeds. For this purpose risk assessment was also conducted for animal health by comparing the estimated daily exposure levels in main livestock and with species-specific toxicity reference dose (NOAEL, LOAEL, BMDL, etc.). Detection frequency of DON in compound feeds was 98% which means most compound feeds were contaminated with DON. In case of feed ingredients, detection frequency of DON was determined as 71%. In general, poultry was exposed to the highest level of DON. The chronic and acute exposure rate were 16% and 45.9%, respectively. Pigs were exposed to the lowest level of DON. Only 6.6 % of pigs were exposed to DON and chronic and acute exposure rate for pigs was 16%. Interestingly, consequences of risk assessment showed different results compared to those of exposure assessment. The chronic and acute risk indexes were 10.7% and 30.6% for pigs and 32.4% and 30.6% for poultry, respectively.

P6

New successes in the elucidation of the Fusarium fujikuroi metabolome

Birgit Arndt[1,2], Slavica Janevska[3], Isabel Krug[1], Bettina Tudzynski[3], Hans-Ulrich Humpf[1,2]*

[1]Institute of Food Chemistry, Westfälische Wilhelms-Universität Münster, Münster, Germany; [2]NRW Graduate School of Chemistry, Westfälische Wilhelms-Universität Münster, Münster, Germany; [3]Institute of Plant Biology and Biotechnology, Westfälische Wilhelms-Universität Münster, Münster, Germany

* Corresponding author: humpf@wwu.de

Secondary metabolites of molds are important targets to discover, because of their toxic properties or their medicinal benefits, and especially so far unknown compounds should be in the main focus. Therefore the genome of the rice pathogen Fusarium fujikuroi was elucidated and potential secondary metabolism gene clusters were identified [1]. Different mutant strains were created by genetic engineering, including overexpression and deletion mutants of biosynthetic genes and/or global regulator genes.

We analyzed the metabolomes via HPLC-UV-HRMS and compared the secondary metabolite profiles with the software tool MZmine 2 [2], resulting in the identification of new or missing peaks. Several metabolites, including the products of a dimethylallyltryptophansynthase, a sesquiterpene cyclase and two polyketide synthases were isolated, followed by detailed structure elucidation by NMR and fragmentation experiments with HPLC-HRMS [3]. If possible, the isolated compounds were tested in a cytotoxicity assay using Hep G2 cells. With this procedure, known secondary metabolites as well as new, yet unknown compounds were identified, and corresponding gene clusters characterized.

Literature:

[1] P. Wiemann, C. M. K. Sieber, K. W. von Bargen, L. Studt, E.-M. Niehaus, J. J. Espino, K. Huß, C. B. Michielse, S. Albermann, D. Wagner, et al., PLoS Pathog. **2013**, 9, 1–36.

[2] T. Pluskal, S. Castillo, A. Villar-Briones, M. Oresic, BMC Bioinformatics **2010**, 11, 395–406.

[3] B. Arndt, L. Studt, P. Wiemann, H. Osmanov, K. Kleigrewe, J. Köhler, I. Krug, B. Tudzynski, H.-U. Humpf, Fungal Genet. Biol. **2015**, 84, 26–36.

Financial support by the DFG (Project HU 730/9-3) and the NRW Graduate School of Chemistry is gratefully acknowledged.

P7

The role of water activity in mycotoxin production: are results obtained with solutions of sodium chloride and glycerol reliable?

Vincent Ayugi, Katharina Pfohl, Petr Karlovsky*

Molecular Phytopathology and Mycotoxin Research Unit, Georg-August-University, Göttingen, Germany

* Corresponding author: pkarlov@gwdg.de

Fungal growth and physiology are controlled by the genotype and environmental factors, most important of which are temperature, nutrients, humidity and light. Water availability is a key factor affecting fungal growth and their mycotoxin production, with water activity (a_w) being most frequently used in studies of fungal growth and mycotoxin production.

A common strategy used in these studies is to amend the culture media with high concentrations of solutes such as sodium chloride or glycerol to adjust the water activity, thereafter monitor with commercial humidity sensors. However, these strategies are fundamentally flawed since liquid media cannot adequately substitute solid substrates such as grains or plant debris, while the solutes in the media can affect the fungal growth and mycotoxin production.

In the current study, a newly-developed system is used to determine the effect of water activity (relative humidity) and temperature on the growth and mycotoxin production of a representative fungal species (Aspergillus parasiticus) on grains without contact between solute and fungus. The fungal cultures are incubated in an atmosphere with defined humidity between substrate and air with relative humidity set to the value corresponding to desired water activity by use of vapor pressure above a saturated solution of glycerin in water.

P8

Effects of a lipopolysaccharide (LPS) stimulus on the kinetics of zearalenone (ZEN) and its metabolites in pigs chronically exposed to dietary ZEN

Erik Bannert[1], Tanja Tesch[1], Jeannette Kluess[1]*, Jana Frahm[1], Susanne Kersten[1], Stefan Kahlert[2], Lydia Renner[2], Hermann-Josef Rothkötter[2], Sven Dänicke[1]

[1]Friedrich-Loeffler Institute, Brunswick, Germany; [2]Otto-von-Guericke University, Magdeburg, Germany

* Corresponding author: Jeannette.Kluess@fli.bund.de

We investigated the kinetics of ZEN and its metabolites α-zearalenol (α-ZEL), β-ZEL, zearalanone (ZAN), α-zearalanol (α-ZAL) and β-ZAL in endotoxemic pigs differing in site of LPS entry, either pre- or post-hepatic.

Fifteen barrows were fed a mycotoxin-contaminated diet (0.22mg ZEN/kg feed) for 28d and equipped with jugular and portal catheters. Infusion of 7.5µg LPS/kg BW or control 0.9% NaCl resulted in three groups: CONjug-CONpor, CONjug-LPSpor, LPSjug-CONpor. Blood samples were taken at −30, +15, +30, +45, +60, +75, +90, +120, +150 and +180min relative to infusion start, pigs were sacrificed at 195min and liver, bile, urine and liquor cerebrospinalis collected. Samples were analyzed for ZEN and its metabolites simultaneously by in-house-validated LC-MS/MS (Agilent Technologies, Böblingen, Germany). Samples were either treated or non-treated with β-glucuronidase in order to investigate free, total and conjugated ZEN/metabolites. Plasma data were evaluated using PROC MIXED (SAS 6.1, Cary, USA) with group, catheter and time as main factors and their interactions. Data of bile, urine, liquor and liver were evaluated using non-parametric procedures (StatSoft Inc., Tulsa, USA).

ZEN, α-ZEL and β-ZEL were detected in all matrices, except in liquor and β-ZEL in plasma and other metabolites were only sporadically detected. ZEN was highest in bile and lowest in plasma (bile>urine>liver>plasma). ZEN and α-ZEL were nearly completely conjugated in all matrices. Portal and jugular plasma ZEN concentrations were significantly higher in LPS-infused animals at +120min compared to control group ($p \leq 0.05$). This effect was more pronounced in portal compared to jugular LPS-infused animals at +150min ($p \leq 0.05$) in portal blood. There were no significant effects due to LPS-entry site in the other matrices. ZEN, α-ZEL and β-ZEL levels were lower in LPS-treated pigs in liver ($p \leq 0.05$), slightly higher in bile and urine compared to control group.

P9

Application of an enzyme immunoassay for the detection of Paxilline in beer

Julia I. Bauer*, Madeleine Gross, Ewald Usleber

Justus-Liebig-University, Dairy Sciences, Giessen, Germany

* Corresponding author: julia.bauer@vetmed.uni-giessen.de

Paxilline (PAX, Figure) is a tremorgenic mycotoxin, structurally belonging to the group of diterpene alkaloids that contain an indole moiety. Toxins of this group (e.g. PAX, Lolitrems, Penitrems) have been demonstrated to cause tremors and other neurological disorders, also known as "staggers-syndromes", in mammals and ruminants. PAX was first discovered in *Penicillium paxilli* cultures, isolated from insect-damaged pecans, in 1974. In addition to further *Penicillium* spp., it has also been detected in a variety of fungal species belonging to different genera, including *Emericella* spp., endophytic *Neotyphodium lolii*, and *Claviceps paspali*. This suggests that PAX may occur widespread on or in plant materials; however, data on the occurrence of PAX in food and feed are very scarce. In order to provide a convenient screening tool to study occurrence of PAX in food and feed, we have developed a competitive indirect enzyme immunoassay (EIA), using polyclonal antibodies for its detection. This EIA is highly specific for PAX and has a standard curve detection limit in the ng/L range. In a first application study, international and domestic samples of beer are analyzed by the PAX EIA. The list of beer ingredients covers a wide range of cereals and other plant/fruit additions. The results of this study will be presented in this contribution.

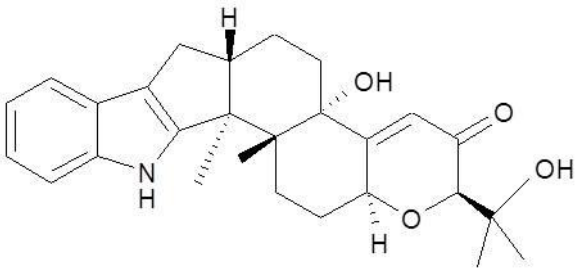


Figure: Structure of Paxilline

This work is financially supported by the Brigitte and Wolfram Gedek-Stiftung (Ismaning, Germany).

P10

Dietary supplements: occurrence of ochratoxin A in brewer`s yeast

Barbara Biermaier[1]*, Christoph Gottschalk[1], Madeleine Gross[2], Karin Schwaiger[1], Manfred Gareis[1]

[1]Chair of Food Safety, Ludwig-Maximilians-University, Munich, Germany; [2]Institute of Veterinary Food Science, Justus Liebig University, Giessen, Germany

* Corresponding author: Barbara.Biermaier@lmu.de

Brewer's yeasts are recommended as valuable food supplements for special population groups such as pregnant women, children, adolescents, and people with high physical activity, because they are rich in vitamins and other nutritive factors. Besides, certain strains of brewer's yeast proved to be capable of adsorbing mycotoxins (1). Therefore, these yeasts are regarded as having positive effects in food, beverage, and feed technology (2). However, the ability to bind mycotoxins such as ochratoxin A (OTA) can subsequently lead to a contamination of yeast products used as food supplements (3).

In the present study, 46 samples of brewer's yeasts purchased in retail stage between 2010 and 2014 were analyzed by HPLC-FLD, and by LC-MS/MS in order to reveal a probable occurrence of OTA. Almost 90 % of the samples were contaminated with OTA, with levels ranging from the limit of detection (LOD, 0.01 µg/kg) to 4.2 µg/kg. The mean and median levels of contamination were 0.49 µg/kg and 0.27 µg/kg, respectively (4).

Based on these results and the recommended intake of brewer’s yeast products, the additional weekly OTA exposure ranged from 9.3 % (mean case) to 114 % (worst case) of the mean weekly OTA intake in Germany (adults 279.3 ng, children 195.3 ng), depending on different subpopulations and levels of contamination (5). At present, there are no maximum levels for OTA in nutritional supplements like brewer's yeast. It is questionable whether targeted consumer groups including pregnants and adolescents should not avoid any additional OTA exposure. Based on our results, mycotoxin analyses in ongoing and future selfmonitoring programs and in product quality checks are recommended for producers of these dietary supplements.

1. Moslehi-Jenabian S, Lindegaard L, Jespersen L (2010) Beneficial effects of probiotic and food borne yeasts on human health Nutrients 2:449-473
2. Kapetanakou AE, Kollias JN, Drosinos EH, Skandamis PN (2012) Inhibition of A. carbonarius growth and reduction of ochratoxin A by bacteria and yeast composites of technological importance in culture media and beverages International Journal of Food Microbiology 152:91-99 doi:10.1016/j.ijfoodmicro.2011.09.010
3. Gareis M (2002) Ochratoxin A in Brewer's Yeast Used as Nutrient Supplement Mycotoxin research 18 Suppl 2:128-131 doi:10.1007/bf02946080
4. Gottschalk C, Biermaier B, Gross M, Schwaiger K, Gareis M (2016) Ochratoxin A in brewer's yeast used as food supplement Mycotoxin Res 32:1-5 doi:10.1007/s12550-015-0230-x
5. Cholmakov-Bodechtel C et al. (2000) Ochratoxin A: Representative food consumption survey and epidemiological analysis Archiv Fur Lebensmittelhygiene 51:111-115

This work was financially supported by the Brigitte and Wolfram Gedek foundation (Ismaning, Germany).

P11

Impact of Deoxynivalenol (DON) and DON-sulfates on oxidative stress and the redox-sensitive Nrf2/ARE signalling pathway

Dominik Braun[1]*, Lydia Wölflingseder[1], Giorgia Del Favero[1], Gudrun Pahlke[1], Benedikt Warth[1], Philipp Fruhmann[2], Rudolf Krska[3], Rainer Schuhmacher[3], Gerhard Adam[4], Doris Marko[1]

[1]Dept. of Food Chemistry and Toxicology, UniVie, Vienna, Austria; [2]Institute of Applied Synthetic Chemistry, TU Wien, Vienna, Austria; [3]Department IFA-Tulln, BOKU, Tulln, Austria; [4]Dept. of Applied Genetics and Cell Biology, BOKU, Tulln, Austria

* Corresponding author: dominik.braun@univie.ac.at

DON is a trichothecene mycotoxin produced by various Fusarium spp. that can colonize food and feed worldwide. For this reason, the European Food Safety Authority (EFSA 2013) suggested a provisional maximum tolerable daily intake (PMTDI) for DON and its acetylated metabolites of 1 µg/kg body weight and many countries introduced regulatory limits to decrease the entrance of DON-contaminated food into the market. Recently, new DON metabolites have been identified, namely DON-3-sulfate (DON3S) and DON-15-sulfate (DON15S). Their biological characterization is of crucial importance to understand if they have to be considered as toxicologically relevant. As one aspect of DON-mediated toxicity, the influence of the DON-sulfates on the onset of oxidative stress and the activation of the Nrf2/ARE pathway has been investigated. The impact of the three compounds on ROS production in the human adenocarcinoma cell line HT-29 was assessed by DCF assay. Both DON (10 µM) and DON3S (1 µM and 10 µM) induced a fast and transient increase of the intracellular ROS level. After the initial response, cells recovered to basal level within 2 h of incubation. In order to verify if the increase of the ROS level was sufficient to activate the Nrf2/ARE pathway and induce a cellular inflammatory response, qPCR experiments were performed on selected genes (Nrf2, Keap 1, ɣ-GCL, NQO-1, IL-8, COX-2). After 24 h of incubation 1 µM DON triggered a significant increase of the relative gene transcription of IL-8 and ɣ-GCL, the latter sustained even at 10 µM DON. Moreover, these concentration decreased NQO-1 gene transcription significantly. The two DON-sulfates did not influence the relative gene transcription of any of the tested genes. In conclusion, an oxidative response is triggered very fast by higher concentrations, but only DON is affecting the transcription of the selected Nrf2-dependent genes. These data indicate that with respect to inflammatory responses at least on the transcriptional level sulfation is associated with detoxification of the parent compound DON. Thereby, the provided data give new insights for the continual discussion regarding the toxicology of DON and its family class.

The authors thank the Austrian Science Fund (project SFB Fusarium F3701, F3702, F3706, F3718) and the City of Vienna Jubilee Funds for the University of Natural Resources and Life Sciences, Vienna (project MycoMarker) for the financial support.

P12

Incidence of Fusarium (modified) mycotoxins in Nigerian maize

Cynthia Adaku Chilaka[1]*, Marthe De Boevre[1], Olusegun Atanda[2], Sarah De Saeger[1]

[1]Laboratory of Food Analysis, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium; [2]Department of Biological Sciences, McPherson University, Seriki Sotayo, Ogun State, Nigeria

* Corresponding author: Cynthia.Chilaka@ugent.be

Maize is the most important cereal in Africa serving various purposes of economic importance especially as a staple diet. Unfortunately, this commodity serves as a suitable substrate for fungi development, which often results in the production of secondary toxic metabolites. Fusarium fungi are common plant pathogens causing several plant diseases and subsequent production of toxins which include fumonisins (FB), zearalenone (ZEN) and trichothecenes (TH). These mycotoxins have been implicated to cause a variety of toxic effects in humans and animals ranging from acute to chronic. It is of food safety concern that these free mycotoxins may co-exist with structurally related compounds generated by plant or fungi metabolism, or through food processing, referred to as modified mycotoxins. Modified mycotoxins may contribute toxic effects on humans and animals, as they may possibly hydrolyse into free toxins in the digestive tract of mammals. However, little attention has been paid to the occurrence of Fusarium mycotoxins and their modified forms in Nigeria food system. The present study seeks to determine the incidence of Fusarium mycotoxins and modified forms in maize from Nigerian markets. A total of 136 maize samples were collected in 2015 from randomly selected markets in four different agro-ecological zones of Nigeria (Southern Guinea Savanna, Northern Guinea Savanna, Derived Savanna and Sudan Savanna). A multi-mycotoxin LC-MS/MS method was used to analyse and quantify 16 Fusarium mycotoxins. Preliminary results reveal high incidence of FB (75%) in the samples, with ranges from 20 µg/kg to 8169 µg/kg, 26 µg/kg to 7197 µg/kg and 26 µg/kg to 2281 µg/kg of FB_1, FB_2 and FB_3, respectively. Fusarenon-X, ZEN, α-zearalenol, β-zearalenol and nivalenol were found in the samples at 52% (range: 18-188 µg/kg), 18% (range: 18-47 µg/kg), 20% (range: 18-88 µg/kg), 10% (range: 18-44 µg/kg) and 3% (range: 53-980 µg/kg), respectively. Co-occurrence of mycotoxins was observed in 81% of the samples which suggests that a possible synergistic toxic effect may occur. High levels of FB and mycotoxins co-occurrence as observed in this study represent a health risk to the Nigeria population. Further analysis using in-vitro digestion method will be done to determine possible occurrence of hidden FB in the samples.

Key words: Fusarium (modified) mycotoxin, LC-MS/MS, Maize, Nigeria

Authors would like to acknowledge Ghent University Special Research Fund (BOF-01W01014) for funding first author's doctoral studies.

P13

Nano-immuno detection of Ochratoxin with gold nanoparticles as labels

Priyanka Choudhari, Jyutika Rajwade, Dhananjay Bodas, Vandana Ghormade*

Agharkar Research Institute, Pune, India

* Corresponding author: vandanaghormade@aripune.org

Ochratoxin is the secondary metabolite produced by the fungi, Aspergillus ochraceus. Consumption of ochratoxin contaminated food causes health problems for animal and humans such as abdominal pains, hepato-, nephro-toxicity and carcinogenecity. Mycotoxin detection is carried out mostly by HPLC, LC- or GC-MS characterization of microbial metabolites, PCR based molecular methods and serodiagnostic kits which are equipment specific and are unsuitable for on-site application. The application of nanoparticles as labels, which are known to improve the detection sensitivity by virtue of their size related properties was investigated for development of a lateral flow immunoassay. Nanoparticles, due to their size and shape dependent physical and chemical properties have potential in development of chromogenic, on-site diagnostic assays. Conjugation of nanoparticles to antibodies allows for promising applications in signal amplification and detection.

Gold nanoparticles (24nm) were synthesised and conjugated to the commercial anti-Ochratoxin polyclonal antibody as labels for toxin detection. The culture conditions for A. ochraceus growth and ochratoxin production was optimised in liquid and solid state fermentations. HPTLC method could detect of ochratoxin above 50ng. A sandwich ELISA platform was devised for detection of ochratoxin with gold nanoparticles conjugated antibodies followed by silver enhancement. The ELISA platform was translated on to the nitrocellulose strip. A lateral flow assay was developed with gold-nanoparticles conjugated commercial anti-Ochratoxin polyclonal antibody that was able to detect ochratoxin upto 50ng. Such a nano-diagnostic immunoassay has potential for development as a cost-effective, indigenous, easy to use, rapid and sensitive ochraotxin detection platform.

The authors thank Department of Biotechnology for Funding.

P14

Comparison of blood sampling methods for investigating the concentrations and ratios of sphingoid bases and their phosphates as fumonisin biomarkers in pigs

Sonia Colicchia *, Franz Berthiller, Heidi Elisabeth Schwartz-Zimmermann

Department for Agrobiotechnology (IFA-Tulln), Tulln, Austria

* Corresponding author: sonia.colicchia@boku.ac.at

The quantification of biomarkers is a powerful tool to assess mycotoxin exposure in animals. Fumonisins are potent inhibitors of ceramide synthase, an enzyme involved in the sphingolipid metabolism. As a consequence of the ceramide synthase inhibition, the concentrations of the free sphingoid bases sphinganine (Sa) and – to a lesser extent - also sphingosine (So) increases. The Sa/So ratio can be used as a blood biomarker of effect to assess fumonisin exposure in pigs. Recent studies in pigs revealed that also the concentration of sphinganine-1-phosphate (Sa-1-P) in blood increases upon exposure to fumonisins.

The collection of blood is one of the key points during the design and development of animal trials. The aim of this study is the development and validation of LC-MS/MS based methods for the quantification of Sa, So, Sa-1-P and So-1-P in whole blood of pigs. To this end, protein cards for dried blood spot sampling and MitraTM microsampling devices were evaluated for collecting samples. Different solvents were tested for extracting sphingolipids from both devices. The method performance was evaluated by assessing the apparent recovery (RA), the signal suppression/enhancement (SSE), the recovery of the extraction step (RE), limits of detection and quantification and the repeatability.

Our results showed negligible SSE for So and Sa. The REs were higher for Mitra™ than for protein cards, resulting in higher RAs for Mitra™ than for protein cards. For Sa-1-P and So-1-P severe matrix enhancement was observed. The combination of matrix enhancement and RE of approximately 40% resulted in RAs between 124% and 218%. In addition to the unsatisfactory RA, the analysis of the phosphates was accompanied by further difficulties: Sa-1-P is not readily soluble in aqueous or organic solvents and the preparation of the respective standard solutions is tricky. Furthermore, during the LC-MS/MS analysis, So-1-P and Sa-1-P were susceptible to carry-over. Nevertheless, our results show that protein cards and MitraTM microsampling devices are suitable to collect, store and analyze animal blood in order to assess the Sa/So ratio as biomarkers of effect for fumonisin exposure.

P15

The evaluation of DON and D3G content of native contaminated pig feed before and after in vitro digestibility test with HPLC–ESI–MS/MS method

Krisztián Czompó, Orsolya Vida, Edit Deák*

Bábolna Feed Ltd., Nagyigmánd, Hungary

* Corresponding author: edit.deak@btakarmany.bonafarm.hu

According to analytical results 74% of the European samples from the 2014 harvest were contaminated with DON.

Deoxynivalenol (DON) is a naturally occurring toxic secondary metabolite, predominantly produced by Fusarium species (F. graminearum and F. culmorum). Plants can metabolize the Fusarium mycotoxin deoxynivalenol (DON) by forming the masked mycotoxin deoxynivalenol -3-ß-D-glucoside (D3G). More than 50% of the amount of free mycotoxins is considered to exist in commodities in a masked form.

However, there is a recommendation by the European Commission (2006/576/EC) for complementary and complete feedstuffs regarding DON, masked mycotoxins are not included. For most of the species the guidance value for DON is 5 ppm excluded pigs (0.9 ppm) because of their susceptibility. An in vitro study indicated that D3G is of toxicological relevance as it may become bioavailable again due to hydrolysis. The aim of our study was to evaluate the amount of DON and D3G content of native contaminated pig feed before and after in vitro digestibility test.

The masked mycotoxins due to their different chemical behaviour cannot easily determined by routine analyses; therefore we have developed an HPLC–ESI–MS/MS method for qualitative and quantitative analysis of mycotoxins in our study. QTRAP 6500 triple quadrupole-linear ion trap mass spectrometer (SCIEX) was used in multiple reaction monitoring (MRM) mode. The HPLC-ESI-MS/MS coupling was achieved by using an Agilent 1290 HPLC system.

P16

Role of fumonisins in the colonization of maize, sorghum, rice and beet seedlings with Fusarium verticillioides

Raana Dastjerdi[1], Petr Karlovsky[2]*

[1]Horticultural Science Research Institute (HSRI), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran, Karaj, Iran; [2]Molecular Phytopathology and Mycotoxin Research, Georg-August-University Göttingen, Grisebachstrasse 6, Göttingen, Germany, Goettingen, Germany

* Corresponding author: pkarlov@gwdg.de

The function of fumonisins, as the most widespread toxins reported from F. verticillioides strains in virulence is still not conclusively elucidated. Two fumonisin-nonproducing strains of Fusarium verticillioides and their progenitors were tested for aggressiveness toward maize, sorghum, rice, and garden beet seedlings under greenhouse conditions. The plants were inoculated by dipping their roots into suspension of fungal conidia. None of the plants showed obvious disease symptoms following root inoculation. The quantity of species-specific fungal biomass (pg mg-1) in plant tissue was determined by real-time PCR and used as an indicator of fungal aggressiveness. The lowest DNA standard that was employed in qPCR assays considered as the limit of quantification (LOQ) and it was 85 pg mg-1 for root samples and 51 pg mg-1 for the internodes. No significant differences (P = 0.05) between fumonisin-producing strains and their non-producing mutants were found in systemic colonization of the plants. F. verticillioides was not detected in any of the non-inoculated control plants. Fungal transmission from roots to the first two internodes/leaves in maize and beet occurred following the root dip inoculation regardless of fumonisin production, but a low systemic infection rate was observed for the rice and sorghum cultivars. The lack of differences between fumonisin-producing strains and non-producing strains in tissue colonization indicates that fumonisins are not virulent factors in root infection in maize, sorghum, rice and garden beet.

P17

An in vitro model to assess the adsorption of drugs to mycotoxin binders in a buffered, feed matrix

Thomas De Mil, Mathias Devreese*, Patrick De Backer, Siska Croubels

Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

* Corresponding author: mathias.devreese@ugent.be

Mycotoxin binders are feed additives which are mixed in the feed to adsorb mycotoxins and thereby reducing their toxic effects on animals. Interactions with orally administered veterinary medicinal products, such as antimicrobials, or other feed additives, such as coccidiostats, have been reported in animal studies. The study describes an in vitro model to screen for interactions between mycotoxin binders and veterinary drugs. It is designed as a static setup using single concentration of the adsorbate (veterinary drug) and adsorbent (mycotoxin binder) in a feed-containing matrix, buffered at different pH-levels. The model was applied to two frequently used antimicrobials in veterinary medicine, namely doxycycline (DOX) and tylosin (TYL). Proportions of feed, DOX or TYL and mycotoxin binder are equivalent to the in vivo situation for broiler chickens, and pH and volume of the buffer are representative for the gastro-intestinal tract of chickens as well. Conditions can easily be adapted for other target species, adsorbents and adsorbates. Results indicate lower free DOX concentrations as from an inclusion rate of binder of 20 g/kg feed or higher. For TYL, similar results as for DOX were obtained except for clay 3, which showed an interaction as from an inclusion rate of 5 and 10 mg/g feed for pH 6.5 and 8.0, respectively. Results are consistent with previously reported in vivo results, therefore, this model is suitable to assess the safety and binding potential of mycotoxin binders for DOX and TYL and eventually other veterinary drugs.

P18

Development of an LC-MS/MS method for the simultaneous determination of beauvericin, enniatins (A, A1, B, B1) and cereulid in cereal and cereal-based food matrices

Marlies Decleer[1]*, Andreja Rajkovic[2], Benedikt Sas[4], Annemieke Madder[3], Sarah De Saeger[1]

[1]Faculty of Pharmaceutical Sciences, Laboratory of Food Analysis, Ghent University, Ghent, Belgium; [2]Faculty of Bioscience Engineering, Laboratory of Food Microbiology and Food Preservation, Ghent University, Ghent , Belgium; [3]Organic and Biomimetic Chemistry Research Group, Ghent University, Ghent, Belgium; [4]Faculty of Bioscience Engineering, Food2Know, Ghent University, Ghent, Belgium

* Corresponding author: marlies.decleer@ugent.be

Beauvericin and the related enniatins are mycotoxins, secondary metabolites mainly produced by different Fusarium species that occur naturally on cereal and cereal-based foods and feeds. The emetic toxin cereulide is produced by the specific strains of Bacillus cereus. Structurally, these toxins are cyclic depsipeptides with ionophoric properties. Their toxicity is mediated by the ability to initiate cation transport across the cell membrane, disrupting normal intracellular cation levels, leading to apoptosis which is accompanied by DNA fragmentation. They are highly resistant to heat, acidification and proteolytic enzymes. Although these emerging foodborne toxins have different microbial origin, the striking structural and functional similarities enable and provide rationale for their concurrent detection in food matrices. Due to their prevalence in food and feed, and their toxicity it became an imperative to create a new tool in microbial food diagnostics for their reliable detection and quantification. The use of (tandem) mass spectrometry (MS), enabled the sensitive detection and quantification in order to better assess the co-occurrence of toxins. To the best of our knowledge this is a first report of a validated UPLC-MS/MS method for the simultaneous determination of beauvericin and the related enniatins, together with cereulide, in cereal and cereal-based food matrices such as wheat, maize, rice and pasta. A Waters Acquity UPLC system coupled to a Waters Quattro Premier XE™ Mass Spectrometer operating in ESI+ mode was employed. Sample pretreatment involved simple liquid extraction of the target toxins without any further clean-up step. The simple extraction, together with a fast chromatographic separation of only 7 min allowed substantial saving costs and time. The validation of the developed method was performed based on Commission Regulation No. 401/2006/EC and included determination of selectivity, repeatability, limit of detection (LOD), limit of quantification (LOQ), recovery and linearity. The obtained LODs ranged from 0.62 to 3.91 µg/kg and the LOQs from 1.24 to 7.83 µg/kg. The obtained RSD for repeatability was within 5 and 12 % and the obtained RSD for intermediate precision was within 5 and 8 %. The apparent recovery varied from 89 to 110 %. For all compounds the extraction recovery varied between 85% and 105 % in the different matrices. The highly sensitive and repeatable validated method was applied to naturally contaminated Belgian samples (n=127) allowing detection of sub-clinical doses of the toxins. Consequently, the influence of matrix on the thermal stability of the target toxins under different conditions was investigated using the developed LC-MS/MS method.

Acknowledgement: This work was financially supported by the BOF Special Research Fund from Ghent University, GOA project no. 01G02213 and FWO mandate of Prof.dr. Andreja Rajkovic.

P19

Dilute and shoot – enough to pass a PT?

Kay Dietrichkeit*, Lilli Reinhold

Lower Saxony State Office for Consumer Protection, Braunschweig, Germany

* Corresponding author: kay.dietrichkeit@laves.niedersachsen.de

Currently a variety of multi-methods exist. As well as other laboratories the Lower Saxony State Office for Consumer Protection and Food Safety has established an own in-house multi-method. This method is based on the "dilute and shoot" principle with LC-MS/MS. Multi-methods has to comply with validation requirements (e.g. Regulation (EG) 401/2006, Regulation (EG) 882/2004) as well as specific methods (like HPLC-FLD). This multi-method is validated for dried cereal products. 20 mycotoxins and 2 tropane alkaloids can be detected simultaneously. The question is: Are the results generated with the multi-method able to pass a proficiency test (PT)?

The present work shows the sample preparation and measurement parameters of the in-house multi-method. Furthermore advantages and disadvantages are compared. As part of several PTs results from specific methods are compared with the results of the multi-method.

P20

Biomarkers of effect prove the in vivo efficacy of a fumonisin degrading enzyme (FUMzyme®) in swine

Barbara Doupovec[1]*, Ilse Dohnal[1], Doris Hartinger[1], Corinna Kern[1], Roger Berrios[2], Dian Schatzmayr[1]

[1]BIOMIN Research Center, Technopark 1, 3430 Tulln, Austria; [2]BIOMIN Holding GmbH, Erber Campus 1, 3131 Getzersdorf, Austria

* Corresponding author: barbara.doupovec@biomin.net

Fumonisins (FUM) are a group of mycotoxins mainly produced by Fusarium fungi. They represent a serious threat to swine production as this livestock species is the most sensitive to FUM. They have multiple effects in swine. Intoxication with high doses of fumonisins leads to porcine pulmonary edema. Even low contaminations of fumonisins predispose pigs to lung pneumonitis. Fumonisins (FUM) disrupt the sphingolipid metabolism by blocking ceramide synthase leading to accumulation of free sphinganine (Sa). As a consequence, the production of complex sphingolipids, necessary components of nerves, muscles and also membranes, is interrupted. The sphinganine to sphingosine ratio (Sa/So) is used as a biomarker for fumonisin effects - an increase indicates a negative impact of fumonisins on the exposed animal.

The aim of this study was to evaluate the negative effect of fumonisins and the respective counteracting effect of a FUM-degrading enzyme (FUMzyme®) in feed by measuring the Sa/So ratio in serum of pigs.

18 weaning piglets were allocated to three experimental groups of 6 animals according to weight and gender. The control group received no FUM and no additive in the diet, the toxin group received 5 mg/kg FUM and the additive group received 5 mg/kg FUM and the fumonisin degrading enzyme (10 U/kg feed). All animals had free access to feed and water during the whole trial period of 42 days. Performance parameters were recorded and the Sa/So ratio in serum samples was analyzed by HPLC-MS/MS.

In the course of the trial a negative impact of FUM on weight, weight gain and FCR of the animals was observed. The Sa/So-ratio, measured at day 42, was significantly increased in the toxin group ($P<0.05$) compared to both, the control and the additive group. By the addition of the fumonisin degrading enzyme the Sa/So ratio was reduced to the control level. We conclude that FUMzyme was effective in preventing a toxic effect of fumonisin contaminated feed in pigs.

Keywords: fumonisins, biomarker, feed additive, serum, pig

P21

Mycotoxin Screening of Brewing Barley by Optical Spectroscopy and Ion Mobility Spectrometry (IMS)

Alexander Erler, Dennis Klier, Toralf Beitz*, Michael Kumke, Hans-Gerd Löhmannsröben

Physical Chemistry, University of Potsdam, Potsdam, Germany

* Corresponding author: beitz@uni-potsdam.de

Aim of the project OptiScreen is the development of a grain screening sensor to detect mycotoxins during industrial grain sorting. An optical sensor for 100%-screening of a grain stream will enable sorting out contaminated grains. Additionally, an IMS-sensor will sample the headspace of grain silos for marker species of fungal decay.

Spectroscopic methods offer an outstanding potential for the in-situ detection of mycotoxins in agricultural products such as grain. Due to the highly complex matrix of grain, a combination of different spectroscopic techniques, e.g. reflection and fluorescence spectroscopy, is required for the sensitive and selective detection of contaminants. Towards the demand of single grain detection, a combination of fast optical multiparameter detection and chemometrics is envisaged.

The influence of the spectroscopic parameters, e.g. matrix autofluorescence or scattering effects, was studied in detail in order to further improve the selectivity based on principle component analysis. Therefore, artificially inoculated grains and representative “real” samples were examined. The artificial inoculation was performed using an AFM as a nano pipette for locally highly defined “labelling” of single grains. First results of inoculated single grain experiments proved the potential of the approach based on ratiometric analysis of spectroscopic data by differentiating mycotoxin-contaminated from non-contaminated grains.

In the subproject IMS analytics, a headspace analysis is developed with the aim of identifying mycotoxin-forming fungi and obtaining semiquantitative information about the contamination status in silos. In a first step, different fungi are bred on agar and brewing barley (TU Berlin, Brewing Technology) and investigated with regard to characteristic volatile metabolites. The headspace analysis is based on sampling by SPME/TD and subsequent investigation by GC-MS. Two mass spectrometers are used: a classical EI quadrupole MS for identification of metabolites and an ion trap MS with a novel APCI source based on soft x-rays reproducing the ionization chemistry in IMS.

The project OptiScreen was financially supported by the Federal Ministry of Food and Agriculture (FKZ: 2814801811).

P22

Determination of Fumonisin B1 and B2 in different temperature conditions using autosampler in needle derivatization by HPLC/FLD

Hunor Farkas*, Biljana Marosanovic, Milena Jaksic, Svetlana Cujic, Dragana Marinkovic

SP LABORATORIJA AD, Becej, Serbia

* Corresponding author: Hunor.Farkas@victoriagroup.rs

Fumonisin B1 and B2 are mycotoxins produced by Fusarium species and which are prevalent in cereals and other agricultural products. These mycotoxins have been pointed to as a natural cause of Leukoencephalomalacia, porcine pulmonary edema and human esophageal cancer. Determination of fumonisins in various samples is very common analysis, according to food and feed safety regulations. Fumonisins are polar structures, based on long carbon-chain. For simple and easy determination and quantification by liquid chromatography, fumonisins type B must be chemically modified by derivatization. The most common analysis of fumonisins is standard method SRPS CEN/TS 16187/2012, performed with reverse-phased liquid chromatography with fluorescent detector and pre-column derivatization with o-phthaldialdehyde (OPA) reagent. Standard method does not determine mode of derivatization and, in the routine handling, with a large number of samples, autosampler -in-needle- derivatization mode is most effective. Aim of this study was to find the optimal working conditions for autosampler in-needle derivatization, which will fulfill the performance for peak area precision, RSD≤1,5% (1). The experiment included analysis of standard mix solution of Fumonisin B1 and B2 in concentration of 2µg/ml and 0.5µg/ml, retrospectively, in five different series. Five injections of each series were carried out at different temperature in autosampler: 5, 10, 15, 20 and 25°C. According to obtained results, we observed an increased peak response due to autosampler temperature increasing, which was expected in accordance with basic thermodynamic principles. Also, we noticed clear difference in repeatability of peak area among examined series. Namely, repeatability of peak area, expressed as RSD% within series of five injections at the same autosampler temperature, was in decreasing order with increasing the temperature. Values for RSD% were: 8.37, 6.39, 8.14, 1.48, 0.87 for Fumonisin B1 and 8.79, 6.76, 8.85, 1.31, 1.74 for Fumonisin B2, for temperatures of 5, 10, 15, 20, 25°C, retrospectively. During this study we came to significant conclusions that are applicable and useful in routine analyzes of fumonisins. Namely, we found that the optimal autosampler temperature, at which we noticed lowest deviation, that fulfill our equipment performance qualification during autosampler in needle derivatization is 20°C.

Reference:

Qualification of Equipment, Annex 1: Qualification of HPLC equipment PA/PH/OMCL (11) 04

P23

Ochratoxin A and aflatoxins in selected herbal medical supplements

Anna Filipiak-Szok[2]*, Magdalena Twarużek[1], Marzanna Kurzawa [2], Katarzyna Kuźmińska[1], Anna Błajet-Kosicka[1], Edward Szłyk[2], Jan Grajewski[1]

[1]Kazimierz Wielki University, Bydgoszcz, Poland; [2]Nicolaus Copernicus University, Toruń, Poland

* Corresponding author: ania_fsz@chem.umk.pl

The increasing consumption of dietary supplements and tea containing herbs in Poland and Europe, is determined by their health-related properties (e.g. presence of antioxidants), universal availability and strong advertisement. The majority of reports, based solely on in vitro studies, concern plants usage in popular Chinese medicine, suggesting that the plant materials can be an antidote to a wide range of diseases. The therapeutic effect of different plant species, connected with the content of bioactive compounds has been well studied. However, the plant material does not contain healthy elements only, but also others which can have adverse effect on our health. Unfortunately, in case of dietary supplements, food producers are obligated to present only mycological analysis and there are no regulations concerning the analysis of mycotoxins or other toxic compounds contamination.

With regard to the above, the aim of this study was determination of mycotoxins (ochratoxin A and aflatoxins) in plant material used in Traditional Chinese Medicine and Ayurveda, such as: Bacopa monnieri (4), Curcuma longa (2), Ocimum sanctum (2), Schisandra sinensis (2), Mucuna pruriens (2) as well as in dietary supplements containing these plants. Materials for the study consisted of 5 types of dietary supplements, available on the Polish market (chemist's, herbal shops, online stores). The products were: powder and capsules with powdered content.

The extractions of ochratoxin A (OTA) and aflatoxins (AF) were done on the immunoaffinity columns Ochraprep (R-Biopharm Rhône Ltd) and AflaTest HPLC (Vicam), respectively. Mycotoxins were analyzed with HPLC-FLD (Merck-Hitachi). In 10 to 12 of the tested samples OTA was detected in the range from 0.52 to 198 ppb, while aflatoxin was detected in 2 of the 12 analyzed samples below the LOQ. Average OTA contamination of the samples was 56,0 ppb and median 11,7 ppb. The highest concentration of OTA was found in the supplement Bacopa monnieri at the level of 198 ppb.

Supplement	OTA [ppb]	AF [ppb]
Bacopa monnieri 1 - powder	n.d.	n.d.
Bacopa monnieri 2 - capsules	198	n.d.
Bacopa monnieri 3 - capsules	187	n.d.
Bacopa monnieri 4 - capsules	83,8	n.d.
Curcuma longa 1 - powder	2,32	n.d.
Curcuma longa 2 - capsules	24,2	n.d.
Ocimum sanctum 1 - powder	14,1	n.d.
Ocimum sanctum 2 - capsules	n.d.	B_1 < 0,18
Schisandra sinensis 1 - powder	0,85	n.d.
Schisandra sinensis 2 - capsules	0,52	n.d.
Mucuna pruriens 1 - powder	152	n.d.
Mucuna pruriens 2 - capsules	9,33	B_2 <0,09, B_1, G_1, G_2 – n.d.

n.d. – not detected

Table: Results of OTA and AF contamination (ppb) in herbal medical supplements

P24

Intestinal metabolism of plant metabolites of mycotoxins ("masked mycotoxins") in the pig cecum model

Christine Focke, Hans-Ulrich Humpf*

Institute of Food Chemistry, Münster, Germany

* Corresponding author: humpf@wwu.de

Mycotoxins are toxic secondary metabolites produced by various fungi which can commonly be found in food. In addition to the native forms plant metabolites of mycotoxins ("masked mycotoxins") are also a source of mycotoxin exposure for humans and animals. Masked mycotoxins are formed in plants as part of their defence against xenobiotics as well as during food processing. The main reaction is the formation of polar metabolites, especially glucose and sulphate conjugates. In risk assessment studies masked mycotoxins have not been considered so far, due to a lack of knowledge concerning their occurrence, toxicity, bioavailability and metabolism. In general, masked mycotoxins are of lower toxicity in comparison to their native forms. However, it is assumed that masked mycotoxins can be cleaved in the digestive tract to liberate the native mycotoxin which can then exert their toxic effects.

In the course of our study, the bacterial metabolism of masked mycotoxins of several Fusarium mycotoxins was studied. Therefore, the pig cecum model as an in vitro metabolism model was used. The pig cecum model is a powerful tool to mimic the human intestinal metabolism due to the similarities between pig and human regarding anatomy, physiology, metabolism and composition of the gut microbiota with its more than 400 bacterial species.

Our results demonstrate that masked mycotoxins are easily and rapidly cleaved to the native mycotoxins by the gut microbiota.

P25

Zearalenone and enzymatic hydrolysis products – correlation of the estrogenic potential in vitro and in vivo

Sebastian Fruhauf[1]*, Andreas Höbartner[1], Bertrand Grenier[1], Elisavet Kunz-Vekiru[2], Gerhard Adam[3], Gerd Schatzmayr[1], Wulf-Dieter Moll[1], Michaela Thamhesl[1]

[1]BIOMIN Research Center, Tulln, Austria; [2]CD-Laboratory for Mycotoxin Metabolism (IFA-Tulln), Tulln, Austria; [3]DAGZ BOKU Vienna, Tulln, Austria

* Corresponding author: sebastian.fruhauf@biomin.net

Zearalenone (ZEN) is a mycotoxin produced by different Fusarium species which are powerful plant pathogens and infect major crop plants around the world. Acute toxicity of ZEN is low, but due to its structural similarity to β-estradiol, it binds to estrogen receptors and interferes with the hormonal balance. This can cause symptoms like hyperestrogenism and reproductive disorders, which are particularly seen in female swine.

Approaches to minimize the effects of ZEN include biodegradation by microorganisms or enzymes active in the gastrointestinal tract. Enzymes capable of degrading ZEN (e. g. ZHD101 of G. roseum or ZenA of R. erythropolis) are able to cleave the lactone ring of ZEN leading to hydrolysed ZEN (HZEN), which is then spontaneously converted to decarboxylated hydrolysed ZEN (DHZEN).

The estrogenic potential of these metabolites, HZEN and DHZEN, was assessed using two different in vitro assays and results were compared to in vivo data.
Estrogen-sensitive MCF-7 cells were exposed to ZEN, HZEN or DHZEN. No proliferation was detected with HZEN or DHZEN, whereas concentration-dependent proliferation was seen with ZEN. These results were confirmed with the estrogen reporter yeast strain YZHB 817.
To further investigate the estrogenicity of the ZEN degradation products, a feeding trial with 36 female piglets divided into six groups (n=6 per group) was carried out. The animals were fed with feed supplemented with ZEN in three different concentrations (0.1 mg/kg, 1 mg/kg and 4 mg/kg) or HZEN and DHZEN in concentrations equimolar to 4 mg/kg ZEN, or control (without ZEN or ZEN-derived metabolites) for 26 days. Body weight and vulva size were measured during the trial. At the end, the reproductive tract was removed and weighed. The results revealed same levels of vulva size and reproductive tract weight for the control, HZEN and DZEN group. In ZEN fed animals, however, at concentrations of 1.0 mg/kg and 4.0 mg/kg these parameters were increased clearly. Together, in vitro and in vivo studies suggest that there is no estrogenic effect of the ZEN degradation products HZEN and DHZEN.

P26

A study on Fusarium mycotoxins in donkey (Equus asinus) feed and milk

Madeleine Gross*, Julia Bauer, Christian Puck Ploetz

Justus-Liebig-University, Dairy Sciences, Giessen, Germany

* Corresponding author: madeleine.gross@vetmed.uni-giessen.de

The total world population of donkeys (Equus asinus) is about 43 millions, the vast majority (98%) lives in Africa, Asia, and middle/south America (http://faostat3.fao.org). In Europe, agricultural use of donkey meat and milk is of very minor importance. However, in the Mediterranean and in the Balkan area, a limited amount of donkey meat, donkey milk (powder) and donkey milk cheese (pule) is produced. With regard to mycotoxins in donkey feed and a possible transmission into milk, very little is known so far.
The feed given to a group of six female, lactating Baudet du Poitou donkeys, consisting of oat grains and pellets (containing oat fibre, wheat bran, and maize) was analysed by competitive enzyme immunoassays (EIA) for several Fusarium mycotoxins, including T-2 toxin, T-2/HT-2 toxin (sum), deoxynivalenol (DON), and zearalenone (ZEA). The detection limits were 6 µg/kg (DON), 0.7 µg/kg (T-2 toxin), 1 µg/kg (T-2/HT-2 toxin), and 5 µg/kg (ZEA). In oats, low levels of DON (72 µg/kg), T-2 toxin (12.3 µg/kg), T-2/HT-2 toxin (15.8 µg/kg) and zearalenone (7.15 µg/kg) were detected. In pellets, moderate levels of DON (259 µg/kg) and ZEA (182 µg/kg) were found, plus low levels of T-2 toxin (7.66 µg/kg) and T-2/HT-2 toxin (7.02 µg/kg). Milk samples from all animals were collected for mycotoxin analysis. Although the enzyme immunoassays had been successfully used to analyse cows milk, method adaptation and validation of the enzyme immunoassays was required for donkey milk because of the strongly different composition. First results show that no ZEA could be found in milk (1 µg/L), further results will be presented in this contribution.

P27

Optimized synthesis of butenolide: a journey towards isotope labelled butenolide

Maximilian Haider[1], Philipp Fruhmann[1,2,3]*, Julia Weber[1], Philipp Skrinjar[1], Christian Hametner[1], Hannes Mikula[1], Johannes Fröhlich[1], Gerhard Adam[2]

[1]Institute of Applied Synthetic Chemistry, TU Wien, Vienna, Austria; [2]Dept. of Applied Genetics and Cell Biology, BOKU, Tulln, Austria; [3]Center for Electrochemical Surface Technologies, Wiener Neustadt, Austria

* Corresponding author: philipp.fruhmann@tuwien.ac.at

4-Acetamido-4-hydroxy-2-butenoic acid-γ-lactone (Butenolide) is a widespread mycotoxin produced by several Fusarium species. It can be frequently detected in cereal grains and animal feeds and represents a potential threat to animal and human health. Up to now there are a few studies dealing with the toxicity and especially with its role as intracellular glutathione (GSH) scavenger. In order to gain deeper insights into its metabolism and the corresponding modes of action at a cellular level it is highly desirable to have larger amounts of butenolide and especially its isotope labelled form available.

Although butenolide was first isolated in 1967 (Yates et. al, Tetrahedron Letters 621) and already synthesized in the same year (Burkhardt et al, Tetrahedron, 24, 1225-1229), there is surprisingly no reported protocol which provides isolated yields higher than 10 %. Based on this several protocols were considered within an extensive reaction and parameter screening which finally lead to an optimized protocol with a reproducible and isolated yield of more than 50 % for butenolide.

Beside the development of a reliable protocol, we further evaluated different possibilities towards the synthesis of isotopically labeled butenolide. Based on previous results and economic considerations as well as chemical availability, we optimized the introduction of acetamide, in order to perform a triple labelling (2x13C and 1x15N) within one key step. In addition to the synthetic protocol, a short overview about the numerous research possibilities with our synthesized butenolide will be given.

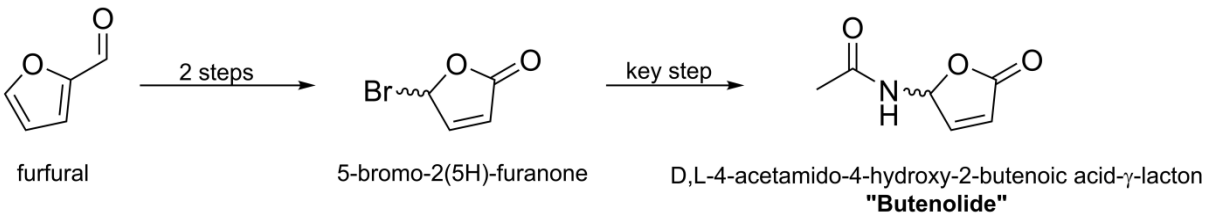


Figure 1: Synthetic route towards butenolide starting with cheap furfural and the key step.

The authors would like to thank the Austrian Science Fund (project SFB Fusarium #F3701) for the financial support.

P28

Deoxynivalenol, deoxynivalenol-3-glucoside and 3-acetyldeoxynivalenol in processed oat: effect of steaming, flaking and cooking on mycotoxin level

Anastasia Hole[1]*, Ida Rud[1], Stefan Sahlstrøm[1], Lada Ivanova[2], Gunnar Sundstøl Eriksen[2], Hege Hvattum Divon[2]

[1]Nofima, Aas, Norway; [2]Norwegian Veterinary Institute, Oslo, Norway

* Corresponding author: anastasia.hole@nofima.no

Deoxynivalenol (DON) and its conjugated forms deoxynivalenol-3-glucoside (DON-3G) and 3-acetyldeoxynivalenol (3-AcDON) commonly occur in oats due to infection by Fusarium spp. and can contaminate processed foods meant for human consumption. This study aimed to assess the influence of sequential softening regimes, flaking and cooking on levels of DON, DON-3G and 3-AcDON in naturally contaminated oat grain. Before flaking oat was dehulled and either traditionally steam softened or conditioned at room temperature to obtain kernels with 20% moisture. Both softening regimes caused to 20 – 70 % reduction of DON, DON-3G and 3-AcDON. Interestingly, retention of DON and 3-AcDON in flakes was dependent on softening regime applied before flaking, but not for DON-3G: 3-AcDON level was lower in flakes prepared from conditioned oat groat, while content of DON was lower in flakes prepared from steam softened oat groats.

Further cooking of flakes generally had no effect on the levels of DON and its conjugated forms. Noteworthy, although flake thickness had no important role for retention of DON, DON-3G and 3-AcDON in flakes, concentrations of DON and 3-AcDON in final porridges varied with flake thickness.

In summary, the study shows that flaking significantly reduces content of DON and its masked forms DON-3G and 3-AcDON in oat flakes, with mycotoxin retention being dependent on softening regime applied to oat groats before flaking. Levels of DON and its conjugates in flakes seem not to be significantly affected by further porridge cooking. It is not clear, however, whether DON and its conjugates are degraded during processing or covalently bound to the food matrix. Further investigations are therefore needed.

P29

Mycotoxin co-occurence in subsistence farmed maize from Zimbabwe and related risk assessment

Melody Hove[1]*, Marthe De Boevre[1], Carl Lachat[2], Liesbeth Jacxsens[2], Loveness Nyanga[3], Sarah De Saeger[1]

[1]Laboratory of Food Analysis, Ghent University, Ghent, Belgium; [2]Department of Food Safety and Food Quality, Ghent, Belgium; [3]Institute of Food, Nutrition and Family Sciences, Harare, Zimbabwe

* Corresponding author: Melody.Hove@Ugent.be

Mycotoxins pose risk to food security and human health. To date, studies on mycotoxin occurrence in food from Zimbabwe are insufficient to draw meaningful conclusions human exposure. Maize is the staple food of Zimbabweans and is consumed daily in the majority of households due to poor dietary diversity. The aim was to determine mycotoxin contamination in maize produced by subsistence farmers in Zimbabwe, the related exposure and corresponding human health risk. 95 maize meal samples were collected from the household stores of randomly selected subsistence farming households in 2014 from two provinces of Zimbabwe. Agronomic practices of the households were investigated using a questionnaire and field observations. Analysis and quantification of mycotoxins in maize was performed using a multi-mycotoxin LC-MS/MS method. Fumonisin B1 (FB1), FB2, deoxynivalenol and zearalenone were detected in 95, 31, 24 and 15 % of the samples at mean levels of 242, 120, 217 and 110 µg/kg respectively. FB3, aflatoxin B1 (AFB1), AFB2, diacetoxyscirpenol, nivalenol, 15-acetyl-deoxynivalenol and alternariol-methylether were found in less than 5 % of the samples at mean levels of 57, 11, 3, 14, 330, 88 and 60 µg/kg respectively. The highest incidence and levels of various mycotoxins in the maize were from the agro-ecological zone with the highest annual rainfall and cooler temperatures. Only FB1 had a median value above limit of detection and was considered in the exposure and risk assessment. Dietary exposure was derived from combining maize intake data and median FB1 contamination. Maize intake data was obtained by means of a quantitative food frequency questionnaire. Mean maize intake was calculated to be 26.8, 9.6, 13.4, 15.5 and 11.0 g/kg bw/day and FB1 exposure was calculated to be 3.91, 1.40, 1.96, 2.26 and 1.61 µg/kg bw/day for toddlers, children, adolescents, adults and the elderly respectively. Exposure to FB1 through maize intake exceeded the provisional maximum tolerable daily intake (2 µg/kg bw/day) by 96, and 13 % for toddlers and adults respectively. In conclusion toddlers and adults from subsistence farming communities in Zimbabwe are at risk of high exposure to FB1.

The authors would like to acknowledge the special research fund for candidates from developing countries (Ghent BOF grant no. 01W02813), the Department of Agriculture and Extension Services (AGRITEX), Zimbabwe and Kudzai Ndemera (Forestry Commission, Zimbabwe).

P30

Comparison of liquid chromatography coupled with fluorescence and mass spectrometry detection in the determination of aflatoxin M1 in dairy products

Piotr Jedziniak*, Marta Piątkowska, Olga Burek, Katarzyna Pietruszka

National Veterinary Research Institute, Department of Pharmacology and Toxicology, Pulawy, Poland

* Corresponding author: piotr.jedziniak@piwet.pulawy.pl

Aflatoxin M1 (AFLM1) is the metabolite of aflatoxin B1 that can be found in animal and human breast milk. AFLM1 presence in milk is considered as the potential risk to human health because of its carcinogenicity potential and thus a need for regular monitoring of milk and dairy product. The European Union and several other countries have set up an acceptable level of AFLM1 in milk and milk products at 0.05 µg/kg. Thus sensitive methods for determination of its metabolites is required. Since many years, different analytical techniques were used for determination of AFLM1 (e.g. ELISA). Nowadays, confirmatory analysis based on the liquid chromatography coupled with fluorescence or mass spectrometry detectors, give a high degree of results certainty.

The aim of this work was a comparison of HPLC-FLD (with and without the pre-column derivatisation) and UPLC-MS/MS techniques as analytical tools for the determination of AFLM1 in dairy products. The milk and cheese samples were spiked with AFLM1 standard solution on the ML level (0.05 µg/kg), extracted and cleaned up with immunoaffinity columns. Next, underivatized analyte was determined with HPLC-FLD and LC-MS/MS technique. The derivatized AFLM1 was determined also with HPLC-FLD technique.

The HPLC-FLD conditions were following: the separation was performed on the Inertsil (150×4.6 mm, 5 µm) column, the mobile phase, in isocratic mode, was consisted of acetonitrile-water or water-propanol-acetonitrile after pre-column derivatization. The flow rate was 1 mL/min and the injection volume was 20 µL. The fluorescence detector excitation wavelength was 360 nm and emission wavelength was 430 nm. The UPLC-MS/MS conditions were following: the chromatographic separation of mycotoxins was performed with the Luna Phenyl column (150 × 2.1 mm; particle size 3.0 µm,) using 0.3 mL/min of constant flow and oven temperature 40°C. The mobile consisted of 0.01M ammonium acetate –methanol in gradient elution. The injection volume was 10 µL. The mass spectrometer was operated in electrospray positive (ESI+) ionisation mode and two multiple reaction monitoring (MRM) transitions for aflatoxin M1 was monitored (m/z: 329->273 and 329->229).

The results show comparable reproducibility of all studies techniques. Despite the highest sensitivity of UPLC-MS/MS determination, HPLC-FLD determination (derivatized and non-derivatized aflatoxin M1) was sensitive enough for the unequivocal determination of AFLM1. In the HPLC-FLD determination, pre-column derivatization of aflatoxin M1 gives higher signal –to –noise ratio in comparison to non-derivatized AFLM1.

The results show that HPLC-FLD and UPLC-MS/MS technique has comparable performance as confirmatory technique.

Funded by KNOW (Leading National Research Centre) Scientific Consortium

P31

Development and validation of HPLC-MS/MS method for determination of deoxynivalenol, de-epoxy-deoxynivalenol and their 3α-sulfates in freeze-dried eggs

Nada Jurisic, Heidi Elisabeth Schwartz-Zimmermann[1], Elisavet Kunz-Vekiru[1], Dian Schatzmayr[2], Franz Berthiller[1]*

[1]Christian Doppler Laboratory for Mycotoxin Metabolism and Center for Analytical Chemistry, Department for Agrobiotechnology, Tulln, Austria; [2]BIOMIN Holding GmbH, Tulln, Austria

* Corresponding author: franz.berthiller@boku.ac.at

Potential carryover of mycotoxins into animal tissues is of significance for human and animal health. The Food and Agriculture Organization of the United Nations (FAO) has established maximum levels of specific mycotoxins in milk, meat, and their products. Maximum limits for mycotoxins in eggs have not yet been proposed because too few data are available. In the present work, an analytical method for the determination of deoxynivalenol (DON), de-epoxy-deoxynivalenol and their 3α-sulfates in freeze-dried eggs of laying hens has been developed and validated. Sample extraction using acetonitrile/water/acetic acid 79/20/1 (v/v/v) was followed by SPE clean-up with phospholipid removal columns and LC-ESI-MS/MS analysis. The developed method was successfully applied to freeze-dried eggs collected during a 10-week animal trial conducted at the Department of Poultry Science, Texas A&M University. Laying hens were assigned into the following dietary treatment groups: 1) control; 2) ~3-4 mg of DON/kg of diet; 3) ~7-8 mg of DON/kg of diet. The only metabolite found in concentrations above the limit of quantification (2.9 µg/kg) was deoxynivalenol-3α-sulfate (D3S). Its average content in the samples collected on three sampling occasions from the dietary treatment 2 was 17.1 ± 1.1 µg/kg, 22.7 ± 1.1 µg/kg, 25.1 ± 1.7 µg/kg, as well as from the dietary treatment 3 was 30.9 ± 4.9 µg/kg, 34.9 ± 2.5 µg/kg, 38.1 ± 3.3 µg/kg. Hence, we discovered a significant carryover of D3S into eggs, which is in line with literature reporting that D3S is the main metabolite of deoxynivalenol in chickens.

P32

Influence of mycotoxin DON on LPS distribution and clearance in pigs

Stefan Kahlert[1]*, Lydia Renner[1], Erik Bannert[2], Tanja Tesch[2], Jeannette Kluess[2], Constanze Nossol[1], Anikó Barta-Böszörményi[1], Hermann-Josef Rothkötter[1], Sven Dänicke[2]

[1]Institute of Anatomy, Otto von Guericke University, Magdeburg, Germany; [2]Institute of Animal Nutrition, FLI, Braunschweig, Germany

* Corresponding author: stefan.kahlert@med.ovgu.de

Deoxynivalenol (DON) is a trichothecene mycotoxin produced by Fusarium graminearum and is found as contamination in various cereals. We analysed the lipopolysaccharide (LPS) distribution and clearance in pigs fed with DON contaminated (4.6 mg DON/kg) or control feed. After 4 weeks feeding the animals received LPS (7.5 µg/kg BW, LPS) or saline (control, CON) systemically for 1 h via a jugular vein (ju) or portal vein (po) catheter. Samples were collected from a catheter in the carotid artery (ca) and ju / po catheter at 30 min before and 15, 60, 90 and 180 min after beginning of LPS infusion. LPS content was then analysed by the limulus amebocyte lysate (LAL) test adapted for the analysis of LPS in serum samples. The experimental set up allowed different combinations of LPS application and sampling. Kinetics of the following configurations were analysed: 1. jugular application / portal sampling; 2. po / ju; 3. po / ca; 4. ju / ca. The kinetics of all configurations showed a broad range of individual response pattern. Initial concentrations (-30 min) ranged between 0 (not detectable) and 52.7 pg/mL. The LPS level increased continuously and peak concentrations after 60 min infusion of LPS were found between 1.5 and 1720 pg/mL. Serum LPS levels decreased generally within 15 min after the end of the infusion. The serum LPS was still elevated in most pigs in comparison to initial concentrations 120 min after the end of the LPS infusion. The serum LPS was not correlated with position of LPS application. Furthermore, the LPS kinetic did not differ between DON and CON fed animals. The feeding of DON contaminated grain for 4 weeks did not modify the handling of blood LPS in pigs.

The work was supported by the DFG (RO 743/3-3 and DA 558/1-4).

P33

Indole-based alkaloids from *Penicillium expansum* and *Penicillium crustosum*

Svetlana Kalinina[1,2], Annika Jagels[1], Sebastian Hickert[1,2], Benedikt Cramer[1], Hans-Ulrich Humpf[1,2]*

[1]Institute of Food Chemistry, Münster, Germany; [2]NRW Graduate School of Chemistry, Münster, Germany

* Corresponding author: humpf@wwu.de

The genus *Penicillium* contains many toxigenic species (approximately 100), and the range of mycotoxin classes produced by this genus is much broader than that of any others. *Penicillium* toxins can be divided in two wide groups based on toxic effect: those that effect liver and kidney function and that are neurotoxins [1]. The most important of them are: patulin, ochratoxin A, citrinin, penicillic acid, citreoviridin, penitrems, roquefortines, verruculogen, chaetoglobosins, PR-toxin and cyclopiazonic acid. Moreover, over the last decades, a large family of fungal tremorgenic indole-based diterpenes including penitrems, paxilline and its analogues - lolitrems, aflatrems and janthitrems drag an attention due to their toxic effects and food contamination [2].

In the course of our study of toxins from *Penicillium* species in food, penitrems were successfully isolated. Also, effects of various stress-factors (oxidative stress, salinity, nitrogen and carbon sources, pH, temperature and light intensity) on the fungal growth and production of the indole-based diterpens were analyzed. New rapid and effective method of extraction and isolation of these toxins was developed. Thus, fungal cultures were extracted with acetonitrile, followed by separation of compounds using flash chromatography (normal-phase column SNAP-25) with a cyclohexane/ethyl acetate gradient. Final purification of indole-based alkaloids was carried out with preparative HPLC using isocratic mode of appropriate solvent. The structures of isolated compounds were elucidated by NMR and HPLC-MS experiments. This approach facilitates further research on the fungal indole-based alkaloids, their toxic effects and food contamination.

Literature:

[1] M.J. Sweeney, A.D.W. Dobson, Int J Food Microbiol 1998, 43.3, 141-158.

[2] G.S. Eriksen et al., Med. Mycol. 2010, 48, 188-196.

Supported by NRW Graduate School of Chemistry

P34

Determination of fumonisin B1 and B2 in corn and corn products by HPLC-FLD with automatic pre-column derivatization after strong anion exchange clean-up

Florian Kaltner[1], Corina Rampl[2], Michael Rychlik[3], Thomas Zimmermann[2], Alexander Rohe[2]*

[1]Chair of Food Safety, Ludwig-Maximilians-University, Oberschleissheim, Germany; [2]Central Institute of the Bundeswehr Medicinal Service Munich, Garching-Hochbrueck, Germany; [3]Chair of Analytical Food Chemistry, Freising-Weihenstephan, Germany

* Corresponding author: alexanderrohe@bundeswehr.org

Fumonisins, a type of mycotoxins produced by Fusarium species, can cause fatal diseases in animals and humans. Therefore, regulatory limits for fumonisins were established by many governments. Herein, a sensitive, valid and fast analytical method for quantitative determination of the relevant fumonisins B1 (FB1) and B2 (FB2) in sweet corn and corn products is described. It is based on reversed phase high performance liquid chromatography with fluorescence detection (RP-HPLC-FLD). As opposed to most comparable methods strong anion exchange (SAX) cartridges were used instead of widespread immuno-affinity chromatography (IAC) cartridges. The separation of analytes with retention times of 9.8 min for FB1 and 13.8 min for FB2 was obtained using a Zorbax Eclipse® XDB C8 column with a gradient elution. Solvents were methanol and 0.1 M phosphate buffer at pH 3.15. The limits of detection (LOD) for FB1 and FB2 were 29.2 µg/kg and 17.5 µg/kg, respectively. Obtained recoveries ranged from 79.4 % to 98.0 % for both fumonisins. The inter-day-precision achieved was 16.0 % for FB1 and 16.5 % for FB2, while the intra-day-precision was below 6 %. Positive confirmation of the developed and validated method was achieved by participation in an interlaboratory proficiency testing. Moreover, 19 samples of sweet corn and corn products were analyzed with the validated method. The results showed a widespread contamination with fumonisins at mean contents of 422.7 µg/kg in corn grits and 367.6 µg/kg in corn flour. In contrast, no fumonisins could be detected in sweet corn samples.

P35

Phase II-Metabolism of Alternariol and Alternariol Monomethyl Ether in Carrot and Tomato Explant Cultures

Beate N. Kohn*, Hannah Renner, Andreas A. Hildebrand, Erika Pfeiffer, Manfred Metzler, Mirko Bunzel

Karlsruhe Institute of Technology, Karlsruhe, Germany

* Corresponding author: beate.kohn@kit.edu

The two most prevalent Alternaria toxins found in food and feed commodities are alternariol (AOH) and alternariol-9-O-methyl ether (AME). Due to their free hydroxyl groups, AOH and AME can be conjugated as part of the xenobiotic metabolism of the plant without prior phase 1 functionalization reactions. These “masked” mycotoxins may be hydrolysed in the digestive tract of humans or animals, which results in the release of the parental toxin, increasing the total exposure to the toxin. Routine analysis methods cannot detect masked mycotoxins if the applied methods exclusively focus on the detection of the parental compounds. Recently, we have studied the metabolism of AOH and AME in tobacco BY-2 suspension cells. Five conjugates of AOH and four of AME were identified and were available as reference compounds (Hildebrand, 2015).

The aim of this study was to investigate the conjugation of AOH and AME in carrot and tomato fruits. Therefore we incubated pieces of tomatoes and carrots (in media) with 50 µM toxin for two days and the freeze-dried pieces were analyzed.

AOH was taken up and extensively metabolized in both explant cultures. In the carrot explant culture β-D-glucopyranosides and their 6’-malonyl derivatives were formed comparable to the reference compounds. An additional metabolite was also observed, which was tentatively identified as (p-coumaryl-glycosyl)AOH by LC-MS/MS-analysis. In comparison, in the tomato pieces only the two β-D-glucopyranosides were detected. AME was also found in the carrot and the tomato to a high extent but in contrast to AOH it was metabolized only to an insignificant extent in both cultures.

Our in vitro study demonstrates for the first time that AOH is efficiently conjugated in carrot and tomato fruits. Furthermore, major differences between the two species were observed, demonstrating that different pathways or enzyme expression levels of plants result in different metabolite patterns.

Hildebrand, A.A.; Kohn, B.N.; Pfeiffer, E.; Wefers, D.; Metzler, M.; Bunzel, M.; 2015; Journal of Agricultural and Food Chemistry; 63; 4728-36

P36

Mycotoxins influence on yeast growth and productivity in alcoholic fermentation

Daniela Kotz, Stephan Rose, Dian Schatzmayr*, Gerd Schatzmayr

BIOMIN Research Center, Tulln, Austria

* Corresponding author: dian.schatzmayr@biomin.net

Saccharomyces cerevisiae plays a central role in the production of alcohol from cereals such as corn. In order to achieve high ethanol yields, it is essential to avoid yeast stress factors such as suboptimal temperature and pH, high sugar, sulfite, phytic acid or sodium concentrations, bacterial contamination of the fermentation and mycotoxins. Mycotoxins often contaminate cereals, which are the raw material for the production of bioethanol. Mycotoxins are concentrated during the bioethanol process in the byproducts such as Distiller Dried Grains with Solubles (DDGS), and can impair the yeast during fermentation. Possible negative effects on the yeast can range from declined growth and reduced productivity to unfinished fermentations.

The objective of this study was to systematically evaluate the effects of different concentrations of fumonisin B1 (FB1) and deoxynivalenol (DON) on growth and productivity of Saccharomyces cerevisiae in anaerobic fermentations under controlled conditions in lab-scale bioreactor cultivations using chemically defined yeast media.

FB1 was tested at three concentrations (5, 50 and 100 µg/mL) in comparison to the control without fumonisin addition. Yeast viability which was assessed by flow cytometry after live/dead staining, was significantly reduced at 50 and 100 µg/mL FB1 ($P<0.05$). Likewise, fermentation lasted 36% longer at 50 µg/mL FB1 and was almost doubled in presence of 100 µg/mL. Ethanol concentration was significantly decreased for all three tested FB1 concentrations, leading to an ethanol loss of 5.1%, 17.2% and 16.1% at 5, 50 and 100 µg/mL FB1 respectively.

For DON, a decrease in ethanol concentration after 7 h of anaerobic fermentation was detected for media contaminated with 5, 50 or 100 µg/mL DON.

These results suggest that on industrial scale mycotoxins like fumonisin or deoxynivalenol are not only a problem as they are concentrated in the byproduct DDGS, but that mycotoxins can actually decrease ethanol yields and yeast viability and increase fermentation time, leading to direct economic losses for the bioethanol producer.

P37

^{13}C labeled standards – a reliable tool to overcome matrix effects in LC-MS/MS analysis

Lilian Kuster*

Romer Labs Division Holding GmbH, Getzersdorf, Austria

* Corresponding author: lilian.kuster@romerlabs.com

A crucial point for the analysis of mycotoxins is the sample matrix that influences the results obtained by the applied analytical technique. This matrix effect might be an issue in LC-MS/MS analysis that severely influences the results by ion enhancement or suppression during the ionization step, leading to over- or underestimates of the amount of target analyte.

Various methods can be applied to overcome these matrix effects, including matrix-matched calibration, standard addition to each sample or the application of internal standards. The first two possibilities are a good and feasible alternative, but are tedious, in terms of sample preparation and additional runs, and tend to be more expensive due to the additional effort. Internal standards in contrast can be either chemically related compounds or similar compounds that only differ in the mass of the atoms, but behave identically during the ionization process. Stable isotope labeling involves the use of non-radioactive isotopes like ^{2}H, ^{13}C or ^{15}N to replace the naturally occurring atoms, which leads to a defined shift of the atom mass. This shift then allows to distinguish between the naturally occurring and isotope labeled, added mycotoxin. Fully ^{13}C stable isotope labeled standards present the best option, as carbon is present in all mycotoxins. Replacing a naturally occurring ^{12}C atom by a ^{13}C atom leads to only a slight change in the atom mass. The replacement of ^{1}H by ^{2}H doubles the atom mass and might possibly lead to retention time shifts. In detail, ^{13}C labeled analogues retain the same characteristics as their naturally occurring ^{12}C counterparts, which means that they elute at the same retention time and are affected by the same ionization effects. The mass difference allows the separation of analyte and standard by the mass spectrometer. The known quantity of the added ^{13}C isotope can then be used to calculate the unknown amount of the analyte and thereby correcting possible enhancement and suppression effects.

^{13}C labeled standards are normally associated with high costs. This talk will give insight into the application of ^{13}C labeled standards in LC-MS/MS multimycotoxin methods. The possible points of addition of the standards and the corresponding advantages and disadvantages will be discussed. Furthermore, some cost examples will be shown to highlight the fact that the usage of ^{13}C labeled standards and the associated costs are highly dependent on the sensitivity of the system, the factors one wants to correct and the number of analyzed mycotoxins.

P38

Effects of graded levels of deoxynivalenol in an artificially contaminated diet on body weight, feed and water intake in broiler chickens

Annegret Lucke[1]*, Barbara Doupovec[2], Qendrim Zebeli[1], Josef Böhm[1]

[1]Institute of Animal Nutrition and Functional Plant Compounds Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Vienna, Austria; [2]BIOMIN Research Center, Technopark 1, 3430 Tulln, Austria

* Corresponding author: annegret.lucke@vetmeduni.ac.at

The mycotoxin deoxynivalenol (DON) is mainly produced by Fusarium species and results in worldwide economic losses in husbandry production. According to an EFSA-report 2013 (1), DON is mainly found in wheat, maize and oat grains, the main components in poultry feeds. Chickens generally are considered to be less sensitive to DON than for example swine.

The aim of this study was to elucidate the effects of four different low to moderate DON-doses in feed on economic performance parameters of broiler chickens.

Therefore, a total of 160 Ross 308 chicks were randomly allocated to four equal feeding groups. The trial was performed in three experimental runs with 32, 64 and 64 broilers each. The animals were kept in flat-deck cages. In each treatment group, half of the animals were slaughtered in week three, while the other half of the birds were slaughtered in week five. Birds had free access to drinking water and feed during the whole period of the trial. The feed ration based on a commercial wheat/soy ration and was artificially contaminated with the following target-concentrations of 2.5, 5 and 10 mg DON/kg feed and a control diet.

Feed intake and water consumption were measured daily, body weight was recorded weekly. Data were analyzed by PROC MIXED of SAS. The model accounted for fixed effects of run and treatment, random effect of animal within run, and repeated measures taken over time on the same animal.

The overall dry matter intake per bird was reduced in all of the three contaminated feeding-groups compared to the control group. Furthermore, the ratio of water intake to dry matter intake was increased in the DON-groups, whereas the absolute water intake per bird was not affected by the treatment. The overall body weight was decreased in the three DON contaminated feeding groups compared to the control group.

The conclusions are that in opposite to EU and US limits with 5 ppm for poultry feeds even lower (2.5 ppm) DON-contaminated rations for broilers may already lead to economic losses concerning feed intake and body weight gain in broilers.The increased ratio of water to dry matter intake might be an indicator for possible effects of DON on the kidney function. Further research is needed to elucidate this issue.

The research is funded by the FFG (FFG-Nr. 848446) and Biomin GmbH.

(1) European Food Safety Authority, 2013. Deoxynivalenol in food and feed: occurrence and exposure. EFSA Journal 2013;11(10):3379, 56 pp. doi:10.2903/j.efsa.2013.3379

P39

Effects of citric and lactic acid treatment on common mycotoxins in artificially contaminated feed samples

Annegret Lucke[1]*, Hauke Harder[1], Elke Humer[1], Josef Böhm[1], Barbara Metzler-Zebeli[2], Qendrim Zebeli[1]

[1]Institute of Animal Nutrition and Functional Plant Compounds, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Vienna, Austria;
[2]University Clinic for Swine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Vienna, Austria

* Corresponding author: annegret.lucke@vetmeduni.ac.at

Exposure to feeds contaminated with mycotoxins especially Fusarium toxins e.g. deoxynivalenol (DON) and other trichothecene derivatives is a real concern in animal production. This has increased the interest to develop cost-effective methods of mycotoxin decontamination. Organic acids such as citric (CA) and lactic acids (LA) have been used in feed processing, but their potential role in mycotoxin decontamination has not been evaluated yet. The aim of this study was to elucidate the effects of CA and LA on common mycotoxins in a cereal-based compound feed artificially contaminated with DON. Average concentrations of main mycotoxins in the contaminated feed samples (n=12) were: 6.1±0.58 mg DON, 5.3±0.64 mg aurofusarin, 0.8±0.20 mg fusarin C, and 0.33 ±0.05 mg zearalenone (ZEA) per kg dry matter. Three randomly taken replicates of contaminated feed samples were processed either with 5% CA or 5% LA solution in a ratio of 1:1.2 (wt/vol), and stored at -20°C until analysis. The analysis of mycotoxins was conducted using liquid chromatography–tandem mass spectrometric method for multiple mycotoxin metabolites. Data were processed by PROC MIXED of SAS using a model accounting for fixed effect of treatment and random effect of replicate. Data showed that treating the feed with CA and LA lowered the concentrations of DON, whereby 5% LA lowered the original DON concentration in the contaminated feed samples by half. Similar lowering effects were observed for concentrations of the DON derivative 15-acetyl-DON and trichothecene metabolite sambucinol and for culmorin derivative 5-hydroxyculmorin. The concentration of nivalenol was only lowered by LA treatment. In contrast, the treatments with CA and LA showed no effect on the concentrations of several mycotoxins or their derivatives including ZEA as well as aurofusarin, fusarin C and culmorin.

In conclusion, the study indicates that 5% LA and CA treatment did not fully decontaminate the artificially contaminated feed but lowered the concentration of common mycotoxins especially of DON and its derivative 15-acetyl-DON and nivalenol. Further research is warranted to validate these findings and also determine their significance for animal feeds and nutrition.

P40

Respiratory toxicity of indoor aspergilli and penicillia

Maria Majorosova, Elena Pieckova*, Marta Hurbankova, Sona Wimmerova

Slovak Medical University, Bratislava, Slovakia

* Corresponding author: elena.pieckova@szu.sk

Sick building syndrome (SBS) is a complex of health disorders and general body dyscomfort related to the stay in buildings of low indoor (air) quality. The dampness is a limited indoor factor related to SBS. Moist building materials might be colonized by mycobiota producing detectable amounts of mycotoxins – could be aerosolized – and VOCs as well. All the indoor fungal metabolites could contribute to occupational ill health. Acute toxicity of the common airborne fungi under Slovak housing conditions: Aspergillus versicolor – synthesizes sterigmatocystin, colonizing 1/3 of mouldy dwellings, A. ustus – its products cotanins were found in contaminated building materials, no biological effect related to them is known yet, present in 5 % of, Penicillium expansum and P. chrysogenum – present in >90% of hygienically debilitated apartments, producing also additional adverse health affecting haemolysins (chrysolysin), were studied in 3-d-experiments. In vitro toxicity was tested by a chick tracheal culture bioassay, combined with histologic (coarsing and lysis of the cells) and biochemical (concentration of alkaline phosphatase) changes in rat lung tissue and cell cultures (lung cells type II, Clara cells). In vivo toxicity was indicated by cytotoxic and inflammatory parameters after intratracheal instillation to Wistar rat males (ca 200 g). Solvent (2 % dimethylsulphoxide – negative control) and mycotoxin standards (sterigmatocystin for both aspergilli, patulin for P. expansum and ochratoxin A for P. chrysogenum; 20 or 4 microg/mL) – positive controls were applied in the systems. Cytotoxic (phagocytic activity and viability of lung alveolar macrophages - AM, activity of cytoplasmic enzymes cathepsin D, lactatedehydrogenase and acid phosphatase) biomarkers and the ones of macroorganism inflammatory response (total cell count, AM and granulocyte in bronchoalveolar lavage fluid – BALF, AM portion from the BAL cells) were measured in the BALF. All fungal metabolites tested proved to be concentration and cell origin (exo- or endometabolites) dependent toxic. The ones produced by A. versicolor (its sterigmatocystin production detected by LC/MS/MS) showed the highest toxic potential. In general, exometabolites produced into the cultivation medium damaged the upper and lower respiratory tract of the animals in vitro and in vivo more significantly. Longlasting repeated expozition to indoor fungi and their metabolites in dwellings may lead to serious, even irreversible, health disorders in the occupants, esp. of the youngest age subpopulations.

The publication resulted from the project realization

P41

Phomopsins in legumes – a multidimensional approach

Ronald Maul[1]*, Svenja Schloß[1], Corinna Herz[2], Evelyn Lamy[2], Matthias Koch[3], Sascha Rohn[1]

[1]Hamburg School of Food Science, Hamburg, Germany; [2]Institute for Prevention and Cancer Epidemiology, Freiburg, Germany; [3]Bundesanstalt für Materialforschung und –prüfung, Berlin, Germany

* Corresponding author: ronald.maul@chemie.uni-hamburg.de

Besides those mycotoxins regulated by law, a multitude of other secondary fungal metabolites exists that also belong to the class of mycotoxins. Several of these compounds are considered as being especially important in terms of food and feed safety ("emerging" mycotoxins), such as the peptidic secondary metabolite phomopsin A produced by the fungus Diaporthe toxica. As only limited data is available about the occurrence of PHO-A in food and feed, fast and sensitive methods are requested e.g. for (contaminant) monitoring programs by the EU mandate M/520. Recently, we developed a biosynthetic approach to gain access to 15N-isotopically labelled PHO-A as internal standard (IS) for matrix independent quantification of PHO-A in various commodities. Aim of the present work was to investigate the phomopsin formation on various legume seeds as well as plant material using HPLC-MS/MS and hrMS.

The HPLC-MS/MS method using $^{15}N_6$-PHO-A as IS showed good performance data and sensitivity with recoveries between 97 and 103%, RSD below 3% and LODs between 0.5 and 1.0 µg/kg depending on the matrix. Severe toxin formation could be proven for all seed and plant matrices with 220 - 440 mg/kg PHO-A being determined in the different commodities. Moreover, hrMS indicated the presence of a novel phomopsin derivative showing a mass shift of +14.0156 amu compared to PHO-A. The novel methylated PHO-A derivative accounted for 27 to 43% of the total phomopsins detected in the samples. Unlike on regular reversed phase HPLC columns on a modified HILIC column a semi-preparative separation and cleanup of the methylated derivative was feasible. Initial comparative in vitro investigation of the novel compound and PHO-A in cultured HepG2 cells indicate a significant decrease in cell viability in the low micromolar range for both substances. In summary, the applicability of the HPLC-MS/MS method could be proven and the potential of D. toxica to produce high amounts of phomopsins under unfavorable conditions on lupins and – even more remarkably – on other legume seeds could be shown. Moreover, a novel toxic methylated PHO A derivative was unveiled in natural samples infested with *D. toxica*.

P42

Glucosylation of trichothecene toxins by plant UDP-glucosyltransferases

Herbert Michlmayr[1]*, Elisabeth Varga[2], Alexandra Malachova[2], Wolfgang Schweiger[3], Philipp Fruhmann[4], Christian Hametner[4], Rudolf Krska[2], Franz Berthiller[2], Gerhard Adam[1]

[1]Department of Applied Genetics and Cell Biology, BOKU, Tulln, Austria; [2]Center for Analytical Chemistry, IFA-Tulln, BOKU, Tulln, Austria; [3]Biotechnology in Plant Production, IFA Tulln, BOKU, Tulln, Austria; [4]Institute of Applied Synthetic Chemistry, Vienna University of Technology, Vienna, Austria

* Corresponding author: herbert.michlmayr@boku.ac.at

Glycosylation is an important plant defense mechanism and glucoconjugates of Fusarium toxins often co-occur with their parent compounds in cereal based food and feed. Of particular importance is deoxynivalenol-3-O-ß-D-glucopyranoside, but glucosides of other relevant trichothecenes such as nivalenol (NIV), T-2 (T2) and HT-2 (HT2) toxins have been identified as well. The toxicological relevance of trichothecene glucosides is not fully understood and it is crucial to synthesize such compounds in sufficient amounts to make toxicological studies possible. Standards of glucosides of NIV, T2 and HT2 toxins are so far not commercially available, which limits screening of their occurrences in cereal samples. Better understanding the role of glycosylation in plant-pathogen interaction with strains producing different toxins may also be of interest for breeders aiming to increase Fusarium resistance of cereal crops. Previously, our group has identified and studied several DON-conjugating plant UDP-glucosyltransferases (UGTs). A rice UGT (OsUGT79) was expressed in E. coli and biochemically characterized. In vitro biochemical assays showed that the recombinant enzyme is able to equally conjugate DON, NIV and HT2, but is inactive with C4-acetylated derivatives such as T2 and Fusarenon X. Interestingly, a related UGT from barley (HvUGT13248) prefers NIV over DON as substrate which indicates a possible tolerance to substitutions at C4. Indeed, preliminary assays showed that HvUGT13248 is able to glucosylate T2 and Fusarenon X.

OsUGT79 was used to synthesize mg quantities of DON-3-glucoside, NIV-3-glucoside and HT2-3-glucoside. NMR spectroscopy confirmed the formation of a trichothecene-3-O-ß-D-conjugate in each case. The ability to synthesize these compounds is a crucial step forward in evaluating the natural occurrence and toxicity of these masked mycotoxins in animal feeding trials.

Funded by the Austrian Science Fund SFB Fusarium (F3706, F3708) and Vienna Science and Technology Fund (WWTF LS12-021).

P43

Heterokaryon incompatibility/compatibility and phenotypic characterisation of *Aspergillus flavus* isolates in low and high risk zones in Kenya

Alfred Mitema[1]*, Rafudeen Suhail [1], Okoth Sheila [2], Iyer Revel[1]

[1]University of Cape Town, Cape Town, South Africa; [2]University of Nairobi, Nairobi, Kenya

* Corresponding author: alfmite@yahoo.com

Globally, food spoilage and poisoning pathogens leads to food insecurity, ill-health and death. Aflatoxins are a series of highly toxic polyketides produced by several species of Aspergillus. The most commonly occurring aflatoxin, aflatoxin B1, is a genotoxin known to be carcinogenic, teratogenic for both humans and animals. Crops infected by aflatoxin producing fungi frequently become contaminated with aflatoxins. Aspergillus flavus has two morphotypes; S- and L-type strains that differ in aflatoxin producing ability and other characteristics. S-type strains have repeatedly been associated with acute aflatoxicosis. Kenya experienced the worst outbreak of aflatoxin poisoning in 2004, 2006 and 2010 where 317 cases and 215 deaths reported. In this study, diagonal transect random household sample collection was conducted between November and December 2013. Maize kernels were collected from different counties in Kenya. Isolated A. flavus strains were phenotypically characterised, genetic diversity studied through vegetative compatibility groups (VCGs) and metabolic fingerprinting profile of mycotoxins analysed. Out of thirty seven isolates, the nitrate non-utilising auxotrophs (nit mutants) complementation test revealed 20 VCGs. Vegetative Compatibility Group were designated with a prefix (KVCG1); (K) Kenya, (VCG) Vegetative Compatibility Group and 1 represent the VCG number. KVCG14 and KVCG15 (n=13; 10.8%) had high percentage frequency distribution whereas KVCG10 and KVCG20 (n=1; 0.8%) the least respectively. The isolates were found to be disseminated 2-6 in a single VCG (n=4; 10.8%): Nandi (n=3; 33.3%) and Homa Bay (n=1; 10%). VCGs diversity index was lowest in Nandi (H'= 0.108), Homa Bay (H'= 0.216), Makueni (H'=0.270) and Kisumu (H'= 0.324) counties. The findings showed that, all isolates from each county were more self-compatible within the county but incompatible between the counties with a few exceptions. Strong heterokaryon incompatibility was observed between Nandi county isolates (n=6; 67%) and Makueni county (n=3; 33.3%) isolates. Isolated strains from Nandi, Kisumu, and Homa Bay were more vegetatively compatible, forming dense hyaline mycelia at the point of intersection. Kisumu and Makueni county isolates were widely distributed and found to belong to more than one VCGs group. High mould prevalence infection in Makueni (78%, n=7) was noted. Mycotoxin detection by coconut cream agar discovered 37 isolates screened under UV light (365 nm) fluoresced blue (57%, n=21) and green (43%, n=16). Makueni, Nandi, Kisumu and Homa Bay counties fluoresced blue (78%, n=7; 33%, n=3; 67%, n=6; and 50%, n=5) and fluoresced green (22%, n=2; 67%, n=6; 33%, n=3; and 50%, n=5) respectively. The study revealed that fifty seven percent (n=21; 57%) of the isolates from the four counties were L-type strains, S-type (n=2; 7%), L/S- type (n=8; 22%) and non-sclerotia producers (n=7; 19%). The findings suggest that strains from Makueni might be producers of aflatoxin AFB1, AFB2, the most potent carcinogen as compared to other counties. This corresponds to previous studies indicating a high risk of constant aflatoxicosis in Makueni.

This work is part of my MSc. thesis in molecular and cell biology. I sincerely acknowledge and thank my supervisors for their professional guidance and critique throughout the research period and fellow Plant Stress Lab group members. I also acknowledge University of Cape Town for admitting me into their study programme, University of Nairobi in Kenya and USHEPiA for funding my studies.

P44

Feed from Austria and Germany tested for more than 380 mycotoxins and secondary metabolites

Michele Muccio[1]*, Christina Schwab-Andics[1], Karin Naehrer[1], Paula Kovalsky[1], Rudolf Krska[2], Michael Sulyok[2]

[1]BIOMIN Holding GmbH, Getzersdorf, Austria; [2]IFA-Tulln, University of Natural Resources and Life Sciences , Vienna , Austria

* Corresponding author: michele.muccio@biomin.net

Fungi and their mycotoxins were recently described as the largest threat to animal production of all classes of pathogens. There is evidence that mycotoxin occurrence is increasing over time and this may be due to factors such as climate change. This situation brings up the need to conduct deeper analysis and to focus on new and less characterized mycotoxins to assess their toxicity. For this purpose, a LC-MS/MS method (Vishwanath et al. 2009) was used to screen 357 feed and raw material samples for more than 380 mycotoxins and other secondary metabolites. The samples were collected from Austria and Germany in 2015.

The mean number of mycotoxins and metabolites per sample was 24.5 with 31% of samples containing between 10 to 19 of the compounds. The most frequent occurring secondary metabolite was cyclo (L-Pro-L-Tyr), present in more than 90% of the samples analyzed with a mean concentration of 554.1 ppb for all positive samples, followed by the emerging mycotoxin emodin, detected in 76% of the samples at a mean concentration of 187.4 ppb. Other detected emerging mycotoxins were: enniatin A1/B/B1 (mean concentrations 18, 40.5 and 44.8 ppb respectively), rugulosin (mean concentration 136.6 ppb) and moniliformin (mean concentration 17 ppb). Deoxynivalenol, nivalenol and the masked mycotoxin deoxynivalenol-3-glucoside were detected in more than 50% of the samples as well (mean concentrations 746.6, 84.7 and 79.5 ppb, respectively). Zearalenone was detected in 54% of the samples (mean concentration 227.2 ppb).

These results show the power and accuracy of LC-MS/MS based methods for multi-mycotoxin analysis. The analysis also clearly demonstrated the frequent complex co-contamination of feed and raw-materials including a wide range of metabolites that are not characterized yet in terms of toxic effect. In order to allow an accurate assessment of the risk associated with the presence of these contaminants, more data is needed on their metabolic fate, toxicities and possible synergistic interactions with other mycotoxins present.

P45

Phenotypic and phylogenetic characterization of Alternaria infectoria isolated from wheat in Germany and Russia

Müller Marina E.H.[1]*, Kahl Sandra M.[2], Ulrich Andreas [1], Kirichenko Albina A.[3]

[1]Leibniz Center for Agricultural Landscape Research, Müncheberg, Germany; [2]University of Potsdam, Potsdam, Germany; [3]Novosibirsk State Agricultural University, Novosibirsk, Russia

* Corresponding author: mmueller@zalf.de

A classification of small-spored Alternaria strains usually results from the identification of morphological characters, like growth rates on different nutrient media, coloration of colonies and numbers and shape of conidia. A polyphasic approach including macro- and micromorphological, molecular and chemotaxonomic features could provide some prospects of success concerning a taxonomic system of sections, species groups, subspecies and pathotypes within the genus Alternaria. The aim of this study is to characterize Alternaria strains isolated from wheat in the Northeast of Germany in comparison with isolates from cereals in the Novosibirsk region in Russia. Using a combination of macro- and micromorphological and genetic characters as well as mycotoxin profiling, a cluster analysis of these 95 isolates should elucidate the relationships among the isolates and the possibility of an unambiguous taxonomic classification. The examination of toxin profiles and the phylogenetic features based on sequence variation in translation elongation factor 1-α (TEF1-α) resulted in two distinct clusters, in each case containing A. infectoria isolates (92% and 96%, respectively) in the first and the A. alternata, A. arborescens and A. tenuissima isolates (77% and 79%, respectively) in the other combined cluster (Kahl et al. 2015). The production of Alternariol, Altertoxin and Altenuene has not been reported previously in the A. infectoria species group. The isolates from Germany and Russia differ slightly in species composition and mycotoxin production capacity. We identified that the A. infectoria species group can be differentiated from the A. alternata, A. arborescens and A. tenuissima species group by color, low mycotoxin production and by the sequence variation in TEF1-α gene. These results allow a reliable toxic risk assessment when detecting different Alternaria fungi on cereals.

Kahl, S.M., Ulrich, A., Kirichenko, A.A., Müller, M.E.H. (2015) J.Appl. Microbiol 119: 1637-1650.

P46

Is plastic mulching a risk for Deoxynivalenol/Nivalenol soil contamination in strawberry cultivation?

Katherine A. Muñoz[1]*, Markus Schmidt-Heydt[2], Dörte Diehl[1], Rolf Geisen[2], Gabriele E. Schaumann[1]

[1]University of Koblenz-Landau, Landau, Germany; [2]Max Rubner-Institut, Karlsruhe, Germany

* Corresponding author: munoz@uni-landau.de

Mulching is a widely used management in agriculture due to its effects on soil temperature and humidity regulation, which results in improved yield rates and product quality. In this study we investigated the Deoxynivalenol/Nivalenol (DON/NIV) content in soil samples from two different mulching systems: organic (OM) vs. plastic (PM). The last was studied in 2-years (2-y PM) and 4-years (4-y PM) coverage. In total 36 soil samples were collected from irrigated strawberry-fields. Data was correlated with mycobiome abundance and soil physicochemical parameters. Mycotoxin content in dried soil was analysed via LC-HRMS. Total colony-forming unit (CFU) was indicative of fungal biomass. Soil physicochemical parameters (water content, dissolved organic carbon (DOC), pH, total C and N were analysed using previously validated method.

In general, DON/NIV concentrations in soil samples from PM were higher than those from OM. Particularly in the 2-y PM treatment with conc. ranging from 1.6-20.9 and 0.9-9.6 (ng/g dry soil) for DON and NIV, respectively (10/12 positive samples). In the 4-y PM, only 50% of the samples tested positively, ranging from 1.9 – 7.4 for DON and 1.0 – 4.1 (ng/g dry soil) for NIV. In OM, the lowest mycotoxin concentrations and detection frequency were observed: Range: 1.1 – 2.4 (ng/g dry soil), 4/12 positive samples. Contradictory, the highest CFU values were observed in OM samples: $5.7 \times 10^5 \pm 1.3 \times 10^4$ (CFU/g dry soil). In the 2-y PM, the mycobiome decreased significantly, but seems to recover in the 4-y PM treatment: $2.7 \times 10^5 \pm 7.0 \times 10^4$ (CFU/g dry soil). Variations on mycobiome statistically correlated with DOC, C_{tot} and pH ($p < 0.05$). The mycotoxin concentration in soil under PM was associated with variations on DOC, total C and N.

The type and duration of the mulching influence the soil physicochemical parameters, resulting in a reduced in soil fungal abundance after 2-y PM, but in a high DON/NIV concentration. Further studies are needed to assess whether this findings may be reflected in commodities cultivated under plastic.

The authors want to thank the financial support by the Prof B. Gedek and W. Gedek foundation

P47

Analysis of fourteen mycotoxins in corn and corn derived-products during wet-milling by LC-MS/MS

Hee-Min Noh[1], Woori Kim[1], Chanvorleak Phat[1], Dong-Ho Kim[2], Chan Lee[1]*

[1]Brain Korea 21 PLUS Project for Department of Food Science and Technology, Chung-Ang University, Anseong, Republic of Korea; [2]National Agricultural Products Quality Management Service, Gimcheon, Republic of Korea

* Corresponding author: chanlee@cau.ac.kr

Co-occurrence of Fusarium and Aspergillus mycotoxins such as deoxynivalenol (DON), fumonisins (FUMs) and aflatoxins have been reported in many fungal contaminated foods and feeds. These mycotoxins are relatively highly contaminated in corn more than other grains. The distribution of fourteen mycotoxins including aflatoxins (B1, B2, G1, G2), ochratoxin A, fumonisins (B1, B2), deoxynivalenol, zearalenone, T-2 toxin and HT-2 toxin was analyzed in corns and corn derived-products to investigate co-occurrence and transferring of mycotoxins in wet- milled maize fractions in Korea. Fifty samples were collected from three different factories producing corn starch with by-products such as corn bran, corn gluten, corn gluten feed, corn germ, corn steep liquor (CSL) and light steep water (LSW). The levels of all mycotoxins were analyzed by liquid chromatography coupled triple quadrupole mass spectrometry (LC-MS/MS) simultaneously using IAC (immunoaffinity column) for pretreatment. The coefficients of correlation (R2) in standards for AFB1, FB1, ZEN were 1.0000 and those of other toxins were between 0.9992 and 0.9999. Overall, the recovery ratio of most mycotoxins was in range of 73.9%~133.0%. Most mycotoxins could be recovered within the range of the guidelines of codex (the ratio 75~120% at the level of 10 ug/kg~100 ug/kg) and AOAC (the ratio 80%~110% at the level of 100 ug/g). As a results of simultaneous analysis, the levels of all mycotoxins were at the maximum in corn gluten feed samples and were at the minimum in corn starch samples. Among the fourteen toxins, the contamination level of DON was the highest of 7,410 ug/kg. DON and FUMs were contaminated heavily in corn gluten, CSL, LSW and it mainly transferred to corn gluten feed. Whereas, ZEN was more contaminated in corn gluten compared with DON and FUMs showing various distribution patterns. However, the levels of other mycotoxins, such as aflatoxins, ochratoxin A, T-2 and HT-2 toxin were very low in all fractions. Except for CSL and LSW, the levels of fourteen mycotoxins were under the guideline for mycotoxin level of food standards of European Food Safety Authority and Codex.

P48

Initial response to low deoxynivalenol (DON) doses in combination with low lipopolysaccharide (LPS) concentration and their consequence on interleukin production

Constanze Nossol[1]*, Anikò Barta-Böszörményi[1], Michael Oster[2], Klaus Wimmers[2], Lydia Renner[1], Stefan Kahlert[1], Sven Dänicke[3], Hermann-Josef Rothkötter[1]

[1]Institute of Anatomy, Magdeburg, Germany; [2]Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany; [3]Institute of Animal Nutrition, Braunschweig, Germany

* Corresponding author: constanze.nossol@med.ovgu.de

DON is a common cereal contaminant. It is known for its immuno-modulating properties Therefore, the exposure to this toxin might pose a health risk for both human and farm animals. Pigs are particularly susceptible to DON and show poisoning symptoms after DON-intake like feed refusal, reduction in feed intake and vomiting (1). In our study we analysed the effect of low DON (50 ng/mL) and LPS (10 ng/mL) doses and their combination via microarray analyses after 3 hours of incubation on porcine monocyte derived dendritic cells (MoDCs). 4 Treatment groups were defined: 1. control, 2. 50 ng/mL DON, 3. 10 ng/mL LPS E. coli and 4. 50 ng/mL DON + 10 ng/mL LPS E. coli. Overall, we found 1639 genes which are significantly regulated in the comparison of treatment groups to control. Furthermore, 636 genes were significantly up- und 1003 genes were significantly down regulated. The comparison between control and DON treatment group, resulted in only 3 different regulated genes e.g. GRP78 is significantly up-regulated. In contrast, 1376 genes were significantly regulated in CON vs. DON/LPS and 1023 in CON vs. LPS. In further analyses of CON vs. DON/LPS and CON vs. LPS, we found 760 genes which are the same in both comparisons to control group. IL-1B, Il-6 and TNFα are 3 interleukins which are high significantly up-regulated in the treatment groups in both comparisons CON vs. DON/LPS and CON vs. LPS. Functional analyses of IL-6 production showed also a significant increase in comparison to control. Due to its role as an important mediator of the immune reaction in the innate immune system response, IL-8 was also examined. In all treatment groups an up-regulation was found in the microarray. This corresponds with our functional analyses. Here, we found a synergistic effect of both low DON and LPS doses.

References: [1] Rotter BA, Prelusky DB, Pestja JJ (1996). Toxicology of deoxynivalenol. (vomitoxin). J Toxicol Environ Health 48(1):1-34

P49

Optimization of Total Aflatoxin Recovery Levels in Overparticular Matrices Using a Twenty-Minute ELISA by Dilution Normalization and Two Incubation Steps

Antonios Ntantasios*, Sotirios Athanasiou , Christos Chatzoglou, Nikolaos Natsaridis

R&D Department, Prognosis Biotech Ltd, Larisa, Greece

* Corresponding author: lab@prognosis-biotech.com

Introduction: There have been many problems with total aflatoxin recovery levels using most commercial competitive ELISAs for grains. Especially, corn germ, corn silage, cottonseed, ginger, paprika and walnuts require clean-up pre-columns because when extracted, form overparticular matrices. Extracts usually constitute one-third of the incubated mixture affecting negatively the HRP and competitive step. Although most immunossays run better after pre-column treatment, unsatisfactory recovery levels are unavoidable.

Purpose: The purpose of this work was an alternative way to improve the recovery levels of total aflatoxin in overparticular matrices avoiding the pre-column treatment.

Methods: Fifty grams of the above samples were extracted with 70% methanol and after filtration the total aflatoxin was determined by a direct competitive ELISA with or without pre-column treatment. Micoplates were coated with a high affinity monoclonal antibody produced after mice immunisation, preparation of hybridomas, clones screening and protein G purification. The immunoassay had two incubation steps (in contrast with other tests), the first incubation of 20% extract and 80% HRP-free buffer mixture for 10 minutes followed by washing steps, the second incubation of aflatoxin-HRP for 5 minutes followed by washing steps and the addition of chromogen for 5 minutes followed by the addition acidic solution. The aflatoxin-HRP and the immunogens were produced by organic modification of hapten, active ester biochemical conjugation, optimization of conjugates molarity and gel filtration purification.

Results: The immunoassay requires only 20% of extract and the sensitivity remains in increased levels giving the maximum range of quantification. Due to increased dilution normalization of matrix and enzyme protection from methanol by using two incubation steps, the recovery levels were more acceptable without pre-column step.

Significance: This ELISA constitutes a very valuable tool in the total aflatoxin analysis since optimize the recovery levels leading in an effortless and rapid immunoassay without pre-column treatment.

P50

Variability in accumulation of *Fusarium* mycotoxins in grain of winter wheat breeding lines artificially inoculated with *F.culmorum* in 2015

Piotr Ochodzki[1]*, T. Góral[1], H. Wiśniewska[2], I. Grzeszczak[1], M. Kwiatek[2]

[1]Department of Plant Pathology, Plant Breeding and Acclimatization Institute-NRI, Radzikow, Blonie, Poland; [2]Institute of Plant Genetics, Polish Academy of Sciences, Poznan, Poland

* Corresponding author: p.ochodzki@ihar.edu.pl

Fusarium head blight (FHB) is a disease of grain caused by the complex of toxinogenic *Fusarium* fungi. The most important species are highly pathogenic: F. graminearum and F. culmorum producing deoxynivalenol and zearalenone, which can cause severe epidemics of FHB.

One of the most efficient ways leading to reduction of mycotoxin contamination of food products is breeding of cultivars with improved resistance to FHB. The aim of our study was to evaluate selected winter wheat breeding lines in terms of mycotoxin accumulation.

In the field experiments in two locations: Radzików (Central Poland) and Cerekwica (Western Poland) the heads of 70 winter-type wheat lines were artificially inoculated with F.culmorum at flowering stage. Grain of 35 genotypes with elevated resistance to FHB was analyzed for content of mycotoxins: deoxynivalenol (DON), zearalenone (ZEA) and ergosterol (ERG).

The mean content of ERG was relatively low (2.1 ppm), and was 4 fold higher in Western Poland (4.1 ppm) than in Central Poland (0.9 ppm).

The deoxynivalenol (DON) content in wheat grain varied from 480 to 38065 ppb (average 15621 ppb) the level of DON in Western Poland was 20 fold higher than in Central Poland (21114 ppb and 848 ppb respectively).

The content of ZEA was very low (43 ppb). The range of variation of 0 to 148 ppb. In Cerekwica, ZEA content was more than 10 times higher than in Radzików.

This year's conditions were unfavorable for the development of FHB, as indicated by the low value of analyzed compounds. Only the DON content was high in Western Poland, except that twice lower compared to 2014. Despite the unfavorable weather conditions, obtained results showed that in the winter wheat population coming from Polish breeding programs, there is variability in resistance to the accumulation of *Fusarium* mycotoxins in the grain. It has been that significant relationship between damage to the grains and the content of toxins were found. As a result, it seems possible to select resistant genotypes combining different types of resistance. Such genotypes are stable under various weather and exhibit good resistance not only to decreases in grain yield and contaminate the grain which by toxins caused by Fusarium blight.

Acknowledgements

This project was financially supported by Ministry of Agriculture and Rural Development (project: HORhn 801-12/14, no. 6)

P51

Fusarium graminearum infection via wounding promotes mycotoxin contamination of maize ears

Elisabeth Oldenburg[1]*, Frank Ellner[2]

[1]Julius Kühn-Institut, Braunschweig, Germany; [2]Julius Kühn-Institut, Berlin, Germany

* Corresponding author: elisabeth.oldenburg@jki.bund.de

The infection of maize ears may either result from fungal entry via the silks at the stage of flowering or via injuries caused by insects, animals or damage from storm or hail. The European corn borer (Ostrinia nubilalis), a major pest of maize (Zea mays L.) known to promote infections of Fusarium spp. of Liseola section and, therefore, the formation of mycotoxins in maize grain, has spread to northern regions of Central Europe, where Fusarium spp. of Discolor and Roseum sections are the predominant pathogenic species. In order to investigate whether physical injuries may increase the risk of mycotoxin contamination resulting from pathogens of the Discolor section, a field study was conducted infecting maize ears with Fusarium graminearum by simulating either entry of the pathogen via the silks or via damage of the female inflorescence.

The experimental field was located at the research facility of the Julius Kühn-Institut and conducted during the vegetation periods of 2012 and 2013. The maize cultivar 'Oldham' was grown in plots consisting of 6 rows, 3 m long with 0.75 m row spacing and a density of 10 plants m^2. Ten main ears each were inoculated at the stage of female flowering (BBCH 65) with 1 x 10^5 conidia of F. graminearum according to the following variants: (A) placement into the silk channel without wounding the inflorescence, (B) placement with a syringe by wounding the inflorescence in the upper half of the ear. Each of the two inoculation-series was manually harvested in September of both years, when ears showed heavy outer signs of ear rot symptoms. After removing the husks the upper half of each of the ten ears were freeze-dried for 3 days before ground to a fine powder and analysed for deoxynivalenol (DON), 3-/15-acetyldeoxynivalenol (3-/15-acDON) and zearalenone (ZON) by LC-MS (5500 QTrap).

In both years of investigation, DON and 15-acDON were the dominant toxins produced. When the ears were inoculated via the silks, DON concentrations ranged from 4.9 - 26 mg kg^{-1} (yearly average) which was considerably lower than in wounded ears, with 108 and 270 mg kg^{-1} in both years, respectively. The same trend was observed with respect to 15-acDON showing lower concentrations (<1 - 35 mg kg^{-1}) after infection via the silks compared with wounding (4.5 - 215 mg kg^{-1}). 3-acDON was only occasionally detected at levels ≤ 1 mg kg^{-1}, whereas high ZON concentrations up to 50 mg kg^{-1} were only found after wounding in 2013.

It is suggested that the risk of mycotoxin contamination due to F. graminearum infection is considerably increased, when wounding facilitates penetration of the pathogen into internal tissues of the ear, e.g. via entry points resulting from European corn borer infestation.

P52

Recent information about mycotoxins and the IARC classification of human carcinogens

Vladimir Ostry [1]*, Frantisek Malir[2], Yann Grosse[3], Jakub Toman[2]

[1]National Institute of Public Health, Center for Health, Nutrition and Food, Brno, Czech Republic; [2]University of Hradec Kralove, Faculty of Science, Hradec Kralove, Czech Republic; [3]International Agency for Research on Cancer, Lyon, France

* Corresponding author: ostry@chpr.szu.cz

Mycotoxins have been implicated in a range of human diseases and occur in foodstuffs of plant and animal origin. The ingestion of mycotoxins can produce both acute and chronic toxicities ranging from death to chronic interferences with functions of the central nervous, cardiovascular and pulmonary systems, and of the gastrointestinal tract. Some mycotoxins are carcinogenic, mutagenic, teratogenic and immunosuppressive.

The agents evaluated by the IARC (International Agency for Research on Cancer) are classified into five groups based on the existing scientific evidence for their carcinogenicity: group 1: "Carcinogenic to humans" there is sufficient evidence to conclude that it can cause cancer in humans; group 2A: "Probably carcinogenic to humans" there is strong evidence from human, experimental animals and/or mechanistic data that it can cause cancer in humans, but at present it remains insufficient; group 2B: "Possibly carcinogenic to humans" there is some evidence from human, experimental animals and/or mechanistic data that it can cause cancer in humans, but at present it is still far from being conclusive; group 3: "Unclassifiable as to carcinogenicity in humans" there is insufficient, inadequate or no data to classify the agent, and group 4: "Probably not carcinogenic to humans" there is strong evidence that it does not cause cancer in humans.

Mycotoxins are currently classified by the IARC (as of January 28, 2016) as follows: aflatoxins, group 1 in 2012; citrinin, group 3 in 1987; cyclochlorotine, group 3 in 1987; deoxynivalenol, group 3 in 1993; fumonisin B1, group 2B in 2002; fumonisin B2, group 2B in 1993; fusarenone X, group 3 in 1993; kojic acid, group 3 in 2001; luteoskyrin, group 3 in 1987; nivalenol, group 3 in 1993; ochratoxin A (OTA), group 2B in 1993; patulin, group 3 in 1987; penicillic acid, group 3 in 1987; rugulosin, group 3 in 1987; sterigmatocystin, group 2B in 1987; T-2 toxin, group 3 in 1993; and zearalenone, group 3 in 1993.

Even though the classification of OTA in group 2B is based on solid ground (sufficient evidence of carcinogenicity in experimental animals), the mechanisms of carcinogenicity involved have often been controversial. However, new information regarding the genotoxicity of OTA (formation of OTA-DNA adducts), its role in oxidative stress, and the identification of epigenetic factors involved in OTA carcinogenesis, could provide strong evidence that OTA carcinogenicity is mediated by a mechanism that also operates in humans, which could lead to an upgrade from 2B to group 2A.

Supported by Ministry of Health, Czech Republic - conceptual development of research organization („National Institute of Public Health – NIPH, IN 75010330“) and by the specific research project (no. 2118/2015) of the Faculty of Science, University Hradec Kralove, Czech Republic

P53

Application of an aqueous, time-saving, simple, extraction method for multiple mycotoxins.

Elise Palmer*, John Davenport, Xinwen Wang

VICAM - A Waters Business, Milford, USA

* Corresponding author: elise_palmer@waters.com

Amongst the most common mycotoxins are deoxynivalenol (DON) and aflatoxin (AF), both of which can have adverse health effects. Simple, swift, and effective extraction is essential for rapid detection using on-site screening. The analysis of mycotoxins has been a challenge largely due to the complexity of food matrices and desired low detection limits. As such, current extraction methods have relied on the use of environmentally harmful organic solvents, and multiple manipulation steps by largely untrained personnel. In this study, we describe the use of a simple, single, sampling and extraction process, giving quantitative results for multiple mycotoxins in five minutes.

AFLA-V® and DON-V® strip tests were used, with a Vertu® reader, to accurately detect and measure total aflatoxins (B1, B2, G1 and G2), and total deoxynivalenol, in grain, using a single, water-based sample extraction procedure. 20 g corn was stirred for 2 minutes in 100 mL AQUA® solution, filtered, and applied to each test. For DON-V®, this involved a single dilution of the extract, and application to the strip, followed by a rapid 3 minute development time; for AFLA-V® this was direct application of extract, and a short 5 minute development time. Quantitative values for both toxins were obtained on the Vertu® reader.

Naturally contaminated AF and DON, and clean corn samples, were all tested using the above method, and results were correlated with HPLC data. All data fell within the relevant GIPSA specifications and requirements, with detection limits for total AF and DON below the EU and US regulatory limits in corn. The time required from dry sample to result, inclusive of both toxins, is less than 10 minutes.

The results indicate that this new time-saving, water-based single extraction method for multiple mycotoxins, minimizes risk to the operator and environment, and can be used in conjunction with Vertu® products to accurately quantify potentially harmful mycotoxin contamination in food and feed.

P54

Risk assessment of fumonisin B1 and B2 in feed stuff for livestock

JuHee Park[1], JooWon Lim[1], Hansub Jang[2], Chan Lee[1]

[1]Brain Korea 21 PLUS Project for Department of Food Science and Technology, Chung-Ang University, Anseong, Republic of Korea; [2]National Agricultural Products Quality Management Service, Gimcheon, Republic of Korea

* Corresponding author: chanlee@cau.ac.kr

Fumonisin B1 and B2 produced in naturally contaminated foods and feeds have harmful effects on human and livestock by mycotoxin intoxications such as porcine pulmonary edema, vomiting, feed intake refusal, reduced body weight gain and reproduction, and even death. So far, the permitted levels of the fumonisin B1 and B2 in livestock have not been established in South Korea. Therefore, the contamination of Fusarium mycotoxins, fumonisin B1 and B2 were monitored to evaluate the level of mycotoxin in 754 animal feed samples (578 compound feed and 176 feed ingredients) produced in domestic in years 2009-2014. In addition, daily dietary exposure of these mycotoxins for cows, pigs, and chickens were estimated for to set the regulation level of fumonisin B1 and B2 in feeds. Furthermore, risk assessment was also performed by comparing the estimated daily exposure levels in main livestock with the species-specific toxicity reference doses (NOAEL, LOAEL, BMDL, etc.). Detection frequencies of fumonisin B1 and B2 in compound feeds were 91% and 59%, respectively. This result explains that most compound feeds were contaminated with fumonisins. In case of feed ingredients, detection frequency of fumonisin B1 and B2 were determined as 62%.and 43%, respectively. In general, poultry was exposed to the highest level of fumonisin B1 and B2. The chronic and acute exposure rates of fumonsin B1 were 106.1% and 296.7%, and those of B2 were 21.8% and 61.8% respectively. Dairy cows were exposed to the lowest level of fumonisin B1. The chronic exposure rate of fumonosin B1 was was 19.6% and acute exposure rate was 47.2%. Interestingly, pigs were exposed to the lowest level of fumonisin B2 showing chronic and acute exposure rates of 0.8% and 2.7%, respectively. Based upon the monitoring results and data of risk assessment, the chronic and acute risk indexes of fumonisins B1 were calculated as 22.0% and 107.3% for pigs and 5.3% and 14.8% for poultry, respectively. Fumonisin B2 can be regarded as safe with no risk because of low chronic and acute exposure rates under 5%.

P55

Complex formation of cyclodextrins with zearalenone as a tool to decrease toxin exposure

Miklós Poór[1]*, Beáta Lemli[2], Sándor Kunsági-Máté[2], Nikolett Sali[3], Tamás Kőszegi[3], Lajos Szente[4]

[1]Department of Pharmacodynamics, Pécs, Hungary; [2]Department of General and Physical Chemistry, Pécs, Hungary; [3]Department of Laboratory Medicine, Pécs, Hungary; [4]CycloLab Ltd., Budapest, Hungary

* Corresponding author: poor.miklos@pte.hu

Mycotoxins are secondary metabolic products of fungi. They occur in foods, drinks, and animal feed causing health problems for humans and animals. Zearalenone (ZEA) is a mycotoxin produced mainly by Fusarium species. ZEA is a xenoestrogen therefore it cause reproductive disorders in farm animals and hyperoestrogenic syndromes in humans. Cyclodextrins (CDs) are built up from glucopyranose units, they have a conical structure with a hydrophobic interior and a hydrophilic exterior space. CDs are host molecules, the internal cavity can include a guest molecule forming non-covalent inclusion complexes. These host-guest interactions can be strongly influenced by the chemical modification of CDs.

In our study, the complex formation of ZEA with native and chemically modified β-CDs was investigated. ZEA is a fluorescent mycotoxin therefore fluorescence spectroscopy was applied to determine the binding constants of ZEA-CD complexes. Thereafter, in vitro cell experiments were performed as well, in order to examine the potential effect of CDs on the cellular uptake of the mycotoxin. Finally, removal of ZEA from toxin-exposed aqueous solutions by β-CD bead polymer was tested.

In the presence of β-CDs, strong increase of the fluorescence signal (15-20-fold) of ZEA was observed. The native β-CD form stable complex with ZEA (K ~ 10.000 L/mol), however, the complex stability of heptakis-2,6-di-O-methyl-β-cyclodextrin (DIMEB) with ZEA is approximately 6-fold higher compared to ZEA-β-CD complex. During the cell experiments, β-CD was found to effectively alleviate the ZEA-induced toxicity, on the other hand, DIMEB failed to protect the cells despite of its stronger complex stability with the mycotoxin. This phenomenon possibly can be explained by the strong affinity of DIMEB toward cholesterol which is a very important constituent of cell membranes. Even small amounts of β-CD bead polymer are suitable to effectively remove ZEA from aqueous solution. The beads (with the bound mycotoxin) can be easily removed by centrifugation or filtration. The above listed observations highlight that cyclodextrin technology may be a suitable tool in the future to decrease the exposure with ZEA.

Financial support of the University of Pécs, Medical School (PTE ÁOK-KA-2013/15) and the "Environmental industry related innovative trans- and interdisciplinary research team development in the University of Pécs knowledge base" (SROP-4.2.2.D-15/1/Konv-2015-0015) project are highly appreciated.

P56

Development and first applications of an LC-ESI-MS/MS multi-method for the quantification of Alternaria toxins

Hannes Puntscher[1]*, Benedikt Warth[1], Philipp Skrinjar[2], Doris Ellmer[2], Hannes Mikula[2], Doris Marko[1]

[1]Dept. Food Chemistry and Toxicology, Univie, Vienna, Austria; [2]Inst. Applied Synthetic Chemistry, Vienna UT, Vienna, Austria

* Corresponding author: hannes.puntscher@univie.ac.at

Alternaria toxins are secondary metabolites produced by different species of the fungal genus Alternaria. Even though several studies have already shown the acute toxicity, mutagenicity and genotoxicity of some of these Alternaria toxins, still no guideline limits have been defined by any regulatory authority. Because of the possible contamination of food and feed, these compounds may be of relevant concern in respect of human and animal health (EFSA, 2011).

In this study, a new high-performance liquid chromatography tandem mass spectrometric method was developed for the simultaneous quantification of 15 Alternaria toxins including the most abundant parent compounds (Alternariol (AOH), Alternariol monomethyl ether (AME), Tenuazonic acid, Altenuene, Tentoxin, Altertoxin I, Altertoxin II, Stemphyltoxin III and Alterperylenol) and some selected phase I (4-Hydroxy-AOH and 4-Hydroxy-AME) and phase II metabolites (AOH-3-glucoside, AOH-9-glucoside, AOH-3-sulfate, AME-3-glucoside).

The presented method was based on a recently published method by Tölgyesi et al., 2015, and shall be applied for the detection of Alternaria toxins in different sample matrices like food, feed, cell extracts and biological fluids. First examples and preliminary data on sample preparation procedures will be presented.

EFSA, 2011. Panel on contaminants in the food chain: Scientific opinion on the risks for animal and public health related to the presence of Alternaria toxins in feed and food. EFSA Journal 9, 2407.

Tölgyesi, Á., Stroka, J., Tamosiunas, V., Zwickel, T., 2015. Simultaneous analysis of Alternaria toxins and citrinin in tomato: an optimised method using liquid chromatography-tandem mass spectrometry. Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment 32, 1512-1522.

P57

Determination of total fumonisins and aflatoxins levels in some grain sorghum lines in Iran

Vahid Rahjoo[1]*, Mohammad Taghi Feizbakhsh[2]

[1]Seed and Plant Improvement Institute (SPII), Karaj, Iran; [2] Agricultural Research Center of Golestan Provin, Gorgan, Iran

* Corresponding author: vrahjoo@yahoo.com

Grain mold is an important disease of sorghum in warm and humid regions of the world and Iran. A complex of fungi especially Fusarium spp. and Aspergillus spp. cause moldy disease on grain sorghum. Some Fusarium species especially Fusarium verticillioides produces potent mycotoxins known as fumonisins. The aflatoxins are mainly produced by Aspergillus flavus and have an important economic impact on the grain quality. In order to determine total fumonisins and aflatoxins production on different grain sorghum lines, a field trial was carried out based on a randomized complete block design with 20 treatments and three replications for each mycotoxin at two locations: Karaj (near the capital, Tehran) and Gorgan (in the north of Iran) stations in 2015. The Sorghum panicles were inoculated by spraying of spore suspension isolates of each fungus at flowering stage. All infected kernels were evaluated by ELISA kits (Celer FUMO & Celer AFLA, Tecna, Italy) for their total fumonisins and aflatoxins production at the physiological maturing stage. All genotypes and locations showed statistically significant difference in their fumonisins and aflatoxins production. The results of fumonisins analysis obtained from ELISA test showed that total fumonisins amount of sorghum genotypes ranged from 0.75 to 3.31 ppm, and from 1 to 60 ppm in Karaj and Gorgan respectively, suggesting that fumonisin occurrence is probably related to humid conditions in the north of Iran. Total aflatoxins amount of sorghum genotypes ranged from 2 to 24.23 ppb, and from 2.7 to 80 ppb in Karaj and Gorgan respectively, suggesting that aflatoxins are also produce more under humid conditions in the north of Iran.

This work was supported by Seed and Plant Improvement Institute (SPII) and the Ministry of Jihad-e-Agriculture of the Islamic Republic of Iran.

P58

Reduction of Ochratoxin A in beer by using genetically improved lager brewing yeasts.

Sandra Rainieri*

AZTI, Food Research Division, Derio, Spain

* Corresponding author: srainieri@azti.es

Ochratoxin A (OTA) is one of the most widespread and toxic mycotoxins. This type of mycotoxin has been detected in foods and beverages, including barley, malt and beer. Given the widespread consumption of beer worldwide, the presence of OTA and other mycotoxins in this foodstuff is a relevant problem of food safety. OTA occurrence in beer has been related to the contamination of malt barley with ochratoxigenic species, particularly Penicillium verrucosum. OTA produced in grains is carried to wort and, although fermentation decreases the concentration, the toxin is not eliminated. Besides reducing the fungal contamination of malt barley, additional strategies to further reduce the concentration of OTA in contaminated samples should be developed. In this work the construction of lager brewing yeasts specifically designed to reduce the concentration of OTA is described. Lager brewing yeasts, currently classified as Saccharomyces pastorianus, are considered hybrids between two different species; Saccharomyces cerevisiae and Saccharomyces eubayanus or S. bayanus. By hybridizing yeasts belonging to the two species it is possible to construct genetically improved lager brewing strains. In this work three different S. cerevisiae strains previously selected for being able to reduce OTA in wine (Caridi et al., 2012) have been hybridized to a strain of S. bayanus previously used to construct artificial lager brewing yeasts (Rainieri et al., 2006). Three lager brewing strains were obtained. Those strains were used to ferment wort spiked with OTA (200 ug/L). After the fermentation the concentration of OTA was considerably reduced. Additionally, the beer obtained showed good organoleptic characteristics demonstrating that this is a good strategy to reduce the content of mycotoxins in lager beer.

References:

Caridi et al. J. Gen. Appl. Microbiol., 58, 225-233 (2012)

Rainieri et al., Yeast Volume: 24: S1 – S38, 01-41

Acknowledgments to Prof. Paolo Giudici and Prof. Andrea Pulvirenti (University of Modena and Reggio Emilia, Italy) for providing strains and for scientific advice. Study funded by the Government of the Basque Country.

P59

Development of in vitro model plant system to study the detoxification ability of different wheat varieties

Laura Righetti[1]*, Enrico Rolli[2], Chiara Dall'Asta[1]

[1]Department of Food Science, University of Parma, Parma, Italy; [2]Department of Biosciences, University of Parma, Parma, Italy

* Corresponding author: laura.righetti1@studenti.unipr.it

Fusarium head blight (FBH) is one of the most important fungal wheat diseases worldwide. Several types of resistance have been described to FHB, classified as type I (resistance towards initial infection of spikelets), type II (resistance against spread of pathogen within spike) and type III (resistance to toxin accumulation in grains). Focusing on type III resistance, mycotoxin detoxification through glycosylation, has been already described in bread wheat and barley. However, data are scarily reported so far about durum wheat, a key cereal in the pasta production chain. The objective of this project was to study the ability of two different durum wheat varieties, Kofa and Svevo, to detoxify and convert DON and ZEN into their modified forms.

To address this issue, in vitro model plant system was developed exploiting a micropropagation technique. In addition, in order to determine the detoxification capacity of the different plant organs, three experiments were separately set up; (i) micropropagated detached roots, (ii) stems and (iii) leaves.

The parent mycotoxins DON and ZEN were added into the growing medium and the system was placed at controlled temperature and light for 14 days. Growing media were sampled 5 times during 24 hours (t1=0, t2=1h, t3=6h, t4=12h, t5=24h) in order to highlight the detoxification ability in the first phases, then after 7 days. In the end, after 14 days, detached roots, stems and leaves, together with the remaining medium were collected and processed.

Results showed that Kofa exhibited a higher detoxification ability compared to Svevo. Two strategies were used to confirm modified forms: (i) the biosynthesis of DON3Glc was confirmed using its respective commercially available reference standard, and (ii) ZEN modifications ZEN14Glc, ZEN16Glc, ZEN-Sulf, α and βZEL, α and βZEL-Glc were identified and confirmed using Orbitrap high-resolution mass spectrometry.

P60

Variety specific control of fusarium head blight and mycotoxin reduction in winter wheat by the use of fungicides

Bernd Rodemann *, Tim Baumgarten

Julius Kühn-Institute; , Braunschweig, Germany

* Corresponding author: bernd.rodemann@jki.bund.de

The occurrence of numerous Fusarium species in various crop species often leads to plant damage and is connected with the production of mycotoxins in the plant tissue. In addition to yield damage and quality losses the contamination of the grains limits the use for human consumption and animal exploitation.

To inhibit the infection and toxin formation in the locations with a significant hazard risk the efficacy of fungicide use and tank mixtures against fusarium head blight were evaluated under field conditions. Additionally the winter wheat varieties Toras, SU Anapolis, Colonia, Julius, Tobak and Kurt, fitted with different resistance properties but similar beginning of flowering were included in the investigations. The inoculation was done at GS 61-63 once with a suspension of 5 x 105 conidia of Fusarium culmorum at a water volume rate of 600 L / ha. Under field conditions the FHB nursery was investigated at several times 22, 28 and 33 dpi. Furthermore, the FHB and AUDPC were calculated using both incidence and severity. In all treatments the DON contamination of the grains was also analyzed.

Active substances with different target sites for inhibition mycotoxin synthesis were tested in the field studies of 2014 /2015. The applied azoles were metconazole, epoxiconazole, thiophanate-methyl and prochloraz. These have been applied 48 hours after inoculation. It were assessed the effectiveness avoiding the infection of the ear and reduction of toxins (deoxynivalenol, zearalenone, T2/HT-2 toxin). In all treatments FHB symptoms between 2 to 65% were assessed 33 days after inoculation. The first results confirm the official classification of the varieties in the susceptibility for Fusarium head blight: Toras < SU Anapolis < Colonia = Julius < Tobak < Kurt.

Using the low susceptible variety Toras primary infections could be determined only at individual florets and the fusarium head blight could be reduced by 75%. Applying additional the fungicides in cv. Toras the effectiveness was increased up to 92%. In mean of all varieties, the visual ear infection could be prevented most effectively combining metconazole + epoxiconazole + thiophanate-methyl between 60-90%.

Analysing the harvested grains revealed DON contents up to 7.4 mg/kg. A very strong correlation coefficient (r=0.95**) was observed between the visual disease symptoms and the mycotoxin content in grains.

The highest DON reduction of the cultivars was achieved by cv. Toras without fungicide on a level of 83% compared to the high susceptible cv. Tobak. Applying the tankmix of metconazole + epoxiconazole + thiophanate-methyl the efficacy has been increased to 90% by a range from 60-94%. In the susceptible cv. Kurt and Tobak levels between 48% and 60% were accessible.

These two year field studies demonstrated that a fusarium specific inhibition of mycelium growth was coupled with a lower concentration of deoxynivalenol in the grain. In summary, an optimized use of fungicides as part of an integrated pest management system can be achieved by comparative studies of fungicide performance and taking into account the resistance type of varieties.

P61

Deoxynivalenol and its modified forms in Croatian wheat harvested in 2015

Bojan Šarkanj[1]*, Lidija Brodar[1], Michael Sulyok[2], Rudolf Krska[2], Tomislav Klapec[1]

[1]Faculty of Food Technology, Osijek, Croatia; [2]Department of Agrobiotechnology, IFA-Tulln, BOKU, Tulln, Austria

* Corresponding author: bsarkanj@ptfos.hr

Deoxynivalenol (DON) and its modified forms 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), deepoxy-deoxynivalenol (DOM-1), deoxynivalenol-3-glucoside (D3G), deoxynivalenol-3-sulfate and deoxynivalenol-15-sulfate were determined in wheat samples from Croatia applying a liquid chromatography–tandem mass spectrometry multi-mycotoxin method. Considering DON is one of the most frequently occurring trichothecene mycotoxin produced by various Fusarium species, it greatly affects agriculture causing yield & technological quality loss through Fuasrium head blight (FHB) disease. Plants can metabolise DON to conjugated, masked mycotoxins D3G, deoxynivalenol-3-sulfate and deoxynivalenol-15-sulfate which are not legally regulated. However, free DON can be released from conjugates during digestion so it is important to monitor their concentrations. In this survey 62 wheat samples were collected from all Croatian regions. DON was detected in 31 samples (50%), with the maximum concentration 1065.3 ppb and the median was 123.4 ppb. D3G was detected in 26 samples (42%), with the maximum concentration 182.1 ppb, and the median 31.0 ppb, and 3-AcDON in 7 samples (11%) with the maximum concentration 10.6 ppb, and the median 4.7 ppb. DON-sulfates, DOM-1 and 15-AcDON were not detected in any of the samples. Most of the samples with detected DON and the highest detected concentrations originated from eastern Croatian regions. This can be related with weather conditions during wheat flowering. Air temperature and amount of rain in May were above average in eastern Croatia and that created favourable conditions for Fusarium infection. Conversely, the wheat grown in south Croatian regions at the coast did not have high levels of DON (up to 10.6 ppb), and no modified DONs were detected.

Key words: deoxynivalenol, modified deoxynivalenol, LC-MS/MS multi-mycotoxin detection, occurrence, Croatia

Acknowledgments: This research was financially supported by the European Structural and Investment Funds, European Social Fund (ESF), Power of Development – Human resources development, CroMycoScreen HR.3.2.01-0274

P62

Reduction of T-2 and HT-2 toxins in oats by combined mechanical and thermal energies

Henning S. Schmidt, Stefanie Becker, Benedikt Cramer, Hans-Ulrich Humpf*

Institute of Food Chemistry, Münster, Germany

* Corresponding author: humpf@wwu.de

The secondary fungal metabolites T-2 toxin (T2) and HT-2 toxin (HT2) are Fusarium mycotoxins that are classified into the group of type-A trichothecenes with T2 being regarded as the most acute cytotoxic toxin amongst the group of trichothecene mycotoxins. Both occur frequently in oats which is in times of a growing interest on oat-derived products for a healthy and balanced diet considered as an increasing problem due to the lack of maximum levels in the European Union.

Strategies affecting the mycotoxin-content in food range from agricultural management to prevent fungal infestation to food processing steps that either remove the toxins or destroy the mycotoxins' molecular structure. Thermal food processes were shown to reduce the content of some mycotoxins in food efficiently. Extrusion cooking is a high-temperature short-time (HTST) process, providing high rates of thermal and mechanical energy and is therefore a promising technology to lower the T2 and HT2 concentrations in extruded oats.

We therefore investigated the degradation of T2 and HT2 during extrusion cooking on a laboratory food extruder using toxin-spiked and naturally contaminated oatmeal. In naturally contaminated samples we also considered the stability of the masked forms of T2 and HT2.

P63

Necessity of different moieties of the ochratoxin A molecule to influence collagen formation or gene expression: structure-activity-studies with synthesized derivatives in comparison to natural OTA

Gerald Schwerdt[1]*, Ulrike Rottkord[2], Marie-Christin Schulz[1], Hans-Ulrich Humpf[2], Michael Gekle[1]

[1]Julius-Bernstein-Institute of Physiology, Halle, Germany; [2]Institute of Food Chemistry, Münster, Germany

* Corresponding author: gerald.schwerdt@medizin.uni-halle.de

Although many studies have been done to unravel the toxic mechanisms of the ubiquitous mycotoxin ochratoxin A (OTA) the underlying mode of its action on cells is still unknown. Whatever this may be, a prerequisite for the adverse effects of OTA is its interaction with target molecules. This interaction needs specific properties of either the target molecules (most probably proteins) but also of the OTA molecule itself. Looking at the OTA molecule, some of its moieties may be of importance for its interaction as e.g. the amino acid moiety (phenylalanine) or the halogen (chlorine). Therefore, to test the necessity of these parts of the molecule, a set of OTA derivatives was used, in which either the amino acid was exchanged against another (tyrosine or alanine) or the halogen against another (fluorine, bromine or iodine) or against both, the amino acid and the halogen. These derivatives were administered to human renal cells (RPTEC, HK2 and HEK-T) and the influence on collagen formation and on gene expression (exemplary that of an in the WISP1 gene located RNA transcript that is highly inducible by OTA) was determined in comparison to the effects of natural OTA. These studies revealed that the exchange of the halogen had almost the same effect on collagen formation and on gene expression as OTA has. In contrast, the exchange of the amino acid moiety led to less enhanced collagen formation and also to less enhanced expression of the WISP1-RNA. Therefore, we conclude that the amino acid phenylalanine is essential for the interaction with at least those molecules which are in some way involved in collagen formation or gene expression. Therefore, based on our observations it seems worthwhile to search for proteins which handle with a phenylalanine moiety of their interaction partner in order to identify probable candidates involved in the adverse effects of OTA.

Supported by the Deutsche Forschungsgemeinschaft (DFG) SCHW1515/2-1 and HU 730/12-1)

P64

How does the Fhb1 Locus of Wheat Affect the Ability to Detoxify DON – a Hypothesis

Gerald Siegwart[1], Wolfgang Schweiger[2], Benedikt Warth[3], Herbert Michlmayr[1], Sonia Vautrin[4], Barbara Steiner[2], Rudolf Krska[3], Rainer Schuhmacher[3], Helene Berges[4], Hermann Buerstmayr[2], Gerhard Adam[1]*

[1]Dep. of Applied Genetics and Cell Biology, BOKU, Vienna, Austria; [2]Inst. of Biotechnology in Plant Prod., BOKU (IFA), Tulln, Austria; [3]Center for Analytical Chemistry, BOKU (IFA), Tulln, Austria; [4]INRA – CNRGV, Castanet Tolosan, France

* Corresponding author: gerhard.adam@boku.ac.at

The mycotoxin deoxynivalenol (DON) is a virulence factor of Fusarium graminearum required for spreading of the pathogen in the infected wheat head. The major spreading resistance QTL of wheat, the Fhb1 locus, has been associated with increased ability to detoxify DON by formation of a glucose conjugate, leading to the hypothesis that Fhb1 either encodes a UDP-glycosyltransferase (UGT) or a gene regulating its activity. Within the Fhb1 interval of the sequenced susceptible cultivar Chinese Spring no UDP-glycosyltransferase (UGT), which might catalyze this detoxification-reaction, was found.

We have determined the Km value (concentration of half-maximal enzyme activity) of a recombinant UGT capable of detoxifying DON into DON-3-O-glucoside for the UGT co-substrate UDP-glucose. The observed Km value of 2.2 mM is about 10x higher than the concentration found in wheat heads at anthesis in Fhb1 lines, suggesting that the availability of the co-substrate UDP-glucose is limiting the detoxification ability. Wheat lines containing Fhb1 show about 2.5-3x higher levels of UDP-glucose than lines lacking it. A gene biochemically linked to UDP-glucose was found in the Fhb1 interval of Chinese Spring, potentially encoding a truncated UDP-glucose dehydrogenase (UGDH), presumably a nonfunctional pseudogene. Both enzymes, UGT and UGDH, use UDP-glucose as a co-substrate - the first to detoxify DON into DON-3-O-glucoside, the latter to produce UDP-glucuronic acid, a precursor for activated sugar donors for cell wall biosynthesis. Co-expression of functional UGDH and UGT in yeast leads to a decrease of UDP-glucose levels and to sensitivity against DON compared to yeast expressing the UGT alone. We were therefore interested to learn what is encoded in the Fhb1 interval of a resistant wheat cultivar. A comparison of the corresponding region between the Fhb1 containing cultivar CM-82036 with Chinese Spring revealed that the pseudo-UGDH is absent in the Fhb1 cultivar due to a 41.5 kb deletion.
The mechanism how the pseudogene lowers the UDP-glucose level is under investigation. A hypothesis how an absent pseudogene can confer a dominant resistance phenotype will be presented.

Funded by the Austrian Science Fund FWF (SFB Fusarium, F3706, F3708 and F3711)

P65

Deoxynivalenol and its metabolite deepoxy-deoxynivalenol: multi-endpoint analysis for the evaluation of cellular effects

Alexandra Springler, Sabine Hessenberger, Nicole Reisinger, Corinna Kern, Veronika Nagl, Gerd Schatzmayr, Elisabeth Mayer*

Biomin Holding GmbH, Tulln an der Donau, Austria

* Corresponding author: alexandra.springler@biomin.net

The mycotoxin deoxynivalenol (DON) contaminates agricultural commodities worldwide, posing health threats to humans and animals. Associated with DON, are derivatives, such as deepoxy-deoxynivalenol (DOM-1), produced by enzymatic transformation of certain intestinal bacteria. Particular relevance of DOM-1 in monogastric animals, where bacterial transformation of DON to DOM-1 is unlikely to occur prior to reaching the small intestine, arises due to the use of a feed additive, containing DON to DOM-1 transforming bacteria. The present study compared effects of DON and DOM-1, using differentiated porcine intestinal epithelial cells (IPEC-J2), in a detailed cytotoxicity study based on lysosomal activity, total protein content, membrane integrity, mitochondrial metabolism, and ATP synthesis. Furthermore, it investigated the ability of DON and DOM-1 to induce apoptosis, mitogen activated protein kinase (MAPK) signaling, oxidative events, and alterations of mitochondrial structure. DON differentially influenced IPEC-J2 viability, depending on the cellular endpoint being analyzed. DON compromised viability according to assays measuring lysosomal activity, total protein content, and membrane integrity, however to strikingly different degrees. DON however increased viability according to assays based on mitochondrial metabolism and ATP synthesis. DON dose-dependently induced cleaved caspase-3 (maximum induction 3.9-fold) and MAPK p38 (maximum induction 1.46-fold), altered mitochondrial morphology, but did not increase intracellular ROS. Its de-epoxy metabolite DOM-1 however, left cells entirely unaffected at equimolar concentrations. The dependence of the degree of DON toxicity on the chosen cytotoxicity parameter highlights the need for meticulous evaluation of the method used for in vitro assessment of cell viability. It encourages multi-endpoint evaluation of toxicants in general, such as to avoid the possibility of considerable over- or underestimation of the toxicological relevance of tested compounds.

The study extends current knowledge regarding toxicological effects of DON and its metabolite DOM-1 and therefore indirectly provides important information regarding the safety of DON to DOM-1 transforming feed additives.

P66

Rapid dust screening by means of LC-MS/MS to obtain occurrence patterns of *Fusarium*, *Aspergillus*, *Penicillium* and *Alternaria* toxins in corn

Simone Staiger*, Mareike Reichel, and Scarlett Biselli

Eurofins WEJ Contaminants, Hamburg, Germany

* Corresponding author: SimoneStaiger@eurofins.de

Multiple factors favour the pre- and postharvest infection of corn with moulds. Main mycotoxin producing genera are *Fusarium*, *Aspergillus*, *Penicillium* and *Alternaria*. According to the prevalent strain and environmental conditions these moulds produce a wide range of toxins. Multi-mycotoxin screening methods are best suited to reveal contamination pattern of corn lots. Results are needed at the beginning of each harvest season to set up sufficient risk-based sampling plans and identify the right toxins to focus on in daily control. Furthermore, occurrence pattern can help to obtain data on emerging toxins and deduce the risk for a specific cropping region.

Mycotoxins accumulate on small particles in food or feed bulk. Consequently, dusts are often higher contaminated with mycotoxins than the grain kernels. By using the advantages of dust sampling, a dilute and shoot LC-MS/MS approach was set up to screen corn samples for more than 30 mycotoxins including A- and B-type trichothecenes, zearalenone, fumonisins, enniatins, ochratoxin A, aflatoxins, and alternaria toxins.

More than 100 samples from Germany, Poland, Romania, France and further European countries harvested in 2011-2015 were analysed. All mycotoxin concentrations were plotted in colour intensity charts to reveal occurrence patterns. While middle European samples showed mainly *Fusarium* toxins, a prevalence of *Aspergillus* toxins was observed in southern European corn in 2013 harvest. *Fusarium* toxins always comprised deoxynivalenol, often accompanied by its 15-acetyl and sometimes also 3-acetyl derivative as well as nivalenol. Many samples contained also zearalenone and fumonisins. Besides, enniatins were detected in every sample with beauvericin showing highest concentrations. In contrast, fusarenon X was hardly ever observed. Aflatoxins often co-occurred with ochratoxin A but also sterigmatocystin, cyclopiazonic and mycophenolic acid. The latter was also found together with roquefortine C in absence of aflatoxins in wet corn intended for silage. Alternaria toxins were observed in many samples as well, in which tenuazonic acid dominated followed by alternariol and alternariol monomethyl ether.

In conclusion, dust sampling combined with LC-MS/MS multitoxin screening was shown to give a comprehensive overview on mycotoxin co-occurrence patterns in corn. Hence, this technology has a huge impact on cost management for risk assessment since sampling strategy and toxin analysis can be focused on identified toxins.

P67

Co-occurrence of Fusarium, Aspergillus, Penicillium, Alternaria toxins and Ergot alkaloids in European wheat and rye samples screened by means of dust analyses

Simone Staiger*, Mareike Reichel, and Scarlett Biselli

Eurofins WEJ Contaminants, Hamburg, Germany

* Corresponding author: SimoneStaiger@eurofins.de

Often grain is naturally contaminated with several mycotoxins. Hence, the co-occurrence of toxins has become of main toxicological interest. Also from an analytical point of view occurrence patterns help to identify potential indicator-toxins or design risk-based test plans.

Many mycotoxins are predominantly produced in the outer kernel layers. Hence, abraded particles from the surface are often higher contaminated with mycotoxins compared to the grain kernels. Other toxins as ergot alkaloids accumulate on small particles in the bulk due to their higher surface to volume ratio. The natural enhancement of toxins in the dust can facilitate analysis and enable simple dilute and shoot LC-MS/MS screening methods.

By means of dust sampling combined with LC-MS/MS multitoxin screening a comprehensive overview of co-occurrence of mycotoxins in wheat and rye samples could be given. More than 100 wheat and ~40 rye samples from Germany, Poland, France and further European countries harvested in 2011-2015 were analysed. Occurrence patterns of 40 toxins were shown in colour intensity charts based on concentrations in the grain dust.

Fusarium toxins were found in every sample with deoxynivalenol dominating. In addition to the latter, the samples often contained also 3- acetyl and/ or 15-acetyl-deoxynivalenol and/ or nivalenol. However, amount and occurrence was hardly correlated with the former. Also, zearalenone occurrence could not be deduced from deoxynivalenol, although no sample exclusively containing zearalenone was found. Whereas T2- and HT2-toxin were observed in many samples, fusarenone X as well as mono- and diacetoxyscirpenol were hardly detected. In contrast, enniatins were found to be ubiquitous. Of the latter, enniatin B prevailed. Aflatoxins do not play a role in European wheat and rye samples. In contrast ochratoxin A analyses are always crucial. Traces of *Alternaria* toxins were found in many wheat samples. Within the Ergot alkaloids no marker toxin could be identified. In fact, Ergot alkaloid patterns differed from sample to sample showing the need for specific methods including all derivatives.

Summing up, detailed multi-toxin analyses remain the only possibility to assess the contamination of a grain sample correctly. Thereby, the presented quick and cost-efficient pre-screening using the dust sample can help to focus on the right toxins. Toxins not detected in the enriched dust fraction are extremely unlikely to be present in the grain.

P68

Fusarium resistance evaluation in the set of winter wheat varieties from Common EU catalogue

Lenka Štěrbová*, Jana Chrpová

Crop Research Institute, Prague, Czech Republic

* Corresponding author: sterbova@vurv.cz

The FHB resistance evaluation is an important part of the wheat variety registration process in the Czech Republic, that is guaranteed by CISTA agency (Central Institute for Supervising and Testing in Agriculture). New varieties are characterized regarding their FHB resistance in the three consecutive years and the results are spread to the farmers by means of annual reports. Nowadays, EU has a common catalog of varieties and the varieties do not need any further examination by CISTA agency before being distributed in the Czech Republic. Regarding this situation, the evaluation of the FHB resistance under the same condition of artificial infection pressure as in national registration process is highly desirable since it produces unified information and facilitate the comparison of variety value for farmers.

The field tests of 40 varieties were conducted at the Research Institute of Crop Production in Prague-Ruzyně in hill plots (three replications). Artificial inoculation of spikes with highly pathogenic isolate B of F. culmorum was performed at mid-flowering Head blight symptoms were evaluated in three terms (usually 14, 21 and 28 days after inoculation) on a 1–9 scale. Visual symptom scores (VSS) are based on average value of the three measurements.

Determination of other resistance traits was based on seed samples obtained in each plot from inoculated spikes, which were threshed at a low wind not to lose light infected scabby grains. Fusarium damaged (scabby) grains (FDG) were calculated as percentage by total seed number. The content of DON was determined by ELISA with the use of RIDASCREEN® FAST DON kits from R-Biopharm GmbH, Darmstadt, Germany. DON content accumulated under infection pressure ranged from 1.3 to 190 ppm enabling differentiation of varieties. Additionally, we phenotyped anther retention, plant height and flowering date to analyze their association with resistance.

This work was supported by The Ministry of Agriculture of The Czech Republic (project no. NAZV QJ1210189) and The Ministry of Education Youth and Sport project. no. LD14087)

P69

WGS Sequencing of the citrinin producer Penicillium citrinum and subsequent characterization of the citrinin gene cluster

Dominic Stoll, Najim Touhami, Rolf Geisen, Markus Schmidt-Heydt*

Max Rubner-Institut, Karlsruhe, Germany

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

Penicillium citrinum is a fungal species which produces very high amounts of the nephrotoxic mycotoxin citrinin and shows worldwide-distribution. In this work we analyzed the citrinin biosynthesis gene cluster from P. citrinum and compared it with the citrinin clusters of the closely related species P. expansum, P. verrucosum (which has been recently sequenced by us, as well. NCBI LAKW00000000.1.) and Monascus purpureus.

The genome of the filamentous ascomycete Penicillium citrinum BFE575 has been sequenced for the first time using shotgun Illumina sequencing technology on a MiSeq sequencer. The approximately 32 mega base containing draft whole genome shotgun sequence has a coverage of about 64X and a GC-content of 46 % +/- 2.84. The assembled sequence contains in total 976 genomic contigs and supercontigs spanning a length between 621bp to 285,182bp. The WGS sequence is available at NCBI under the accession number LKUP00000000

In subsequent analyses of this WGS at least 39 secondary metabolite gene clusters (SMGC) could be identified using the SMURF algorithm. Of these gene clusters 10 belong to the PKS-, 9 to the NRPS-, 1 to the indole-, 4 to the hybrid PKS/NRPS-, 1 to the lantipeptide- and 1 to the siderophore type. Ten need further investigation. Furthermore 57 SMGC backbone genes could be identified and aligned using the MUSCLE algorithm provided by CLC Sequence Viewer Version 7.6 and visualized using the Neighborhood Joining algorithm. Within the genome sequence of P. citrinum BFE575 we could identify the complete gene cluster for citrinin biosynthesis, which we comparatively analyzed with respect to other known citrinin gene clusters in related fungi. A neighborhood phylogenetic tree based on the DNA-sequences of the complete gene clusters for CIT biosynthesis was constructed. The respective similarities could be determined as followed: P. citrinum vs. M. purpureus: CIT-cluster Identity/Similarity 56,4 %; Gaps 27,9 %; Score 45.121,0; P. citrinum vs. P. expansum: CIT-cluster Identity/Similarity 56,4 %; Gaps 27,3 %; Score 43.166,5; P. citrinum vs. P. verrucosum: CIT-cluster Identity/Similarity 62,0 %; Gaps 19,7 %; Score 42.809,5. Interestingly the syntheny of the 4 citrinin gene clusters is homologous, which suggest an early common ancestral species or recent horizontal gene transfer.

This work was supported by the Brigitte and Wolfram Gedek Foundation.

P70

Improving the determination of aflatoxins – a never ending story for the analyst?

Joerg Stroka*, Stefanka Bratinova

Institute for Reference Materials and Measurements, Geel, Belgium

* Corresponding author: joerg.stroka@ec.europa.eu

In 2003 worldwide 61 countries had specific regulatory levels for aflatoxin B1 in food while 76 regulated total aflatoxins (B1, B2, G1 and G2). These levels vary globally from 1 – 20 µg/kg for AfB1 and from 0 -35 µg/kg for total aflatoxins and it is remarkable that a number of countries regulate AfB1 next to total aflatoxins. The 2003 FAO report on worldwide regulations for mycotoxins in food and feed [1] already mentions that: "Whether a regulatory level for the sum of the aflatoxins, which requires more analytical work than for aflatoxin B1 alone, contributes significantly to better protection of public health than a regulatory level for aflatoxin B1 alone is debatable". The author also concluded that it is unlikely that products are contaminated with AfB2, AfG2 or AfG1, but not with AfB1, while general contamination pattern question the need for regulating both for public health safety.

However this - long known - issue seems to have not been sufficiently relevant to trigger any investigation on the fitness-for-purpose of such double regulation and it seems difficult to make steps to simplify the situation, unless in a common way on a global scale, in order to avoid trade disputes. Another issue is that legislation is in general more difficult to change than laboratory practice.

Taking into account that many routine laboratories still use HPLC with fluorescence for the determination of aflatoxins a simple approach is presented that on one hand will not require omitting current "double" regulation, while offering a significant reduction of workload for the analyst. The approach is based on the observation that for given HPLC systems responses for all four aflatoxins are fairly stable, in some cases over more than 3 years with deviations of less than 5%. To what degree such an approach is feasible for other chromatographic methods is still unknown.

On the basis of data published in the RASFF database [2] for aflatoxins, the general fitness-for-purpose is demonstrated, while future proficiency tests of the EURL for mycotoxins will focus on this topic to further investigate the specific suitability for individual laboratories.

Authors want to thank Dr. Ivanka Doncheva for her contributions sorting a large scale of the RASFF data entries. Additional thanks goes to all laboratories that supported the EURL for mycotoxins in gathering data from calibration graphs for a variety of chromatographic systems.

1. Food and Agriculture Organization of the United Nations, Worldwide regulations for mycotoxins in food and feed in 2003, Rome, Italy

2. RASFF – the Rapid Alert System for Food and Feed:
http://ec.europa.eu/food/safety/rasff/portal/index_en.htm

P71

Purification of enniatins from F. tricinctum cultures on solid white bean medium

Rosine Ghislaine Suchfort, Petr Karlovsky*

Molecular Phytopathology and Mycotoxin Research, Georg-August University Göttingen, Göttingen, Germany

* Corresponding author: pkarlov@gwdg.de

Strains F. tricinctum DSM 23357 and F. tricinctum O32 were examined for the production of enniatins A, A1, B and B1 on solid white bean (Phaseolus vulgaris) (Wang et al. 2013) medium at 25°C for 30 d. A method was established for the quantification of enniatins content using HPLC-DAD with an RP column, elution with a methanol-water gradient and detection by light absorption at 210 nm. The enniatin content increased up to day 24. Total enniatin content of F. tricinctum O32 culture reached 614 mg/kg, which was about two times more as compared to F. tricinctum DSM 23357. F. tricinctum O32 was therefore used for the production of enniatins in 9 kg while been cultures. Enniatins were extracted with ethyl acetate and the extract was fractionated using flash chromatography on a reverse phase column with a step gradient consisting of water-methanol mixtures. Fractions containing enniatins (about 3 g per kg culture) were combined and further purified using recrystallization from methanol-water. 1.2 g enniatins containing about 83% enniatin B, 13% enniatin B1, 4% enniatin A1 and 0.25% A were obtained per 1 kg culture.

Keywords: Enniatins, F. tricinctum, production, flash chromatography

Wang JP, Debbab A, Hemphill CF, Proksch P (2013): Optimization of enniatin production by solid- phase fermentation of Fusarium tricinctum , Zeitschrift für Naturforschung C 68 (5-6), 223-30

P72

Functional characterization of ethylene biosynthesis candidate genes of Fusarium graminearum

Thomas Svoboda[1], Alexandra Parich[2], Alexandra Malachova[2], Maria Doppler[2], Clemens Schmeitzl[1], Gerlinde Wiesenberger[1], Rainer Schuhmacher[2], Gerhard Adam[1]*

[1]BOKU - Dept. of Applied Genetics and Cell Biology, Tulln, Austria; [2]BOKU - Dept. for Agrobiotechnology (IFA-Tulln), Tulln, Austria

* Corresponding author: gerhard.adam@boku.ac.at

Fusarium graminearum is a plant pathogen with the ability to infect many economically important plant species. It has been reported that Fusarium graminearum can interfere with ethylene signalling to enhance the susceptibility of the host plants. Furthermore, deoxynivalenol accumulation was shown to be strongly reduced in ethylene insensitive barley (Chen et al., 2009). Ethylene, a plant hormone, is formed in response to biotic and abiotic stress. In planta the biosynthesis starts with methionine which is converted by AdoMet-synthase to S-adenosyl-L-methionine (AdoMet) which is further metabolized to 1-aminocyclopropane carboxylate by ACC-synthase. ACC is then oxidized by ACC oxidase to ethylene, but it can also be degraded by ACC deaminase to α-ketobutyrate (KBA). In our study we focused on characterization of ACC synthase and ACC deaminase candidate genes from Fusarium graminarum. Based on sequence similarity three putative ACC synthases and two ACC deaminases were identified. We have characterized one ACC deaminase (ACD2) of Fusarium graminearum by using in vivo and in vitro methods. This enzyme clearly showed ACC deaminase activity, while the second deaminase candidate gene was inactive but had D-cysteine desulfhydrase activity. For testing activity of ACC synthase candidate genes an in vivo assay in a modified E. coli T7-express strain was used. Single knock out mutants of Fusarium graminearum were prepared. Different wheat infections with ACD2 knock out mutants were performed to determine effects on virulence and DON production, ACC- and KBA production, as well as different metabolites. Preliminary results show that the ACC-deaminase is dispensable for virulence of F. graminearum on wheat.

P73

Relationship between body core temperature and inflammatory markers in pigs exposed to oral deoxynivalenol (DON) and systemic lipopolysaccharide (LPS)

Tesch Tanja[1]*, Bannert Erik[1], Kluess Jeannette[1], Frahm Jana[1], Hüther Liane[1], Kersten Susanne[1], Renner Lydia[2], Kahlert Stefan[2], Rothkötter Hermann-Josef[2], Dänicke Sven[1]

[1]Friedrich-Loeffler-Institut, Braunschweig, Germany; [2]Otto von Guericke Universität, Magdeburg, Germany

* Corresponding author: tanja.tesch@fli.bund.de

Body temperature is a stable parameter in healthy conditions and an important sign for systemic inflammation besides other inflammatory indicators such as are leukocytes or TNF-α, but their interrelationship is not yet fully clarified. Thus, we investigated their relationship in healthy as well as in pigs exposed to DON and LPS.

Barrows (n=44) were fed either a DON-(4.59mg DON/kg feed) or a control-(CON)diet and surgically equipped with a temperature logger and permanent portal and jugular catheters for simultaneous infusion (60min) with 0.9%NaCl (CON) and LPS (7.5µg/kg BW) and blood sampling (every 15min). Combination of diet and infusion created six groups: CON_CON_{jug}-CON_{por}, CON_CON_{jug}-LPS_{por}, CON_LPS_{jug}-CON_{por}, DON_CON_{jug}-CON_{por}, DON_CON_{jug}-LPS_{por}, DON_LPS_{jug}-CON_{por}. Blood samples were analysed for leukocyte counts, TNF-α and kynurenine-tryptophan (-30min to +180min). Data were evaluated by SAS PROC MIXED with group, time and their interaction and PROC CORR.

Leukocytes: DON-feeding alone caused higher leukocyte counts (p=0.04). LPS-induced leukopenia and temperature-rise changed contemporaneous from 15min p.i. and both reached a plateau at 60min p.i.. LPS and DON yielded an earlier leukopenia ($p<0.05$) and lower temperature rise (~0.5°C, p=0.08) in DON_LPS_{jug}-CON_{por} compared to CON_LPS_{jug}-CON_{por}. A significantly positive correlation between both parameters was found in DON_CON_{jug}-CON_{por}, while LPS groups were negatively correlated ($p<0.001$). TNF-α: LPS induced a sudden increase in TNF-α with a peak at 60min p.i.. Significantly positive correlations with temperature were found in all LPS groups ($p<0.05$). Kyn-Trp ratio: LPS induced an increased ratio at 180min p.i. ($p<0.001$). Significantly positive correlations were found in both DON-LPS groups.

Our data confirm a significant relationship between body core temperature and leukocytes, Kyn-Trp ratio and TNF-α in descending order under pathophysiological conditions and with the strongest dependency in CON_LPS_{jug}-CON_{por}.

The authors would like to thank all colleagues of the Institute of Animal Nutrition, Friedrich-Loeffler-Institute Braunschweig, Germany, who have actively supported the experimental trail as well as the sample analysis and the “Deutsche Forschungsgemeinschaft” (DFG) for financial support (DA 558/1-4).

P74

Prevalence of ochratoxin A and ochratoxigenic microfungi in medicinal herbs and teas

Jakub Toman[1]*, Frantisek Malir[1], Vladimir Ostry[2], Vladimir Dvorak[1], Katerina Kotalova[1], Tomas Roubal[3]

[1]University of Hradec Kralove, Faculty of Science, Hradec Kralove, Czech Republic; [2]National Institute of Public Health in Prague, Brno, Czech Republic; [3]Institute of Public Health in Usti nad Labem, Hradec Kralove, Czech Republic

* Corresponding author: jakub.toman.1@uhk.cz

OTA is a very important mycotoxin produced as a secondary metabolite by several toxigenic microfungi of the genera of Aspergillus (e.g. A. carbonarius, A. foetidus, A. lacticoffeatus, A. ochraceus, A. niger, A. sclerotioniger, A. steynii, A. tubingensis, A. westerdijkiae) and Penicillium (P. nordicum, P. verrucosum). OTA represents significant risk to human health. OTA has been detected in foodstuffs of both plant, and animal origin.

Attention in this study was focused on teas from Turkish Black Sea Region and medicinal herbs where is root used as drug: Common turmeric (Curcuma longa L.), Mongolian Milkvetch (Astragalus mongholicus Bunge), Licorice (Glycyrrhiza glabra L.), Ashwagandha (Withania somnifera (L.) Dunal), Ginger (Zingiber officinale Rosc.) and Ginseng (Panax ginseng C. A. Mey.).

Twenty samples of black teas were collected from Turkish Black Sea Region in the January 2015. It represents 12 samples of loose tea and 8 samples of bag tea. Total 36 different samples of medicinal herbs were collected from the biggest Czech distributor of medicinal herbs during 10 months in 2015.

Validated and accredited ultra-trace HPLC-FLD method was employed for OTA determination. Tea and herb samples were cleaned by means on immunoaffinity columns (OCHRAPREP® columns, R-Biopharm, Germany). Limit of quantification of the method (LOQ) was 0.35 ng/g. The total count of ochratoxigenic microfungi was determined by horizontal method (CSN ISO 21527-2:2008).

OTA levels in medicinal herbs in Mongolian Milkvetch (Astragalus mongholicus) ranged from 313.5 to 1110 ng/g, (average 659.8 ng/g), in Licorice (Glycyrrhiza glabra) from 8 to 16 ng/g (average 12.0 ng/g). All results of the black tea samples and other herbs were under LOQ 0.35 ng/g. Aspergillus section Nigri, commonly known as the black aspergilli, was determined in 9 (45%) black teas and in 10 (28%) medicinal herbs

Supported by the Ministry of Health, Czech Republic - conceptual development of research organization („National Institute of Public Health – NIPH, IN 75010330“) and by the specific research project (no. 2118/2015) of the Faculty of Science, University Hradec Kralove, Czech Republic.

P75

Ochratoxin A and aflatoxin B1 in breakfast cereals from Serbian market

Ljilja Torovic[1]*, Gorica Vukovic[2]

[1]Institute of Public Health of Vojvodina, Novi Sad, Serbia; [2]Institute of Public Health of Belgrade, Belgrade, Serbia

* Corresponding author: ljilja.torovic@izjzv.org.rs

As part of risk management strategy, maximum levels have been established for certain mycotoxins in different foodstuffs, including cereals and cereal-derived products: 3 µg/kg of ochratoxin A (OTA), 2 µg/kg of aflatoxin B1 (AFB1) and 4 µg/kg of total aflatoxins. The aim of this study was to estimate the presence of ochratoxin A and aflatoxins in breakfast cereals commonly consumed in Serbia. Samples were collected from retail stores, in consumer-sized packages, in 2015. Breakfast cereals, 54 samples in total, were chategorized as multicereal (40), with wheat, oat, barley, rye and rice tipycally listed as ingredients, including samples with addition of cinnamon, chocolate, dried fruits, nuts, dried fruits and nuts, or as corn flakes (14). OTA and AFs were simultaneously extracted from the samples, while the clean-up procedures included monoclonal antibodies for OTA or AFs. Liquid chromatography with fluorescence detection methods were applid for the analysis, with detection limits of 0.07 µg/kg OTA and 0.04 µg/kg AFB1. According to the results, OTA was detected in 6 out of 40 samples of multigrain cereals (15%), all of which contained dried fruits and/or nuts, and only one corn flakes sample (7%). The maximum level was not exceeded, and overall mean of positive samples was 0.48 µg/kg. Regarding AFB1, 11% of the samples were positive: one (2.5%) in the group of multicereal products, and 5 (36%) among the corn flakes. With the highest concentration of 0.15 µg/kg no sample exceeded the maximum level, and no sample presented detectable levels of the other aflatoxins (AFB2, AFG1, AFG2). With respect to possible influence of other parameters, it was observed that all positive samples were characterized with normal fiber content, while whole grain/non-whole grain ratio of positive samples was 0.75 and 0.5 in case of OTA and AFB1, respectively. Only one corn flakes sample showed detectable amounts of both examined mycotoxins. Daily intake of OTA, estimated only for orientation purpose, based on producer recommended portion of breakfast cereal (30 g) and overall mean content of mycotoxin, presented less than 1% of tolerable intake and thus negligible risk for consumers.

P76

New plasmids allowing positive-negative selection for fungal transformation and efficient Cre-loxP mediated marker recycling

Krisztian Twaruschek*, Pia Spörhase, Gerlinde Wiesenberger, Gerhard Adam

University of Natural Resources and Life Sciences, 3430 Tulln, Konrad Lorenz Str. 24, Austria

* Corresponding author: krisztian.twaruschek@boku.ac.at

In filamentous fungi, disruption of multiple genes of interest in the same strain (e.g. to test for redundant gene function) is a difficult task due to the limited availability of different reliable and efficient selection markers. In S. cerevisiae, the Cre-lox system can be efficiently used to remove loxP-flanked resistance cassettes. Activation of Cre gene expression from an inducible promoter (PXYN1) was inefficient to evict the selection marker in a self-excising cassette in Fusarium graminearum. To reduce the screening effort, we have created a series of hybrid fusion genes which allow positive selection of transformants in the first step and subsequent negative selection for marker removal. The constructs consist of Herpes simplex thymidine kinase (HSV-TK) to which the commonly used drug resistance markers hph, nptII and nat1 (conferring resistance to hygromycin B, geneticin and nourseothricin) were fused c-terminally. For removal of the loxP flanked resistance cassettes, protoplasts of transformants were directly treated with purified Cre recombinase protein. Loss of HSV-TK containing cassette can be selected by restoration of resistance to 5-fluoro-2-deoxyuridine (5-F2DU). The fusion genes expressed under the Trichoderma PKI promoter are also conferring antibiotic resistance in E. coli, allowing straightforward construction of disruption plasmids (e.g. by Gibson assembly).

P77

Evaluation of cytotoxicity and mould contamination of selected meadow plants

Magdalena Twarużek [1]*, Romuald Dembek [2], Ewelina Soszczyńska[1], Jan Grajewski[1]

[1]Kazimierz Wielki University, Bydgoszcz, Poland; [2]University of Science and Technology, Bydgoszcz, Poland

* Corresponding author: twarmag@ukw.edu.pl

Current knowledge of the dietary value of meadow plants requires verification, primarily with regard to the concentration of anti-dietary compounds as well as the plants' susceptibility to fungal pathogens. The aim of this study was to evaluate the cytotoxicity of selected meadow plants using the MTT cytotoxicity assay and to determine the level of mould contamination.

Plant material was harvested from meadows located in the valley of the Bydgoszcz Canal. 21 plant species were harvested in 2014 upon reaching cutting maturity: Alopecurus pratensis, Arrhenatherum elatius, Carex acutiformis, Carex nigra, Dactylis glomerata, Deschampsia caespitosa, Eqisetum palustre, Festuca arundinacea, Glyceria maxima, Holcus lanatus, Lolium perenne, Molinia caereluea, Ostericum palustre, Phalaris arundinacea, Plantago lanceolata, Poa pratensis, Ranunculus acris, Ranunculus repens, Rhinanthus serotinus, Trifolium repens and Odontites serotinus. Mycological examination of grass samples was carried out on YGC agar medium (yeast extract and glucose with chloramphenicol), after 5-7 days incubation in $25^0C \pm 1^0C$. The results are expressed as the number of colony forming units (cfu) per gram of a sample. The identification of moulds was done to genus. Qualitative and quantitative mycological analysis was carried out according to PN ISO 7954: 1999. Cytotoxicity evaluation was performed using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium salt) test with swine kidney (SK) cells.

The experimental plot was dominated by moulds belonging to the following genus: Humicola – 34%, Cladosporium – 16%, Alternaria – 11%, Torula – 11%, Fusarium – 5%, Pythium – 4%, Rhizopus – 1%, Nigrospora – 1%, and unidentified colonies – 15%. The remaining 2% was comprised of moulds belonging to the genus: Mucor, Epicoccum, Chrysosporium, Chaetomium, Drechslera, Morteriella, Aspergillus, Botryotrichum, Aureobasidium, Penicillium, Arthrinium, Stemphylium, Diplodia, Bipolaris, Phoma, Ulocladium, Colletotrichum. The highest level of mould contamination was observed for Carex acutiformis – 8.5×10^5 cfu/g, and Molinia caereluea – 2.0×10^5 cfu/g, while the lowest for Ranunculus repens <20 cfu/g and Ranunculus acris – 6.1×10^2 cfu/g. MTT test showed that the most cytotoxic species were Ranunculus repens (IC50 3.125 mg/ml), Plantago lanceolata, Arrhenatherum elatius (IC50 6.25 mg/ml) as well as Ranunculus acris and Odontites serotinus (IC50 12.5mg/ml). The plants that showed high cytotoxicity were not heavily contaminated with moulds. Such high cytotoxicity can be attributed to natural compounds such as cyanogenic glycosides (Ranunculus acris) and saponins (Arrhenatherum elatius), which are responsible for the increasing resistance to pests and pathogens. However, their presence in hay could have a negative impact on the rumen microflora.

P78

Mycological contamination and cytotoxic evaluation of used cars' dust filters

Magdalena Twarużek*, Ewelina Soszczyńska, Justyna Kwiatkowska, Jan Grajewski

Kazimierz Wielki University, Bydgoszcz, Poland

* Corresponding author: twarmag@ukw.edu.pl

Cabin dust filter, is one of the most important components of the air conditioning system, which each air-conditioned car is equipped with. The filter should be changed regularly, at least once a year or after 15 thousand km. Regular exchange of the filter prevents the release of dust, soot and mould inside the vehicle. The growth of bacteria and fungi or clogging filters may be indicated by steamed windows and unpleasant smell from the conditioning system. Prolonged growth of microorganisms colonies, which may degrade, reduces the effectiveness of filtration and shortens the time of use filter's. Moreover, prolonged exposure to mould spores can develop cough, chronic sneezing, acute breathing problems and allergic diseases (allergic rhinitis, allergic conjunctivitis, bronchial asthma, allergic alveolitis) and feeling of being unwell. The study material included 57 used dust filters, obtained from the authorized car service stations. Mycological examination of dust filter was carried out on YGC agar medium (yeast extract and glucose with chloramphenicol), 5-7 days incubation in $25^0C \pm 1^0C$. The results are expressed as the number of colony forming units per gram of a sample. Identification of moulds was done to genus. Cytotoxicity of the samples was evaluated using the MTT cytotoxicity test, with swine kidney cells (SK). Prior to the mycological evaluation the filters were divided into 3 groups depending on the time of use, respectively: less than 1 year (n=12), 1 to 2 years (n=23) and more than 2 years (n=20). The average contamination of moulds in the filters was: in the first group 1.2 x 10^5 cfu/g, in the second group - 3.1 x 10^5 cfu/g, and in the third - 7.5 x 10^5 cfu/g, respectively. The highest values were detected in the third group of filters used for more than 2 years. The moulds dominating turned out to be: Cladosporium spp. (I - 83%, II - 83%, III - 79%), Alternaria spp. (I - 4%, II - 4%, III - 3%) and Acremonium spp. (II – 10%, III – 12%) respectively. The analysis of the MTT test results showed 43% of samples with low cytotoxicity (IC50 250-125 mg/ml), 14% of the samples of medium cytotoxicity (IC50 62,5-15,6 mg/ml) and 4% of the samples with a high cytotoxicity (IC50 7.8 - 1 9 mg/ml). The samples with IC50 were ranging from 1000 to 500 mg/ml were classified as non-toxic (28%).

P79

Green young barley as potential source of moulds and mycotoxins

Magdalena Twarużek[1]*, Robert Kosicki[1], Anna Błajet-Kosicka[1], Iwona Ałtyn [1], Josef Böhm[2], Jan Grajewski[1]

[1]Kazimierz Wielki University, Bydgoszcz, Poland; [2]University of Veterinary Medicine, Vienna, Austria

* Corresponding author: twarmag@ukw.edu.pl

Green barley has become a new ingredient in nutrition. It's high content of vitamins and minerals has made it one of the most desirable dietary supplements to improve the health, fitness and appearance. The aim of this study was to evaluate moulds and mycotoxins contamination of green barley and food supplements containing green barley, traded in Poland. The material consisted of 26 green barley samples, which were divided into 3 groups: powder (n=14), tablets (n=8) and capsules (n=4).

Mycological examination was performed according to PN ISO7954, 1999. Mycotoxins determination was performed using HPLC-FLD method (immunoaffinity column AflaTest from Vicam-AF; immunoaffinity column OchraPrep from R-Biophram Rhône Ltd–OTA; CitriTest from Vicam-CIT) and HPLC-MS/MS method (Bond Elut®Mycotoxin column from Agilent - DON, NIV, ZEN, T-2 and HT-2).

The mycological analysis of green barley powder showed significant levels of moulds content (an average of 1,9 $x10^4$ cfu/g) and it was genus Mucor that prevailed (67%). In case of dietary supplements containing green barley, the average mould contamination was 8,4 x 10^2 cfu/g (tablets) with the prevalence of Eurotium (77%) and Rhizopus (22%) genus. The lowest level of moulds was found in capsules (<10 cfu/g).

The most frequently detected mycotoxins were DON and ZEN - 69% and 100%, respectively. The highest level of those mycotoxins was (DON_{med} =<3 ppb, DON_{max} = 531 ppb; ZEN_{med} = 2,8 ppb, ZEN_{max} = 27,8 ppb). 8% of the samples contained NIV, and the highest concentration reached the value of <3 ppb (powder). The studied samples were hardly contaminated by T-2 and HT-2. The highest concentration was in tablets: 1,01 ppb and 0,5 ppb on average, respectively. DAS was not detected in any of the green barley samples and food supplements containing green barley extract. The least contaminated turned out to be capsules. There were no AF, CIT or OTA found in the material.

In 65% of the samples NO_3- ions, ranging from 0,71 mg/g to 3,57 mg/g per sample, were also detected.

Financial support: Austrian-Polish Cooperation 2015-2017 Project No PL/AT 3/2015

P80

Prevalence of *Aspergillus* section *fumigati* in Portuguese slaughterhouses: a fungal and mycotoxin concern

Carla Viegas[1], Tiago Faria[1], Raquel Sabino[2], Anita Quintal Gomes[1], Susana Viegas[1]*

[1]ESTeSL/IPL - Environment and Health Research Group, Lisbon, Portugal; [2]Mycology Laboratory, INSA, Lisbon, Portugal

* Corresponding author: susana.viegas@estesl.ipl.pt

Within the Aspergillus genus, Aspergillus fumigatus species is one of the most ubiquitous saprophytic fungi and have clinical relevance since is the frequent cause of invasive aspergillosis. Additionally, the most abundant metabolite produced by this fungus is gliotoxin.

The aim of this work was to determine the prevalence of Aspergillus section Fumigati by cultural and molecular methods in different types of slaughterhouses.

Air and surface samples were collected and subject to further macro and microscopic observations. We collected additional air samples to perform real-time quantitative PCR (qPCR) amplification of genes from A. fumigatus complex.

Aspergillus section Fumigati was present only in the air from 3 workplaces of the Poultry Slaughterhouse (10 – 30 CFU.m^3), and in 1 workplace in the Bovine Slaughterhouse (10 CFU.m^3). Molecular tools amplified successfully DNA from the A. fumigatus complex in more 6 workplaces.

Besides suggesting A. fumigatus complex as an indicator of harmful fungal contamination, this study also indicates the complementarity of conventional and molecular tools.

Results pinpoint also to mycotoxins co-exposure: Aflatoxin B_1 has been detected in Poultry Slaughterhouse and gliotoxin can also be a reality.

Keywords: Aspergillus; section Fumigati; gliotoxin; co-exposure; slaughterhouses

P81

Co-exposure to mycotoxins – the case of waste management workers

Susana Viegas[1]*, Bernd Osteresch[2], Susana Almeida[1], Benedikt Cramer[2], Hans-Ulrich Humpf[2], Carla Viegas[1]

[1]ESTeSL/IPL - Environment and Health Research Group, Lisbon, Portugal; [2]University of Münster, Münster, Germany

* Corresponding author: susana.viegas@estesl.ipl.pt

Occupational exposures are complex and normally associated to co-exposure to several contaminants by different exposure routes.

Recently, it was reported occupational exposure to Aflatoxin B1 (AFB1) in one Portuguese waste management unit.

The aim of the present work was to know if the workers were exposed to other mycotoxins besides AFB1.

The same serum samples from the workers were consider (n=39) in order to detect other mycotoxins using a multibiomarker approach.

From the analytical procedure was quantified more two mycotoxins: Enniatin B (EnB) and Ochratoxin A (OTA). OTA results showed a median value of 0.62 ng/ml (0.36 - 4.99 ng/ml). EnB results were lower and presented a median value of 0.04 ng/ml (0.01 - 0.15 ng/ml).

Waste management workers were exposed simultaneously to AFB1, OTA and EnB, probably with different exposure routes. Interaction between mycotoxins should be considered to perform (cumulative) risk assessment.

Keywords: mycotoxins; occupational exposure; co-exposure; risk assessment

P82

Toxigenic fungi in coffee samples: a menace to public health

Carla Viegas, Cátia Pacifico, Ana Cebola de Oliveira, Ana Pintão, Anita Quintal Gomes, Susana Viegas*

ESTeSL/IPL - Environment and Health Research Group, Lisbon, Portugal

* Corresponding author: susana.viegas@estesl.ipl.pt

Mycotoxin contamination was reported to occur in some food and commodities, such as coffee, particularly due to the presence of toxigenic fungi such as Aspergillus, Penicillium and Fusarium spp.. Aspergilli are known to produce high levels of mycotoxins, such as ochratoxin and aflatoxin. Aspergillus ochraceus has been proposed as the major cause of ochratoxin A contamination in coffee beans.
The aim of this work was to evaluate the prevalence of Aspergillus sections Circumdati, Flavi and Fumigati in 28 green coffee samples to be used by Portuguese coffee industry, from Coffea arabica (Arabica coffee) and Coffea canephora (Robusta coffee) species from different origins. Twenty grams of coffee beans were resuspended in 180 mL of distilled water and homogenized during 20 minutes at 200 rpm. The washed supernatant was then processed for DNA extraction using the ZR Fungal/Bacterial DNA MiniPrep Kit and the above mentioned Aspergillus sections were detected through real-time quantitative PCR (qPCR). Molecular tools were able to successfully identify the presence of fungal contamination from the Fumigati and Circumdati sections. From the 28 coffee samples analyzed, 27 were contaminated, particularly by Fumigati section (59.3%), followed by Fumigati and Circumdati simultaneously (37.0%) and Circumdati alone (3.7%). Data obtained most probably indicates the presence of ochratoxin and gliotoxin, which will be confirmed in future studies This raise the concern regarding the co-exposure to multiple mycotoxins by diet, being coffee a good example.

Keywords: Coffee; toxigenic fungi; mycotoxins contamination

This research project is still ongoing and have the financial support of Lisbon School of Health Technology.

P83

Assessment of toxigenic fungi in poultry feed

Carla Viegas, Cátia Pacifico, Tiago Faria, Ana Cebola de Oliveira, Anita Quintal Gomes, Susana Viegas*

Environment and Health Research Group, Lisbon Sch, Lisbon, Portugal

* Corresponding author: susana.viegas@estesl.ipl.pt

Feed supplies the necessary nutrients for the growth of healthy animals, which are a part of the human diet. The presence of toxigenic fungi in animal feed such as Aspergillus spp. may contribute to 1) the loss of nutritional value of feedstuff, since fungi will assimilate the most readily available nutrients present in the feed, and 2) the development of mycotoxicoses and chronic conditions, which can raise economic issues due to animal disease and contamination of animal derived products.

The goal of this work was to evaluate the incidence of Aspergilli, particularly from the Circumdati, Flavi and Fumigati sections, through real-time quantitative PCR (qPCR) in 11 feed samples. Feed samples (20g) were resuspended in 180 mL of distilled water and homogenized during 20 minutes at 200 rpm. The washed supernatant was then processed for DNA extraction using the ZR Fungal/Bacterial DNA MiniPrep Kit and subsequent amplification and detection of the target DNA fragments.

Six of the eleven feed samples (55%) analyzed were positive for the presence of DNA from the A. fumigatus complex, however Flavi and Circumdati sections were not detected. The results point out for the possible presence of gliotoxin and other mycotoxins which are commonly produced by Fumigati section. Due to the easiness of fungal detection, fungi are often used as an indirect indicator of mycotoxins presence. However, since mycotoxins can be present in the different matrix long after fungal elimination, the results obtained do not exclude the need to screen for the presence of these compounds.

Keywords: poultry feed; toxigenic fungy; mycotoxins

This work is still ongoing and have the financial support of Lisbon School of Health Technology and Politec&ID.

P84

Aflatoxins and ochratoxin A in different types of flours marketed in Serbia

Gorica Vukovic[1]*, Ljilja Torovic[2]

[1]Institute of Public Health of Belgrade, Belgrade, Serbia; [2]Institute of Public Health of Vojvodina, Novi Sad, Serbia

* Corresponding author: goricavukovic@yahoo.com

Cereals and cereal-detived products represent very important food source in Serbia, but nowadays they are also considered as potential source of mycotoxin exposure.

The main objective of present investigation was to estimate occurence of aflatoxins (AFs) and ochratoxin A (OTA) in different types of flours. Promotion of healty life-styles turned people's attention to different types of plant species, apart from wheat: buckwheat, rye, oat, barley (source of fibers, whole grain products), and rice and corn (glute-free products). Respective flour samples were obtained on retail market, as a single bag, in a way to represent available products and producers from Serbia in 2015, with the exception of commonly used refined wheat flours. A total of 33 samples were evaluated, using separate methods consisted of immunoaffinity chromatography clean-up and HPLC-FLD determination, with UV postcolumn derivatization for AFs.

The study revealed presence of aflatoxins (AFB1, AFB2 and AFG1) in 3 samples (9%), two rice and one corn flour, with two of them contaminated with AFB1 above the maximum level (4.7 µg/kg in rice and 8.8 µg/kg in corn flour; ML 2 µg/kg). Flours of other cereals were free of detectable amounts of AFs. Ochratoxin A showed wider occurence, with 30% of positive samples (buckewheat, rye, wheat and corn flours), although only one corn flour (5.7 µg/kg) exceeded the maximum level of 3 µg/kg. Intrestingly, the same corn flour was non-compliant with respect both to AFB1 and OTA. Presence of mycotoxins in non-compliant samples was confirmed by LC-MS/MS. In addition to the type of cereal used in manufacturing, the influence of other parameters was studied. Whole grain flours, representing 45% of total samples, showed no presence of aflatoxins and two-fold lower occurence of OTA compared to refined flours. Only one sample was available from organic agriculture, characterized with detectable amount of OTA (0.07 µg/kg) and no aflatoxins detected. In conclusion, to achieve a high level of public health protection, stricter controls should be enforced towards mycotoxin contamination of food.

This study was carried out with the project TR31018 supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

P85

DATEX.CZ – Natural occurrence of mycotoxins in foodstuffs in year 2011-2014

Jiri Vyslouzil, Vladimir Ostry*, Marcela Dofkova, Irena Rehurkova, Jiri Ruprich

National Institute of Public Health, Center for Health, Nutrition and Food, Brno, Czech Republic

* Corresponding author: ostry@chpr.szu.cz

"DATEX.CZ" is a group of Czech specialists from National Institute of Public Health for chemical contaminants data collection, standardization, analysis and submission to European Food Safety Authority (EFSA). These data are contributory factor to food safety and crucial for health risk assessment. The data were collected from providers (control bodies) in the Czech Republic. "DATEX.CZ" database includes also data on mycotoxins occurrence in foodstuffs. Submitting analytical data on mycotoxins to EFSA should follow the requirements of EFSA's Guidance on Standard Sample Description (SSD) for food and feed. SSD specifies the data elements, the sample data structure and the format for analytical results for mycotoxins in food and feed included in monitoring and control programmes.

In total 6692 analytical data for 13 mycotoxins were gathered in years 2011-2014.

The results of mycotoxins in foodstuffs are presented (n = total samples, n+% = per cent of positive sample, arithmetic mean, median and range) as follows: aflatoxin B1 (n = 1337, n+ = 8.6%, 1.9 ng/g, 0.05 ng/g, 0.025–638 ng/g); aflatoxin B2 (n = 953, n+ = 2.5%, 0.4 ng/g, 0.1 ng/g, 0.05–75 ng/g); aflatoxin G1 (n = 953, n+ = 3%, 0.3 ng/g, 0.1 ng/g, 0.05–48 ng/g); aflatoxin G2 (n = 953, n+ = 1%, 0.2 ng/g, 0.1 ng/g, 0.05–5.9 ng/g); aflatoxin M1 (n = 208, n+ = 0.5%, 0.005 ng/g, 0.0025 ng/g, 0.0025-0.011 ng/g); deoxynivalenol (n = 344, n+ = 14.2%, 146 ng/g, 50 ng/g, 25–11091 ng/g); fumonisin B1 (n = 173, n+ = 3.5%, 149 ng/g, 120 ng/g, 10–3762 ng/g); fumonisin B2 (n = 173, n+ = 2.9%, 49 ng/g, 50 ng/g, 10–699 ng/g); HT-2 toxin (n = 179, n+ = 7.3%, 52 ng/g, 2.5 ng/g, 0.045–933 ng/g); ochratoxin A (n = 783, n+ = 20.9%, 5.7 ng/g, 0.5 ng/g, 0.005–1610 ng/g); patulin (n = 144, n+ = 8.3%, 2.9 ng/g, 2.5 ng/g, 1.5–40 ng/g); T-2 toxin (n = 179, n+ = 6.1%, 36 ng/g, 2.5 ng/g, 0.015–1408 ng/g); zearalenone (n = 313, n+ = 8.3%, 15 ng/g, 10 ng/g, 0.25–1068 ng/g).

Data collection of mycotoxins is only a part of regular call for continuous collection of chemical contaminants occurrence data in food and feed. EFSA data management system extracts and uses the data to create scientific opinions and reports. In the following years, the SSD2 model with upgraded FoodEx2 (EFSA food classification and description system for exposure assessment version 2) catalogue will be implemented into the data submission procedure.

Supported by Ministry of Health, Czech Republic - conceptual development of research organization („National Institute of Public Health – NIPH, IN 75010330")

P86

Optimized Synthesis of Deoxynivalenol-15-β-D-glycosides

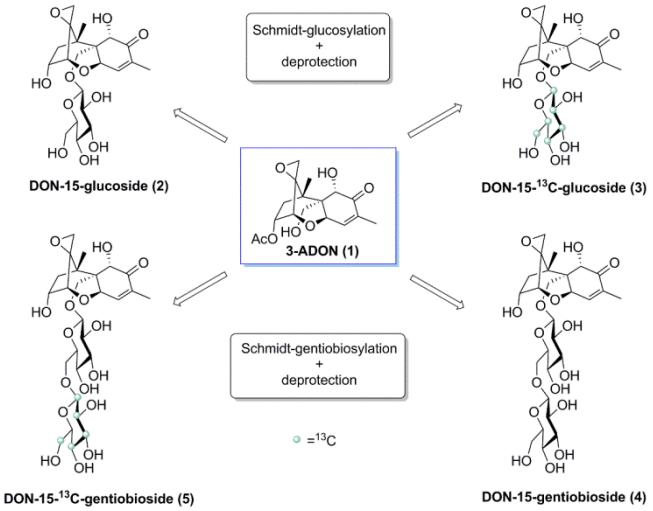
Julia Weber[1]*, Philipp Fruhmann[1], Christian Hametner[1], Alois Schiessl[2], Georg Häubl[2], Johannes Fröhlich[1], Hannes Mikula[1]

[1]Institute of Applied Synthetic Chemistry, Vienna, Austria; [2]ROMER Labs, Tulln, Austria

* Corresponding author: julia.weber@tuwien.ac.at

Deoxynivalenol-conjugates are formed as part of the detoxification process in plants but can also be generated during food processing. As these conjugates can release their parent toxic precursors during digestion, they are harmful to mammals. Recently, DON-15-glucoside was detected as novel DON-metabolite in wheat and DON-dihexosides were found in cereal-based food [1].

For further investigations in terms of bioanalytics and toxicologic properties, these compounds are needed as reference materials. Therefore we decided to optimize the protocol by Savard et al. for the synthesis of DON-15-glucoside [2]. Using 3-Acetyldeoxynivalenol **(1)** as starting material in a Schmidt-glycosylation reaction, acetyl-protected 3-ADON-15-glucoside **(2)** could be produced in good yields and with only minor formation of 3,15-diADON as side product. This optimized procedure could then also be applied successfully for the synthesis of acetyl-protected DON-15-gentiobioside **(4)** as well as for the ^{13}C-labelled glycosides **(3)** and (**5**). Deprotection of the acetyl-protected DON-15-conjugates was achieved in nearly quantitative yields using mild basic conditions to prevent formation of iso-DON derivatives. The isotopic labeled compounds serve as internal standards for LC/MS analysis and thereby enable accurate quantification of these mycotoxin-conjugates in food samples.
The detailed synthesis and chromatographic data of these substances will be presented within the poster.



Synthesis of DON-15-glycosides

(1) Schmeitzl, C.; Warth, B.; Fruhmann, P.; Michlmayr, H.; Malachová, A.; Berthiller, F.; Schuhmacher, R.; Krska, R.; Adam, G. Toxins (Basel). 2015, 7 (8), 3112–3126. (2) Savard, M. E. J. Agric. Food Chem. 1991, 39 (3), 570–574.

P87

Mechanism of the genotoxic potential of Alternaria mycotoxins

Nadin Wedler*, Stefanie Fleck, Ann Liza Piberger, Andrea Hartwig

Karlsruhe Institute of Technology, Karlsruhe, Germany

* Corresponding author: nadin.wedler@kit.edu

Moulds of the kind Alternaria are ubiquitous in the environment and are responsible for spoilage of fruits, vegetables and wheats. The resulting loss of yields in agriculture represent an enormous problem. Furthermore Alternaria produces numerous secondary metabolites toxic to humans. In the chinese province of Linxian an increased incidence of esophageal cancer was ascribed to uptake of Alternaria toxins. With respect to their health risks it is a critical task to examine and control the contamination of food and animal feed by moulds. However, according to the EFSA the present data is not sufficient for risk assessment of Alternaria toxins. Also the BfR criticizes the insufficient data and currently the health risks to humans cannot be evaluated. Therefore further investigation of Alternaria toxins is urgently required, including studies of the potential genotoxic mechanisms. Many Alternaria toxins, e.g. Altertoxin II (ATX II), Stemphyltoxin III (STTX III) and Alternariol (AOH) induce DNA strand breaks, which can have many origins: imaginable are DNA single strand breaks (SSBs), DNA double strand breaks (DSBs), intermediates of DNA repair and alkali labile DNA adducts. DNA strand breaks have not been investigated so far. Therefore a wide variety of analytic methods was employed to characterize the DNA strand breaks, induced by ATX II, STTX III and AOH. In our study we observed that STTX III induces poly(ADP-ribose), which is considered as an indicator for the induction of SSBs. DNA strand breaks induced by AOH were identified as DSBs via γ-H2AX-immunofluorescence labeling. ATX II, in contrast, induces neither SSBs nor DSBs. Very likely the detected DNA strand breaks can be explained by the formation of alkali labile DNA adducts and resulting alkali labile AP sites, which is reasonable due to the presence of the reactive epoxide in the mycotoxine. The evaluation of the proposed mechanism is the aim of further investigations. Thus, the Alternaria toxins drastically differ in nature of their induced damage. However, for the assessment of health risks further investigation is mandatory. Further research will be focused on formation of DNA adducts and the mutagenic potential.

P88

In Situ Analysis of Trichodiene as a Volatile Biomarker for the Fast and Non-Invasive Gas-Phase Analysis of Cereal Mycotoxins

Martina Werneburg[1]*, Julian Gebauer[2], Matthias Koch[1]

[1]Bundesanstalt für Materialfoschung und -prüfung, Berlin, Germany; [2]ASCA GmbH Angewandte Synthesechemie Adlershof, Berlin, Germany

* Corresponding author: martina.werneburg@bam.de

Typically, mycotoxigenic moulds and consequently small percentages of extremely contaminated portions (“hot spots”) are randomly distributed in a cereal lot. Therefore, an efficient sampling procedure for mycotoxin analysis represents a complex challenge for operators involving invasive and cost intensive steps. Establishing an in situ analysis of mycotoxins from the homogeneous gas phase above cereal crops instead of analysing random samples could address this difficult issue. During studies for microbial volatile organic compounds (MVOCs) indicating an infection with Fusarium, trichodiene was identified as a unique biosynthesis intermediate of trichothecenes - one of the largest groups of the mycotoxin family. The sesquiterpene trichodiene is the only volatile biogenic precursor of the trichothecenes, thus, early and fast in situ detection of this biomarker might be of interest for a potential trichothecene infestation. However, there is no commercial trichodiene standard available needed for the quantification of trichodiene in cereal grains.

The aim of the current project is to develop a fast, easy-to-handle and non-invasive gas-phase quantification of trichodiene in the field. Therefore, trichodiene was prepared by total synthesis based on a tandem orthoester Claisen rearrangement - oxidation - Robinson annulation strategy providing the racemic natural product in 9 steps and 8 % overall yield. Its structure was fully elucidated by NMR and MS. With the reference standard in hand, a protocol was established for the quantitative headspace analysis of trichodiene above crop spikes by GC/MS in the < 10 µg/kg range. Besides a sample survey and a trichodiene - trichothecene correlation study, it is aimed to transfer the validated analytical method from the laboratory into a field-portable analytical system.

The fast and reliable in situ detection of trichodiene as a volatile biomarker for trichothecene mycotoxins will contribute to a reduction of food production/analysis costs and to an improvement of food safety.

P89

Non-toxic degradation products of NX-2 and NX-3

Gerlinde Wiesenberger[1]*, Elisabeth Varga[2], Christian Hametner[3], Gerhard Adam[1], Franz Berthiller[2]

[1]DAGZ, BOKU, Tulln, Austria; [2]IFA Tulln, BOKU, Tulln, Austria; [3]TU Wien, Vienna, Austria

* Corresponding author: gerlinde.wiesenberger@boku.ac.at

Fusarium graminearum, one of the economically most important plant pathogens causing diseases such as Fusarium Head Blight (FHB) of small grain cereals and ear rot of maize, produces deoxynivalenol (DON), which is a virulence factor on wheat and most likely on other hosts. During a large scale survey of Fusarium graminearum (sensu strictu) in the northern United States strains (termed N-strains) had been discovered (Liang et al., 2014), which show normal aggressiveness and produce a so far unknown type A trichothecene termed NX-2. This mycotoxin is identical to 3-ADON with the exception that it lacks the keto group at C-8 (Varga and Wiesenberger et al., 2015). On plants and in plant extracts NX-2 is de-acetylated to NX-3.

During purification and characterization of NX-2 we co-purified a second substance termed NX-1 (NX-2-M1), which differentiates from NX-2 by an opened epoxide ring resulting in a bridge between C-10 and C-12 and the addition of water. A similar degradation/rearrangement product (DAS-M1) had previously been described as less-toxic degradation product of DAS (Shams et al., 2011). Furthermore, an analogous product of NX-3 (termed NX-3-M1) has been identified, which is generated rapidly by treatment of NX-3 at high pH (≥ 9) and/or elevated temperature (65°C).

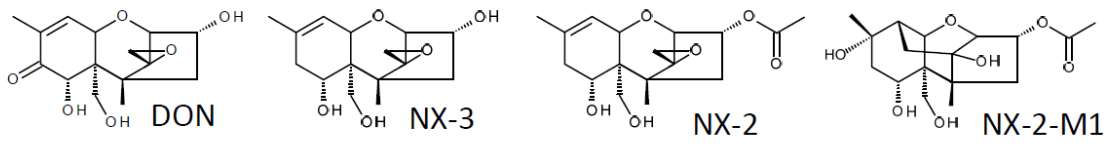
The rearranged products are not inhibitory for animal and plant ribosomes.

References:

Liang, J.M., Xayamongkhon, H., Broz, K., Dong, Y., McCormick, S.P., Abramova, S., Ward, T.J., Ma, Z.H., Kistler, H.C., 2014. Fungal Genetics and Biology 73, 83-92. DOI: http://dx.doi.org/10.1016/j.fgb.2014.10.002

Shams, M., Mitterbauer, R., Corradini, R., Wiesenberger, G., Dall'Asta, C., Schuhmacher, R., Krska, R., Adam, G., Berthiller, F., 2011. Journal of agricultural and food chemistry 59, 9709-9714. DOI: 10.1021/jf2022176

Varga, E. and Wiesenberger, G., Hametner, C., Ward, T.J., Dong, Y., Schofbeck, D., McCormick, S., Broz, K., Stückler, R., Schuhmacher, R., Krska, R., Kistler, H.C., Berthiller, F., Adam, G., 2015. Environmental microbiology 17, 2588-2600. DOI: 10.1111/1462-2920.12718



Research was funded by FWF (SFB Fusarium F3702 and F3706) and Christian Doppler Society.

P90

Resistance of winter triticale breeding lines to accumulation of *Fusarium* metabolites in grain

Halina Wiśniewska[1]*, Tomasz Góral [2], Piotr Ochodzki [2], Michał Kwiatek[1], Maciej Majka [1], Dorota Walentyn-Góral[2], Iga Grzeszczak[2], Zofia Banaszak[3], Mirosław Pojmaj[3], Danuta Kurleto [3], Marcin Konieczny[3], Grzegosz Budzianowski[4], Alicja Cicha[4], Kazimierz Paizert[4], Janina Woś[4]

[1]Institute of Plant Genetics, Poznań, Poland; [2]Plant Breeding and Acclimatization Institute , Radzików, Poland; [3]Danko Plant Breeders Ltd., Choryń, Poland; [4]Plant Breeding Strzelce Ltd., Strzelce, Poland

* Corresponding author: hwis@igr.poznan.pl

Fusarium head blight (FHB) is a disease of cereals caused by fungi of the Fusarium genus. These fungi can produce toxic secondary metabolites referred to as mycotoxins, which accumulate in the grain. Resistance to *Fusarium* head blight of 30 Polish winter triticale lines and three cultivars (Fredro, Meloman and Tomko) was tested in 2015. Triticale lines were sown in field experiments in two locations. At flowering stage triticale heads were inoculated with three *Fusarium culmorum* isolates. FHB index was scored and after the harvest percentage of Fusarium damaged kernels was evaluated. Grain was analyzed for a content of trichothecenes B: deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA) using gas chromatography technique (trichothecenes) and AgraQuant® ZON test kit (zearalenone) and ergosterol (ERG) using HPLC technique. The average FHB index was 9.1% and ranged from 4.3 (DS.9) to 21.5% (BOHD 554). Percentage of *Fusarium* damaged kernels (%FDK) was on average 13.4%. It ranged from 4.5 (DS.9) to 22.8 (Fredro). ERG was detected in grain of all lines tested in two locations and the average amount was 4.9 mg/kg. It ranged from 1.4 mg/kg (BOH 534-4) to 8.1 mg/kg (MAH 34068-5). Nivalenol (NIV) was not detected in analyzed grain. The average content of deoxynivalenol (DON) amounted to 11.98 ppm, ranging from 1.9 (BOHD 1025-2) to 39.5 ppm (Danko 1). The content of the zearalenone (ZEA) in the grain was lower and amounted to 41 ppb. It ranged from 10 ppb (DL 446/08) 188 ppb (Danko 8). Relationships between %FDK and ERG, ZEA and DON were found. FDK percentages correlated with ERG (r = 0.511), and concentration of DON (r = 0.414) and ZEA (r = 0.558). DON correlated significantly with ZEA content in grain (r = 0.665).

This work was supported by Ministry Agriculture and Rural - grant reference HOR hn 078/PB/29/15.

P91

Aurofusarin, a secondary metabolite of *Fusarium* fungi, induces oxidative stress and DNA damage in HT29 colon carcinoma cells

Konstantin Wolters[1], Benedikt Warth[1], Gudrun Palke[1], Michael Sulyok[2], Doris Marko[1*]

[1]Department of Food Chemistry and Toxicology, Vienna, Austria; [2]Center for Analytical Chemistry (IFA Tulln), Tulln, Austria

* Corresponding author: doris.marko@univie.ac.at

Aurofusarin (AURO) is a secondary metabolite produced by *Fusarium* spp. Although it is an ubiquitous contaminant of a variety of food items, data about its toxicity are scarce. Previously, AURO was reported to induce cytotoxicity in colon cells1. In order to investigate further potential detrimental effects, the toxicological impact of AURO was investigated in HT29 colon carcinoma cells. As basis for the experiments in cell culture the chemical stability of AURO was examined under varying storage times and conditions using a newly developed LC-MS/MS method. The stability of the compound proved to be strongly light- and temperature-dependent, with a pronounced disintegration visible already after one day at RT. Despite this instability AURO induced significant cytotoxicity in HT29 cells after 24h. Additionally, the potential of AURO to induce DNA damage was evaluated in the comet assay. After 1h of incubation a significant increase of DNA strand breaks was observed, raising the question about the underlying mechanism of action. In the decatenation assay AURO was found to potently inhibit the activity of topoisomerase II (topo), enzymes crucial for the topology of DNA. In the catalytic cycle of topo different ways of interference and impact on DNA integrity are possible. In the ICE assay AURO did not affect the stability of the covalent DNA-topo-intermediates, formed during the catalytic cycle, thus arguing for a role as a catalytic topo inhibitor. In addition, in the DCF assay significant ROS formation was observed, which could be linked to a potential onset of oxidative stress, visible as a shift of the GSSG/GSH ratio after 3h and 24h. Taken together, the observed effects induced by AURO in HT29 cells suggest that the secondary metabolite may be considered as a mycotoxin.

1 Vejdovszky K, Warth B, Sulyok M, Marko D. Non-synergistic cytotoxic effects of Fusarium and Alternaria toxin combinations in Caco-2 cells. Toxicology Letters 2016; 241:1-8.

P92

Interaction between fungivorous Collembola and mycotoxin-producting Fusarium species

Yang Xu, Katharina Pfohl, Petr Karlovsky*

Georg-August-University Göttingen, Göttingen, Germany

* Corresponding author: pkarlov@gwdg.de

Folsomia candida is a fungivorous Collembolan species. We studied the effect of feeding damage of *Fusarium graminearum* on the production of mycotoxins zearalenone (ZEN), deoxynivalenol (DON) and its acetylated derivatives 3-ADON and 15-ADON. Real-time PCR was used to quantify fungal biomass and HPLC for determining the mycotoxin content. The fungal amount after feeding were decreased, while feeding damage stimulated the production of ZEN and suppressed production of DON, 3-ADON and 15-ADON.

Preference of Folsomia candida for certain fungal species is well established. In traditional food preference experiments, several fungal colonies species are placed at a distance in an arena (usually a Petri dish) and the number of collembolans feeding on the cololonies is monitored. In natural environment, colonization of organic substrates by a mixture of different fungi is common. It is therefore questionable whether food preferences determined with spacially separated colonies of pure fungal strains reflect the behavior of collembolans in natural environments. In our study, we first determined food preference if *F. candida* for *F. graminearum* and *F. verticilliodes* in the traditional way with separated colonies. *F. verticillioides* turned out to be a preferred food source; colonies of *F. graminearum* were avoided. When then fed the animals with a mixture of mycelia of both species and determined food consumption by species-specific real-time PCR. We found that *F. candida* still preferred *F. verticillioides* but the consumption of *F. graminearum* was much higher than under conditions of spatially separated colonies. Our results indicate that traditional food choice experiments with spatially separated colonies of fungal isolates do not adequately model the behavior of fungivorous arthropods in natural environments.

List of authors

A	
Abdallah, Mohamed Fathi	P1, P2
Achten, Elisabeth	P3
Adam, Gerhard	L13, L29, P11, P25, P27, P42, P64, P72, P76, P89
Akinyemi, Adeyemi	L8
Aleksic, Brankica	L18
Ali, Nurshad	L22, P4
Almeida, Ana	L19
Almeida, Susana	P81
Ałtyn, Iwona	P79
Alvito, Paula	L21
Ammann, Harriet	L16
An, TaeKyung	P5
Antonissen, Gunther	L33
Arndt, Birgit	P6
Assunção, Ricardo	L21
Atanda, Olusegun	P12
Athanasiou, Sotirios	P49
Ayugi, Vincent	P7

B	
Bader, Oliver	L5
Bailly, Jean-Denis	L18, L34
Bailly, Sylviane	L18
Banaszak, Zofia	P90
Bannert, Erik	L28, P8, P32, P73
Barta-Böszörményi, Anikó	P32, P48
Bauer, Julia I.	P9, P26
Baumgarten, Tim	P60
Baydar, Terken	P1
Becker, Stefanie	P62
Beitz, Toralf	P21
Beloglazova, Natalia	L2
Berges, Helene	P64
Berrios, Roger	P20
Berthiller, Franz	L29, P14, P31, P42, P89
Bertuzzi, Terenzio	L8
Biermaier, Barbara	L5, L17, P10
Biselli, Scarlett	P66, P67
Błajet-Kosicka, Anna	P23, P79
Blaszkewicz, Meinolf	P4
Bodas, Dhananjay	P13
Böhm, Josef	P38, P39, P79
Bouten, Katrien	L1
Bracarense, Ana Paula	L30

Bratinova, Stefanka	P70
Braun, Dominik	P11
Brodar, Lidija	P61
Broekaert, Nathan	L29
Budzianowski, Grzegosz	P90
Buerstmayr, Hermann	P64
Bunzel, Mirko	P35
Burek, Olga	P30

C

Caceres, Isaura	L34
Carolino, Elisabete	L19
Cebola de Oliveira, Ana	P82, P83
Chatzoglou, Christos	P49
Chilaka, Cynthia Adaku	P12
Chobot, Vladimir	L35
Choudhari, Priyanka	P13
Chrpová, Jana	P68
Cicha, Alicja	P90
Colicchia, Sonia	P14
Cossalter, Anne Marie	L31
Cramer, Benedikt	L4, P33, P62, P81
Croubels, Siska	L29, L32, L33, P17
Cubero Leon, Elena	L1
Cujic, Svetlana	P22
Czompó, Krisztián	P15

D

Dall'Asta, Chiara	L26, P59
Dänicke, Sven	L25, L28, P8, P32, P48,P73
Dastjerdi, Raana	P16
Davenport, John	P53
De Backer, Patrick	P17
De Baere, Siegrid	L33
De Boevre, Marthe	L29, P12, P29
De Mil, Thomas	L32, P17
De Saeger, Sarah	L2, L29, L32, P12, P18,P29
Deák, Edit	P15
Decleer, Marlies	P18
Degen, Gisela H.	L22, P4
Del Favero, Giorgia	L13, P11
Dellafiora, Luca	L26
Dembek, Romuald	P77
Demeyere, Kristel	L29
Devreese, Mathias	L29, L32, L33, P17
Diehl, Dörte	P46
Dietrichkeit, Kay	P19

Divon, Hege Hvattum	L11, P28
Dofkova, Marcela	P85
Dohnal, Ilse	P20
Doppler, Maria	P72
Dopstadt, Julian	L35
dos Santos, Mateus	L19
Doupovec, Barbara	P20
Draghi, Marjorie	L18
Dvorak, Vladimir	P74

E

Edler, Lutz	L6
Eeckhout, Mia	L32
El Khoury, Rhoda	L34
Ellmer, Doris	P56
Ellner, Frank	P51
Eriksen, Gunnar Sundstøl	P28
Erler, Alexander	P21

F

Fæste, Christiane	L11
Faria, Tiago	P80, P83
Farkas, Hunor	P22
Feizbakhsh, Mohammad Taghi	P57
Filipiak-Szok, Anna	P23
Fleck, Stefanie	P87
Focke, Christine	P24
Föllmann, Wolfram	P4
Foubert, Astrid	L2
Fraeyman, Sophie	L33
Frahm, Jana	L25, L28, P8, P73
Fröhlich, Johannes	P27, P86
Fromme, Hermann	L17
Fruhauf, Sebastian	P25
Fruhmann, Philipp	L13, P11, P27, P42, P86

G

Galaverna, Gianni	L26
Gareis, Manfred	L5, L17, P10
Gebauer, Julian	P88
Geisen, Rolf	P46, P69
Gekle, Michael	L23, P63
Ghormade, Vandana	P13
Girgin, Gözde	P1
Góral, Tomasz	P50, P90
Gottschalk, Christoph	L5, L17, P10
Gourbeyre, Pascal	L31

Grajewski, Jan	P23, P77, P78, P79
Grenier, Bertrand	P25
Gross, Madeleine	L10, P9, P10, P26
Grosse, Yann	P52
Grzeszczak, Iga	P50, P90

H

Haase, Hajo	L3
Hadacek, Franz	L35
Hahn, Kathrin	L7
Haider, Maximilian	P27
Hametner, Christian	P27, P42, P86, P89
Harder, Hauke	P39
Harms, Katrin	L36
Hartel, Mathias	L36
Hartinger, Doris	P20
Hartwig, Andrea	P87
Häubl, Georg	P86
Herz, Corinna	P41
Hessenberger, Sabine	P65
Hickert, Sebastian	L4, P33
Hildebrand, Andreas A.	P35
Höbartner, Andreas	P25
Hole, Anastasia	L11, P28
Hong, SeungRan	P5
Hove, Melody	P29
Humer, Elke	P39
Humpf, Hans-Ulrich	L4, L23, L35, P6, P24, P33, P62,P63,P81
Hurbankova, Marta	L20, P40
Hüther, Liane	P73

I

Ibrahem-Aroud, Husam	P3
Ivanova, Lada	L11, P28

J

Jacxsens, Liesbeth	P29
Jagels, Annika	P33
Jaksic, Milena	P22
Janevska, Slavica	P6
Jang, Han Sub	P5, P54
Jedziniak, Piotr	P30
Jitjak, Wuttiwat	L35
Johanning, Eckardt	L15, L17
Jurisic, Nada	P31

K

Kahl, Sandra M.	P45
Kahlert, Stefan	L28, P8, P32, P48, P73
Kalinina, Svetlana	P33
Kaltner, Florian	P34
Karlovsky, Petr	L14, L35, P7, P16, P71,P92
Keller, Julia	L3
Kern, Corinna	P20, P65
Kersten, Susanne	L28, P8, P73
Kilicarslan, Bilge	P1
Kim, Dong-Ho	P47
Kim, Woori	P47
Kirichenko, Albina A.	P45
Klaffke, Horst	L12
Klapec, Tomislav	P61
Klier, Dennis	P21
Kluess, Jeannette	L25, L28, P8, P32, P73
Koch, Matthias	L3, P41, P88
Kohn, Beate N.	P35
Konieczny, Marcin	P90
Korn, Ulrike	L9
Kosicki, Robert	P79
Kőszegi, Tamás	P55
Kotalova, Katerina	P74
Kotz, Daniela	P36
Kovalsky, Paula	P44
Krska, Rudolf	L13, P2, P11, P42, P44, P61, P64
Krug, Isabel	P6
Kumke, Michael	P21
Kunsági-Máté, Sándor	P55
Kunz-Vekiru, Elisavet	P25, P31
Kurleto, Danuta	P90
Kurzawa, Marzanna	P23
Kuster, Lilian	P37
Kuźmińska, Katarzyna	P23
Kwiatek, Michal	P50, P90
Kwiatkowska, Justyna	P78

L

Lachat, Carl	P29
Lacroix, Marlene	L18
Laffitte, Joelle	L31
Lahrssen-Wiederholt, Monika	P3
Lamy, Evelyn	P41
Lebuhn, Michael	L36
Lee, Chan	P5, P47, P54
Lemli, Beáta	P55

Letzel, Matthias	L4
Lichti, Fabian	L36
Lim, JooWon	P54
Lippi, Yannick	L31
Liskova, Aurelia	L20
Löhmannsröben, Hans-Gerd	P21
López de Cerain, Adela	L24
Loureiro, Susana	L21
Lucke, Annegret	P38, P39

M

Madder, Annemieke	L2, P18
Majka, Maciej	P90
Majorosova, Maria	L20, P40
Malachová, Alexandra	L29, P42, P72
Malir, Frantisek	P52, P74
Marinkovic, Dragana	P22
Marko, Doris	L7, L13, P11, P56, P91
Marosanovic, Biljana	P22
Martins, Carla	L21
Maruo, Viviane Mayumi	L30
Maul, Ronald	P41
Mayer, Elisabeth	P65
Métayer, Jean-Paul	L30
Metzler, Manfred	P35
Metzler-Zebeli, Barbara	P39
Meyer, Evelyn	L29
Meyer, Karsten	L36
Michlmayr, Herbert	L29, P42, P64
Mikula, Hannes	P27, P56, P86
Mischke, Carsten	L1
Mitema, Alfred	P43
Moll, Wulf-Dieter	P25
Muccio, Michele	P44
Müller, Marina E.H.	L12, P45
Munk, Bernhard	L36
Muñoz, Katherine A.	P46

N

Naehrer, Karin	P44
Nagl, Veronika	P65
Natsaridis, Nikolaos	P49
Noh, Hee-Min	P47
Nossol, Constanze	P32, P48
Ntantasios, Antonios	P49
Nyanga, Loveness	P29

O	
Ochodzki, Piotr	P50, P90
Okoth, Sheila	P43
Oldenburg, Elisabeth	P51
Oster, Michael	P48
Osteresch, Bernd	P81
Ostertag, Johannes	L36
Ostry, Vladimir	P52, P74, P85
Oswald, Isabelle P.	L18, L30, L31, L34

P	
Pacifico, Cátia	P82, P83
Pahlke, Gudrun	L13, P11, P91
Paizert, Kazimierz	P90
Palmer, Elise	P53
Parich, Alexandra	P72
Park, JuHee	P54
Pastor, Laura	L24
Paulick, Marleen	L25
Payros, Delphine	L31
Pfeiffer, Erika	P35
Pfohl, Katharina	P7, P92
Phat, Chanvorleak	P47
Piątkowska, Marta	P30
Piberger, Ann Liza	P87
Pieckova, Elena	L20, P40
Pierron, Alix	L31
Pietri, Amedeo	L8
Pietruszka, Katarzyna	P30
Pietsch, Constanze	L27
Pinhão, Mariana	L21
Pintão, Ana	P82
Pinton, Philippe	L30, L31
Pistol, Gina	L25
Ploetz, Christian Puck	P26
Pojmaj, Mirosław	P90
Poór, Miklós	P55
Puel, Olivier	L34
Puntscher, Hannes	P56

Q	
Quintal Gomes, Anita	P80, P82, P83

R	
Rahjoo, Vahid	P57
Rainieri, Sandra	P58
Rajkovic, Andreja	L2, P18

Rajwade, Jyutika	P13
Rampl, Corina	P34
Rehurkova, Irena	P85
Reichel, Mareike	P66, P67
Reinhold, Lilli	P19
Reisinger, Nicole	P65
Renner, Hannah	P35
Renner, Lydia	L28, P8, P32, P48, P73
Revel, Iyer	P43
Riedl, Janet	P3
Righetti, Laura	P59
Ritoux, Sébastien	L18
Robine, Enric	L18
Rodemann, Bernd	P60
Rohe, Alexander	P34
Rohn, Sascha	P41
Rolli, Enrico	P59
Rose, Stephan	P36
Rothkötter, Hermann-Josef	L28, P8, P32, P48, P73
Rottkord, Ulrike	L23, P63
Roubal, Tomas	P74
Rud, Ida	L11, P28
Ruprich, Jiri	P85
Rychlik, Michael	L12, L33, P34

S

Sabino, Raquel	P80
Sahlstrøm, Stefan	L11, P28
Sali, Nikolett	P55
Sanoamuang, Niwat	L35
Šarkanj, Bojan	P61
Sas, Benedikt	L2, P18
Schafft, Helmut	P3
Schatzmayr, Dian	P20, P31, P36
Schatzmayr, Gerd	P25, P36, P65
Schaumann, Gabriele E.	P46
Schauvliege, Stijn	L29
Schiessl, Alois	P86
Schloß, Svenja	P41
Schmeitzl, Clemens	P72
Schmidt, Henning S.	P62
Schmidt-Heydt, Markus	P46, P69
Schuhmacher, Rainer	L13, P11, P64, P72
Schulz, Marie-Christin	P63
Schwab-Andics, Christina	P44
Schwaiger, Karin	L5, P10
Schwartz-Zimmermann, Heidi Elisabeth	P14, P31

Schweiger, Wolfgang	P42, P64
Schwerdt, Gerald	L23, P63
Siegwart, Gerald	P64
Silva, Maria João	L21
Skrinjar, Philipp	P27, P56
Somorin, Yinka	L8
Soszczyńska, Ewelina	P77, P78
Spörhase, Pia	P76
Springler, Alexandra	P65
Staiger, Simone	P66, P67
Steiner, Barbara	P64
Štěrbová, Lenka	P68
Stoll, Dominic	P69
Straubinger, Reinhard	L5
Stroka, Joerg	L1, P70
Suchfort, Rosine Ghislaine	L14, P71
Suhail, Rafudeen	P43
Sulyok, Michael	P2, P44, P61, P91
Svoboda, Thomas	P72
Szente, Lajos	P55
Szłyk, Edward	P23

T

Tesch, Tanja	L28, P8, P32, P73
Thamhesl, Michaela	P25
Toman, Jakub	P52, P74
Torovic, Ljilja	P75, P84
Touhami, Najim	P69
Tran, Anh Tuan	L25
Tudzynski, Bettina	P6
Twaruschek, Krisztian	P76
Twarużek, Magdalena	P23, P77, P78, P79

U

Uhlig, Silvio	L11
Ulrich, Andreas	P45
Ulrich, Sebastian	L5
Usleber, Ewald	L10, P9

V

van Bergen, Thomas	L29
Vanhaecke, Lynn	L29
Varga, Elisabeth	P42, P89
Vasco, Elsa	L21
Vautrin, Sonia	P64
Veiga, Luísa	L19
Vejdovszky, Katharina	L7

Vermeulen, An	L29
Vettorazzi, Ariane	L24
Vida, Orsolya	P15
Viegas, Carla	L19, P80, P81, P82, P83
Viegas, Susana	L19, P80, P81, P82, P83
Völkel, Wolfgang	L17
Vukovic, Gorica	P75, P84
Vyslouzil, Jiri	P85

W

Walentyn-Góral, Dorota	P90
Wang, Xinwen	P53
Warth, Benedikt	L7, L13, P11, P56, P64,P91
Weber, Julia	P27, P86
Wedler, Nadin	P87
Werneburg, Martina	P88
Wiesenberger, Gerlinde	L13, P72, P76, P89
Wimmerova, Sona	L20, P40
Wimmers, Klaus	P48
Wiśniewska, Halina	P50, P90
Wolf, Georg	L5
Wolf, Sandra M.	L10
Wölflingseder, Lydia	L13, P11
Wolters, Konstantin	P91
Woś, Janina	P90

X

Xu, Yang	P92

Z

Zebeli, Qendrim	P38, P39
Zimmermann, Thomas	P34
Zupaniec, Milena	P3
Zwickel, Theresa	L12

European Commission

© THPStock - Fotolia.com

European Union Reference Laboratory for Mycotoxins

We work on:
Aflatoxins - Ochratoxin A - Patulin - Deoxynivalenol - Zearalenone
Fumonisins B1 & B2 - T-2 & HT-2 toxin - Ergot alkaloids

by:
Organising interlaboratory comparisons to validate methods
Testing proficiency of laboratories
Providing training

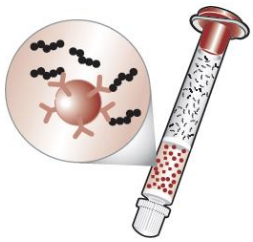
The JRC also produces certified reference materials for mycotoxin testing

JRC Science Hub
ec.europa.eu/jrc

Science@EC JRCAudiovisuals JRC Company Page EU_ScienceHub

Joint Research Centre

Leading Mycotoxin Testing Solutions to protect and enhance the global food chain



Proven solutions
Precise results

VICAM's portfolio of approved tests provide validated monitoring solutions for companies seeking to minimize the costly consequences of mycotoxin contamination. Regulatory compliance and risk management decisions demand proven solutions to provide accurate, precise results. For onsite or preliminary lab screening, VICAM's rapid mycotoxins test kits are the most powerful and cost effective way to give your quality assurance program a head start on cost effective compliance.

- **Quantitative immunoassays for Fluorometer or HPLC**
- **Multi-Analyte-Columns for LC/MS/MS**
- **Qualitative test strips for rapid screening**

VICAM's mycotoxin monitoring solutions are designed to empower users to detect target mycotoxins that threaten the quality, safety, and marketability of agricultural products.

Technical Service and Support
Tel +49 40 819 768 0
info@ruttmann.de
www.ruttmann.de

Ruttmann sales and marketing

Testing Solutions

AflaTest
AflaTest WB
AflaCHECK
Afla M1 FL+
Afla M1 HPLC

CitriTest HPLC

DONtest HPLC
DONtest WB
DONCHECK

FumoniTest
FumoniTest WB

OchraTest
OchraTest WB

T-2test HPLC

ZearalaTest
Zearalatest WB

Multi-Analyte Testing Solution

AflaOchra HPLC
AOZ HPLC
DON-NIV WB
Myco 6in1
T-2/HT-2 HPLC

Try our mycotoxin regulations tool at www.commodityregs.com
twitter@vicamwaters
facebook.com/vicamwaters
linkedin.com/company/vicam
youtube.com/vicamwaters

VICAM®
A Waters Business