

**10-12 NOVEMBER 2014**

**THE**  
**World**  
**Mycotoxin**  
**THE 8<sup>TH</sup> Forum<sup>®</sup>**  
**CONFERENCE**

**Mycotoxin control:  
*the systems approach***

**VIENNA MARRIOTT HOTEL**

**AUSTRIA**

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

**THE  
*World  
Mycotoxin  
Forum*<sup>®</sup>**

**8TH CONFERENCE**

**MYCOTOXIN CONTROL: *THE SYSTEMS APPROACH***

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international conference and networking event for  
the food and feed industry, public health sector,  
regulatory authorities and academia

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**10-12 November 2014  
Vienna, Austria**

# THE WORLD MYCOTOXIN FORUM® – 8TH CONFERENCE

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## WELCOME IN VIENNA!

The occurrence of mycotoxins in various crops affects food and feed safety, food security, public health and international trade. Mycotoxin contamination is due to a series of events, including weather conditions, land use and crop rotation, varieties, cropping practices, harvest and post-harvest handling practices, and possible effects of climate change. Even though a lot of research has been carried out, no single approach has proven to be a successful strategy to cope with the challenge of mycotoxins. Therefore, efforts should be directed at the implementation of an integrated or 'systems' approach to control mycotoxin contamination along the food and feed supply.

The 8th conference of **The World Mycotoxin Forum**<sup>®</sup> initiates the systems approach to minimise contamination of raw materials and provide management solutions for safe use from the farm to the fork. The conference will offer an excellent way to network, share ideas and formulate conclusions on how the systems approach might be achieved.

The 8th conference of **The World Mycotoxin Forum**<sup>®</sup> takes place in Vienna, Austria, on 10-12 November 2014 and includes:

- presentations and discussions in plenary meetings and parallel sessions
- poster sessions
- spotlight presentations covering a wide range of topics, including case studies and industry updates
- workshops and demonstrations
- a concurrent instrument/manufacturers exhibition providing information on products, equipment and services.

As a comprehensive overview, the 8th conference of **The World Mycotoxin Forum**<sup>®</sup> is a vital source of acquiring knowledge and information for everyone involved in the food and feed chain. High-quality speakers, ample time for discussions, and every opportunity to establish rewarding contacts are conference values **The World Mycotoxin Forum**<sup>®</sup> wants to uphold.

You are invited to take part in the discussions with participants from different disciplines and meet business relations in your area. The members of the Advisory Committee and Organising Committee wish you an active and fruitful meeting!

### About The World Mycotoxin Forum<sup>®</sup>

**The World Mycotoxin Forum**<sup>®</sup> is the leading international meeting series on mycotoxins where representatives from the food and feed industry and public health sector meet with people from universities and governments from around the world. The main objectives of **The World Mycotoxin Forum**<sup>®</sup> are:

- to provide a unique platform for the food and feed industry, public health sector, regulatory authorities and science;
- to exchange information and experiences on the various aspects of mycotoxins;
- to review current knowledge related to mycotoxins in food and feed;
- to discuss strategies for prevention and control of mycotoxin contamination ensuring safety and security of food and feed supply; and
- to initiate integrated approaches for the control of mycotoxin contamination along conventional and organic supply chains.

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Key to the abstracts of lectures and posters:

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

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## PROGRAMME AT A GLANCE

### Sunday 9 November 2014

18:00 – 19:30	Welcome reception sponsored by R-Biopharm
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### Monday 10 November 2014

09:00 – 10:15	Conference opening	Exhibition
10:45 – 12:45	Plenary meeting <i>Recent issues – Why did they happen, why couldn't they be prevented?</i>	
14:00 – 17:45	Session 1 <i>Tackling human and animal health risks</i>	
17:45	Poster viewing Wine tasting sponsored by Biomin	

### Tuesday 11 November 2014

08:45 – 10:30	Session 2 <i>Update on sampling and analysis</i>	Session 4 <i>Mycotoxins and EFSA risk assessments – safer food and feed</i>	Exhibition
11:00 – 12:30	Session 3 <i>The power of metabolomics in mycotoxin research</i>	Session 5 <i>Mycotoxins – a wide array of effects</i>	
12:30 – 13:03	Workshops & poster viewing		
14:00 – 17:00	Session 6 <i>Prevention and control of mycotoxins: pre- and postharvest interventions</i>	Session 7 <i>Mycotoxin solutions in the spotlight: industry updates</i>	
		Session 8 <i>Speed presentations</i>	
17:00 – 18:00	Poster viewing & drinks		
20:00	Conference dinner (reservations only)		

### Wednesday 12 November 2014

08:45 – 10:30	Session 9 <i>Highlights of the 'World Mycotoxin Journal' special issue on rapid methods</i>	Session 10 <i>Environmental and agricultural issues</i>	Exhibition
11:00 – 13:00	Plenary meeting <i>The systems approach</i>		

## CONFERENCE PROGRAMME

MONDAY 10 NOVEMBER 2014

### CONFERENCE OPENING

- 09:00 *Welcome!*  
Prof.dr. Rudolf Krska, Department IFA-Tulln, BOKU Vienna, Austria
- 09:15 *Mycotoxins: a global threat to food security and food safety – FAO's perspectives and activities*  
Dr. Renata Clarke, Food and Agriculture Organization of the United Nations (FAO), Italy
- 09:45 *Significance of mycotoxins in food safety compared to other hazards*  
Dr. Diane Benford, Chair of the Panel on Contaminants in the Food Chain, European Food Safety Authority (EFSA), Italy
- 10:15 **Networking break & exhibition**

### PLENARY MEETING

#### Recent issues – Why did they happen, why couldn't they be prevented?

- Chair: Hans van Egmond, M.Sc., RIKILT Wageningen UR, the Netherlands
- 10:45 *Recent issue in the Balkan related to aflatoxins in maize used for feed, and aflatoxin M1 in milk and milk products*  
Prof.dr. Ferenc Bagi, University of Novi Sad, Serbia
- 11:05 *Did the 2013 growing season create a 'perfect storm' for ochratoxin A?*  
Dr. Stefan Wagener, Canadian Grain Commission, Canada
- 11:30 *Recent Fusarium mycotoxin problems in China*  
Dr. Weiwei Zhang, Department of Public Health, Chengdu Medical College, China
- 11:55 *Fusarium head blight epidemic in wheat during the 2012/2013 harvest season in South America*  
Prof.dr. Sofia Chulze, Universidad Nacional de Rio Cuarto, Argentina
- 12:20 *The recent outbreaks of aflatoxicoses in eastern Africa*  
Dr. Amare Ayalew, Partnership for Aflatoxin Control in Africa (PACA), African Union Commission, Ethiopia
- 12:45 **Lunch break**  
**Workshops & poster viewing**

## MONDAY 10 NOVEMBER 2014

### SESSION 1

#### Tackling human and animal health risks

Chair: Prof.dr. Johanna Fink-Gremmels, Utrecht University, the Netherlands

14:00 *Mycotoxin exposure assessment: pros and cons of existing approaches*  
Dr. Paul Turner, University of Maryland, USA

14:30 *Interaction of co-occurring mycotoxins: what do(n't) we know about toxicity?*  
Dr. Isabelle Oswald, INRA, France

14:55 *Mycotoxin mechanisms of action and health effects: in vitro or in vivo tests, that's the question*  
Prof.dr. Federica Cheli, University of Milan, Italy

15:20 *Food, feed ....and then workers safety!*  
Dr. Danièle Jargot, Institut National de Recherche et de Sécurité (INRS), France

#### 15:45 **Networking break & exhibition**

#### Recent exposure assessment studies

16:15 *Assessing the human mycotoxin exposure in Cameroon and Nigeria through an LC-MS/MS based multiple biomarker approach*  
Dr. Chibundu N. Ezekiel, Babcock University, Nigeria

16:30 *Biomarkers as accurate tool for the assessment of mycotoxin exposure at individual levels in Belgium*  
Dr. Ellen Heyndrickx, Ghent University, Belgium

16:45 *Risk assessment of dietary exposure to the conjugated mycotoxin deoxynivalenol-3- $\beta$ -glucoside in the Dutch population*  
Esmée Janssen M.Sc., National Institute for Public Health and the Environment (RIVM) / Wageningen University, the Netherlands

#### Recent toxicity studies

17:00 *Synergistic toxic effects of zearalenone and ochratoxin A in human cells – protective role of glutathione or selenomethionine*  
Prof.dr. Annie Pfohl-Leszkowicz, University of Toulouse, France

17:15 *New tricks of an old enemy: Fusarium graminearum can also produce a type A trichothecene*  
Dr. Elisabeth Varga, Department IFA-Tulln, BOKU Vienna, Austria

17:30 *In silico/in vitro approaches for mycotoxin metabolism and toxic activity in humans: the case-study of zearalenone and its conjugates*  
Dr. Chiara Dall'Asta, University of Parma, Italy

17:45 **Poster viewing**  
**Wine tasting sponsored by Biomin**



**TUESDAY 11 NOVEMBER 2014**

**SESSION 2**

**Update on sampling and analysis**

Chair: Prof.dr. Sarah De Saeger, Ghent University, Belgium

08:45 *Sampling for mycotoxins in food and feed – theoretical simplicity and practical ambiguity*  
Dr. Alexey Solyakov, National Veterinary Institute, Sweden

09:10 *Highlighting new promising developments in analysis*  
Dr. Franz Berthiller, Department IFA-Tulln, BOKU Vienna, Austria

**Case studies**

09:35 *Aflatoxin contamination in maize: is the sampling procedure fit for purpose?*  
Theo de Rijk, RIKILT Wageningen UR, the Netherlands

09:55 *The lab-on-mobile-device platform for the quantitative detection and tracking of aflatoxins*  
Dr. Donald Cooper, University of Colorado Boulder / Mobile Assay, Inc., USA

10:15 *Potential of front-face fluorescence to assess mycotoxin content in cereals*  
Dr. Pierre Lacotte, Spectralys Innovation, France

10:30 **Networking break** sponsored by the European Food Safety authority (EFSA)  
**Exhibition**

**SESSION 3**

**The power of metabolomics in mycotoxin research**

Chair: Prof.dr. Rudolf Krska, Department IFA-Tulln, BOKU Vienna, Austria

11:00 *How does deoxynivalenol affect the wheat metabolome?*  
Dr. Benedikt Warth, Department IFA-Tulln, BOKU Vienna, Austria

11:15 *Using high resolution mass spectrometry and metabolomics to enable discovery and identification of fungal metabolites*  
Dr. José Diana di Mavungu, Ghent University, Belgium

11:30 *Metabolomics as an effective tool for early prediction of deoxynivalenol/masked deoxynivalenol content in on-field barley*  
Prof.dr. Jana Hajšlová, Institute of Chemical Technology, Czech Republic

11:45 *Stable isotopic labelling-assisted untargeted metabolic profiling reveals novel conjugates of mycotoxins in wheat*  
Prof.dr. Rainer Schuhmacher, Department IFA-Tulln, BOKU Vienna, Austria

12:00 *Alternaria jesenskae, a source of valuable secondary metabolites including mycotoxins sensu stricto*  
Dr. Roman Labuda, Romer Labs, Austria

12:15 Discussion / Q&A

12:30 **Lunch break**  
**Workshops & poster viewing**

## TUESDAY 11 NOVEMBER 2014

### SESSION 4

#### **Mycotoxins and EFSA risk assessments – safer food and feed**

Chair: Frans Verstraete, M.Sc., European Commission, Belgium

08:45 *Introduction to the European Food Safety Authority (EFSA)*

Dr. Mari Eskola, EFSA BIOCONTAM Unit, Italy

08:50 *Modelling the influence of climate change on mycotoxin production in European cereal crops*

Prof.dr. Paola Battilani, Università Cattolica del Sacro Cuore, Italy

09:15 *EFSA CONTAM Panel risk assessments of mycotoxins – recent examples of Fusarium toxins*

Dr. Mari Eskola, EFSA BIOCONTAM Unit, Italy

09:40 *Collation of mycotoxins occurrence data for exposure assessment: focus on deoxynivalenol*

Fanny Héraud, M.Sc., EFSA DATA Unit, Italy

10:05 *The assessment of feed additives for reduction of the contamination of feed by mycotoxins*

Dr. Jaume Galobart, EFSA FEED Unit, Italy

10:30 **Networking break** sponsored by the European Food Safety Authority (EFSA)  
**Exhibition**

### SESSION 5

#### **Mycotoxins – a wide array of effects**

Chair: Dr. Joseph Shebuski, Cargill, USA

11:00 *Mycotoxins in human diets – unpredicted concerns?*

Prof.dr. Johanna Fink-Gremmels, Utrecht University, the Netherlands

11:20 *The impact of mycotoxins on nutritional behaviour*

Prof.dr. Jean Denis Troadec, Aix-Marseille University, France

11:40 *Mycotoxins exposure and child growth: the joint effect of aflatoxins and fumonisins*

Dr. Yun Yun Gong, Queen's University, UK

12:00 *Don't forget the fish: organ damage and hepatic lipid accumulation in carp after feedborne exposure to deoxynivalenol*

Dr. Constanze Pietsch, University of Basel, Switzerland

12:15 *Risk of mycotoxin contamination of edible mealworms*

Sarah van Broekhoven M.Sc., Wageningen University, the Netherlands

12.30 **Lunch break**  
**Workshops & poster viewing**

**TUESDAY 11 NOVEMBER 2014**

**SESSION 6**

**Prevention and control of mycotoxins – pre- and postharvest interventions**

Chair: Dr. Deepak Bhatnagar, U.S. Department of Agriculture, USA

13:30 *Breeding healthy cereals: genetic improvement of Fusarium resistance and consequences for mycotoxins*

Prof.dr. Hermann Burstmayr, Department IFA-Tulln, BOKU Vienna, Austria

13:50 *Unravelling the mysteries of Aspergillus flavus, beyond aflatoxins*

Dr. Deepak Bhatnagar, U.S. Department of Agriculture, USA

14:10 *How to reduce mycotoxin risks through food processing? An ILSI perspective*

Dr. Michele Suman, Barilla, Italy

14:30 *Mycotoxin detoxification by enzymatic biotransformation – present state and future perspectives*

Dr. Wulf-Dieter Moll, Biomin, Austria

14:50 *Mycotoxins in silage: checkpoints for effective management and control*

Prof.dr. Vittorio Dell'Orto, University of Milan, Italy

15:10 Mycotoxin prevention and control measures in China

Prof.dr. Yang Liu, Chinese Academy of Agricultural Sciences, China

15:30 **Networking break & exhibition**

**Case studies**

16:00 *Breeding, marker-assisted selection and RNAi technologies for developing resistance in maize to aflatoxin contamination*

Dr. Robert Brown, U.S. Department of Agriculture, USA

16:15 *Fusarium species dynamics – challenges in the development of a strategy to reduce mycotoxins in oats*

Dr. Ingerd Skow Hofgaard, The Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Norway

16:30 *Fusarium mycotoxins in naturally contaminated wheat mill fractions*

Dr. Ronald Maul, BAM Federal Institute for Materials Research and Testing, Germany

16:45 *Code of practice to minimise sclerotia and ergot alkaloids along the food chain*

Dr. Christine Schwake-Anduschus, Max Rubner-Institut, Germany

17:00 – 18:00 **Poster viewing & drinks**

**TUESDAY 11 NOVEMBER 2014**

**SESSION 7**

**Mycotoxin solutions in the spotlight – industry updates**

Chair: Dr. Lei Bao, AQSIQ, China

13:30 – 15:30

*From 'sample' to 'analysis' – critical points & practical solutions*

Ronald Niemeijer, M.Sc., R-Biopharm, Germany

*New LC-MS/MS multimycotoxin testing concept – implementation of the new method <sup>13</sup>C-MycoSpin in routine testing labs to meet ISO 17025 standards*

Alois Schiessl, Romer Labs, Austria

*Toxicity of type B trichothecenes: beyond deoxynivalenol*

Julia Laurain, M.Sc., Olmix Europe, France

*Tools for monitoring aflatoxin M1 in the dairy production chain*

Dr. Stephen Powers, Vicam, A Waters Business, USA

*Environmentally-friendly mycotoxin testing using water extraction technology*

Mark Tess, Charm Sciences, USA

*Recent advances in mycotoxin diagnostics*

Mary Gadola, Neogen, USA

*Improving on-site mycotoxin measurements by integrated procedures based on dust sampling*

Mareike Reichel, Eurofins WEJ Contaminants, Germany

*Styrene and methacrylic acid-based ergotamine polymer: effect of pH, temperature, contact time and initial concentration on isothermal sorption properties towards ergot alkaloids*

Dr. Alexandros Yiannikouris, Alltech, USA

*Solid phase extraction clean-up based on molecularly imprinted polymers for single mycotoxin and multimycotoxins analysis*

Dr. Sami Bayouhd, Polyintell, France

*The ToxiMet system – a revolutionary approach to the control of mycotoxins*

Prof. Ray Coker, ToxiMet, UK

*Addition of multiple matrices to EnviroLogix' DON3 and Aflatoxin FREE assays*

Dr. Breck Parker, EnviroLogix, Inc., USA

*Addressing the routine mycotoxin analysis challenges with accurate mass MS/MS*

Dr. Michal Godula, Special Solutions Center, Thermo Scientific, Germany

*Applicability of two new immunoassays to the screening of mycotoxins in feed: biochip-based immunoassay for a multi-analytical approach and ELISA for the detection of aflatoxin B1*

Raymond Devlin, Randox Food Diagnostics, UK

15:30 **Networking break & exhibition**

**SESSION 8**

**Speed presentations**

Chair: Dr. Franz Berthiller, Department IFA-Tulln, BOKU Vienna, Austria

16:00 *Launch 2<sup>nd</sup> EC CEN Mandate to harmonise methods for mycotoxin analysis*

Marcel de Vreeze, Netherlands Standardization Institute (NEN), the Netherlands

16:15 – 17:00

Short presentations by selected poster presenters (**see page 55**)

17:00 – 18:00 **Poster viewing & drinks**

**WEDNESDAY 12 NOVEMBER 2014**

**SESSION 9**

**Highlights of the 'World Mycotoxin Journal' special issue on rapid methods**

Chair: Dr. Michele Suman, Barilla, Italy

08:45 *Rapid testing and regulating for mycotoxin concerns: a perspective from developing countries*  
Dr. Gordon Shephard, Cape Peninsula University of Technology, South Africa

09:05 *Cross-reactivity in immunochemistry-based methods applied in mycotoxins analysis*  
Dr. Milena Zachariášová, Institute of Chemical Technology, Czech Republic

09:25 *Fluorescence polarisation immunoassay for rapid, accurate and sensitive determination of mycotoxins*  
Dr. Vincenzo Lippolis, Institute of Sciences of Food Production, National Research Council Italy, Italy

09:50 *Near- and mid-infrared spectroscopy as efficient tools for detection of fungal and mycotoxin contamination in agricultural commodities*  
Prof.dr. Tetsuhisa Goto, Shinshu University, Japan

10:10 *Using commercial immunoassay kits for mycotoxins: 'joys and sorrows'*  
Dr. Susie Dai, Texas A&M University, USA

10:30 **Networking break & exhibition**

**SESSION 10**

**Environmental and agricultural issues**

Chair: Dr. Jörg Stroka, Joint Research Centre, Institute for Reference Materials and Measurements, Belgium

08:45 *Mycotoxins: environmental micropollutants of concern?*  
Dr. Felix Wettstein, Agroscope Institute for Sustainability Sciences (ISS), Switzerland

09:10 *Transferring innovation into practical agriculture to manage mycotoxins*  
Dr. Vittorio Rossi, Università Cattolica del Sacro Cuore and Horta Srl., Italy

09:35 *Systems approach to integrate toxin gene expression, growth and fumonisin B<sub>1</sub> and B<sub>2</sub> production by *Fusarium verticillioides* under different environmental factors*  
Dr. Angel Medina, Cranfield University, UK

10:00 *Ice toxin: monitoring the accumulation of ochratoxin A during on-farm storage*  
Prof.dr. Art Schaafsma / Dr. Victor Limay-Rios, University of Guelph, Canada

10:30 **Networking break & exhibition**

**WEDNESDAY 12 NOVEMBER 2014**

**PLENARY MEETING**

**The systems approach**

Chair: Dr. Stefan Wagener, Canadian Grain Commission, Canada

11:00 *Mycotoxin control: an industry perspective on the systems approach*  
Dr. Robert Baker, Mars Incorporated, China

11:25 *Integrating public health goals into systems management of staple crops to limit mycotoxin exposure*  
Prof.dr. J. David Miller, Carleton University, Canada

11:50 *Managing food risk communication in the social media era: practical lessons learnt!*  
Adrian Moss, Focus Business Communications, UK

12:15 *Is the systems approach the answer? Top five answers learned at WMF2014!*  
by Prof.dr. Rudolf Krska and Hans van Egmond, M.Sc.

12:40 Poster Award hand out by Prof.dr. Rudolf Krska

12:45 Closing remarks of the 8<sup>th</sup> conference of **The World Mycotoxin Forum®** and looking forward to the **WMFmeetsIUPAC2016** conference by Dr. Stefan Wagener

13:00 Take your packed lunch to eat along the way!



**The World Mycotoxin Forum®** and the **IUPAC International Symposium on Mycotoxins and Phycotoxins** proudly announce that their next joint conference will take place in Winnipeg, Canada, on **6-9 June 2016**.

This unique combined event – **WMFmeetsIUPAC2016** – will build on the success of the previous conference that was held in Rotterdam, the Netherlands in 2012.

For all information, please contact the Secretariat of **WMFmeetsIUPAC** by email:

[WMF@bastiaanse-communication.com](mailto:WMF@bastiaanse-communication.com)

## **WORKSHOP PROGRAMME**

**MONDAY 10 NOVEMBER 2014**

12:30 – 13:30 **Workshop 1**

**Innovative rapid detection methods as part of a HACCP system for managing mycotoxins**

**Sponsored and presented by R-Biopharm**

Mycotoxin contamination of food and feed products imposes a risk to human and animal health and has serious economic impact. Since mycotoxins are natural occurring toxins, exposure cannot be 100% controlled. To meet international regulations and guidelines products are tested for the amount of mycotoxins. Yet, instead of testing large numbers of end products a more pro-active approach would have many benefits. To assure safe food and feed various quality assurance tools can be applied, such as GAP and GMP.

During the production process of food and feed critical steps can be identified where it is possible to minimise the risk of unacceptable mycotoxin concentrations in the end product. For this HACCP-based approach for mycotoxin management, a measurement tool to monitor mycotoxins at critical steps is needed. Rapid and on-site decision making with quantitative lateral flow tests can be an excellent tool for that. In this workshop, R-Biopharm will present the latest developments in this field.

**MONDAY 10 NOVEMBER 2014**

12:30 – 13:30 **Workshop 2**

**Mycotoxins risk management in animal nutrition**

**Sponsored and presented by Olmix**

Mycotoxins involve many disorders on animals, mainly immune depression and digestive and reproduction troubles. These effects occur with high contamination but also under chronic exposure to low levels of several mycotoxins. Detection of mycotoxin-related troubles is not easy. Mycotoxins are invisible and odourless. Moreover, mycotoxicosis symptoms can be associated to an infectious pathogen instead of the presence of mycotoxins in the feed. As a consequence, mycotoxins require specific attention. Mycotoxins risk management tools may be used to address this issue.

This workshop will be focused on different tools set up by Olmix to evaluate the risk of mycotoxins on a farm (Mycotoxin Risk Evaluator), better understand mycotoxin analysis (Mycotoxin Essentials) and optimise the solution to implement (MT.X+ Calculator).

**TUESDAY 11 NOVEMBER 2014**

12:30 – 13:30 **Workshop 3**

**Unravelling the mycotoxin challenge: a 360° perspective on science, sampling and innovative testing solutions**

**Sponsored by Romer Labs**

In this workshop, Romer Labs provides a complete picture on state-of-the-art analysis of food and feed for mycotoxins throughout various stages of production.

At several stations, participants can gather insights and practical examples regarding correct and regulatory compliant sampling, efficient and fast sample preparation, rapid test methodologies for quick and reliable result generation not only applicable in laboratories but particularly in the field, reference testing methods for accurate low-level quantification of contaminants and analytical services provided by its ISO 17025-certified laboratories. The latest R&D findings with special focus on water-based extraction for LFDs, implementation of the new SIDASpin method for multimycotoxin testing with LC-MS/MS, and complex matrix purification by MycoSpin™ 400 multitoxin column in combination with <sup>13</sup>C internal standards will be presented. Moreover, the workshop will be an opportunity to discover masked mycotoxins and other 'hot topics' in the field of mycotoxins.

**TUESDAY 11 NOVEMBER 2014**

12:30 – 13:30 **Workshop 4**

**Challenges to further increase effectiveness nebo throughput of food safety control: integration of pesticide residues and natural toxins analysis into a single run**

**Sponsored by Thermo Scientific, Germany**

Presenter: Prof. Jana Hajšlová, Department of Food Chemistry and Analysis, Institute of Chemical Technology, Czech Republic

The occurrence of pesticide residues, mycotoxins and other emerging chemical substances in foods has become a major concern both from public as well as regulatory agencies around the world. Effective monitoring of the large groups of possible contaminants and residues is therefore required. Although in most existing studies multi-analyte methods were employed, typically only one group of the above hazardous compounds was targeted. In the presented study, we merged target analysis of 323 pesticide residues, 56 mycotoxins and 11 pyrrolizidine alkaloids into a single method. For sample preparation, QuEChERS-like extraction method was used. The extracts were then examined by ultra-high performance liquid chromatography coupled with tandem high resolution mass spectrometric detection (UHPLC-HRAM-MS/MS) employing quadrupole-Orbitrap hybrid mass spectrometer Q Exactive™. Advanced data dependent MS/MS algorithm was used to trigger automatically MS/MS acquisition based on the fullscan MS survey scans. This allowed simultaneous screening, quantitation and confirmation of all analytes within a single analytical run.



# LECTURES

MONDAY 10 NOVEMBER 2014 – Conference opening

## **Mycotoxins: a global threat to food security and food safety – FAO's perspectives and activities**

**Renata Clarke**

Food and Agriculture Organization of the United Nations (FAO), Italy; [renata.clarke@fao.org](mailto:renata.clarke@fao.org)

Promoting the prevention and control of mycotoxin contamination of food commodities has been an important area of work within the food safety programme of the Food and Agriculture Organization of the United Nations (FAO) for many years. Addressing the challenges posed by mycotoxins is an imperative for FAO given its core mandate to improve food security: these toxins can affect staple foods such as maize and sorghum with important consequences for food security of poor and vulnerable populations. In many countries, particularly in Africa, dietary exposure to mycotoxins remains unacceptably high and the mounting evidence of the possible linkage between aflatoxins and stunting further underlines the urgency of improved control of mycotoxin contamination in order to achieve target food and nutrition security outcomes. Mycotoxin contamination also leads to food/feed loss and has major negative economic consequences.

FAO, in collaboration with other partners, carries out a range of activities that contribute to the prevention and control of mycotoxins globally, nationally and locally. The areas of FAO work relate primarily to: (i) the development of science-based international standards and guidelines, and (ii) support to countries to develop and implement national programmes.

Despite decades of effort in promoting mycotoxin control, it remains a major global problem: the limited success of the past underlines the importance of concerted system-wide action that pays adequate attention to economic and technical feasibility as well as social acceptability.

## **Significance of mycotoxins in food safety compared to other hazards**

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The major causes of death around the world are ischaemic heart disease, stroke, lower respiratory infections and chronic obstructive lung disease. An unhealthy diet and insufficient exercise are associated with ischaemic heart disease and stroke, and the number of deaths per year attributable to these conditions are increasing, as is that for the related condition of diabetes. Furthermore, the prevalence of obesity has tripled in European countries over the past three decades. In contrast to these dietary-related health problems, issues related to food safety appear to contribute much less to human illness. There are comparatively smaller numbers of deaths attributable to foodborne pathogens and allergens than for heart disease and stroke, although the bouts of illness are still appreciable in terms of suffering and decreased productivity of workers.

In the context of these established health issues, should we be concerned about contaminants in food? Key contaminants of concern include heavy metals, persistent organic pollutants (POPs), which are ubiquitous in the environment and therefore in food, process contaminants formed during cooking and other types of food processing, and natural toxins produced by plants and microorganisms. Many of these contaminants have the potential to cause chronic effects arising from prolonged exposure, which makes it difficult to make a direct association between the exposure and ill health.

Risk assessments indicate that dietary exposure to lead, cadmium and inorganic arsenic is at a level of concern in some population groups, but it is not possible to estimate incidences of ill health attributable to these metals. Amongst the POPs, exposure to dioxins, and to some pesticides that are no longer approved, has decreased markedly over past decades and is becoming less of a concern. Trends in exposure to contaminants that have been more recently designated as POPs are less clear, and there is possible concern about exposure to some of them. Acrylamide is a process contaminant

formed during high temperature cooking of starchy foods. Based on its carcinogenicity in animal studies, and knowledge of its mode of action, it is probable that it can cause cancer in humans, and dietary exposure is at a level that is of concern. However, acrylamide has not been proven to be a human carcinogen, possibly due to limitations in the power of the epidemiological studies, and therefore attribution of potential cancer incidence to acrylamide exposure is uncertain.

The EFSA Panel on Contaminants in the Food Chain (CONTAM) has produced a series of opinions on mycotoxins, including aflatoxins, ochratoxin A, zearalenone, deoxynivalenol, nivalenol and other *Fusarium* toxins, *Alternaria* toxins and ergot alkaloids. Some of these toxin groups are regulated, but the established limits do not necessarily include all relevant individual toxins in the group. Other toxin groups are not currently regulated. The CONTAM Panel risk assessments have indicated possible health concern for some population groups with respect to dietary exposure to a number of mycotoxins, including aflatoxins, zearalenone, citrinin and sterigmatocystin. In many cases, the information on toxicity of, or exposure to, relevant toxins were insufficient for conclusions to be drawn. Therefore, although it is generally not possible to attribute numbers of deaths or ill health to mycotoxins, it should not be concluded that they are having no effect. Furthermore, these EFSA assessments relate specifically to European countries. In some other regions of the world there is potential for exposure to mycotoxins to be higher, and for populations to be more sensitive to the toxicological effects, for example in the case of increased risk of liver cancer from aflatoxin exposure in regions of chronic hepatitis B virus infection.

An additional consideration is the frequent occurrence of foods found to exceed regulatory limits for mycotoxins. Monitoring for mycotoxins, and subsequent action to remove non-compliant food products from the market places a financial burden on food authorities and food businesses. Thus, there is potential for both public health and economic benefits from reducing mycotoxin contamination of foodstuffs.

## Recent issue in the Balkan related to aflatoxins in maize used for feed, and aflatoxin M1 in milk and dairy products

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Aflatoxin B1 contamination of maize harvested in 2012, resulting in feed and milk contamination, caused significant economic losses in Serbia, as well as public concern which caused consequences at the state level. During 2012, temperatures above long-term average and a long-term lack of precipitation significantly reduced the vitality of maize crops making them vulnerable to *Aspergillus* spp. Infections were also favoured by damages caused by the European corn borer (*Ostrinia nubilalis*). In autumn 2012, RASFF notifications pointed to the increased content of aflatoxin B1 in exported maize, and public attention to milk contamination with aflatoxin M1 was attracted by information that appeared in February 2013. Inconsistent information on the content of aflatoxin M1 in milk was provided to the public, which caused even more concern.

In February 2013, the government of the Republic of Serbia decided to increase the legal limit for aflatoxin M1 in milk to 0.5 µg/kg. The Plant Protection Department of the Ministry of Agriculture organised monitoring of aflatoxin B1 in maize in 2013 in three phases: in the field, during harvest and during storage. The Extension Service of the Republic of Serbia monitored flight and population abundance of the European corn borer, as well as occurrence of disease symptoms on ears. According to the monitoring plan, in the first phase control was conducted at 246 locations that were marked as potentially risky by visual control conducted by the Extension Service. Control and sampling were conducted during harvest and at selling sites prior to storage. A total of 232 samples were delivered to authorised laboratories for aflatoxin B1 analysis. Higher level was found in 11 samples, out of which in 9 samples aflatoxin B1 was at the level that restricted use of the concerned lots of maize in the human diet, while still enabling feed use. In 2 samples, the aflatoxin level was too high for the maize to be used for food and feed.

The second aflatoxin monitoring phase started in December 2013, and was finished in February 2014. Control and sampling were conducted during the storage period in silos and maize cribs. A total of 272 farms were included in the control and 447 samples were collected. According to laboratory analyses, 14 samples had increased level of aflatoxin B1 and the maize concerned could only be used in feed and not in human diets. On the whole, in monitoring aflatoxin B1 in maize in 2013, 679 reports of laboratory analyses of aflatoxin B1 were filed, based on which 25 samples were found to have an increased content of aflatoxin B1. The level of aflatoxin B1 found in 23 samples analysed (3.4%) restricted the use of maize in the human diet, however, it could be used for feed. In 2 samples (0.3%), the level of aflatoxin B1 was too high for the maize to be used for food or feed. In the remaining samples, the level of aflatoxin was below the permitted limit in Serbia.

In March 2014, a ministerial decision on the decrease of the aflatoxin B1 limit in the regulation on animal feed quality was taken to bring it in harmony with legislation in the European Union (EU). Since 1 July 2014, the limit for aflatoxin M1 in milk has been brought to the level of the EU limit of 0.05 µg/kg milk. However, 15 days after the regulation came into force; it was decided to change the aflatoxin M1 level in milk to 0.25 µg/kg until the end of the year, as a consequence of a request filed by the Serbian Milk Producers Association. The reason for this temporary derogation was the fact that 23% of the milk could not meet the standard of 0.05 µg/kg. The Serbian Milk Producers Association claimed that after the Regulation requiring a limit of 0.05 µg/kg came into force, 100 tons of milk had to be destroyed within two days, and 30-40% farmers still could not meet the demands. Consequently, tens of thousands of family farms would lose their incomes. The argument of the milk producers was also the fact that in many regions in the world, the legal limit for aflatoxin M1 amounts to 0.5 µg/kg milk. A problem in milk production and animal feed quality control in Serbia is certainly the fragmentation of farm holdings (the number of cows per farm). Therefore, it is necessary to conduct self-control of food by producers, besides legal control procedures of animal feed. The Ministry of Agriculture plans to apply the EU standard for aflatoxin M1 in milk from 1 January 2015.

## Did the 2013 growing season create a 'perfect storm' for ochratoxin A in Canadian wheat?

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In western Canada, the major wheat growing area of the country, 2013 was a very productive year for grain. For example, the 2013 wheat harvest was approximately 40% larger than in previous years. The pulse of grain into the Canadian grain handling system led to challenges with storage and transportation within the system. The bulk of Canadian wheat is exported, and must be transported from the growing region of the Canadian Prairies in the centre of the country to ports on the Pacific coast in the west and on the St Lawrence Seaway in the east. However, other factors, including weather conditions in late 2013 and early 2014, as well as business changes within the grain industry, also contributed to the pressure of storing and transporting the 2013 harvested grain.

The challenges brought about by these varied factors included the use of more temporary, and in turn, less ideal, storage for grain. Temporary grain storage included open air piles, large piles covered with tarps, and plastic grain bags. These storage options can be more open to insect infestation and access to other pests, as well as precipitation. It is also more likely that grain stored using these options is not aerated to keep cool and dry, as is grain stored in modern metal bins. This resulted in an increased possibility of *Penicillium verrucosum* – the relevant organism that can affect stored grain and produce the mycotoxin ochratoxin A under specific conditions in temperate climates – flourishing in the grain stored in less than ideal conditions.

The annual increase in ochratoxin A concentrations in grain shipments in late summer was observed again in 2014, suggesting that the various factors ranging from business changes to weather, all played a role in the recent presence of this mycotoxin in Canadian wheat.

## Recent *Fusarium* mycotoxin problems in China

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*Fusarium* mycotoxins, such as trichothecenes, zearalenone (ZEA) and fumonisins, are widely distributed in grain and animal feed, and cause hazards to human and animal health. China, one of the largest producers of agricultural products and animal feed, always faces challenges regarding *Fusarium* mycotoxins problems. The objective of this presentation is to review the recent issues that existed in China, including main areas and samples of contamination, major types of *Fusarium* mycotoxins, (probable) causes of these problems, and updated regulations. The introduction and analysis of these problems is of interest to many stakeholders, and provides information to all who wish to understand more about current challenges for combating *Fusarium* mycotoxin contamination of grain and feed in China.

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## ***Fusarium* head blight epidemic in wheat during the 2012/2013 harvest season in South America**

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*Fusarium* head blight (FHB) is one of the most devastating diseases of wheat worldwide and of increasing concern in South America due to sporadic epidemics with economic losses caused by yield reduction and rejection of grain contaminated with the mycotoxin deoxynivalenol (DON). The disease is mainly caused by species within the *Fusarium graminearum* complex, which comprises several biogeographically structured and phylogenetically distinct species. In South America, *F. graminearum sensu stricto* is the dominant species associated with FHB epidemics in wheat, but other species have also been isolated, such as *F. meridionale*, *F. cortaderiae*, *F. brasilicum*, *F. austroamericanum*. These species produce a range of mycotoxins but the most significant are those of the type B trichothecene class, such as DON, nivalenol (NIV), and the DON-acetylated forms. The dependence of FHB on weather factors, its nature and epidemiology and its sporadic manifestation have determined that control measures are not always successful. Possible factors explaining the FHB increase can be due to the increase in the not till area, climatic conditions, since epidemics occur in crop years dominated by humid and warm weather conditions, and pathogen aggressivity. This sporadic disease continues to challenge researchers from South America for breeding for FHB resistance, and to apply models for predicting disease incidence, infection risk, disease severity or DON levels. These models also help to decide if fungicide application is necessary or not. Biological control is another strategy under evaluation to reduce the impact of the disease and DON accumulation. Regulations on the maximum levels of DON in wheat and wheat-based products have also been established to reduce the entry of this toxin into the food and feed chain.

## **Recent outbreaks of aflatoxicoses in eastern Africa**

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Aflatoxins are carcinogenic and acutely toxic fungal metabolites. Evidence is accumulating on the association of aflatoxin exposure with childhood stunting. Aflatoxin contamination of agricultural produce adversely affects food security and nutrition and is a major constraint to trade in affected commodities. This paper analyses outbreaks of acute aflatoxin poisoning in eastern Africa, mainly based on the extensive reports from Kenya. Lethal aflatoxicoses of humans have occurred for the last three decades in Kenya in association with consumption of contaminated maize. The biggest episode that occurred in 2004 claimed more than 125 lives. Studies consistently show high average levels of contamination ranging from 16-75% of maize samples testing above the allowable limit of aflatoxin. S-strain of *Aspergillus flavus* (phylogenetically distinct from other regions of the world), capable of producing large quantities of the more toxic B aflatoxins, has been associated with lethal aflatoxicoses. Erratic weather conditions, such as end of season drought, could expose the crop to stress leading to higher toxin accumulation. Moreover, in drought conditions fear of theft may derive farmers to harvest too early without adequate field drying. On the other hand, long rains can prevent drying properly which can result in harvesting prior to crop maturity. Improper crop production and postharvest handling practices aggravate contamination. The widespread occurrence of the toxin in maize and other dietary staples in the region suggests that the acute poisoning and tragic losses of human life could be the 'tip-of-the-iceberg' as compared to more pervasive effects of chronic exposure to aflatoxins. Holistic approaches integrating prevention of aflatoxin contamination through integrated value chain controls, effective regulatory mechanism and awareness generation are urgently needed. Further research towards increased understanding of the problem and developing appropriate and affordable technologies is warranted. Partnership between multiple players and avoiding duplication of efforts are key to dealing with the complex aflatoxin problem.

## Mycotoxin exposure assessment: pros and cons of existing approaches

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Mycotoxin exposure assessment may be conducted for various reasons, including routine bio-monitoring, epidemiological investigations and assessing the efficacy of interventions to restrict exposure. In order to make valuable assessments of exposures to any biological or chemical agent, you need reliable tools, and mycotoxin assessment is no exception. Exposure estimates may involve classical dietary questionnaire and or food diary approaches with random food sampling and mycotoxin measurements. This approach provides useful data, though is not always ideal. Exposure biomarkers offer the promise of improved exposure assessment over traditional methods, though when developed and used, the research community needs to carefully consider the end goal for the data collected and what the term 'reliable tools' means. Reliability refers to more than simple analytical accuracy and reproducibility. We need to have some estimate of the ability of that measurement to predict the actual exposure. There is increasing use of bio-specimens for measuring various mycotoxins and or their metabolites. This review, focusing on exposure biomarkers, will discuss the strengths and weaknesses of these measures, both from a single analyte and multiple analyte perspective.

## Interaction of co-occurring mycotoxins: what do(n't) we know about toxicity?

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Most fungi are able to produce several mycotoxins simultaneously; moreover food and feed can be contaminated by several fungi species at the same time; complete diet is made from various different commodities. Thus, humans and animals are generally not exposed to one mycotoxin but to several toxins at the same time. This is supported by global surveys underlying the multicontamination.

The toxicity of combinations of mycotoxins cannot always be predicted based upon their individual toxicities. The data on the combined toxic effects of mycotoxins are limited and therefore, the health risk from exposure to a combination of mycotoxins is incomplete. Most of the studies concerning the toxicological effect of mycotoxins have been carried out taking into account only one mycotoxin. Interactions between concomitantly occurring mycotoxins can be antagonistic, additive, or synergistic. Three main methodological approaches have been used to determine the interaction between mycotoxins; the arithmetic model of additivity, factorial designs and the theoretical biology-based models of additivity. These latter models are the most advanced. In this respect, the Chou-Talalay method, that is not linked to mechanistic considerations, appears more reliable and present the advantage to allow a quantitative assessment of the interaction. Using this model in several cellular systems, we have observed a synergistic interaction for trichothecenes, especially when used at low concentration [1,2].

The synergistic effects observed after cell exposure to a mixture of low concentrations of mycotoxins could pose a significant threat to public health. New risk assessment strategies should take into account the toxicological interactions of mycotoxins in food and feed.

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## **Mycotoxin mechanisms of action and health effects: *in vitro* or *in vivo* tests, that's the question**

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Mycotoxins are toxic secondary metabolites of fungi and represent significant food safety challenges. Despite efforts to control fungal contamination, mycotoxins can contaminate food and feed materials, enter the body via ingestion of contaminated foods, and elicit acute and chronic adverse effects, as determined by animal studies, *in vitro* bioassays, and human epidemiological studies. It is difficult to get a complete understanding and an adequate modelling of mycotoxin health effects due to the complexity of the interactions between the numerous factors affecting the magnitude of mycotoxin toxicity, such as species sensitivity, level and time of exposure, individual sensitivity, age, health, nutritional status, bioaccessibility, mechanisms/modes of action, metabolism, and defense mechanisms. *In vivo* investigations may provide information on mycotoxin net effects in whole animals, whereas cell-specific answers may result from *in vitro* investigations. Within *in vitro* systems, cell-based tests, representing simplified biological systems, have become more and more realistic and representative of *in vivo* condition, and, therefore, may offer a suitable alternative to *in vivo* animal testing. The presentation will show examples of *in vivo* and *in vitro* tests for mycotoxin research. Advantages, drawbacks and technical problems regarding specific applications of each model test, the link between *in vitro* versus *in vivo* tests, the relevance of *in vitro* tests compared to *in vivo* test, and the predictive efficacy of *in vitro* tests will be discussed. The translation of *in vitro* data into meaningful *in vivo* effects remains an unsolved problem. Results of *in vitro* studies indicate that there is only partial agreement with those obtained in *in vivo* experiments. However, some emerging evidence of good *in vitro* tests is arising. Although we cannot yet simply extrapolate the results obtained in *in vitro* study to the *in vivo* exposure of humans and animals to mycotoxins, *in vitro* tests provide insights into how mycotoxins in food might enter into the organism and the mechanisms of action to attain health effects. An understanding of the mode of action in simple *in vitro* systems can provide a rational basis for predicting health effect of single and multi-mycotoxin contamination before any animal or human clinical studies. In conclusion, *in vitro* and *in vivo* tests are complementary approaches to understand the 'whole mycotoxin picture', and lead to improved understanding of dietary relevant mycotoxin exposure and risk scenarios.

## **Food, feed .....and then workers safety!**

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If the potential health risks to animals and humans posed by food- and feedborne mycotoxin intoxication have been largely recognized [1]..... the hazard for the workers posed by airborne mycotoxins are less well-known! However, these dangerous substances justify the assessment of occupational exposure indeed. Methods have been experimentally validated for the most frequently occurring airborne mycotoxins and now proposed with a specific air sampling strategy and a detailed analytical protocol. They meet the criteria required of reproducible and reliable methods for personal workplace sampling in dusty environments and have been adopted to directly measure a minimum air concentration of 0.5 pg/m<sup>3</sup> (aflatoxins) to 1 ng/m<sup>3</sup> (fumonisins). The inhalable dust fraction, which approximates to the airborne particles that enter the nose and mouth during breathing, is collected. As on-line or real-time sampling is unsuitable for occupational measurements, the air sampler had to be reliable for a 8 hour-work shift sampling, small and lightweight to be attached to the worker and commonly used in industry for measuring dust, fibres or moulds in 'real-life' sampling conditions. Measurement results are given and confirm that workers could be exposed when storing, loading, handling or milling contaminated cereals, root vegetables, blends of spices, liquorice powder and pork-based products, such as French 'saucissons'. Airborne mycotoxins during ximenia kernel extraction or waste treatment were also a concern.

Significant airborne particle contamination levels have been detected whereas the commodity contamination was below the regulatory limits. Occupational measurements, relevant to operator

awareness raising purposes, aim at assessing the need for future research on the action mechanisms of inhaled mycotoxins.

We still try to improve the user-friendliness of our analytical method by testing the use of multi-mycotoxin immunoaffinity columns and two molecular recognition based-sorbents. We checked whether these possibly miniaturized tools should have constituted an alternative to the cost-effective antibodies-based sorbent that is currently used [2]. In addition, biological monitoring will be approached in a near future as a complementary action to make 'your job' safer [3].

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3. Our goal, make your job safer (<http://en.inrs.fr/>).

## Assessing the human mycotoxin exposure in Cameroon and Nigeria through an LC-MS/MS based multiple biomarker approach

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Mycotoxin exposure in humans can be to either individual or to mixtures of toxins, and the latter may cause modulation of toxic effects compared to any individual mycotoxins exposure. Human exposure to multiple dietary mycotoxins has been predicted in sub-Saharan Africa, mainly by evaluating mycotoxin occurrence data in variety of foods and calculating exposure assessments using food data. More recently, an LC-MS/MS based multi-mycotoxin biomarker method was developed for the simultaneous detection of 15 mycotoxins/mycotoxins metabolites in urine in order to improve bio-monitoring at the individual level. This method was applied in two separate studies involving individuals from rural areas in Cameroon and Nigeria. A total of 175 urine samples from adults in Cameroon and 120 urine samples from children, adolescents and adults in Nigeria were measured. Eleven and eight analytes were detected singly or in combinations in 63% and 51% of the studied urine samples from Cameroon and Nigeria, respectively. In both countries, regardless of age category, aflatoxin M1, fumonisin B1 and ochratoxin A were the most frequently occurring biomarkers of exposure, in addition to deoxynivalenol in urine from Cameroon. Co-exposures to mycotoxins were found in 18 and 13% of urine from Cameroon and Nigeria, respectively, while family exposures were evident in samples from Nigeria. Urinary levels of several mycotoxins were not only found to directly correlate with dietary estimates, but were predicted to exceed tolerable daily intakes in some individuals of the studied populations. Both studies implicate a public health risk from multiple dietary mycotoxins in rural Nigeria and Cameroon.

## Biomarkers as accurate tool for the assessment of mycotoxin exposure at individual levels in Belgium

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Human exposure assessment to mycotoxins is often based on calculations combining occurrence data



in food with population data on food consumption. Because of limitations inherent to that approach, biomarkers have been proposed as a suitable alternative. Individual variation in absorption, distribution, metabolism and excretion is integrated into the formation of the biomarker, whereby a more accurate assessment of exposure at individual level can be performed.

The BIOMYCO study is designed to assess human mycotoxin exposure using urinary biomarkers of exposure. Over different seasons of 2013 and 2014, morning urine was gathered in a representative part of the Belgian population according to a designed study protocol, whereby 140 children and 278 adults were selected. Every participant completed a food frequency questionnaire to assess the consumption of relevant foodstuffs of both the day before the urine collection and the previous month. Validated LC-MS/MS methods were used to analyse aflatoxins, fumonisins, ochratoxin A, trichothecenes, zearalenone and their metabolites in morning urine. The protocol was approved by the ethical committee of the Ghent University Hospital.

The BIOMYCO study is the first study whereby a multitoxin approach is applied for mycotoxin exposure assessment in adults and children on a large-scale. Moreover, it is the first study that describes the exposure to an elaborated set of mycotoxins in the Belgian population. Besides providing descriptive data on mycotoxin exposure, exposure of different subgroups (age, gender, region) were compared. Furthermore, correlations between urinary mycotoxin concentrations and food consumption were made. In more than 60% of the urine samples analysed in the first seasons, deoxynivalenol and its main metabolites, 3- and 15-glucuronide were found. Also citrinin and ochratoxin A were detected in more than 50% of the samples. Study design, methods and results of the whole sampling period will be discussed.

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## **Risk assessment of dietary exposure to the conjugated mycotoxin deoxynivalenol-3- $\beta$ -glucoside in the Dutch population**

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In this study, a risk assessment of the dietary exposure to the conjugated mycotoxin deoxynivalenol-3- $\beta$ -glucoside (D3G) in the Dutch population was conducted. Data on levels of D3G in food products available in the Netherlands are scarce and information related to its occurrence in other countries is limited to grains and grain-based products. Data on co-occurring levels of D3G and deoxynivalenol (DON), its parent compound, were used to estimate a D3G/DON ratio. The occurrence of DON and D3G correlated positively and D3G/DON ratios could be quantitatively determined for several food product categories. This resulted in a D3G/DON ratio of 0.2 (90% CI, 0.03-0.8) in grains, 0.3 (90% CI, 0.04-2.6) in grain-based products and 0.8 (90% CI, 0.4-1.8) in beer. These ratios were applied to the Dutch monitoring data of DON to estimate the D3G concentrations in food products available in the Netherlands. The DON and D3G concentrations were combined with food consumption data of two Dutch National Food Consumption Surveys to assess chronic exposure in young children (2-6 years), children (7-16 years) and adults (17-69 years) using the Monte Carlo Risk Assessment program.

Due to lack of studies on the hazard characterisation of D3G, the intrinsic toxicity of D3G could not be assessed yet. The possible conversion of D3G to DON, resulting in an additional uptake of DON in the human body, could not be confirmed. Nevertheless, the assumption was made that D3G is deconjugated and then fully absorbed as DON. Therefore, chronic exposure levels of DON, D3G and the sum of both compounds (DON+D3G) were compared to the tolerable daily intake (TDI) of 1  $\mu$ g/kg bw/day based on the most critical effect of DON, namely decreased body weight gain. Exposure (P97.5) of the population aged 7-16 years and 17-69 years to DON or D3G separately, did not exceed the TDI. However, exposure to upper bound levels of DON+D3G (i.e., worst-case scenario) in the same age categories (P97.5) exceeded the TDI with a maximum of 1.3  $\mu$ g/kg bw/day. Exposure (P97.5) of the 2-6 year-olds to DON was close to the TDI. Within this group, exposure (P97.5) to upper

bound levels of DON+D3G exceeded the TDI with not more than a factor 2. Although the evaluation of D3G should be treated with caution, due to the lack of bioavailability and toxicological studies, the present study shows the importance of systematic monitoring of D3G.

## **Synergistic toxic effects of zearalenone and ochratoxin A in human cells – protective role of glutathione or selenomethionine**

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Ochratoxin A is a nephrotoxic mycotoxin produced by several penicillia and aspergilli. It contaminates cereals, coffee, beans, and beer. It is implicated in Balkan endemic nephropathy and urinary tract tumours. Zearalenone (ZEA) is an oestrogenic mycotoxin produced by several fusaria. It contaminates cereal, and is often present simultaneously with ochratoxin A (OTA). The aims of this study were to obtain informations about the synergistic effect of ZEA and OTA on human kidney cells (HK2) and human mammary cells (MCF-7). The role of yeasts enriched with glutathione (GSH) and selenomethionine (SE) was also investigated. Cells were treated by increasing amounts of ZEA, OTA or both of them ranging from 10 nM to 100 µM. The cell viability was checked by MTS test. The genotoxicity was evaluated by the detection of DNA adduct using post-labelling method. The metabolic pathway was analysed by HPLC. Cell viability was differently affected depending of the mycotoxins and the range of exposure. A non-monotone dose response (hormesis) was observed when MCF7 were exposed to OTA or ZEA individually or in mixture, indicating an endocrine disrupting effect. In contrast, OTA decrease HK2 cell viability. This decrease was exemplified by ZEA. Interestingly, it was demonstrated that exposure to low concentrations to both toxins simultaneously induced a toxic effect, even though individually the mycotoxins did not induce any effect. OTA and ZEA induced DNA adduct formation in both cell lines. Simultaneous exposure led to increase of OTA specific DNA adduct, notably C8dG OTA. Glutathione significantly reduced DNA adduct formation both in kidney and mammary cells whatever the conditions of exposure (i.e., mycotoxin alone or in mixture). Selenomethionine reduced significantly DNA adduct only in mammary cells, whereas it increased genotoxicity of both mycotoxins in human kidney cells. Biotransformation of OTA into OTHQ (ochratoxin quinone derivative) was linked to genotoxicity. Simultaneous presence of ZEA shifted the metabolic pathway of OTA. Human kidney cells were more sensitive to OTA whereas the mammary cells were more sensitive to ZEA. The data clearly indicate that exposure to low concentrations of mycotoxins that are considered safe can lead to dramatic effects when they are present together. Until now, regulation does not take into account co-contamination.

## **New tricks of an old enemy: *Fusarium graminearum* can also produce a type A trichothecene**

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The filamentous fungus *Fusarium graminearum* shows a wide-spread occurrence across temperate regions of the world and can produce several mycotoxins on almost every cereal. A large-scale survey of *F. graminearum* (*sensu stricto*) on wheat in the northern United States was conducted to investigate the population dynamics. Some isolated strains produced no deoxynivalenol (DON), nivalenol or acetylated derivatives thereof even though they were classified as 3-acetyl-DON-chemotype based on molecular markers. Rice cultures inoculated with these strains did not contain any of the known trichothecenes. Since the strains were able to produce the volatile trichothecene precursor trichodiene and caused normal symptoms of *Fusarium* head blight on wheat, we investigated whether they produce a novel trichothecene toxin.

In LC-MS measurements, the extracts of rice cultures of these isolates were compared to those of the

previously characterised *F. graminearum* PH-1 and revealed a candidate compound (named NX-2) in significant amounts. By LC-HRMS/MS the sum formula was determined to be C<sub>17</sub>H<sub>26</sub>O<sub>6</sub> and the typical fragmentation pattern of trichothecenes was observed. To elucidate the structure, NX-2 was purified from inoculated rice cultures by normal phase and subsequent reversed phase chromatography and 1D- and 2D-NMR measurements were performed. NX-2 is identical to 3-acetyl-DON with the exception that it lacks the keto group at C-8 and hence is classified as type A trichothecene. Similar to 3-acetyl-DON, we infer that *in planta* NX-2 is rapidly deacetylated and hence we produced the deacetylated form, named NX-3, by alkaline hydrolysis, purified it and could confirm the structure by NMR. Wheat ears inoculated with the isolated strains revealed a ten-fold higher contamination with NX-3 (up to 500 mg/kg) compared to NX-2. The toxicities of the novel mycotoxins were evaluated in two *in vitro* translation assays using plant and mammalian ribosomes. Compared to DON, NX-3 has a slightly decreased potency to inhibit protein biosynthesis, whereas NX-2 is far less toxic and similar to 3-acetyl-DON.

The main genetic difference of known type A trichothecene producing *Fusarium* strains and type B trichothecene producing strains is located in the *TRI1* gene, encoding for a cytochrome P450 monooxygenase. Genetic analysis revealed a different *TRI1* allele in our isolates and we could verify that its gene product is responsible for the hydroxylation of C-7 alone.

Concluding, we have discovered a novel, previously undetected type A trichothecene produced by *F. graminearum* strains, elucidated its biosynthetic pathway and assessed its toxicity regarding the ability to inhibit protein synthesis.

### ***In silico/in vitro* approaches for mycotoxin metabolism and toxic activity in humans: the case-study of zearalenone and its conjugates**

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The interest in identification and quantification of metabolites of free and masked mycotoxins has grown during the past years, on account of the increasing availability of biomarker-based methods for risk assessment. Moreover, knowledge about the conjugative metabolism of xenobiotics is crucial for estimation of toxic effects and intrinsic clearance in humans and livestock.

Metabolic pathways investigations are commonly carried out by means of liver microsomes and/or hepatocytes, followed by mass spectrometry-based analysis. However, despite the larger availability of targeted and untargeted MS methods, metabolite identification is still a time-consuming task, especially when the kinetic of formation of phase I and phase II derivatives has to be considered. In this context, advanced *in silico* techniques can successfully assist data processing, thus moving the bottleneck of metabolite structure elucidation from spectra interpretation to data acquisition. In this frame, the use of smart and friendly tools for metabolite identification, such as Mass-MetaSite (Molecular Discovery Ltd., UK), can be challenging for time saving. Once identified the circulating metabolites, the investigation of possible toxic activities is another demanding task. Also in this case, *in silico* techniques, mainly based on virtual screening, docking and rescoring methods, may help driving *in vitro* tests and act as a priority setting tool to focus further analysis and resources only on worthy candidates of significant concern.

Since zearalenone (ZEA) metabolism in humans has been extensively studied in the past decade and reliable data are actually available in the literature, we decided to apply an integrated *in silico/in vitro* approach to this mycotoxin and its conjugated forms, as a case-study. Our results were in agreement with those reported in the literature, with a notable saving of time. Moreover, the combination of *in silico/in vitro* tests has led to new insights about the potential agonistic activity of ZEA derivatives towards oestrogen receptor  $\alpha$  in humans. Data about possible and controversial estrogenic activity of ZEA-14-glucoside will be thus reported here for the first time and discussed from a molecular point of view.

## Sampling for mycotoxins in food and feed – theoretical simplicity and practical ambiguity

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Mycotoxins are secondary metabolites of diverse moulds and possess some toxic properties. Due to their broad occurrence in feed and food commodities of non-animal origin, both lawmakers and feed/food business operators take special efforts to ensure that feed/food are safe for the human being and animals. The major feature of mycotoxins in general is their severe heterogeneous distribution in the matrix. Bearing that in mind, the lawmaker has stipulated specific procedures for sampling for mycotoxins in the field and for handling of the samples in the testing laboratory. On the EU level, the relevant legislative acts are (i) Commission Regulation (EC) No 401/2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs and (ii) Commission Regulation (EC) No 152/2009 down the methods of sampling and analysis for the official control of feed. The both acts have been recently revised with, for example, new provisions for sampling of very large lots. The major development is introducing the possibility to sample only much smaller part of the whole lot (at least 10% for food commodities), while the analytical result for that sampled portion is supposed to be valid for the whole lot. That approach makes definitely easier professional life of feed/food officials but in some cases is not enthusiastically met by feed/food business operators.

While handling samples in the testing laboratory, the challenge with the extremely heterogeneously distributed mycotoxins is the same while the legislation is much less specific about the adequate way(s) for sample preparation and sub-sampling for the analytical determination. The experience gained up to date shows very different sample preparation and sub-sampling approaches in European official laboratories. Taking into account that every analytical result obtained in a European official testing laboratory is supposed to be accepted by any European authority or feed/food business operator, those differences in sample preparation and sub-sampling approaches may lead to legislative use of non-representative results and thus to potential risks for human health or economic interests of feed/food business operators.

The presentation will touch the latest development of legislative acts as regards sampling for mycotoxins in field and focus on sample preparation and sub-sampling in the testing laboratory and its importance for implication in reality.

## Highlighting new promising developments in analysis

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To ensure nutritional safety for man and animal alike, the detection and quantification of mycotoxins in food and feed remains crucial. Methods used for mycotoxin determination can be grouped into chromatographic methods, immunochemical methods and 'other methods', which include direct spectroscopic methods. The determination of mycotoxins follows general trends in analytical chemistry. These include faster methods, more sensitive methods, as well as the concurrent determination of a large number of analytes in single measurements.

In particular, the tremendous power of liquid chromatography mass spectrometry (LC-MS) instruments regarding selectivity, sensitivity and multiplexing capacity is rendering this technique more and more popular for food contaminant determination – including mycotoxins. While in former times only the most prominent mycotoxins were monitored on a regular basis, nowadays more and more LC-MS based multimethods are developed. These methods often comprise so called 'emerging' and/or 'masked' mycotoxins, which are toxins more frequently found recently or soluble plant metabolites of mycotoxins, respectively. A main advantage of multimethods is to save time and cost by using one method rather than many different ones. More than 300 fungal metabolites can be quantified by such

methods. The rapid improvement of LC-MS instruments is visible in a gain of sensitivity of at least a factor 100 during the last 10 years. Also high resolution mass spectrometric (HR-MS) methods can be employed for the identification and quantification of mycotoxins. Advantages of HR-MS instruments, like QTOFs or Orbitraps, are fast data acquisition and post-acquisition data analysis. The latter is an intriguing option as also contaminants not considered at the time of measurement might be evaluated at a later time. The main disadvantage of HR-MS instrument is that the sensitivity is still about tenfold less than the most modern triple quadrupole LC-MS/MS systems.

A completely different strategy to assess the exposure of individual creatures to mycotoxins is the use of biomarker methods. Such methods are mainly based on LC-MS as well and determine the concentration of mycotoxins, their metabolites (biomarkers of exposure) or other affected endogenous substances (biomarkers of effect) in biological fluids like blood or urine. Finally, there is also a clear need for rapid and easy to use methods which can be used to screen various food matrices without the need of high-end laboratory equipment. These assays are typically based on antibodies and a number of those have been developed recently for the most important toxins.

For comprehensive reviews the interested reader is referred to the series of annual updates on 'Developments in mycotoxin analysis' published in World Mycotoxin Journal [e.g., 1,2].

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## Aflatoxin contamination in maize: is the sampling procedure fit for purpose?

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On 1 March 2013, Germany posted a rapid alert, based on an official control on the market, and related to the genotoxic and carcinogenic aflatoxin B1 (AFB1) contamination in Balkan maize intended for feed (RASFF 2013.0268). The AFB1 concentration ranged from 21 to 204 µg/kg, whereas the legal limit for AFB1 in feed materials is set at 20 µg/kg (Directive 2002/32/EC). Subsequent analysis by the owner of the lot confirmed the results albeit on a lower level: 37.1 µg/kg and 1.9-158.5 µg/kg (RASFF 2013.0281 and RASFF 2013.0316).

As the shipment had passed official controls without a problem a number of questions arose which needed to be answered in order to understand why the official control had failed. And, if necessary, to recommend improvement of monitoring plans, sampling procedures, and analytical methods. The samples originated from a shipment of 45,000 metric tonnes of maize out of which the Netherlands Food and Consumer Products Authority (NVWA) was able to retrieve 1,010 metric tonnes in Rotterdam harbour. Official sampling of the 1,010 metric tonnes was performed according to Commission Regulation (EC) No 691/2013, while at the same time a contract surveyor sampled the shipment according to Commission Regulation (EC) No 152/2009, which was in force at the moment of sampling. In the presentation results and interpretation of individual sample analysis will be shown, and the results of both sampling procedures will be compared. Additionally results will be presented of the influence of laboratory storage conditions on the detection of mycotoxins.

## The lab-on-mobile-device platform for the quantitative detection and tracking of aflatoxins

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Maize is consumed by over 100 million people in East Africa. Aflatoxins are highly toxic to human

health, are correlated with immunosuppression and stunting childhood development, form a barrier to trade, and reduce livestock productivity and contaminate livestock commodities in cases of contaminated feed. Aflatoxins contaminates 25% of the global food supply, primarily in developing countries in the sub-tropics and tropics. There is a need to develop next generation mobile diagnostic devices that are inexpensive, sensitive and easy to use. Recent advances in consumer-based mobile device imaging, computational power, ease of use, connectivity and prevalence have offer a robust platform for building a lab-on-mobile-device platform (LMD) for use with rapid diagnostic tests (RDTs) in the field. RDTs are used worldwide for diagnostics, disease outbreak surveillance. There exists a multitude of such RDT targets (e.g., plant pathogens, malaria, HIV, water quality, pregnancy, toxins, etc.) that are standardised and primarily used for qualitative tests applications. Mobile Assay Inc. (Boulder, CO) has developed an LMD platform capable of boosting the sensitivity of these RDTs up to 100-fold. Mobile Assay's mobile image ratiometry technology provides a powerful diagnostic tool for the quantification of commercially available RDTs for the detection and quantification of mycotoxins and a number of plant and animal pathogens. Cloud-based data storage, analysis and visualisation tools are integral components of the LMD platform that provide the 'big data' enabling integration of climate data with other metadata for predictive modelling of pathogen outbreaks and more targeted interventions. The absence of agricultural data is a serious, but often overlooked problem; however, certain strategies could greatly improve the way data are collected and analysed. Below are several suggested approaches that would transform the state of agriculture data in Africa:

- leveraging mobile technology as data gathering tools;
- developing more accessible data collection systems;
- creating agencies and providing training to monitor progressing;
- integrating crop data with climate data and pest and pathogen data to create data visualisation and predictive models;
- improving data sharing coordination between governmental agencies and nonprofits; and
- standardising data collection and visualisation methods for a common open access platform.

Africa is the second most connected region on the planet and it is projected that by 2016 it will hit the 1 billion mobile phones milestone. However, taking advantage of the growing capability for mobile data gathering there must be integration of mobile technology with existing services, perceived relevance by the end-users and it must be inexpensive and reliable. Although much development and infrastructure has been accomplished in many areas of sub-Saharan Africa, there are significant barriers to commercialising mobile diagnostic technology in the developing world. In this presentation, the development of the technology and the key lessons learned from commercialising mobile diagnostics in sub-Saharan Africa will be discussed.

## **Potential of front-face fluorescence to assess mycotoxin content in cereals**

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Front-face fluorescence spectroscopy and subsequent data analysis using chemometric tools has proven to be a successful diagnosis technique for detection of contaminants in food products (fish, milk, etc.). We demonstrate here how this approach can be applied to mycotoxins content measurement in cereals. Deoxynivalenol (DON) and zearalenone (ZEA) are strictly regulated at the European level, with maximal authorised values being 1,250 and 1,750 µg/kg for DON in wheat and maize, respectively, and 350 µg/kg for ZEA in both cereals for human consumption. Current analytical methods (HPLC, ELISA strips, fluorescence readings, etc.) require prior extraction of mycotoxins, thus restricting their use to laboratory. To answer the need for rapid and simple analysis before silo storage, the potential of front-face fluorescence as non-destructive and real-time analytical technique was investigated.

The Fluoralys® analyser developed by Spectralys Innovation was used for spectral data acquisition on ground wheat and maize. In the frame of this study, 98 maize samples and 162 wheat samples harvested in 2012 were analysed by fluorescence, as well as for DON and ZEA using enzyme-linked immunosorbent assay or high performance liquid chromatography. Multiway spectral decomposition followed by multivariate linear regression between fluorescence variables and mycotoxins concentrations were carried out to obtain calibration models. Maize samples were artificially contaminated with DON concentrations ranging from 0 to 900,000 ppb. To maintain suitable accuracy

throughout this wide range of concentrations, three separate models were built. Prediction of DON values could thus be made precise using models successively, leading to a relative root mean squared error of prediction of 15%. The same methodology was applied for ZEA concentrations ranging from 0 to 4,000 ppb (mean RMSEC<20%). Wheat samples were naturally contaminated and DON values ranged from 0 to 2,000 ppb. ZEA was not detected in these samples. On this concentration range, wheat samples were split in two groups of different genetic properties, and two separate models were built for DON content prediction. Very satisfactory calibration performances were achieved for both models ( $R^2=0.97$  and  $0.94$  / RMSEC=75 ppb and 116 ppb).

This preliminary study reveals the high potential of front-face fluorescence and chemometrics to replace time-consuming and expensive techniques for rapid detection of mycotoxins in cereals.

## How does deoxynivalenol affect the wheat metabolome?

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The trichothecene mycotoxin deoxynivalenol (DON) plays a pivotal role in the development and spread of *Fusarium* head blight (FHB). This plant disease evoked by the fungus *Fusarium graminearum* commonly infects wheat and other grains and might cause severe harvest losses as well as mycotoxin contamination.

The emerging scientific discipline of metabolomics has a great potential to substantially improve the basic understanding of plant-pathogen interactions [1]. Therefore, the presented study was carried out in order to investigate the effect of DON on the metabolome of different wheat genotypes which showed a varying degree of FHB resistance. Treated wheat plants were sampled after 0, 12, 24, 48 and 96 h and were immediately shock frozen in liquid nitrogen. A gas chromatography-mass spectrometry (GC-MS) based targeted metabolomics approach was chosen to detect substances occurring at different concentration levels according to treatment (DON vs. control), time point, and/or wheat genotype [2].

The results indicated that the carbohydrate metabolism as well as the tricarboxylic acid cycle were significantly affected in wheat by DON treatment. Most importantly amino acids and derived amines were stimulated. In addition, the levels of the aromatic amino acids phenylalanine, tyrosine, and tryptophan increased. These amino acids are all synthesised by the shikimate pathway, which plays a crucial role in plant physiology and defense. The presented experiment highlights the major impact of DON in the development of the FHB disease and for the first time demonstrates its great influence on the metabolome level. In the future we intend to merge the metabolite levels determined by the described GC-MS based approach with data obtained from our LC-HRMS platform focusing on semi- and non-polar secondary metabolites [3].

### Acknowledgements

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## Using high resolution mass spectrometry and metabolomics to enable discovery and identification of fungal metabolites

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Mycotoxins are fungal secondary metabolites that are toxic to vertebrates when introduced in small amounts via a natural route. About four hundred mycotoxins have been identified; however, fungi have the genetic machinery to produce many more metabolites than are currently known. For instance,



besides the gene cluster responsible for production of aflatoxins, the most toxic and carcinogenic compounds known from fungi, the genome of the filamentous fungus *Aspergillus flavus* has been shown to harbour 54 other putative secondary metabolic gene clusters. A strategy based on gene knockout and gene overexpression has been applied to determine the function of some of these gene clusters. The role of high resolution mass spectrometry (HRMS) in detecting and identifying the metabolites that are produced by these gene clusters will be demonstrated.

Furthermore, although the primary goal of accurate mass measurement, using HRMS instruments, has been its use for compound identification, there is currently a trend towards the use of this technology to remove interferences in quantitation. We investigated the usefulness of a HRMS workflow involving time-of-flight (TOF)-MS survey scan and information-dependent acquisition (IDA) – MS/MS to enable the simultaneous quantification of major ergot alkaloids and detection of less studied and novel derivatives. Ergot alkaloids are a group of mycotoxins produced mainly by fungi of the genus *Claviceps* and sharing a common tetracyclic ring. The alkaloid pattern and content in contaminated samples vary largely depending on the maturity of sclerotia (ergot alkaloids-containing fungal wintering bodies), the fungal strain, the host plant, the geographical region and weather conditions. This presentation will highlight the fit for purpose of the proposed untargeted HRMS data acquisition approach for correct quantification of target ergot alkaloids in various commodities and to perform at the same time a non-targeted assessment of data, thereby allowing detection and identification of unexpected ergot alkaloids.

## **Metabolomics as an effective tool for early prediction of deoxynivalenol/masked deoxynivalenol content in on-field barley**

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*Fusarium* head blight (FHB) represents a major destructive fungal disease of economically important crops. Infection is commonly caused by several representatives of filamentous fungal species of the *Fusarium* genus. These fungi are known for their active and extensive secondary metabolism within which (not only) mycotoxins are produced. The resistance of particular crop cultivars to FHB is often the key factor associated with formation and accumulation of mycotoxins (production of their free, but also masked forms that are incorporated in the polysaccharide structures within the grain). Thus, the reduction of mycotoxin production through breeding of varieties resistant to their penetration and accumulation is nowadays a challenging task.

In the past decade, metabolomics has emerged and found its application in many research fields, including plant science. The data obtained through a comprehensive metabolomics experiment may provide a deeper insight into biological processes and support the discovery of various biomarkers. Metabolomics-based studies are facilitated by advanced analytical platforms capable of the analysis of numerous chemically diverse metabolites (small molecules up to approx. 1,200 Da) at a large range of concentrations. Depending of their set-up, liquid chromatography-mass spectrometric (LC-MS) techniques are able to detect hundreds of metabolites typically present in plant extracts. Especially, the ultra-high performance liquid chromatography (UHPLC) operated at very high pressures and using sub-2 µm packing columns has allowed a substantial decrease in analysis time and increase in peak capacity, sensitivity, as well as reproducibility of retention times as compared to conventional HPLC. Considering these features for non-target metabolomics of crude plant extracts, the popularity of coupling the UHPLC with high-resolution MS, especially with TOF, Orbitrap, Q-TOF or Q-Orbitrap is steadily growing. Of course, for the data processing, powerful multivariate chemometric tools are necessary to be utilised for gaining the comprehensive information.

In this presentation, several case studies demonstrating metabolomic fingerprinting/profiling of barley and other cereals will be presented. The potential of this approach to predict upcoming mycotoxins contamination, as well as application of metabolomics as a tool for authentication of barley varieties and/or agricultural growing technology, will be documented.

### **Acknowledgements**

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## Stable isotopic labelling-assisted untargeted metabolic profiling reveals novel conjugates of mycotoxins in wheat

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Untargeted metabolic profiling shows great potential to provide a deeper understanding of biological processes including the metabolism of xenobiotics in plants. In this study we describe a novel liquid chromatography- high resolution mass spectrometry (LC-HRMS) based approach for the untargeted screening of metabolism products of <sup>13</sup>C isotope labelled tracer compounds in plants. The approach was successfully applied to wheat plants after treatment with mixtures of the U-<sup>13</sup>C labelled and non-labelled mycotoxins deoxynivalenol (DON), T2- and HT2 toxin in wheat. Untargeted profiling of putative mycotoxin biotransformation products resulted in the assignment of numerous glucose and glutathione derived conjugation products of the applied mycotoxins. For DON, a total of nine different DON conjugates including the well-known DON-3-O-glucoside plus further related metabolites as well as DON-glutathione (GSH), DON-S-cysteinyl-glycine and DON-S-cysteine have been reported for the first time [1]. The molecular structures of the formed conjugates were further characterised by the use of LC-HRMS/MS. In case of T2- and HT2-toxin, preliminary results for the putative identification of biotransformation products in wheat will be presented. For DON, different wheat lines with well characterised levels of resistance against *Fusarium* head blight (FHB) were treated with either DON- or *F. graminearum* and monitored for the formation of DON and its nine conjugates with the aim to associate toxin metabolism with FHB resistance. To this end, the two parent cultivars Remus (susceptible to FHB) and CM-82036 (resistant) plus four near isogenic wheat lines (NILs), which differed in the two major resistance quantitative trait loci (QTLs) against FHB Qfhs.ndsu-3BS and Qfhs.ifa-5A, were challenged at anthesis in two inoculation variants with *F. graminearum* and DON and harvested at 0, 14, 48 and 96 h after inoculation. For each time point and inoculation variant, 5 plants (1 ear per plant) were treated and after the respective inoculation period, treated ears were harvested, immediately frozen in liquid nitrogen, homogenised, extracted and analysed by LC-HRMS. Both, type and relative concentration levels of the produced DON derivatives were studied in detail. The results are presented and discussed in view of the different combinations of resistance QTLs present in the tested near isogenic wheat lines.

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## *Alternaria jesenskae*, a source of valuable secondary metabolites including mycotoxins *sensu stricto*

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A long-spored *Alternaria* species, *A. jesenskae* isolated from seeds of *Fumana procumbens* (a shrubby perennial) gained attention after a recent re-discovery of HC-toxin production by this fungus. HC toxin (a cyclic tetrapeptide) is a host selective toxin for maize lines of genotype hm1/hm1 and hm2/hm2 originally known only from the plant pathogenic fungus *Cochliobolus carbonum* race 1. In addition to HC-toxin production, *A. jesenskae* seems to be very interesting and valuable also due to its ability to produce a variety of secondary toxic metabolites, mycotoxins, namely altertoxins (I-III), altenuene, alternariol and its methylether, altersolaniol, macrosporin, tentoxin and tenuazonic acid. This contribution has a main goal to point at metabolic profile and biology of this unique fungal species as well as to present the data from a study comparing the metabolic (extrolites) profile among *Alternaria* taxa being phylogenetically close to *A. jesenskae*, i.e., so called long-spored *Alternaria* spp. Furthermore, common toxigenic small-spored *Alternaria* species (*A. alternata*, *A. arborescens* and *A. tenuissima*) were included in this study as well.

## Modelling the influence of climate change on mycotoxin production in European cereal crops

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Changing patterns in mycotoxin production in cereals due to climate change must be considered a matter of concern and an emerging issue for food and feed safety. Aflatoxins, secondary metabolites of *Aspergillus flavus* and *A. parasiticus*, are supposed to be the most affected by the climate change, with hotter and dryer summers predicted, therefore conducive for fungi well adapted to tropical/sub-tropical areas. Indeed, extreme high aflatoxin contaminations were detected in maize grains in 2003 in Europe, an area considered usually unsuitable for aflatoxin producing fungi. The aim of this study was to predict aflatoxin contamination in maize, wheat and rice crops, within the next 100 years, under a + 2°C and + 5°C climate change scenarios, applying a predictive modelling approach. The mechanistic model, AFLA-maize was used to predict the risk of aflatoxin contamination in maize; a renewed version was used for predictions in wheat and rice. Data input consist of meteorological data and risk indexes are obtained as output, for each grid point over the EU domain. Regarding meteorological data, a downscaling procedure, based on LARS weather generator, was set up to reproduce the future climate data over the EU domain (2,254 points; 50 km × 50 km). One hundred years of daily weather data including air temperature, humidity and rainfall for three time series for each grid point over EU domain were produced. Risk indexes, computed for each grid point and scenario were calculated. Mean risk indices were presented as a spatial risk distribution for the three scenarios. The + 2°C climate change scenario, actually considered the most reliable in the next 100 years, is related to the most significant increase in the risk of aflatoxin contamination in maize. A wider area of risk and a dramatic increase in the risk level for aflatoxin contamination is depicted. In the + 5°C climate change scenario, the risk of contamination above European legal limit is comparable to, or lower than, the actual one, but spread in a wider area. On the other hand, the risk of contamination in wheat is confirmed as negligible and apparently inexistent in rice. Therefore, aflatoxin in maize is established as an emerging problem in Europe and the modelling/mapping approach used in this study is a useful supporting tool reinforcing aflatoxin detection and control to prevent human and animal health risks.

## EFSA CONTAM Panel risk assessments on mycotoxins – recent examples on *Fusarium* toxins

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The European Food Safety Authority (EFSA) does risk assessments on food and feed safety at the European level. In the European food safety system, risk assessment is done independently from risk management. As the risk assessor, EFSA produces scientific opinions and advice to provide a sound foundation for European policies and legislation. Thus, EFSA supports the European Commission (EC), European Parliament and EU Member States in their risk management decisions. The remit of EFSA covers food and feed safety, nutrition, animal health and welfare, plant protection and plant health. In the process of developing its scientific opinions, EFSA Scientific Panels and Committee have crucial roles. The EFSA Panel on Contaminants in the food chain (CONTAM Panel) carries out risk assessments in the area of chemical contaminants in food and feed, namely process and environmental contaminants, natural toxicants, mycotoxins and residues of unauthorised substances.

This presentation outlines the CONTAM Panel's recently published scientific opinions on the risks to human and animal health related to the presence of *Fusarium* toxins in food and feed. The mycotoxins covered are zearalenone, T-2 toxin, HT-2 toxin, nivalenol, beauvericin and enniatins (<http://www.efsa.europa.eu/en/panels/contam.htm>). The risk assessments on these mycotoxins were all developed upon the requests from the EC. To address the specific scientific areas identified in the

mandates from the EC, the CONTAM Panel set up working groups on zearalenone in food and *Fusarium* toxins comprising scientists with appropriate expertise from the European or international research institutions to prepare the scientific opinions on *Fusarium* toxins.

In the risk assessment of zearalenone in food, the CONTAM Panel concluded that the chronic dietary exposure to zearalenone is not a human health concern. Similarly for T-2 and HT-2 toxins in food, the CONTAM Panel concluded that the exposure to the sum of T-2 and HT-2 toxins is not a human health concern. For farm and companion animals, the outcome of the risk assessment on T-2 and HT-2 toxins was slightly different. For ruminants, fish and rabbits, the CONTAM Panel concluded that the exposure to the sum of T-2 and HT-2 toxins is unlikely a health concern, while for pigs, poultry, dogs and horses the risk of adverse health effects from the exposure to the sum of T-2 and HT-2 toxins in feed is low. For cats, the health risk could not be assessed due to the lack of data. Also for the risk assessment on nivalenol in food, the conclusion of the CONTAM Panel was the same – the dietary exposure to nivalenol is not a health concern for humans. For pigs and poultry, the risk of adverse health effects from the exposure to nivalenol in feed is low, and for ruminants, rabbits, fish, horses, dogs and cats the health risk of the nivalenol exposure could not be assessed due to the lack of data. In the latest *Fusarium* toxin risk assessment on beauvericin and enniatins, the CONTAM Panel concluded that the acute dietary exposure to beauvericin and the sum of enniatins in food do not indicate a concern for human health. However, the chronic exposure to beauvericin and the sum of enniatins in food might be a human health concern but no firm conclusions could be drawn due to the insufficient data. For farm and companion animals, the adverse health effects from the acute exposure to beauvericin and the sum of enniatins in feed were concluded to be unlikely. Similarly the chronic exposure for poultry indicated that adverse health effects from beauvericin and the sum of enniatins in feed are unlikely. For the other farm animals, the lack of data precluded the estimation of chronic health risks from beauvericin and enniatins in animal feed.

The main outcomes of the aforementioned scientific opinions on *Fusarium* toxins are presented. In addition, the future activities of the CONTAM Panel in relation to mycotoxins in food and feed will be outlined.

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The members of the EFSA CONTAM Panel and the members of the CONTAM Working Groups (WG) on zearalenone in food and *Fusarium* toxins.

## **Collation of mycotoxins occurrence data for exposure assessment: focus on deoxynivalenol**

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Assessment of exposure is a central step in any risk assessment of substances whose presence in food can lead to adverse health effects. Dietary exposure to potentially hazardous substances is usually assessed by combining measured chemical occurrence levels in relevant food products with detailed data on their consumption in a population.

Most often, the European Food Safety Authority (EFSA) does not generate any occurrence data for European risk assessments, but collates existing data throughout Europe. An annual call for data on contaminants in food and feed defines a list of 9 types of mycotoxins. In this framework, EFSA received in 2013 around 200,000 analytical results for mycotoxins originating from 25 European countries. To guarantee a good understanding of the information and to facilitate further data handling, EFSA has developed a standard for chemical occurrence data exchange. The EFSA Standard Sample Description (SSD) contains some 80 data elements to fully describe a food sample, e.g., how it has been taken, the chemical measured, the analytical method used, the result obtained and its uncertainty. The SSD is generic and allows handling all kinds of chemical and microbiological hazards. However, not all the data elements are adapted or required for all compounds. For mycotoxins, 20 elements are mandatory and 5 additional elements are recommended in order for EFSA to accept a data transmission. Specific data requirements are also often set during the data analysis and exposure assessment process in order to optimise quality and comparability of the results. Most follow the requirements defined in the European legislation for the data collected in the framework of official monitoring programmes. Overall, the laboratories must be accredited and the analytical method

validated for the compound of interest. According to the compound, the analytical technique to be used, the range of acceptable recoveries or the maximum acceptable limit of quantification by food matrix can also be specified.

Analytical results available for deoxynivalenol (DON) in food have recently been compiled in EFSA scientific outputs [1,2]. Data collected between 2007 and 2012 and reported by 21 European countries – corresponding to 18,804 food samples – were considered. To ensure the comparability of the results regarding the kind of compound measured, only the occurrence data generated with analytical methods based on gas or liquid chromatography were considered. Such a requirement led to the exclusion of around 26% of the data because the analytical technique was not indicated and 15% because they were not generated with gas or liquid chromatography. In addition, to avoid that non quantified results associated with high limit of quantification introduce bias the estimation of DON levels in food, a maximum acceptable limit of quantification was set at 200 µg/kg for grains and grain-based products and 150 µg/kg for other foods. This led to an additional exclusion of 1 % of the data. The remaining data were combined to information from the EFSA Comprehensive European food consumption database, to assess the exposure levels of different European population groups. The average exposure level estimated was in the area of the health-based guidance value (HBGV) for some children population groups. In other population groups, the average exposure level was below the HBGV, but the high exposure level (95th percentile) could fall in the area of the HBGV. Such an assessment shows the importance of having well documented and good quality occurrence data in view of performing a risk assessment.

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## The assessment of feed additives for reduction of the contamination of feed by mycotoxins

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With the creation in 2009 of the new functional group of technological additives ‘substances for reduction of the contamination of feed by mycotoxins’, the European Commission opened the door to the authorisation of additives with the objective to “*suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action*”. It should be noted that these additives can only be used in feed where the levels of mycotoxins are below the maximum or guidance levels established by EU legislation. In order to be authorised the additives need to be assessed by the European Food Safety Authority (EFSA).

EFSA developed a guidance document detailing the requirements for the assessment of the safety and efficacy of these products to help applicants in the preparation of their technical dossiers. As with any other feed additive, the product needs to be properly identified and characterised including purity. The target mycotoxin/s against which it acts should be specified. It should also be demonstrated that the use of the additive does not interfere with the analytical determination of mycotoxins in feed. The safety of additive for the target animals, the consumers of food derived from these animals, the users of the product and for the environment should be demonstrated. The general requirements for technological additives apply, e.g., tolerance studies for the target species, *in vitro* and *in vivo* toxicological studies with laboratory animals for the consumer and user. Simplified requirements apply for microorganisms that are considered by EFSA to qualify for the Qualified Presumption of Safety assessment. For those additives that modify the structure of the mycotoxin, the risks associated with the metabolites/degradation products(s) of the mycotoxin should be examined in appropriate studies (metabolic and residue studies, and toxicological studies). For additives that exert their action by binding, it should be studied whether they affect the availability of nutrients, micronutrients and other additives (e.g., coccidiostats).

Since these additives only have an effect in feed after they have been ingested by the animals, the efficacy can only be demonstrated by means of *in vivo* studies with target animals. *In vitro* studies can be used only to support the mode of action but cannot substitute for *in vivo* studies. A minimum of three *in vivo* studies are required and they can be designed as 'short-term' studies. These studies should demonstrate the effect of the additive against the target mycotoxin present in the diet. The experimental design should include, at least, two experimental groups, a control group in which animals are fed a diet contaminated with the target mycotoxin(s) and a treatment group receiving the same contaminated diet with the additive at the minimum recommended dose. One of the key factors in the design of these studies is that the concentration of mycotoxin(s) should be below the official or advisory limits. The end-points to be measured will depend on the target mycotoxin and target species, and should take into account their relevance (close correlation to exposure) and the availability of sensitive analytical methods. In general, the end-points should include mycotoxin/metabolites excretion in faeces/urine, concentration in blood/plasma/serum, tissues or products (milk or eggs) or other relevant biomarkers.

## Mycotoxins in human diets: unexpected concerns?

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Mycotoxin research was dominated in the past largely by the identification and characterisation of genotoxic and potential carcinogenic mycotoxins. At the same time, the refinement of a reliable exposure assessment resulted in the development of sensitive (multitoxin) analytical methods for the control of food commodities, and in the identification of biomarkers of exposure. These methods, together with total diet studies, indicated the widespread contamination of foods with mycotoxins, despite an increasing awareness of their potential adverse health effects [1,2]. Analysis of urine samples, which serve as biomarkers of exposure, seem to demonstrate even a higher frequency of exposure than that deduced from food surveys. For some mycotoxins, such as zearalenone and deoxynivalenol, the incidence of positive urine sample in Europe exceeded 90%, followed by ochratoxin A and fumonisin, whereas aflatoxin M1 was found only in <10% of all investigated urine samples [3]. Although the absolute exposure levels show regional differences, the urine analyses clearly confirm an unexpected high exposure rate, particularly of children, which might even exceed current health-based guidance values.

The unexpected high frequency of exposure of infants and children to various mycotoxins initiated a reconsideration of the toxic effects of mycotoxins and mycotoxin mixtures. Increasing concerns have been expressed that mycotoxins, such as aflatoxins, might be associated with stunting and malnutrition in infants [4]. While these effects seem to be associated with a frequent high intake of mycotoxins, such as aflatoxins, endocrine and immunological effects can be expected already at much lower toxin exposure levels. For example, zearalenone and its metabolites are identified as endocrine disruptors and exposure in the prepubertal phase might result in endocrine effects at later stages of life. Such a delayed onset of toxic effects has been demonstrated in animal studies together with a transgenerational toxicity [5], but recent epidemiological studies in humans are lacking. Deoxynivalenol has been identified as disruptor of the intestinal barrier, which again might not only lead to acute effects, such as the invasion of pathogens, but also to an increased permeability of the intestinal barrier for food allergens, hence contributing to allergic conditions and chronic inflammatory bowel diseases [6]. Finally, it should be reiterated that many mycotoxins exhibit antimicrobial effects and chronic exposure even to low dietary concentrations may modify the gut microbiome and in turn contribute to the development of metabolic and degenerative diseases. However, at the same time, studies unravelling the interaction between mycotoxins and bacterial populations may open a new avenue for mitigation strategies for the reduction of the prevalence of mycotoxins in agricultural products.

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## The impact of mycotoxins on nutritional behaviour

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Physiological regulations of energy balance and body weight imply highly adaptive mechanisms that match caloric intake to caloric expenditure. In the central nervous system (CNS), the regulation of

appetite relies on complex neurocircuitry which disturbance may alter energy balance and result in anorexia or obesity. The trichothecenes deoxynivalenol (DON) and T-2 toxin constitute widespread contaminants of agricultural commodities as well as commercial food. DON and T-2 toxin have been implicated in acute and chronic illnesses in both humans and farm animals involving weight loss. The mechanisms by which these mycotoxins profoundly modify feeding behaviour remain poorly understood, and more broadly their effects on the CNS have received limited attention. Through an extensive characterisation of sickness-like behaviour induced by DON or T-2 toxin, we showed that their *per os* administration affects not only feeding behaviour but also energy expenditure, glycaemia, body temperature and locomotor activity. Using c-Fos expression mapping, we identified the activated brain structures and the neuronal phenotypes recruited in response to these toxins. Interestingly, a part of the activated neuronal pathways resembled those stimulated by inflammatory signals. By real-time PCR, we report a DON-induced central inflammation, attested by the strong up-regulation of IL-1 $\beta$ , IL-6, TNF- $\alpha$ , COX-2 and mPGES-1 mRNA. Unexpectedly, while T-2 toxin induced a strong peripheral inflammation, the brain exhibited limited inflammatory response at a time point when anorexia was ongoing. These results reveal that, despite strong similarities, physiological changes observed after DON and T-2 toxin-intoxication differ. Finally, intracerebroventricular injections of either DON or T-2 toxin resulted in a rapid (<1 h) reduction in food intake. Thus, we hypothesised that during intoxication these toxins could signal to the brain through neuronal and/or humoral pathways.

The present work provides the first demonstration that trichothecenes modify feeding behaviour by specifically interfering with central neuronal networks devoted to central energy balance. In the future, the consequences of such possible central energy balance modulation should be evaluated, especially on vulnerable and predisposed individuals suffering from unwanted body weight loss or eating disorders.

## MYCOTOXINS EXPOSURE AND CHILD GROWTH: CO-EXPOSURE AND THE JOINT EFFECT ON CHILD GROWTH

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Aflatoxins, fumonisins and deoxynivalenol are among the mycotoxins of major public health concern. Exposure through maize-based weaning food may have a detrimental effect on child health in African children. This prospective study assessed dietary exposure to aflatoxin, fumonisin and deoxynivalenol in Tanzanian children and investigated adverse impact of these toxins on child growth. Children (n=166) aged 6 to 14 months were recruited at a maize harvest season from three villages of different regions (Iringa, Tabora and Kilimanjaro) in Tanzania. Each child was followed up at 6 and 12 months after, respectively. At each visit, blood and urine samples were collected for biomarkers of aflatoxin albumin adducts (AF-alb) and urinary fumonisin B1 (UFB1) and urinary deoxynivalenol (UDON). Diet and family socio-economic information were recorded; body weight and recumbent length were measured, and length for age z-score (LAZ), weight for age z-score (WAZ) and weight for length Z-scores (WLZ) were computed. At visit 1, 2 and 3, respectively, AF-alb was detected in 67, 84 and 99% of the children; UDON was detected in 51, 70 and 80% of the children; UFB1 was detected in 98, 96 and 100% of the children ( $p<0.001$ , for each biomarker). Consistently, AF-alb and UDON increased over the 12-months; and children in Tabora had the highest AF-alb but lowest UDON; UFB1 was the lowest at the 2nd visit. Children in Kilimanjaro consistently showed the lowest UFB1 in all three visits. The biomarkers concentrations are shown in Table 1. On average, 44, 55, and 56% of children were stunted (LAZ < -2); 8, 14 and 14% of the children were underweight at visit 1, 2 and 3, respectively. Iringa had the lowest stunting prevalence. UFB1 at visit 1, and by average of the three visits were negatively associated with LAZ ( $p<0.05$ ) at visit 3 and length velocity ( $p<0.05$ ) in the 12 month period ( $p<0.01$ ). There was no significant correlation between AF-alb and child growth, however the interaction between AF-alb and UFB1 was significant.

Table 1. Geometric means (95% CI) of AF-alb, UFB1 and UDON in Tanzanian children.

	Visit 1 (baseline)	Visit 2 (+ 6 months)	Visit 3 (+12 months)
AF-alb (pg.mg)*	4.7 (3.9-5.6)	12.9 (9.9, 16.7)	23.5 (19.9, 27.7)
UFB1 (pg/ml)*	313.9 (257.4, 382.9)	167.3 (135.4, 206.7)	569.5 (464.5, 698.2)
DON (ng/ml)*	1.1 (0.8-1.4)	2.3 (1.7-3.2)	5.7 (4.1-7.9)

\*For all three biomarkers, the differences between visits are significant,  $p<0.001$  in all cases.



In conclusion, young Tanzanian children are frequently exposed to aflatoxin, fumonisin and deoxynivalenol. The exposure plays an adverse role in child stunting either from exposure to one toxin or in combination. Interventions targeting weaning food should be prioritized for improving child health in Tanzania.

## **Don't forget the fish: organ damage and hepatic lipid accumulation in carp after feedborne exposure to DON**

**Constanze Pietsch**

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The occurrence of mycotoxins in fish nutrition increasingly gains attention. One of the most important contaminants in fish feeds is deoxynivalenol (DON). The investigations that will be presented show effects of DON on carp (*Cyprinus carpio* L.) using three different DON concentrations representative for commercial fish feeds including increased lipid peroxidation in liver, head kidney and spleen after four weeks of DON feeding of fish. Most of the effects of DON were reversible by two weeks of feeding the uncontaminated control diet. However, histopathological scoring revealed increased liver damage in DON-treated fish, which persisted even after the recovery phase. Biochemical analyses revealed a significantly higher fat content, and consequently an increased energy content in whole body homogenates of fish treated with the highest DON concentration. This suggests that DON affects nutrient metabolism in carp. Changes of lactate dehydrogenase activity in kidneys and muscle and high lactate levels in serum indicated an effect of DON on anaerobic metabolism. Thus, DON affects liver function and metabolism in carp. Effects on growth and mass of fish were not observed during these 6 weeks lasting experiments, and only marginal DON concentrations were found in muscle and plasma samples. Nevertheless, DON impaired innate immune responses whereby cytotoxic effects of DON partly contributed to this effect. Investigations of acute and chronic responses to DON revealed that the early responses of immune cells include increased expression of pro-inflammatory and anti-inflammatory genes whereas prolonged exposure to DON only led to the activation of anti-inflammatory genes. Consequently, these results raise concern about impaired immune function and animal welfare of fish raised in aquaculture.

## **Risk of mycotoxin contamination of edible mealworms**

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The world population is growing, leading to an increasing demand for animal protein. Several environmental problems are associated with conventional meat production. Insects could be an alternative, more sustainable source of animal protein. Mealworms (*Tenebrio molitor* L.) are being produced in the Netherlands for human consumption. These larvae could be produced more sustainably on diets made from side streams from the food and bio-ethanol industry. However, side streams can be contaminated with mycotoxins. Previous research showed that mycotoxin contamination of mealworm diet had little to no effect on mealworm growth and development. Hence, contamination of edible mealworms could go unnoticed, posing a possible threat to the consumer. Thus far, little is known about possible retention, excretion or detoxification of mycotoxins in edible insects. This study aims to help assess the possible risk of mycotoxin contamination in edible insects. Mealworms were grown on wheat flour naturally contaminated with mycotoxins (predominantly DON), wheat flour spiked with 8 mg/kg DON, and uncontaminated wheat flour. Mealworm survival, weight gain and food consumption on the three diets will be compared. Presence of mycotoxins will be analysed in mealworms and mealworm faeces using LC-MS/MS.

## Breeding healthy cereals: genetic improvement of *Fusarium* resistance and consequences for mycotoxins

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*Fusarium* diseases of cereals, particularly wheat, are a persistent threat to food and feed safety and security. Although several crop plant species may be attacked by *Fusarium* spp. and get thus contaminated with mycotoxins we focus this presentation on wheat, which is one of the world's major crop plants. Apart from yield losses, the contamination of the crop with *Fusarium* mycotoxins is of great concern. The deployment of *Fusarium* resistant cultivars plays a key role in the reduction of mycotoxin contaminations in feed and food.

The essential pre-condition for any plant breeding activity is genetic variation for the character of interest. Fortunately, in the gene pool of bread wheat genetic variation for this resistance to *Fusarium* head blight is large. Numerous breeding lines and/or cultivars with moderate to high resistance have been found. Breeders can choose resistant lines for their crossing and selection programmes. The difficult task for the breeder is to identify novel cultivars with superior trait combinations in their recombined breeding populations, which combine high productivity, environmental plasticity, high nutritional and technological quality and resistance to relevant biotic and abiotic stresses including resistance to *Fusarium*. Selection for the important trait *Fusarium* resistance has traditionally been based on extensive field evaluations under epidemic conditions. In the era of genomics, novel approaches, such as marker-assisted breeding and genomic selection, allow more targeted selection and should lead to increased progress per unit time. All in all plant breeding has made significant progress in supplying farmers with FHB resistant cultivars and will continue to do so. Choosing resistant cultivars is possibly the best option for mycotoxin reduction right at the beginning of the cereal production chain. Resources that are invested in *Fusarium* resistance breeding are an excellent, but long term investment.

## Unravelling the mystery of *Aspergillus flavus*: beyond aflatoxin

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The saprophytic fungus, *Aspergillus flavus*, has received significant attention worldwide because it produces the extremely toxic and carcinogenic secondary metabolites aflatoxins when this fungus invades crops, such as maize, cotton, tree nuts and peanuts prior to harvest. Adverse health effects from ingestion of these toxins have caused regulatory agencies throughout the world to limit the amount of aflatoxins in commercial food or feed products. This causes an undue economic burden on the grower as highly contaminated commodities are destroyed or significantly reduced in value. The genetics of the biosynthesis of this important toxin has been studied and elucidated in great detail. In addition, the genome of *A. flavus* has been sequenced, with the genome size being about 36.3 Mb on eight chromosomes, capable of encoding about 12,000 proteins. Initially the specific goals of the genomics project were to understand the regulation of expression of genes involved in aflatoxin production in response to environmental signals. However, more recently, developing a greater understanding of secondary metabolism and differentiation in this fungus has received greater attention. For *A. flavus*, over 55 putative secondary metabolite gene clusters have been identified, that have either a gene that encodes a backbone polyketide synthase (PKS), nonribosomal peptide synthase (NRPS), hybrid PKS-NRPS, or prenyltransferase (PTR) to catalyse the initial step in metabolite biosynthesis. On the cluster, these backbone genes are surrounded by genes that are predicted to encode 'decorating' enzymes (e.g., oxidoreductases or methyltransferases) as well as transport and regulatory proteins. The strategy for identifying the metabolite(s) produced by the gene clusters is the disruption of specific genes on the cluster followed by liquid chromatography and high resolution mass spectrometry to determine if production of a specific metabolite is prevented. In the case of cryptic clusters, the strategy is to over-express a transcription factor associated with the cluster followed by screening for appearance of a novel metabolite. So far, the aflatoxins (cluster 54),

cyclopiazonic acid (cluster 55), aflatrem (clusters 15, 32), penicillin (cluster 25) and asparosone (cluster 27) have been correlated with particular gene clusters. In addition, clusters (35 and 48) were shown to possess genes for production of two related piperazines which are important for sclerotial development. We used RNA-seq to compare expression of secondary metabolite cluster genes for the closely related fungi *A. parasiticus*, *A. oryzae*, *A. nomius* and *A. flavus* S and L sclerotial morphotypes to determine which genes in the putative cluster are expressed under the conditions of growth investigated. Our results revealed considerable differences in expression of genes by these fungi. Based on these analyses we suggest that the S-strain *A. flavus*, a prevalent contaminant of maize in central Africa and other regions, is more capable of producing toxic metabolites than the L strain. The diversity of secondary metabolites produced by *A. flavus* indicates that their production plays a considerable role in its ecology and therefore has implications in both human health and food safety.

## How to reduce mycotoxin risks through food processing? An ILSI Europe perspective

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Mycotoxins are toxic secondary metabolites produced by organisms of the fungi kingdom that significantly affect the quality, safety and yield of important crops for food and feed use worldwide. Recent evidences suggest that some food production processes lead to the reduction of levels of parent mycotoxins in finished products compared to the corresponding raw materials\ingredients. The International Life Sciences Institute (ILSI) Europe Process-related Compounds and Natural Toxins Task Force completed in 2013 a project dedicated to give a comprehensive overview of the current knowledge on agricultural practices connection with plant metabolites of mycotoxins, also called masked mycotoxins [1].

To understand the possibility in mitigating mycotoxins occurrence, improving correspondently the safety of the feed/food commodities, the same ILSI Europe Task Force started recently (spring 2014) a new project devoted to review the state of the art about mycotoxins reduction by decontamination/detoxification treatments or by processing. It starts from the premise that Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) have been already optimised. In particular, the most promising detoxification and food processing strategies that can be modulated to mitigate the toxicological impact, will be evaluated in detail, providing parallel considerations about technological and organoleptic acceptability and potential impact on nutritional quality of the finished products. Furthermore, these potential approaches for minimising consumer exposure will be examined, keeping the contemporaneous aim of not inducing other unfavourable secondary effects in food (either possible transformation of mycotoxins into other compounds with relevant safety implications or adverse changes in nutrient profiles) through appropriate management of the industrial technologies. Consequences for the risk assessment of mycotoxins in food that is relevant for the finished product as consumed will be considered. Finally, the different problems that could arise depending on the geographical regions will be taken into account, following therefore an international approach, leaving open the door to not only EU legislation and targeting worldwide solutions scientifically available.

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## Mycotoxin detoxification by enzymatic biotransformation – present state and future perspectives

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Mycotoxins are biodegradable molecules. Microbes break mycotoxins down for the purpose of

detoxification or metabolic utilisation. Several microbial genes and enzymes for mycotoxin biodegradation have been identified. Enzymes can be produced at large industrial scale, and enzymatic biotransformation for mycotoxin detoxification has potential to complement other methods of reducing mycotoxin exposure in a systems approach.

We identified and studied a gene cluster of *Sphingopyxis macrogoltabida* MTA144, which enables this Alphaproteobacterium to utilise fumonisins, a class of carcinogenic mycotoxins produced by the maize pathogen *Fusarium verticillioides*. The enzyme that catalyses the first step of the catabolic pathway encoded in this gene cluster is fumonisin esterase FumD. It releases two tricarballylic acid side chains from the core chain of fumonisins, and this biotransformation reduces affinity to the molecular target, ceramide synthase. We developed biomarkers of exposure and biomarkers of effect to study activity of the enzyme on fumonisin-contaminated feed in the gastrointestinal tract of piglets, and we used these biomarkers to optimise enzyme dose and formulation. We also developed a recombinant host strain for enzyme production, a fermentation and formulation process, and quality control procedures. The technology of fumonisin detoxification for pig nutrition, based on fumonisin esterase FumD, is now available in some countries, and it is filed with regulatory authorities in others.

We have attempted to apply the lessons learned from developing fumonisin esterase as feed enzyme to other enzymes for mycotoxin biotransformation and detoxification. Another enzyme from the fumonisin catabolism gene cluster, aminotransferase FumI, proved a difficult target because of requirement for a cofactor and a cosubstrate. In cases with other mycotoxins, detoxification required more than one biotransformation reaction and therefore more than one enzyme, or enzyme activity in the gastrointestinal tract of an animal was poor. Taken together, development of a technological application for mycotoxin detoxification based on an enzyme is a comprehensive task with many pitfalls, and may not be possible for some of the mycotoxin biotransforming enzymes known today. However, our success with fumonisin esterase FumD shows that enzymatic detoxification is possible, and enzymes should certainly be considered in a systems approach to reduce mycotoxin exposure.

## **Mycotoxins in silage: checkpoints for effective management and control**

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Silage making increased considerably from the 1960s. At present, this practice is considered one of the most appropriate to preserve forage over extended time periods and to maintain nutritional value comparable to fresh pastures. Silage is widely used in farms and has a substantial role in animal production systems. Forage silage, as a source of mycotoxigenic fungi and mycotoxins, merits attention. The contribution of silages to total mycotoxin intake could be significant and sometimes greater than that of compound feed in ruminant diet, as forages are the main dry matter component. For dairy ruminants, the problem does not end in animal disease or production losses, as the carry-over to milk and dairy products of mycotoxins or their metabolic products may eventually affect human health. Based on the increasing amounts of research, it is becoming clear that mycotoxins represent an unavoidable risk. When it comes to managing the challenge of moulds and mycotoxins in silages, there are many factors with pre- and postharvest origins to take into account. Pre-harvest is dictated by environment factors, whereas post-harvest (silage making practices, differences in physical properties and environmental conditions within a silo, feed out phase) can be largely controlled by the farmer. Therefore, an effective mycotoxin management and control program should be personalised to each farm at an integrative level all along the silage production chain: crop growth in the field, silage making practices, and feed out phase.

The presentation will summarise the current knowledge regarding mycotoxin occurrence in silage as well as factors affecting their concentrations and distribution at harvest and during ensiling. With a specific focus on maize silage and grass silage, specific information and suggestions for “in field” decision making to precisely manage the mycotoxin burden and evaluate the acceptability for its use as animal feed will be given. The impact of sampling and analysis will also be discussed.

## Mycotoxin prevention and control measures in China

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Mycotoxins contamination in agroproducts is of great concern during the last few decades in China. Mycotoxins are secondary metabolites produced by fungi when they infect the agroproducts. Most common mycotoxin producing fungi seen in China are species of *Aspergillus*, *Fusarium* and *Penicillium* and the toxins produced by them are aflatoxins, deoxynivalenol, fumonisins, ochratoxins, patulin, and zearalenone. The government of China has set several standard limits regarding mycotoxins contamination in agroproducts to prevent them from entering into the food chain. In recent years, several prevention and control measures has been taken in the storage process to ensure food security, food safety and safeguard the national economic interests. At the same time, the government has initiated a special survey across the nation to study the contamination level of mycotoxins in cash crops and food products. Specific interest in the subjects, such as mechanism of mycotoxin-producing fungal floral growth during storage, mycotoxins formation and its effect on storage products, developing early monitoring technologies for mycotoxin contamination in stored grains, and molecular basis of inhibition and reduction of mycotoxins, were explored in recent years across different laboratories in China within the government initiative. New advanced technologies have been developed by various laboratories for the simultaneous rapid detection of mycotoxins in agroproducts. Various detoxification methods were developed to counteract mycotoxins contamination and prevent them from entering into the food chain.

## Breeding, marker-assisted selection, and RNAi technologies for developing resistance in maize to aflatoxin contamination

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Currently, the most widely-used technology for combating pre-harvest aflatoxin contamination of maize is the application of atoxigenic *A. flavus* strains in maize plots to displace toxigenic strains. While this strategy facilitates a high level of displacement, it alone does not render the crop toxin-free, nor lower aflatoxin accumulation to levels safe for trade or consumption. Maize, however, is a genetically diverse crop and, thus, a sound basis exists for the identification and development of host resistance and the discovery of genes conveying resistance. Breeding programs across the U.S. have produced a number of maize inbreds that consistently accumulate low amounts of aflatoxins, but which exist in less-than-desirable genetic backgrounds. Another breeding effort, a collaboration between the International Institute of Tropical Agriculture (IITA; Ibadan, Nigeria) and the Southern Regional Research Center (SRRC; New Orleans, USA) developed six resistant inbreds that combine resistance genes from temperate backgrounds with those from tropical ones, possibly creating new combinations of genes in the resulting germplasm. Investigations employing comparative proteomics to discover gene markers in resistant lines used genetically diverse collections of aflatoxin-resistant and –susceptible maize lines to identify the presence of elevated levels of resistance-associated proteins (RAPs). The identified proteins included those from storage, stress-related, antifungal or putative-regulatory categories. The identification of these RAPs was confirmed and further discovery was enhanced when closely-related breeding lines developed through the IITA-SRRC breeding collaboration and varying in aflatoxin accumulation, were substituted for the diverse collection of maize lines. RAPs have also been further characterised to clarify their potential roles in resistance; investigations involved enzyme assays, antifungal assays, gene expression studies, and gene knockouts using RNA interference (RNAi) technology. Furthermore, several RAP genes have been placed into virtual bins and the chromosomal location pinpointed to which each gene maps, by using the DNA sequence of RAPs and blasting them against the B73 sequence. Information therein obtained was then compared to results of QTL mapping studies which had implicated certain chromosomal regions in resistance. The above-studies provide a better understanding of aflatoxin-resistance and should highlight genes for use as markers in marker-assisted breeding strategies.

Lastly, a study was conducted which lowered maize seed accumulation of aflatoxins in transgenic plants by suppressing a key *A. flavus* colonisation gene through host induced gene silencing (HIGS). Employment of these strategies could lead to development of resistant commercial maize hybrids which when combined with biocontrol strategies and sound cultural practices could lower aflatoxin in maize to levels safe for commerce and consumption.

## ***Fusarium* species dynamics – challenges in the development of a strategy to reduce mycotoxins in oats**

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*Fusarium* infection and associated mycotoxin contamination of oat grains causes challenges for growers, livestock producers and the food and feed industries in Norway. Besides weather factors, such as rainfall and temperature in the critical periods around flowering and pre-harvest, inoculum production and disease development is also influenced by agricultural practices. From 2011, all oat grain lots in Norway have been analysed for deoxynivalenol (DON) at delivery. However, the occurrence of *F. graminearum* and DON of oats grain lots do not generally correlate with the occurrence of *F. langsethiae* and HT2+T2-toxins. Therefore, in order to develop a robust disease management strategy, there is a need to reveal the influence of weather and agricultural practice on disease development in oats for both these fungal species.

Bioforsk and the Norwegian Agricultural Extension Service study the effects of weather, tillage practice, choice of cultivar, and chemical control treatments on development of *Fusarium* and mycotoxins in oats. Our results indicate inconsistency in the influence of cultivar, fungicide treatment, and weather on the development of *F. langsethiae* versus *F. graminearum* and respective mycotoxins in oats.

## ***Fusarium* mycotoxins in naturally contaminated wheat mill fractions**

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Mycotoxins are among the most abundant contaminants in food and feed worldwide. Therefore, in the European Union, maximum levels are established, e.g., for the frequently occurring *Fusarium* toxins deoxynivalenol (DON) and zearalenone (ZEN). Additional to the free forms of these toxins, several so called 'masked mycotoxins' are present in naturally contaminated grain products. Those derivatives of the parent toxins are altered in their chemical structure and therefore, are not detected in standard analytical methods [1]. As masked forms may significantly contribute to the overall exposure of human and animal with mycotoxins, they are increasingly addressed in analytical and technological research [2]. Thus – as for the free toxins - minimising strategies applying (food) technological techniques are moving into the scientific focus. For the development of such strategies, the toxin profile within the kernel is important as by means of fractionated milling the highly contaminated fractions can be excluded from further processing.

Up to now, data on the spatial distribution of many (masked) mycotoxins in the kernels of naturally contaminated grains are missing. The aim of the present study was to investigate the amounts of DON and ZEN as well as their most abundant derivatives DON-3-glucoside (D3G), 3- and 15-acetyl-DON und ZEN-14-sulfate (Z14S) in mill fractions of naturally contaminated wheat lots. The investigated distribution pattern in the ten investigated passages is comparable among the three different wheat lots. Interestingly, DON and D3G were detected to similar amounts in all fractions. This result shows that in both endosperm and bran high amounts of these toxins have to be expected. By contrast, for ZEN and Z14S, a significantly higher amount of toxin is located in the fibre-rich fractions. The relative mass proportion of D3G comprises for only between 2.9 and 11.2% of the free DON, while the relative mass proportion of Z14S accounts for 13.9 up to 343.3 % of free ZEN of the respective fractions. Acetylated DON derivatives contribute to at most 3% to the total DON amount in any fraction.

The experiments conducted show that a significant reduction of the ZEN and Z14S level in wheat flour can be reached by applying milling technology strategies. However, the almost even distribution of DON and D3G in all fractions does not allow for the technological removal of relevant toxin amounts. Furthermore, it is noteworthy that the overall impact of masked mycotoxin derivatives in the investigated wheat lots is higher for ZEN than for DON.

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## Code of practice to minimise sclerotia and ergot alkaloids along the food chain

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Ergot alkaloids (EAs) are a group of secondary metabolites originated from *Claviceps purpurea*, which may infect rye, barley or other small grain and grass. In case of infection the fungi forms a purple to black sclerotium instead of a healthy caryopsis in the ear. The European Safety Authority (EFSA) estimated a low risk of EAs in the food chain without any indicated health concern for the European population [1]. However, the evaluation of occurrence data and consumption pattern in Germany may lead to exceed the group tolerable daily intake (TDI) and the group acute reference dose (ARfD) for high average consumers [2].

To reduce the contamination with sclerotia and EAs the German Federal Ministry of Food and Agriculture had established a task force to develop instructions for reducing EAs in the food chain of rye bread and other cereal based food. The recommendations will be presented; several are as follows. The minimisation of sclerotia and EAs in agricultural production is based upon avoiding the grain kernels to be infected by spores or conidia, e.g., by the optimisation of crop rotation, use of less-susceptible varieties or turning tillage. If sclerotia have reached the crop, cleaning is required as early as possible after harvest to prevent adhering dust of the soft-structured sclerotia on the grain surface. Despite different cleaning technologies, millers do have more options to reduce the EA content in the final product, e.g., removing of higher contaminated flours from the first stage of break (B1) or strictly avoiding the use of filter flours. While most of the bakers are dependent on purchasing safe and healthy flours for their products, some of them have to be aware about the occurrence of sclerotia in whole grain before milling at their bakehouses. Moreover, during baking, the EAs are relatively heat-tolerant even though the epimeric forms may differ in content, e.g., ergotamine vs. ergotaminine [3,4].

In summary, an efficient reduction of ergot alkaloids in ready to eat products can be achieved at nearly all stages of food production from farm to fork or rather baker. Although maximum limits are presently not established, stakeholders should assume responsibility for safe and healthy products by considering these recommendations.

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## From 'sample' to 'analysis' – critical points & practical solutions

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Mycotoxin analysis can be very challenging. In fact, looking at the sources of error, the actual analytical method contributes only a minor part. It is well known that 'sampling' is a crucial step in mycotoxin analysis and may be the largest contributor to sources of error. The performance characteristics of the analytical method are typically also known. However what happens in between – from 'sample' to 'analysis' – may have major impacts on the quality of the results. The intensity of milling is important, the sample size to be extracted plays an important role. Of course the extraction solvent has an influence on the recovery, but one should also bear in mind if the extraction method matches the actual analytic method. In this presentation some of those critical points in sample preparation are discussed and solutions are presented.

## New LC-MS/MS multimycotoxin testing concept – implementation of the new method <sup>13</sup>C-MycoSpin in routine testing labs to meet ISO 17025 standards

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The need for multimycotoxin analyses is constantly rising. LC-MS/MS is the technology of choice and laboratories are using these methods in routine testing, where it is important to meet ISO 17025 standards. With LC-MS/MS, interferences from matrix components leading to differences in analyte ionisation are a problem. Fully <sup>13</sup>C-labelled internal standards can be applied and will correct such interferences to ensure qualified analysis results. <sup>13</sup>C-labelled internal standards have several advantages over alternatives like deuterated (<sup>2</sup>H) internal standards, as the total mass of the atom is only slightly changed by replacing <sup>12</sup>C by <sup>13</sup>C. For deuterium the mass doubles, thus, <sup>2</sup>H-labelled mycotoxins might show retention time shifts, resulting in less accurate LC-MS/MS results.

To meet ISO 17025 standards and to fulfil EU legislation, highly sensitive mycotoxin detection methods are demanded. For the detection of multiple mycotoxins at very low detection limits a sample clean-up step should be implemented in the LC-MS/MS method. Romer Labs has developed a novel rapid multi-mycotoxin clean-up to reduce the matrix effect and thereafter increase the LC-MS/MS method sensitivity. The use of <sup>13</sup>C-isotope-labelled internal standards in conjunction with the MycoSpin™ 400 multitoxin clean-up allows for a method which is applicable to analyse a wide variety of matrices, with a straightforward sample preparation.

This talk presents a new LC-MS/MS multimycotoxin testing concept set up for the application in routine testing labs to meet ISO 17025 standards. Mycotoxins, including aflatoxins (B1, B2, G1 and G2), fumonisins (B1, B2 and B3), diacetoxyscirpenol, T-2 toxin, HT-2 toxin, nivalenol, deoxynivalenol, 3- and 15-acetyldeoxynivalenol, ochratoxin A, fusarenon-X, and zearalenone in various food and feed samples can be analysed. The method combining MycoSpin™ clean-up for a better LC-MS/MS sensitivity on complex sample matrices and <sup>13</sup>C-labelled internal standards will be presented. Furthermore it will be illustrated how important the application of internal standards is in a multimycotoxin methods to meet the standards given by ISO 17025.

## Toxicity of type B trichothecenes: beyond deoxynivalenol

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Trichothecenes are mycotoxins commonly found in cereals worldwide. The most important trichothecene producers is the *Fusarium* genus, i.e., *Fusarium culmorum* and *F. graminearum*, which is abundant in various cereal crops (wheat, maize, barley, oats, and rye) and processed grains (malt, beer and bread). Deoxynivalenol (DON) is the most current trichothecene, but in contaminated cereals 3- and 15-acetyl DON can occur in significant amounts (10-20%) concomitantly with DON. Nivalenol is



also a common type B trichothecene mycotoxin produced by *Fusarium* genus. Trichothecenes exert multiple toxic effects that are variable between toxins and species. In a review, Petska (2007) concluded that all animal species are susceptible to DON according to the rank order pigs>mice>rats>poultry≈ruminants. Nevertheless, there is a lack of data on other type B trichothecenes' (3- and 15-acetyl DON and NIV) toxicity on farm animals. Mice are the most studied animals; different studies measured the mycotoxins LD50 and permitted to conclude that 15-acetylDON is 2.3-fold more toxic than DON (Forsell *et al.*, 1987) and that NIV is twice more toxic than DON (Ryu *et al.*, 1988). No data are available on 3-acetylDON in mice. The few available data show a lower toxicity of 3-acetylDON compared to DON. In fact in 2010, Danick *et al.* measured an LD50 for 3-acetylDON in ruminants of 2.6 Mm compared to 0.5 Mm for DON. In a pig model, Pinton *et al.* (2012) measured the toxicity of type B trichothecenes on pig intestinal epithelium and classified the toxicity as follows: 3-acetylDON<DON<15-acetylDON. According to these data, we can set up some equivalence of toxicity between DON and other mycotoxins to use one common criterion, toxicity in DON equivalence. In this system, we consider that 0.5 3-acetylDON = 2.3 15-acetylDON = 2 NIV = DON toxicity. We use this criterion in a database of 325 samples of raw materials collected worldwide from 2008 to 2013. DON, 3- and 15-acetylDON, and NIV levels were measured by LC-MS/MS (Accréditation COFRAC 1-0632). We could not measure any strong correlation between DON and 3-acetylDON individually ( $r^2=0.12$ ), DON and 15-acetylDON individually ( $r^2=0.39$ ) and NIV individually ( $r^2=0.09$ ). No correlation ( $r^2=0.27$ ) between DON level and the sum of type B trichothecenes converted into DON toxicity was measured. DON is the type B trichothecene mycotoxin that is most often analysed, whereas the other type B trichothecenes are rarely analysed. The others type B trichothecenes could not be estimated from the DON level, while they remain present in significant amounts and are very toxic. Thus, it is important to measure the presence of all type B trichothecenes in raw materials.

## Tools for monitoring aflatoxin M1 in the dairy production chain

### Stephen Powers

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Aflatoxin M1 (AFM1) is a metabolite of found in milk of dairy cattle which have consumed feed contaminated with aflatoxin B1. AFM1 has been reported to be carcinogenic and hepatotoxic. In the USA and a number of other countries, the permissible level of AFM1 in milk is 0.5 ppb. However, the European Commission has established the stricter limits of 50 parts per trillion (ppt) in milk for adult and 25 ppt for infants. Recent recalls of milk contaminated with aflatoxin M1 due to feed contamination suggest that there is value in testing along the entire production chain from milk tanker to finished product.

VICAM has developed a quantitative rapid lateral flow test for comingled raw milk suitable for screening milk tankers. The test is simple and easy to operate, requiring about 15 min from sample preparation to result read out. Briefly, the raw milk sample is filtered and 250  $\mu$ l of filtrate is transferred into a testing vial containing assay reagent and mixed. An AflaM1-V strip is then placed into the vial, and incubated at 48°C for 12 min. The result is read using Vicam's Vertu reader. In milk spiked with AFM1 in the range of 0 to 1000 ppt, results show that the AflaM1-V test has a high degree of linearity ( $r^2=0.994$ ) with a limit of detection about 25 ppt, and a testing range of 0 to 750 ppt.

For analysis of finished products or where certification for export is desired, VICAM has developed methods utilising sample preparation by immunoaffinity column. Samples are quantitated in a single well fluorometer or using LC with fluorometric detection. Limits of detection for these methods are as low as 12.5 ppt with recoveries close to 90% and % CV of <10%.

## Environmentally-friendly mycotoxin testing using water extraction technology

### Mark Tess and W. Kokke

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Mycotoxins are secondary metabolites produced by moulds that are toxic to animals and humans. Traditionally, mycotoxin analyses focused on chromatographic techniques such as HPLC or TLC. Recently, lateral flow devices have gained acceptance as an alternate method of analysis to test mycotoxins without the needs of a traditional laboratory setting. These lateral flow tests are easier to

use, provide results in less time, and are more cost effective than traditional methods. A further advance of rapid testing is a shift away from organic solvent extractions to water-based extractions. Water based extractions improves worker safety, reduces chemical waste, and provides an environmentally-friendly method for the analysis of mycotoxins.

Charm introduced Biodegradable Extraction Solution Technology in 2009 that used biodegradable surfactants dissolved in water for the extraction of mycotoxins; the water-based extraction was coupled with a lateral flow test strip for the qualitative detection of aflatoxin. In 2012, Charm introduced Water Extraction Technology (WET®) and developed quantitative lateral flow tests for aflatoxin, deoxynivalenol, fumonisin, T-2 and HT-2 toxins, and zearalenone. In WET, a pre-weighed, non-hazardous extraction powder is added to the ground sample followed by water for mycotoxin extraction. The extract is diluted with buffer for subsequent addition to the lateral flow test. After 5-min incubation, the test strip is interpreted by the ROSA-M Reader or Charm EZ-M to provide quantitative results. Charm has received USDA-GIPSA (Grain Inspection, Packers and Stockyards Administration) approval for quantitative test kits for aflatoxin and fumonisin. The ROSA WET-S Aflatoxin Quantitative Test is approved for maize and thirteen additional commodities; the ROSA WET Fumonisin Quantitative Test is approved for maize and seven additional commodities.

## Recent advances in mycotoxin diagnostics

### Mary Gadola

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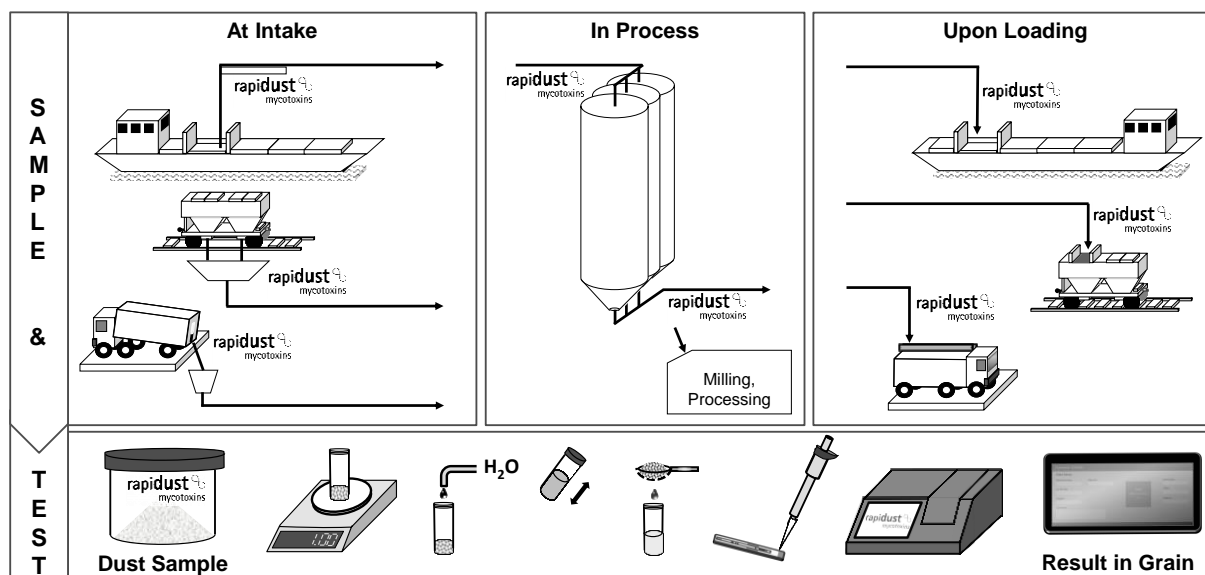
In its 32 year history, Neogen has been a firsthand witness and contributor to the ever evolving field of rapid diagnostics for the detection of mycotoxin residues. In that time, we have seen technology evolve to provide faster, more inexpensive, accurate tools for this purpose while at the same time making them easier and easier to use granting a full skill spectrum of individuals access from the highly trained to the novice. Never before have individuals had so many technology choices for mycotoxin control. With this proliferation of technology, the user has become more sophisticated in their mycotoxin management needs. No longer are test kits alone sufficient for comprehensive mycotoxin management. Many users require conformational services, pure standards, naturally incurred reference materials, proficiency programs, data management with documentation, and predictive modelling. This comprehensive package has become an expectation in today's complex regulatory environment. Neogen would like to share our comprehensive approach to mycotoxin management in addition to recent advances in test kit technology.

## Improving on-site mycotoxin measurements by integrated procedures based on dust sampling

### Mareike Reichel and S. Biselli

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With rapidust® Eurofins is offering an all-round carefree package to operate novel dust sampling and on-site screening technology for mycotoxins in grain lots. The proprietary procedure allows for taking representative samples even out of large batches, simple analysis and reliable results within minutes. Mycotoxins accumulate on small particles in food or feed bulk. Consequently, dusts are often highly contaminated with mycotoxins. Our experts for mycotoxin analyses identified a dust fraction, in which the mycotoxin concentration correlates with the respective mycotoxin content in the grain lot. Hence, the contamination in grains is computable based on concentrations determined in respective dust samples. Dust samplers can easily be installed at grain intake sections, in process or at loading points. Samples are taken by suction and can directly be extracted. No grinding or homogenisation step is needed. A customised lateral flow assay enables simple but sensitive on-site analysis. Results of rapid tests are instantly converted and displayed as contamination of the grain lot.



As integrated approach, rapidust® is the first system for on-site mycotoxin measurements in grains that takes into account both sampling and analysis. Representative results are available within few minutes what facilitates, e.g., fast control of complete trucks at grain intake, gaining comprehensive knowledge on contaminations in stocks or screening of grain quality upon loading of ships. In this way, rapidust® makes mycotoxin control of whole lots a whole lot simpler.

## Styrene and methacrylic acid based ergotamine imprinted polymer: effect of pH, temperature, contact time and initial concentration on isothermal sorption properties towards ergot alkaloids

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Evaluating the distribution of alkaloid in feed commodities is an important analytical challenge in terms of separation and purification to further understand their toxicological impact on livestock health and animal performances. Sorbent technologies may offer effective means to isolate alkaloids and further manage their toxicities. Molecularly imprinted polymers (MIP) can be synthesised with varying degrees of specificity to enable alkaloids interaction. This study is proposing to evaluate different formulations of MIP for their sorption properties to ergotamine. Two different noncovalent copolymers were synthesised from monomers (styrene or methacrylic acid) with a free radical initiator (2,2'-azobis isobutyronitrile) and crosslinker (ethylene glycol dimethacrylate) in toluene as porogen and with ergotamine as template. Corresponding non-imprinted polymers (NIP) were synthesised in the absence of template. An isothermal adsorption experiment was conducted with four synthesised polymers at inclusion rate of 0.01% w/v, using 10 concentrations (range 0.001 to 10 µg/ml) of ergotamine in McDougal's buffer (pH 6.7, 37°C, 90 min). Samples were centrifuged (10,000 g for 10 min) and supernatant was analysed by UPLC-MS/MS for alkaloid concentration to determine adsorption. All the polymers showed fast kinetics and within each monomer type, MIP showed faster kinetics than the corresponding NIP. The pH of the medium had less influence on the binding properties of polymers towards ergotamine at the pH range of 2-10. Additionally, binding was not influenced by variation in temperature ranking from 36 to 42°C. Adsorption equilibrium was assessed by comparing two isothermal equations – Langmuir and Freundlich models. Styrene-based polymer ( $r^2 > 0.93$ ) was better fitted by both model compared to methacrylic acid-based polymer ( $r^2 = 0.62-0.68$ ). For styrene-based polymer, Freundlich model indicated strong adsorption level (n value ~ 3 for both MIP and NIP) and estimated maximum adsorption quantity ( $Q_{max}$ ) of 8.68 and 7.55 µM/mg of MIP and NIP, respectively. Freundlich model indicated stronger adsorption for MIP (n~6.7) than NIP (n~0.4) for methacrylic acid based polymer, with  $r^2$  of ~ 0.62-0.68, and  $Q_{max}$  estimated through Langmuir were 1.38 and 623.73 µM/mg for MIP and NIP, respectively. The sorption process followed a pseudo second order kinetics indicating chemisorptions occurrence.

## **Solid phase extraction clean-up based on molecularly imprinted polymers for single mycotoxin and multimycotoxins analysis**

D. Derrien, O. Lépine, M. Arotçaréna, K. Naraghi and **Sami Bayouhd**  
PolyIntell, France; [contact@polyintell.com](mailto:contact@polyintell.com)

Molecularly imprinted polymer (MIP) is a synthetic material with artificially generated three-dimensional network, able to specifically rebind a target molecule. Based on this technology, we have developed a powerful technique of selective solid-phase extraction and clean-up before analysis for various mycotoxins. Clean-up with MIP products provide clear and unambiguous results at a broad range of concentration levels with very low ion matrix effects. This fast and simple method demonstrates high recovery yields with a low background. Furthermore, these cost-effective products allow wide use for clean-up and enrichment applications before HPLC and LC-MS/MS analysis. This presentation shows results obtained for MIP solid phase extraction (SPE) clean-up for the single analysis of patulin, deoxynivalenol and its derivatives as well as multimycotoxins from food and feed.

## **The ToxiMet system – a revolutionary approach to the control of mycotoxins**

**Ray Coker**

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The ToxiMet system is a revolutionary technology for the highly accurate measurement of mycotoxins in food. It is composed of three main elements: the ToxiSep cartridge that cleans up the sample extracted from the raw commodity; the ToxiTrace cartridge, on which the cleaned-up sample is immobilised; and, the ToxiQuant instrument which rapidly, accurately and simultaneously measures the concentrations of individual toxins which have been immobilised on the ToxiTrace cartridge. The ToxiQuant has been specifically designed to be operated by non-scientists, in warehouses and processing plants throughout the value chain, at ambient temperatures as high as 50°C. The results are reported on an interactive touch screen in less than 4 minutes, and can be readily downloaded onto a laptop.

The ToxiQuant instrument is composed of highly sophisticated, patent protected spectroscopic analysis hardware and software, together with chemometric algorithms which *simultaneously* identify and quantify the individual toxins immobilised on the ToxiTrace cartridge, without physically separating the toxins. This affordable, unique combination delivers outstanding accuracy, at sub-parts per billion levels. Current applications of the system include: the simultaneous and accurate quantification of aflatoxins B1, B2, G1 and G2 in edible nuts, sesame seed, rice, maize, spices, cassava, figs and mixed feeds; the quantification of ochratoxin A in dried vine fruit and coffee; and the simultaneous quantification of the aflatoxins and ochratoxin A in maize, rice and figs. Further applications will follow shortly, including the analysis of *Fusarium* toxins (including zearalenone, deoxynivalenol and fumonisins) in wheat and maize.

The ToxiQuant instrument produces quantitative data that are in excellent agreement with high performance liquid chromatography (HPLC), with an approximately 70% saving in cost. The ToxiQuant's limit of detection (LOD) and limit of quantitation (LOQ) levels have been calculated using a statistical weighted linear regression method. The LOD and LOQ for the quantification of individual aflatoxins, in various commodities, are well below the EU regulatory limits for these commodities (e.g., aflatoxin B1, LOD=0.20 ppb; LOQ=0.67 ppb).

The ToxiMet system is currently in the process of gaining AOAC and Industry Standard (China) certification; and the application of the ToxiMet system to the accurate determination of the aflatoxins, has now been accredited by the UK's Accreditation Service (UKAS).

## **Addition of multiple matrices to EnviroLogix' DON3 and aflatoxin FREE assays**

B. Gow, S. Polakowski, C. Baily, T. Goddard and **Breck O. Parker**  
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Lateral flow devices (LFD) are often the analytical tool of choice when rapid and reliable quantitation of mycotoxins in food or feed products is required. Different matrices vary in extractant absorption,

extraction efficiency, and flow properties, which significantly impact performance, limiting the utility of a single test kit to be applied to a range of food and feed commodities. The expansion of commodity profiles for EnviroLogix' QuickTox kits for DON3 and Aflatoxin FREE, to allow multiple matrices to be tested with a single test kit, has been accomplished using various methods. To overcome matrix challenges with the DON3 assay, which has a water-based extraction and relatively wide analytical range, extraction ratios were adjusted between 4 to 6x (volume to weight) to accommodate different matrices. This approach allows numerous commodities to be quantitated accurately using curves encoded into the barcode on each device. The Aflatoxin FREE assay required a different approach, since sensitivity limited the extraction ratios that could be employed, and the difficulty of solubilising aflatoxins resulted in more variability between matrices. To normalise performance as it relates to extraction efficiency, component solubility (pigments) and extractant absorption, different extractants, extraction ratios and co- factors are utilised for different commodities. In addition, the Quicksan reader allows the user to easily scan in and select from a set of lot-specific quantitation curves for each assay kit. This combination of optimised extraction conditions with improved software utility has enabled the addition of numerous commodities to these assays. The ability to expand matrix offerings for existing assays allows the users to continue running a single, familiar test kit while maintaining industry leading assay run times for core commodities.

## **Addressing the routine mycotoxin analysis challenges with accurate mass MS/MS**

**Michal Godula**

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Screening of mycotoxins and their metabolites is of great importance in regulated environments such as food and animal feed analysis. Due to the broad variability of their physicochemical properties it is critical to employ very simple sample preparation procedure to maintain the recovery of the broad range of analytes. This leads to the fact that final extracts injected into the chromatographic system contain significant amounts of co-extracts. For the chromatographic analysis it is therefore necessary to apply the detection with high selectivity and low achieved detection limits.

Due to limitations of traditional targeted tandem MS approaches there is currently a trend towards applying the full scan MS acquisition experiments using instruments delivering high mass accuracy and resolution. High resolving power of the mass spectrometers based on Orbitrap™ and their ultimate mass accuracy provide unique advantages in the screening and quantitation of low levels of mycotoxins even in complex food matrices. However, to meet regulatory requirements for the confirmatory analyses the necessary approach is to provide at least 2 characteristic masses or produce a fragment ion spectra of an analyte to obtain sufficient number of identification points.

The presentation will summarise how the hybrid quadrupole-Orbitrap Mass Spectrometer provides durable and reliable performance for routine laboratories performing automated screening, profiling and quantification analysis. Full scan confirmation mode and parallel reaction monitoring (PRM) provide reproducible quantitation results and targeted screening capabilities. Variable data-independent analysis (vDIA) provides complete qualitative coverage for unknown screening without compromising proven quantitative attributes.

## **Applicability of two new immunoassays to the screening of mycotoxins in feed: biochip-based immunoassay for a multi-analytical approach and ELISA for the detection of aflatoxin B1**

M. Plotan, **Raymond Devlin**, J. Meneely, J. Porter, R.I. McConnell and S.P. FitzGerald  
Randox Food Diagnostics, UK; [raymond.devlin@randox.com](mailto:raymond.devlin@randox.com)

The determination of mycotoxins is important for animal feed safety as they are undesirable contaminants of cereals and cereal based products and can cause adverse health effects in animals. This study reports the applicability of two new immunoassays to the screening of mycotoxins in feed: a biochip-based immunoassay for the simultaneous detection of multiple mycotoxins (aflatoxins, ochratoxin A, zearalenone, fumonisins, trichothecenes, ergot alkaloids and paxilline) from a single

feed sample and an ELISA for the screening of aflatoxin B1. Methodology: biochip-based immunoassay – simultaneous competitive chemiluminescent immunoassays, defining discrete test sites on the biochip surface and applied to the Evidence Investigator analyser, were employed; ELISA – the assay is a competitive ELISA with short incubation time (20 min in total). Samples were extracted from feed by liquid/liquid extraction. Biochip-based immunoassay results: aflatoxins (B1, B2, G1, G2), fumonisins (FB1, FB2, FB3), ochratoxin A, deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, diacetoxyscirpenol, HT-2 toxin, T-2 toxin, zearalenone and five of its metabolites, nineteen ergot alkaloids and paxilline were detected. The detection limits were at and below the regulatory limits in feed (Directive 2002/32/EC; Commission Recommendation 2206/576/EC). ELISA results: the assay was standardised to aflatoxin B1 (cross-reactivity with aflatoxin B2 7%, with aflatoxin G1 18% and with aflatoxin G2 1%) and showed a detection limit of 1ppb. With both immunoassays, initial authentic feed sample comparisons (n=8) with LS-MS/MS showed 100% agreements for all analytes. The results indicate that these two new immunoassays are applicable to the screening of mycotoxins from feed: the simultaneous detection of a broad range of mycotoxins from a single feed sample with the biochip-based immunoassay and aflatoxin B1 with the ELISA.

## Launch 2<sup>nd</sup> EC CEN Mandate to harmonise methods for mycotoxin analysis

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European Standards are developed by CEN, the European Committee for Standardization (French: Comité Européen de Normalisation). The Members of CEN are the National Standardization Bodies of 33 European countries – including all the member states of the European Union (EU) and other countries that are part of the European Single Market. European standards are developed by teams of experts who have particular knowledge of the specific sector or topic that is being addressed. Each National Standardization Body that is part of the CEN system is obliged to adopt each European Standard as a national standard. Therefore, one European Standard (EN) becomes the national standard in all 33 countries covered by CEN Members. CEN/TC 275/WG 5 'Food analysis – Horizontal method – Biotoxins' is the working group on mycotoxins and plant toxins.

Recently, WG 5 received a second mandate from the European Commission to develop the following standards with validated methods of analysis:

- determination of ergot alkaloids (ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, ergocornine and their epimers) in cereals and cereal products;
- determination of T-2 and HT-2 toxin in cereal based foods for infants and young children by LC-MS/MS;
- determination of zearalenone in vegetable oils including refined maize oil;
- multimethod for determination of zearalenone and trichothecenes at least including deoxynivalenol (DON) and its acetylated derivatives (3-acetyl-DON and 15-acetyl-DON), nivalenol and T-2 and HT-2 toxin in cereals and cereal products by LC-MS/MS;
- multimethod for the screening of ochratoxin A, aflatoxin B1, deoxynivalenol, zearalenone and fumonisin B1 and B2 in foodstuffs, excluding foods for infants and young children, by LC-MS/MS;
- determination of aflatoxins in spices (for which an EU maximum level has been established) other than paprika;
- determination of ochratoxin A in liquorice and spices (for which an EU maximum level has been established) and in cocoa and cocoa products;
- determination of ochratoxin A in meat, meat products and edible offal;
- determination of *Alternaria* toxins (at least including alternariol, alternariol monomethyl ether, tenuazonic acid, tentoxin and altenuene) by LC-MS/MS;
- determination of phomopsis A in lupin seeds and lupin derived products by HPLC-MS/MS; and
- determination of citrinin in food by LC-MS/MS.

An outline will be given on the organisation of the work items and time schedule regarding this mandate and options to participate in interlaboratory studies and standardisation activities for interested parties.

## Speed presentations

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

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*The anti-aflatoxigenic efficacy of Cynara cardunculus L. in sesame seeds (Sesamum indicum)*

**Eleni Kollia**

Department of Food Chemistry, National and Kapodistrian University of Athens, Greece

P96

*Fusarium species fast identification and associated mycotoxins detection by MALDI-TOF*

**Emmanuel Rondags**

Laboratoire Réactions et Génie des Procédés, Université de Lorraine, France

P92

*Selection and characterisation of aptamers for the rapid detection of aflatoxin in maize*

**Viktorija Preiser**

Institute of Chemical Engineering, Vienna University of Technology, Austria

P11

*Fusarium mycotoxins in malting and brewing by-products*

**Kristina Habschied**

Faculty of Food Technology, University of Osijek, Croatia

P159

*Is height a susceptibility trait to Fusarium langsethiae infection on oats?*

**Tijana Stancic**

Harper Adams University College, UK

P143

*Feasibility study on predictive model of aflatoxin B1 via using versicolorin a as a bio-indicator*

**Da-ling Liu**

Biotechnology Department, Ji-Nan University, China

P42

*Exposure assessment of aflatoxin intake by determination of aflatoxin M1 in urine from residents of São Paulo, Brazil*

**Carlos A.F. Oliveira**

Departamento de Engenharia de Alimentos, Universidade de São Paulo, Brazil

P46

*Ochratoxin A induces micronucleus formation and inhibition of DNA repair pathway in human lymphocytes*

**Antonio J. Ramos**

Food Technology Department, Lleida University, Spain



## Rapid testing and regulating for mycotoxin concerns: a perspective from developing countries

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Among the many hurdles faced by developing countries in Africa, food security remains a major challenge. FAO data for 2010-12 show that 27% of the sub-Saharan population suffers from undernourishment in terms of total dietary energy supply. Consequently, food safety with respect to mycotoxin contamination has frequently been sidelined. Few African countries have regulations and, where they do exist, enforcement is poor. Lack of resources, inadequate infrastructure and reliance on imported technology and skills hinder the establishment of local analytical laboratories. Whereas commodity exporters may have the financial means for engaging commercial accredited laboratories, commercial food retailers rely on the quality standards of international or local food processors. However, the greatest challenge is found on rural, predominantly subsistence or smallholder farms, where conventional food surveillance is lacking. Rapid methods, designed for use in field conditions where electricity is lacking or unreliable, can offer some solution to these problems. The World Food Programme's (WFP) 'Blue Box' is an example of how technology can be adapted for these rural areas. Besides conventional grain testing equipment, WFP includes in the 'box' mycotoxin extraction equipment and an appropriate lateral flow device capable of being read in a battery-powered optical reader to provide on-site rapid semi-quantitative analyses. The recent development of temperature stable aptamers, their development into lateral flow dipstick assay formats and smart mobile phone technology may further enhance efforts to provide food safety in these areas. However, sampling remains a challenge, especially in subsistence communities subject to a 'hunger season'. Finally, it must be recognised that mere testing is not an option and that remedial actions need to be available to mitigate possible adverse findings of contaminated crops.

## Cross-reactivity in immunochemistry-based methods applied in mycotoxins analysis

Milena Zachariášová, P. Cuhra and J. Hajšlová

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Rapid methods based on immunochemical reactions in various arrangements pose a good option for high-throughput screening analysis of mycotoxins in food and feed. The most well-known and widespread method is the enzyme-linked immunosorbent assay (ELISA), however, also lateral flow test strips or fluorescence polarisation immunoassay represent a very easy to use approach. Because substantial developments reflecting the demands for multiple mycotoxins measurement are of a growing interest, also immunosensors seem to be a very challenging option. Only recently, high throughput benefits offered by multi-analyte profiling technology, surface plasmon resonance or electrochemical immunosensors have started to be addressed. However, with respect to the quality of the generated data, cross-reactivity of antibody used within these immuno-analytical methods leading to results overestimation has to be mentioned. Both the structurally related (masked) mycotoxins, but also the unknown matrix components can be responsible for that. Cross-reactivity phenomenon may also pose a risk of misinterpretation of the proficiency tests results, when the assigned value is influenced by the over-estimated results generated by immunochemical tests. The problematic of cross-reactivity and the current state of the knowledge on the rapid screening immunochemical methods is reviewed in this paper. A special attention is focused on discussion of cross-reactivities in the ELISA tests because of prevalence of this type of immunoassay in routine practice.

## Acknowledgements

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## **Fluorescence polarisation immunoassays for rapid, accurate and sensitive determination of mycotoxins**

**Vincenzo Lippolis**<sup>1</sup> and C. Maragos<sup>2</sup>

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Analytical methods for the determination of mycotoxins in foods are commonly based on chromatographic techniques (GC, HPLC or LC-MS). Although these methods permit a sensitive and accurate determination of the analyte, they require skilled personnel and are time-consuming, expensive, and are often unsuitable for screening purposes. Simple, rapid, and more effective screening methods for mycotoxins determination are highly demanded.

Fluorescence polarisation immunoassay (FPIA) is a type of homogeneous assay. For low molecular weight antigens, such as mycotoxins, it is based on the competition between an unlabeled antigen and its fluorescent-labelled derivative (tracer) for an antigen-specific antibody. The antigen content is determined by measuring the reduction of fluorescence polarisation signal, which in turn is determined by the reduction of tracer molecules able to bind antibody in solution. The development of a competitive FPIA for mycotoxin analysis has three key elements: an antibody specific for the mycotoxin, the mycotoxin of interest conjugated with a fluorophore as tracer and an instrument able to measure the polarization. The selection of an appropriate antibody-tracer combination is the most important parameter in the development of FPIA because it determines the sensitivity, speed, and accuracy of the assay. Several FPIA methods for the detection of the major mycotoxins, including aflatoxins, fumonisins, ochratoxin A, deoxynivalenol, T-2 and HT-2 toxins and zearalenone in food and beverages, have been developed in the last decade. Basic principles, advantages and limitations of these methods will be described. Their applications vary substantially in sensitivity, the time of analysis, and the degree of sample preparation required. The accuracy of many of the FPIA for mycotoxins has been supported through comparisons between FPIA and HPLC with spiked and naturally contaminated samples. The trueness of FPIA has also been established through the use of certified reference materials. These FPIA methods are simple, readily automated, rapid, and suitable for high-throughput screening, as well as for the reliable quantitative determination of mycotoxins in foods and commodities.

## **Near- and mid-infrared spectroscopy as efficient tools for detection of fungal and mycotoxin contamination in agricultural commodities**

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A wide range of spectroscopic methods is used to determine mycotoxins for both quantitative and qualitative purpose. In most cases, spectroscopic methods are used with combination of other separation techniques, such as liquid chromatography. Among spectroscopic methods, near- and mid-infrared spectroscopy is a method that can identify and quantify compounds in matrices without combination of other separation techniques. So, NIR is recognised as an efficient and non-destructive tool to determine components in agricultural commodities and widely used to estimate certain components in various agricultural products, such as protein in wheat and nitrogen in tea. However, the sensitivity of NIR (MIR) is enough to determine fungal metabolites in cereals directly. Therefore, various mathematical tools are used to estimate mycotoxins in agricultural commodities. With the advancement of chemometrics, NIR (MIR) method has become useful for a quick, non-destructive and inexpensive tool to estimate fungal contamination in agricultural commodities.

## **Using commercial immunoassay kits for mycotoxins: 'joys and sorrows'**

**Susie Y. Dai**, W. Li, T. J. Herrman

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Point-of-sampling rapid test methods are widely used to measure mycotoxins toward decision making

for consumers, industry and government control authorities. The benefit of being able to obtain testing results in minutes has facilitated quick assessment with regard to purchasing decision and safeguard human and animal health. Sound and comparable testing results obtained by commercial testing kit thus play a vital role for government regulation and fair trade. Office of the Texas State Chemist has launched a mycotoxin risk management program so-called 'One Sample Strategy' to standardise sampling and testing of aflatoxin in the grain industry as a co-regulation model between the state agency and United State Department of Agriculture (USDA). The only one testing result for the same sample are accepted by three parties including the industry for purchasing decision, the insurance company for aflatoxin loss compensation, and the government agency for regulation purpose.

Evaluation of kit performance and comparison to a 'gold standard test' of comprehensively validated chromatography method revealed varied performance characteristics of commercial kit products. For some of the kits evaluated, bias has been observed when the kit testing results were compared to an ISO 17025 accredited high performance liquid chromatography method. The performance curve for the overall aflatoxin testing kits used in the maize industry was created to predict the probability of accepting or rejecting of the whole lot of maize. The regulation limit of 20 ppb of aflatoxin in maize was used to evaluate both seller's and buyer's risks.

By implementing the 'One Sample Strategy' programme, the Office of the Texas State Chemist was able to monitor the aflatoxin testing activity for a significant portion of the Texas grain industry (>13,000 truck loads) during the harvest season. The implementation of the 'One Sample Strategy' shows that firms in the field were able to use commercial testing kits to properly measure the aflatoxin concentration in grains provided that a quality programme is implemented.

## Mycotoxins: environmental micropollutants of concern?

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Mycotoxins are naturally occurring secondary metabolites of fungi colonising a variety of cereals, fruits, vegetables and organic material in the soil. They have been studied intensively due to their occurrence in food and feed and, hence, their potential threat to human and animal health. So far, the environmental exposure to mycotoxins has scarcely been studied. Supposed main emission sources of mycotoxins to the aquatic environment are: (i) run-off and drainage water from fields cultivated with wheat or maize; (ii) manure application and excretion from grazing livestock; and (iii) human excretion via sewer system and waste water treatment plants (WWTP).

Extraction methods with LC-MS/MS detection were developed to determine different mycotoxins in drainage water, manure, samples from WWTPs as well as in river water. In drainage water, only the hydrophilic ones or those formed at high concentrations were detected: 3-acetyl-deoxynivalenol (3-AcDON), deoxynivalenol (DON), nivalenol (NIV), beauvericin (BEA), and zearalenone (ZON) [1,2]. DON and NIV exhibited highest concentrations up to a few µg/l. Of the total amounts produced in wheat plants, 0.001-0.12%, were emitted via drainage water. Zearalenone could be quantified in all manure samples in concentrations between 8 and 333 ng/g dry weight [3]. DON was omnipresent in the influent and effluent of three WWTPs in concentrations from 32 to 118 ng/l [4]. Corresponding loads exceed the predicted loads (based on human excretion) by a factor of 2. DON elimination rates in WWTP ranged from 33 to 57%. Of the 33 different mycotoxins surveyed in WWTP effluents [5], only 3-AcDON, DON, NIV, and BEA, were detected. In river water samples from Switzerland, NIV was detected in about 37%, and AcDON, DON, and BEA in 9-36% [5]. Concentrations were river discharge- dependent and mostly in the very low ng/l range, with a maximum of 24.1 ng/l, and 19.0 ng/l for NIV and DON, respectively. River water samples from the United States [6] showed a similar distribution pattern. Exclusively, during snow melt events DON concentrations exceeded 1000 ng/l.

Based on their emission fractions from agricultural areas, frequencies of occurrences and exposure concentrations, which are similar to, e.g., many pesticides, mycotoxins should be considered as environmental and in particular aquatic micropollutants. To evaluate their ecotoxicological risk, effect studies are strongly needed for decision makers.

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## Transferring innovation into practical agriculture to manage mycotoxins

Vittorio Rossi

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In Italy, mycotoxins are of great concern for cereal production in general and for durum wheat and pasta production in particular. In an innovative project, researchers have recently developed procedures for the sustainable production of high quality pasta from high quality durum wheat. A key

procedure addressed by this project is mycotoxin management.

Starting with basic research carried out at the University of Piacenza (Italy) on the biology and epidemiology of the main fungal species involved in the *Fusarium* head blight (FHB) complex, researchers developed a multispecies, mechanistic model that includes the effect of weather (temperature, relative humidity, wetness, and rainfall) on: (i) inoculum production and dispersal; (ii) infection and disease onset; and (iii) mycotoxin (deoxynivalenol and zearalenone) accumulation. The ability of this weather-driven model to predict risk of infection and mycotoxin contamination was validated under a range of epidemiological conditions. The model was then expanded to include those crop management options that significantly modify the risk of FHB and mycotoxin contamination, i.e., plant resistance, previous crop, soil management, and fungicide sprays. The complete FHB model was then included in a web-based Decision Support System (DSS) that was developed by Horta (a spin-off company of the University of Piacenza; [www.horta-srl.com](http://www.horta-srl.com)) and named granoduro.net®. This DSS granoduro.net® provides plot-specific and up-to-date warnings and support information that helps durum wheat farmers make decisions about multiple crop management actions (e.g., sowing, fertilisation, weed and disease control), including those that affect FHB and mycotoxins. Implemented by Barilla, one of the top pasta producers in Italy, the DSS granoduro.net® is currently used by hundreds of farmers to sustainably manage the durum wheat crops used for pasta production. Use of the DSS has reduced external inputs (e.g., fungicides and fertilisers) and costs, maintained or increased crop yield, greatly reduced mycotoxin contamination, reduced emission of greenhouse gasses, and increased farmer income.

New DSS functionalities are being developed, and these include the addition of *Fusarium* species and mycotoxins to the mechanistic model. Similar DSSs are under development for common wheat and barley. DSSs would also be useful for grain buyers. The idea is to develop a scenario-based system for optimising sampling for mycotoxin analyses, depending on the predicted risk in the area where the grain is produced.

## **Systems approach to Integrate toxin gene expression, growth and fumonisin B1 and B2 production by *Fusarium verticillioides* under different environmental factors**

**Angel Medina**<sup>1</sup>, M. Schmidt-Heydt<sup>2</sup>, D.L. Cárdenas-Chávez<sup>3</sup>, R. Parra<sup>3</sup>, R. Geisen<sup>2</sup> and N. Magan<sup>1</sup>  
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The objective of this study was to use a systems biology approach to integrate data on the effect of water activity ( $a_w$ ; 0.995-0.93) and temperature (20-35°C) on activation of the biosynthetic FUM genes, growth and the mycotoxins fumonisin (FB1, FB2) by *Fusarium verticillioides* *in vitro*. The relative expression of 9 biosynthetic cluster genes (*FUM1*, *FUM7*, *FUM10*, *FUM11*, *FUM12*, *FUM13*, *FUM14*, *FUM16*, and *FUM19*) in relation to the environmental factors was determined using a microarray analysis. The expression was related to growth and phenotypic FB<sub>1</sub> and FB<sub>2</sub> production. These data were used to develop a mixed-growth-associated product formation model and link this to a linear combination of the expression data for the 9 genes. The model was then validated by examining data sets outside the model fitting conditions used (35°C). The relationship between the key gene (*FUM1*) and other genes in the cluster (*FUM11*, *FUM13*, *FUM9*, *FUM14*) were examined in relation to  $a_w$ , temperature, FB1 and FB2 production by developing ternary diagrams of relative expression. This model is important in developing an integrated systems approach to develop prevention strategies to control fumonisin biosynthesis in staple food commodities and could also be used to predict the potential impact that climate change factors may have on toxin production.

## **Ice toxin: monitoring the accumulation of ochratoxin A during on-farm storage**

**Victor Limay-Rios and Art Schaafsma**

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Winter wheat has become an important crop for Ontario comprising approximately 73% of Canadian production concentrated in the Southwestern region of the province. A 10-fold increase in the amount

of wheat grain stored on-farm in December can be observed in the last 15 years due to rising agricultural commodity prices, which has facilitated infrastructure investments. Currently, galvanised corrugated steel bins are predominantly used due to being cheaper, easier to mount and do not require painting but they are more expose to fluctuations in weather conditions. Maintaining grain quality and preventing mycotoxin formation in storage are crucial for individual farmers and the grain industry in general. In northern tempered growing areas, ochratoxin A (OTA) produced by *Penicillium verrucosum*, is the most economically important mycotoxin. Health Canada has proposed maximum limits for the presence of OTA in various food commodities including a 5 ng/g maximum level for raw cereal grains and 0.5 ng/g maximum limits for baby foods and processed cereal-based foods for infants and young children. These guidelines approach the current limit of detection so sampling and analytical errors will make it extremely difficult to manage OTA after grains leave the farms and elevators; increasing the likelihood of economic harm to grain producers. The heterogeneous nature of OTA in grains also results in increased surveillance costs with dubious certainty. The development of preventive measures to mitigate the impact of this toxin during production and storage is critical. Managing the potential contamination of grain with OTA requires an understanding of the distribution of *P. verrucosum* in the field, post-harvest and during storage as well as the agronomic and storage conditions that are conducive to toxin accumulation in grains.

A total of 59 unique farms were surveyed during 3 years, 72% indicated spraying triazole fungicide for controlling *Fusarium* head blight during wheat heading. This usage of fungicides in the production of wheat, where the crop is tramped by the sprayer at heading time, can result in soil, seed contact. In a parallel study on 20 farms, soil followed by debris found on harvest equipment, manhole, doors and floor inside the bins and grain found in trampolines were the main source of *P. verrucosum* inoculum ( $p < 0.0001$ ). A total of 83 storage bins were surveyed during the same time. The quantity of cfu in grain collected inside the bin and during sample out-loading is influenced by the location where the sample was taken ( $p = 0.004$ ), grain moisture content at sampling ( $p = 0.0007$ ) and time in storage ( $p = 0.0006$ ). Regression analysis shows that *P. verrucosum* incidence higher than 1000 cfu/g results in samples contaminated with above the proposed maximum levels of OTA for cereal grain ( $r^2 = 0.995$ ;  $p < 0.001$ ). Mould spy sensors for non-invasive monitoring of *P. verrucosum* growth in real-time were validated in localized areas inside an induced OTA hot spot. OTA spatial distribution in the hot spot and during grain out-loading was studied in experimental bins. Colder than usual winter earlier this year resulted in ice cup formation on top of the grain surface inside bins with the consequent production of OTA. The contribution of ice to OTA contamination was also studied in experimental bins and will be presented during this meeting.

**WEDNESDAY 12 NOVEMBER 2014 – Plenary meeting**

## **Mycotoxin control: an industry perspective on the systems approach**

**Robert Baker**

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Food safety goes beyond traditional factory quality management processes and must cover the entire production pipeline ('farm-to-fork approach'). Failure to do so can place your consumers, products and business at risk of a food safety incident. Historical information demonstrates that food safety incidents can be traced back to issues involving raw materials, production, distribution and mishandling prior to consumption. Food safety issues coming from raw materials far outnumber those from other areas accounting for over 60% of the reported incidents (U.S. food data). Mycotoxin contamination (predominantly cereals, fruits and nuts) make up a large portion of the raw material related food safety issues and present a significant challenge to the food industry as a whole. Based on this, it is imperative that a significant focus is needed to manage mycotoxin contamination risks across the raw material pipeline and integrated as part of an overall food safety management programme.

This presentation will provide an overview of mycotoxin risk areas in the food industry, along with providing a framework for managing the risks throughout the supply pipeline.

## **Integrating public health goals into systems management of staple crops to limit mycotoxin exposure**

**J. David Miller**

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Grain production in the USA and Canada operates over a 5,000 km supply chain. In Canada, there are 82,000 farm operators whose principal income is grain production and more than 100,000 farms ship 60,000 tonnes of grains. There are 264 licensed grain dealers, 337 licensed elevators, 7 terminal and 5 transfer elevators. There are >90,000 grain deliveries to the 40 Canadian mills annually. From there the resulting flour goes to consumers, bakers and other end users. The size of the system and the number of actors in the value chain constrain where effective interventions can be made to reduce mycotoxins except at the end user.

Public health law in the USA and Canada is based on the principle that investments must be evidence based, and where the most benefit to the public. More than 100 years ago, the American public health official Dr. Ernest Levy wrote "*It thus becomes the plain duty of health officers, as a body, to determine the actual and relative importance a health issue, to eliminate the useless or less useful, to build up the more useful, and to employ along the lines of greatest public benefit those facilities placed at their disposal.*" Additional costs to that result in price increases need to be justified. Food poverty is not acceptable in modern society. The agrifood system is therefore best served by guidelines based on data with the least uncertainty achievable.

The implementation of a critical path for the management of mycotoxin exposure in a grain handling system brings attention to where food quality objectives can be addressed in the most cost effective fashion. This requires an analysis of the critical points and making investments to develop the tools to manage contamination before the commodity reaches the end user. While this seems obvious, there are few success stories where this has been systematically achieved for the agriculturally important mycotoxins. The alternative has been to manage contamination by default: post market surveillance. Some examples of where research to develop tools that would more effectively address broad public health values lower down the value chain will be discussed.

## **Managing food risk communication in the social media era: practical lessons learnt!**

**Adrian Moss**

Focus Business Communications, UK; [adrian.moss@focusbiz.co.uk](mailto:adrian.moss@focusbiz.co.uk)

In this presentation, I will talk briefly about the history and growth of online social networks and the impact that has had on communication by organisations and governments. The reasons behind the FoodRisC project, the research methods, findings and conclusions drawn will also be explained. In addition, an insight will be given into how it coped with possibly the biggest crisis to face the agriculture and food industry in the last 20 years – the horsemeat ‘scandal’. The continued ramifications in terms of public confidence and sentiment will also be discussed. Insights will be shared from the research into the role of social media and what practical lessons can be learnt about how to prepare for, respond to and recover from a social media crisis.



## POSTERS

### Occurrence

#### P1 – P32

- P1 *Mycotoxin contamination and occurrence of Fusarium ear rot on maize and Fusarium head blight on winter wheat in Kosovo*  
V. Shala-Mayrhofer<sup>1</sup>, E. Varga<sup>2</sup>, R. Marjakaj<sup>3</sup>, **Franz Berthiller**<sup>2</sup>, A. Musolli<sup>1</sup>, D. Berisha<sup>4</sup>, B. Kelmendi<sup>4</sup> and M. Lemmens<sup>1</sup>  
<sup>1</sup>Institute for Biotechnology in Plant Production and <sup>2</sup>Center for Analytical Chemistry and Christian Doppler Laboratory for Mycotoxin Metabolism, Department IFA-Tulln, BOKU Vienna, Austria, <sup>3</sup>Ministry of Agriculture, Forestry and Rural Development and <sup>4</sup>Kosovo Agriculture Institute, Kosovo
- P2 *Aspergillus and Penicillium toxins in chestnuts and derived products produced in Italy*  
**Terenzio Bertuzzi**, S. Rastelli, A. Mulazzi and A. Pietri  
Food and Feed Science and Nutrition Institute, Faculty of Agriculture, Università Cattolica del Sacro Cuore, Italy
- P3 *Occurrence of free and masked fumonisins in maize products available on the Polish market*  
**Marcin Bryła**<sup>1,2</sup>, M. Roszko<sup>1</sup>, K. Szymczyk<sup>1</sup> and R. Jędrzejczak<sup>1</sup>  
<sup>1</sup>Department of Food Analysis, Institute of Agricultural and Food Biotechnology and <sup>2</sup>Faculty of Food Sciences, Warsaw University of Life Sciences, Poland
- P4 *Occurrence of citrinin in food and feed in Belgium*  
C. Thiry, B. Huybrechts and **Alfons Callebaut**  
Unit Toxins and Natural Substances, CODA-CERVA, Belgium
- P5 *Aflatoxin M1 detection in Italian infant formula milk*  
C. Brera, **Sonia Colicchia**, F. Debegnach, E. Gregori and B. De Santis  
GMO and Mycotoxin Unit, Department of Veterinary Public Health and Food Safety, Italian National Institute for Health, Italy
- P6 *Mycotoxin survey in plant and dairy waste streams for feed production: the NOSHAN approach*  
C. Falavigna, **Chiara Dall'Asta**, A. Dossena and S. Sforza  
Department of Food Science, University of Parma, Italy
- P7 *An Italian monitoring study for the evaluation of 'emerging mycotoxins', enniatins, beauvericin and citrinin profile in domestic wheat samples*  
C. Brera, G. Trifirò, **Francesca Debegnach**, E. Gregori, G. Moracci, M.C. Magri, and B. De Santis  
GMO and Mycotoxin Unit, Department of Veterinary Public Health and Food Safety, Italian National Institute for Health, Italy
- P8 *Application of high resolution tandem mass spectrometry in analysis of mycotoxins, pesticides, and pyrrolizidine alkaloids in herbal-based dietary supplements*  
**Zbynek Dzuman**, A. Zachariasova, Z. Veprikova, M. Zachariasova and J. Hajslova  
Department of Food Analysis and Nutrition, Institute of Chemical Technology, Czech Republic
- P9 *Co-occurrence of aflatoxins and fumonisins in cereal mixtures marketed in Brazil*  
**Andreza Maria Fernandes**<sup>1</sup>, N.B. Neres<sup>1</sup>, E.C. Michelin<sup>1</sup>, E. Peluque<sup>1</sup>, T. Alves dos Reis<sup>2</sup>, R.E. Rosim<sup>3</sup>, S.H. Seraphin de Godoy<sup>1</sup>, F.S. Munin<sup>1</sup>, S. Ribeiro de Almeida-Queiroz<sup>1</sup>, C.A. Fernandes de Oliveira<sup>3</sup>, R.L. Moro de Sousa<sup>1</sup> and B. Corrêa<sup>2</sup>  
<sup>1</sup>Department of Veterinary Medicine, Faculty of Animal Science and Food Engineering, University of São Paulo, <sup>2</sup>Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo and <sup>3</sup>Department of Food Engineering, Faculty of Animal Science and Food Engineering, University of São Paulo, Brazil

- P10 *Fusarium graminearum* species complex (FGSC) and *F. verticillioides* incidence in maize roots, crowns, stems and ears  
**Sonia-Mari Greyling**<sup>1</sup>, A. Schoeman<sup>1</sup>, B. Flett<sup>1,2</sup>, N. McLaren<sup>3</sup> and B. Janse van Rensburg<sup>1</sup>  
<sup>1</sup>Grain Crops Institute, Agricultural Research Council, <sup>2</sup>Unit for Environmental Sciences and Management, North-West University and <sup>3</sup>Department of Plant Sciences, University of the Free State, South Africa
- P11 *Fusarium* mycotoxins in malting and brewing by-products  
**Kristina Habschied**<sup>1</sup>, B. Šarkanj<sup>1</sup>, V. Krstanović<sup>1</sup>, N. Velić<sup>1</sup>, M. Novak<sup>2</sup> and K. Mastanjević<sup>1</sup>  
<sup>1</sup>Faculty of Food Technology, University of Osijek and <sup>2</sup>Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia
- P12 *Worldwide mycotoxin occurrence: a ten year investigation*  
**Paula Kovalsky**<sup>1</sup>, Y.-J. Liu<sup>2</sup>, K. Nahrer<sup>1</sup> and E.M. Binder<sup>2</sup>  
<sup>1</sup>Biomin Holding GmbH and <sup>2</sup>Erber AG, Austria
- P13 *Occurrence of mycotoxins in a total diet study (TDS) for adults and children in the Netherlands*  
**Patricia López**<sup>1</sup>, T. de Rijk<sup>1</sup>, M. Alewijn<sup>1</sup>, C. Sprong<sup>2</sup> and J. Castenmiller<sup>3</sup>  
<sup>1</sup>RIKILT Wageningen UR, <sup>2</sup>National Institute for Public Health and the Environment and <sup>3</sup>Netherlands Food and Consumer Product Safety Authority, the Netherlands
- P14 *Occurrence of Alternaria mycotoxins in food products in the Netherlands*  
**Patricia López**<sup>1</sup>, D. Venema<sup>1</sup>, T. de Rijk<sup>1</sup>, A. de Kok<sup>2</sup>, J. Scholten<sup>2</sup>, H. Mol<sup>1</sup> and M. de Nijs<sup>1</sup>  
<sup>1</sup>RIKILT Wageningen UR, <sup>2</sup>National Institute for Public Health and the Environment and <sup>3</sup>Netherlands Food and Consumer Product Safety Authority, the Netherlands
- P15 *Survey of UK retail food products for selected Fusarium mycotoxins including their masked forms*  
**Susan MacDonald**<sup>1</sup>, I. Leon<sup>1</sup>, J. Clough<sup>1</sup>, M. Sehnalova<sup>1</sup>, C. Baskaran<sup>2</sup>, G. Bramley<sup>2</sup>, F. Berthiller<sup>3</sup> and R. Krska<sup>3</sup>  
<sup>1</sup>Food and Environment Research Agency, UK, <sup>2</sup>Food Standards Agency, UK and <sup>3</sup>Department IFA-Tulln, BOKU Vienna, Austria
- P16 *Identification and quantification of mycotoxins in silages: an Irish national survey*  
**Cormac McElhinney**<sup>1,2,3</sup>, M. Danaher<sup>3</sup>, C. Elliott<sup>2</sup> and P. O'Kiely<sup>1</sup>  
<sup>1</sup>Teagasc Animal & Grassland Research and Innovation Centre, Ireland, <sup>2</sup>Institute for Global Food Security, Queens University Belfast, UK and <sup>3</sup>Food Safety Department, Teagasc Food Research Centre, Ireland
- P17 *The Black Sea region is a suitable area for mycotoxins production, with Ukraine as a prime example*  
**Elise Nacer-Khodja** and E. Leroux  
 Neovia Additives, In Vivo NSA Group, France
- P18 *Simple or multicontamination by mycotoxins in French wheat and maize*  
**Elise Nacer-Khodja** and E. Leroux  
 Neovia Additives, In Vivo NSA Group, France
- P19 *Global mycotoxin survey in 2013 and 2014*  
**Karin Naehrer**<sup>1</sup>, P. Kovalsky<sup>1</sup> and D. Schatzmayr<sup>2</sup>  
<sup>1</sup>Biomin Holding GmbH and <sup>2</sup>Biomin Research Center, Austria
- P20 *Mycotoxins and heavy metals in organic commercial cereal based foods sold in supermarkets in Greece*  
 A. Skendi<sup>1</sup>, **Maria Papageorgiou**<sup>1</sup>, M. Irakli<sup>2</sup> and D. Katsantonis<sup>2</sup>  
<sup>1</sup>Department of Food Technology, Alexander Technological Educational Institute of Thessaloniki and <sup>2</sup>Hellenic Agricultural Organization - Demeter, Cereal Institute, Greece
- P21 *Screening and quantification of mycotoxins in various types of feed samples in northern Europe between October 2012 and June 2013*  
**Jog Raj**, A. Bryant, C. Bale, L. Norton and D. Parfitt  
 Micron Bio-Systems Ltd., UK

- P22 *Maize harvest 2013: 72 maize samples from Austria tested for more than 380 mycotoxins and secondary metabolites*  
**Christina Schwab-Andics**<sup>1</sup>, K. Naehrer<sup>1</sup>, P. Kovalsky<sup>1</sup>, R. Krska<sup>2</sup> and M. Sulyok<sup>2</sup>  
<sup>1</sup>BiomIn Holding GmbH and <sup>2</sup>Department IFA-Tulln, BOKU Vienna, Austria
- P23 *Multimycotoxin screening in Latin-American maize and soy samples*  
**Verena Starkl**<sup>1</sup>, P. Kovalsky<sup>1</sup>, M. Sulyok<sup>2</sup>, R. Krska<sup>2</sup>, K. Nährer<sup>1</sup> and U. Hofstetter<sup>1</sup>  
<sup>1</sup>BiomIn Holding GmbH and <sup>2</sup>Department IFA-Tulln, BOKU Vienna, Austria
- P24 *Mycotoxins and other fungal metabolites in grain dust from Norwegian grain elevators and compound feed mills*  
**Anne Straumfors**<sup>1</sup>, S. Uhlig<sup>2</sup>, G.S. Eriksen<sup>2</sup>, K.K. Heldal<sup>1</sup>, W. Eduard<sup>1</sup>, R. Krska<sup>3</sup> and M. Sulyok<sup>3</sup>  
<sup>1</sup>Department of Chemical And Biological Work Environment, National Institute of Occupational Health, Norway, <sup>2</sup>Section for Chemistry and Toxicology, Norwegian Veterinary Institute, Norway and <sup>3</sup>Department IFA, BOKU Vienna, Austria
- P25 *Study of black Aspergillus from Canadian vineyards and the associated risk of ochratoxin A and fumonisin contamination of wine*  
**Mark W. Sumarah** and K.A. Seifert  
 Agriculture and Agri-Food Canada/Agriculture et Agroalimentaire, Canada
- P26 *Trichothecene mycotoxin levels in winter wheat harvested in 2013 from Ontario, Canada*  
**Ljiljana Tamburic-Ilicic**, V. Limay-Rios and J. Brinkman  
 University of Guelph, Ridgetown Campus, Canada
- P27 *Survey on patulin presence in apple juice*  
**Ljilja Torović**<sup>1,2</sup> and N. Dimitrov<sup>1</sup>  
<sup>1</sup>Faculty of Medicine, University of Novi Sad and <sup>2</sup>Institute of Public Health of Vojvodina, Serbia
- P28 *Study on patulin occurrence in fruit products for infants and children*  
 N. Dimitrov<sup>1</sup> and **Ljilja Torović**<sup>1,2</sup>  
<sup>1</sup>Faculty of Medicine, University of Novi Sad and <sup>2</sup>Institute of Public Health of Vojvodina, Serbia
- P29 *Occurrence of mycotoxins and pesticides in products from organic farming*  
**Zdenka Veprikova**, Z. Dzuman, M. Zachariasova and J. Hajslova  
 Department of Food Analysis and Nutrition, Institute of Chemical Technology, Czech Republic
- P30 *Occurrence of (masked) Alternaria toxins – a survey in foodstuffs commercially available on the Belgian market*  
**Jeroen Walravens**<sup>1</sup>, H. Mikula<sup>2</sup>, M. Rychlik<sup>3</sup>, S. Asam<sup>4</sup>, J. Diana Di Mavungu<sup>1</sup>, A. Van Landschoot<sup>5</sup>, L. Vanhaecke<sup>6</sup> and S. De Saeger<sup>1</sup>  
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- P31 *Mycotoxin survey: multimycotoxin analysis and distribution in feed commodities – an holistic approach to risk assessment strategies*  
**Alexandros Yiannikouris**<sup>1</sup>, S. Mobley<sup>2</sup>, A. Weaver<sup>1</sup>, M. Hawkins<sup>1</sup> and N. Adams<sup>3</sup>  
<sup>1</sup>Center for Animal Nutrigenomics and Applied Animal Nutrition, Alltech Inc., USA, <sup>2</sup>Analytical Services Laboratory, Alltech Inc., USA and <sup>3</sup>Alltech House, UK
- P32 *Molecular quantification and genetic diversity of toxigenic Fusarium species in northern Europe and Asia*  
**Tapani Yli-Mattila**<sup>1</sup>, S. Rämö<sup>2</sup>, V. Hietaniemi<sup>2</sup>, T. Hussien<sup>1,3</sup>, O. Gavrilova<sup>4</sup> and T. Gagkaeva<sup>4</sup>  
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## Human and animal health implications

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- P33 *Negative effects of fumonisins in pigs and their effective counteraction*  
**Radka Borutova**<sup>1</sup>, S. Moreland<sup>2</sup>, C.A. Mallman<sup>3</sup>  
<sup>1</sup>NutriAd International, Belgium, <sup>2</sup>NutriAd Inc., USA and <sup>3</sup>Instituto Samitec, Instituto de Soluções Analíticas Microbiológicas e Tecnológicas Ltda., Brazil
- P34 *In vitro effects of fumonisin B1 alone and combined with deoxynivalenol or  $\beta$ -zearalenol on bovine granulosa cell proliferation and steroid production*  
**Francesca Caloni**<sup>1</sup>, L. Schutz<sup>2</sup>, M. Albonico<sup>1</sup>, C. Cortinovic<sup>1</sup> and L.J. Spicer<sup>2</sup>  
<sup>1</sup>Department of Health, Animal Science and Food Safety (VESPA), University of Milan, Italy and <sup>2</sup>Department of Animal Science, Oklahoma State University, USA
- P35 *Effects of mycotoxin exposure on proliferation and apoptosis in the porcine intestinal mucosa*  
**Sophal Cheat**<sup>1,2,3</sup>, M. Younanian<sup>4</sup>, O. Lafaix<sup>1,2</sup>, I. Raymond-Letron<sup>1,2</sup>, J. Cognie<sup>5</sup>, A.-M. Cossalter<sup>1,6</sup>, I.P. Oswald<sup>1,6</sup> and M. Kolf-Clauw<sup>1,2</sup>  
<sup>1</sup>Université de Toulouse, INP-UMR1331, Toxalim, France, <sup>2</sup>Université de Toulouse, INP-ENVT, Ecole Nationale Vétérinaire de Toulouse, France, <sup>3</sup>Faculty of Animal Science and Veterinary Medicine, Royal University of Agriculture, Cambodia, <sup>4</sup>College of Veterinary Medicine, Auburn University, USA, <sup>5</sup>INRA-UMR 6175 PRC 37380, France and <sup>6</sup>INRA-UMR1331, Toxalim, France
- P36 *In silico/in vitro approaches for zearalenone metabolism in human hepatocytes*  
M. Cirlini<sup>1</sup>, A. Valeri<sup>2</sup>, **Chiara Dall'Asta**<sup>1</sup>, R.M. Pellegrino<sup>1</sup> and L. Goracci<sup>2</sup>  
<sup>1</sup>Department of Food Science, University of Parma and <sup>2</sup>Laboratory for Chemoinformatics and Molecular Modelling, Department of Chemistry, Biology and Biotechnologies, University of Perugia, Italy
- P37 *Masked forms of zearalenone mycotoxin and xenoestrogenic activity in MCF7-based assays: computational insight of mechanisms of action*  
L. Dellafiora<sup>1,2</sup>, P. Cozzini<sup>1</sup>, **Gianni Galaverna**<sup>2</sup> and C. Dall'Asta<sup>2</sup>  
<sup>1</sup>Molecular Modelling Laboratory Department of Food Science, University of Parma and <sup>2</sup>Food Chemistry Laboratory, Department of Food Science, University of Parma, Italy
- P38 *Dietary exposure and microbial metabolism of DON and DON-3-glucoside in humans*  
**Silvia W. Gratz**<sup>1</sup>, T. Richardson<sup>1</sup>, G. Holtrop<sup>2</sup> and G. Duncan<sup>1</sup>  
<sup>1</sup>Rowett Institute of Nutrition and Health, University of Aberdeen and <sup>2</sup>Biomathematics and Statistics Scotland, UK
- P39 *Effects of orally administered fumonisin B1, partially hydrolysed fumonisin B1, hydrolysed fumonisin B1 and N-(1-deoxy-D-fructos-1-yl) fumonisin B1 on the sphingolipid metabolism in rats*  
**Irene Hahn**<sup>1</sup>, V. Nagl<sup>1</sup>, H.E. Schwartz-Zimmermann<sup>1</sup>, E. Varga<sup>1</sup>, M. Cirlini<sup>2</sup>, C. Schwarz<sup>3</sup>, R. Krska<sup>1</sup> and F. Berthiller<sup>1</sup>  
<sup>1</sup>Christian Doppler Laboratory for Mycotoxin Metabolism and Center for Analytical Chemistry, Department IFA-Tulln, BOKU Vienna, Austria, <sup>2</sup>Department of Food Science, University of Parma, Italy and <sup>3</sup>Institute of Animal Nutrition, Products, and Nutrition Physiology, Department IFA-Tulln, BOKU Vienna, Austria
- P40 *Porcine, chicken, mouse, and fish derived cell lines as model systems to compare the cytotoxicity of DON and its metabolite after microbial transformation*  
**Elisabeth Mayer**<sup>1</sup>, B. Novak<sup>1</sup>, H. Schwartz-Zimmermann<sup>2</sup>, S. Hessenberger<sup>1</sup> and G. Schatzmayr<sup>1</sup>  
<sup>1</sup>Biomim Research Center and <sup>2</sup>Christian Doppler Laboratory for Mycotoxin Metabolism, Department IFA-Tulln, BOKU Vienna, Austria
- P41 *Immune response and urinary fumonisin B1 excretion in piglets fed low dietary levels of fumonisin*  
P.C.M.C. Souto<sup>1</sup>, A.V. Jager<sup>1</sup>, F.G. Tonin<sup>2</sup>, K. Bordin<sup>1</sup>, A. Pierron<sup>3</sup>, A.M. Cossalter<sup>3</sup>, J. Laffitte<sup>3</sup>, I.P. Oswald<sup>3</sup> and **Carlos A.F. Oliveira**<sup>1</sup>  
<sup>1</sup>Departamento de Engenharia de Alimentos, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Brazil, <sup>2</sup>Departamento de Engenharia de Biossistemas,

Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Brazil and <sup>3</sup> INRA Toxalim, France

- P42 *Exposure assessment of aflatoxin intake by determination of aflatoxin M1 in urine from residents of São Paulo, Brazil*  
A.V. Jager<sup>1</sup>, F.G. Tonin<sup>2</sup>, P.C.M.C. Souto<sup>1</sup>, R.T. Privatti and **Carlos A.F. Oliveira**<sup>1</sup>  
<sup>1</sup>Departamento de Engenharia de Alimentos, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo and <sup>2</sup>Departamento de Engenharia de Biossistemas, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Brazil
- P43 *Genotoxicity of aflatoxin precursors*  
M. Audebert<sup>1,2</sup>, M. Theumeur<sup>1,2,3</sup>, Y. Henneb<sup>1,2</sup>, **Delphine Payros**<sup>1,2</sup>, C. Borin<sup>1,2</sup>, S. Tadrist<sup>1,2</sup>, S.B. Beltz<sup>4</sup>, O. Puel<sup>1,2</sup> and I.P. Oswald<sup>1,2</sup>  
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- P44 *Deoxynivalenol exacerbates DNA damage in rats colonised by Escherichia coli producing the genotoxin colibactin*  
**Delphine Payros**<sup>1,2</sup>, T. Secher<sup>3,4,5</sup>, P. Pinton<sup>1,2</sup>, J. Laffitte<sup>1,2</sup>, M. Boury<sup>3,4,5</sup>, E. Oswald<sup>3,4,5,6</sup> and I.P. Oswald<sup>1,2</sup>.  
<sup>1</sup>INRA-UMR1331, Toxalim, <sup>2</sup>Université de Toulouse, UMR1331, Toxalim, <sup>3</sup>INRA-USC 1043, <sup>4</sup>INSERM-UMR1043, Centre de Physiopathologie Toulouse Purpan, <sup>5</sup>CNRS-UMR 5282 and <sup>6</sup>CHU Toulouse, Hôpital Purpan, Service de bactériologie-Hygiène, France
- P45 *Masked DON and other DON metabolites do not exert intestinal toxicity: transcriptomic analysis of jejunal explants*  
**Alix Pierron**<sup>1,2</sup>, S. Mimoun<sup>1</sup>, Y. Lippi<sup>1</sup>, P. Gourbeyre<sup>1</sup>, L. Liaubet<sup>3</sup>, T. Zhou<sup>4</sup>, F. Berthiller<sup>5</sup>, G. Schatmayr<sup>2</sup>, D. Moll<sup>2</sup> and I.P. Oswald<sup>1</sup>  
<sup>1</sup>INRA-UMR 1331, Toxalim, France, <sup>2</sup>Biomin Research Center, Austria, <sup>3</sup>INRA-UMR444, Laboratoire de Génétique Cellulaire, France, <sup>4</sup>Agriculture and Agri-Food Canada, Guelph Food Research Centre, Canada and <sup>5</sup>Christian Doppler Laboratory for Mycotoxin Metabolism and Center for Analytical Chemistry, Department IFA-Tulln, BOKU Vienna, Austria
- P46 *Ochratoxin A induces micronucleus formation and inhibition of DNA repair pathway in human lymphocytes*  
C.A. González-Arias<sup>1</sup>, **Antonio J. Ramos**<sup>1</sup>, A.B. Benítez-Trinidad<sup>2</sup>, M. Sordo<sup>3</sup>, M.L. Robledo-Marengo<sup>2</sup>, I.M. Medina-Díaz<sup>2</sup>, B.S. Barrón-Vivanco<sup>2</sup>, S. Marín<sup>1</sup>, V. Sanchis<sup>1</sup> and A.E. Rojas-García<sup>2</sup>  
<sup>1</sup>Food Technology Department, Lleida University, Spain, <sup>2</sup>Laboratorio de Contaminación y Toxicología Ambiental, Universidad Autónoma de Nayarit, Mexico and <sup>3</sup>Departamento de Genética y Toxicología Ambiental, Instituto de Investigaciones Biomédicas, U.N.A.M. Ciudad Universitaria, Mexico
- P47 *Risk ranking of mycotoxins for the Austrian population based on data from official control*  
**Elke Rauscher-Gabernig**<sup>1</sup>, S. Scheriau<sup>2</sup>, R. Öhlinger<sup>2</sup> and K. Fuchs<sup>2</sup>  
<sup>1</sup>Business Area Risk Assessment, Data, Statistics and <sup>2</sup>Business Area Food Safety, Austrian Agency for Health and Food Safety (AGES), Austria
- P48 *Assessment of consumer exposure to deoxynivalenol and its acetyl derivatives through consumption of cereal-based food from the Austrian market*  
**Elke Rauscher-Gabernig**<sup>1</sup>, A. Raditschnig<sup>2</sup>, E. Kiendler<sup>2</sup>, W. Brodacz<sup>2</sup> and R. Öhlinger<sup>2</sup>  
<sup>1</sup>Business Area Risk Assessment, Data, Statistics and <sup>2</sup>Business Area Food Safety, Austrian Agency for Health and Food Safety (AGES), Austria
- P49 *Biomarker analysis in serum of pigs fed diets contaminated with fumonisins*  
**Simone Schaumberger**<sup>1</sup>, S. Masching<sup>1</sup>, D. Schatmayr<sup>2</sup> and G. Schatmayr<sup>2</sup>  
<sup>1</sup>Biomin Holding and <sup>2</sup>Biomin Research Center, Austria

- P50 *Examination of the effect of deoxynivalenol (DON) and de-epoxy-deoxy-nivalenol (DOM-1) on intestinal, immune, and hepatic parameters*  
**Alexandra Springler**, S. Hessenberger, E. Mayer, B. Novak and G. Schatzmayr  
 Biomin Research Center, Austria
- P51 *N-deoxyfructosyl-fumonisin B1 may cause DNA damage in porcine mononuclear blood cells*  
 M. Cirlini<sup>1</sup>, C. Falavigna<sup>1</sup>, M. Kachlek<sup>2</sup>, **Judit Szabó-Fodor**<sup>2</sup>, M. Kovács<sup>2</sup> and C. Dall'Asta<sup>1</sup>  
<sup>1</sup>Department of Food Science, University of Parma, Italy and <sup>2</sup>MTA-KE Mycotoxins in the Food Chain Research Group, Faculty of Agricultural and Environmental Sciences, Kaposvár University, Hungary
- P52 *Public health risk associated with the co-occurrence of mycotoxins in spices consumed in Sri Lanka*  
**Pratheeba Yogendrarajah**<sup>1,2</sup>, L. Jacxsens<sup>1</sup>, C. Lachat<sup>1,3</sup>, C. Niroshan Walpita<sup>4</sup>, P. Kolsteren<sup>1</sup>, S. De Saeger<sup>2</sup> and B. De Meulenaer<sup>1</sup>  
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## Analysis

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- P53 *Using stable isotope internal standard for the accurate quantification of cyclopiazonic acid with an HPLC-MS/MS method*  
**Parisa Ansari**, R. Labuda and G. Häubl  
 Romer Labs Division Holding GmbH, Austria
- P54 *Determination of deoxynivalenol and nivalenol in wheat and maize using immunoaffinity column cleanup and RPLC/UV*  
**Lei Bao**, Z. Wu and P. Jing  
 Mycotoxin Reference Laboratory, Shan Dong Entry-Exit Inspection and Quarantine Bureau of China (AQSIQ), China
- P55 *Multimycotoxins analysis using a unique solid phase extraction based on molecularly imprinted polymers*  
 D. Derrien, O. Lépine, M. Arotçaréna, K. Naraghi and **Sami Bayouhd**  
 PolyIntell, France
- P56 *Determination of hidden fumonisins in maize using a phosphate buffer extraction*  
 A. Pietri, S. Rastelli, A. Mulazzi and **Terenzio Bertuzzi**  
 Food and Feed Science and Nutrition Institute, Faculty of Agriculture, Università Cattolica del Sacro Cuore, Italy
- P57 *Evaluation of the Puritox multimycotoxin purification column using LC-MS/MS*  
**Julie L. Brunkhorst**, B.R. Malone, R. Niemeijer and H. Henderson  
 Trilogy Analytical Laboratory, USA
- P58 *New electrochemical immunosensor kits for the determination of ochratoxin A, fumonisin B1 and deoxynivalenol in wine and cereal-based food*  
 A. Ezquerra<sup>1</sup>, J.R. Bertolín<sup>1</sup>, L. Bonel<sup>2</sup>, J.C. Vidal<sup>1</sup>, and **Juan R. Castillo**<sup>1</sup>  
<sup>1</sup>Analytical Spectroscopy and Sensors Group, Institute of Environmental Sciences, University of Zaragoza and <sup>2</sup>Capher Idi S.L., Spain
- P59 *New spectrophotometric immunosensor kits for the determination of ochratoxin A, fumonisin B1 and deoxynivalenol in wine and cereals*  
 A. Ezquerra<sup>1</sup>, J.R. Bertolín<sup>1</sup>, L. Bonel<sup>2</sup>, J.C. Vidal<sup>1</sup>, and **Juan R. Castillo**<sup>1</sup>  
<sup>1</sup>Analytical Spectroscopy and Sensors Group, Institute of Environmental Sciences, University of Zaragoza and <sup>2</sup>Capher Idi S.L., Spain

- P60 *Developments in multimycotoxin testing – MycoSpin™ 400 clean-up and stable <sup>13</sup>C-labelled internal standards improve accuracy and sensitivity in mycotoxin LC-MS/MS methods*  
**Lee Juan Chin**  
 Romer Labs Singapore, Singapore
- P61 *Determination of mycoestrogens in baby food*  
 C. Brera, **Sonia Colicchia**, F. Debegnach, E. Gregori and B. De Santis  
 GMO and Mycotoxin Unit, Department of Veterinary Public Health and Food Safety, Italian National Institute for Health, Italy
- P62 *Challenges in the development of a water-based extraction method for screening of aflatoxins in food and feed*  
**Barbara Cvak**<sup>1</sup>, A. Schiessl<sup>1</sup> and J. Zimmerman<sup>2</sup>  
<sup>1</sup>Romer Labs Division Holding GmbH, Austria and <sup>2</sup>Romer Labs Technology Inc., USA
- P63 *Development of a water-based extraction method for zearalenone in maize*  
**Barbara Cvak**<sup>1</sup>, A. Schiessl<sup>1</sup> and J. Zimmerman<sup>2</sup>  
<sup>1</sup>Romer Labs Division Holding GmbH, Austria and <sup>2</sup>Romer Labs Technology Inc., USA
- P64 *Quantitative analysis of 15 mycotoxins by liquid chromatography electrospray ionisation tandem mass spectrometry (LC-ESI-MS/MS)*  
**Aurélien Desmarchelier**, T. Bessaire and L. Racault  
 Nestlé Research Center, Nestec Ltd., Switzerland
- P65 *Analysis of multiple mycotoxins by LC-MS/MS: in-depth analysis of column selectivity*  
 E.R. Barrey, O.I. Shimelis and **Christine Dumas**  
 Supelco/Sigma-Aldrich, USA
- P66 *Cost-effective methods for detection of deoxynivalenol in wheat at collector intake*  
**Ine van der Fels-Klerx**<sup>1</sup> and C.P.A. van Wagenberg<sup>2</sup>  
<sup>1</sup>RIKILT Wageningen UR and <sup>2</sup>LEI Wageningen UR, the Netherlands
- P67 *Metabolomic technology to decipher maize resistance to Fusarium graminearum infection and trichothecenes accumulation*  
**Léa Gauthier**<sup>1</sup>, V. Atanasova-Penichon<sup>1</sup>, S. Bernillon<sup>2</sup>, M. Maucourt<sup>2</sup>, A. Moing<sup>2</sup>, G. Mey<sup>3</sup>, A. Surault<sup>3</sup>, F. Chopin<sup>4</sup>, R. Fouquet<sup>4</sup> and F. Forget<sup>1</sup>  
<sup>1</sup>INRA, UR 1264-MycSA, <sup>2</sup>INRA, UMR 1332-BFP, <sup>3</sup>Euralis Semences and <sup>4</sup>Monsanto, France
- P68 *Determination of sterigmatocystin in grains using gas chromatography-mass spectrometry with on-column injector*  
 M.Z. Hossain and **Tetsu Goto**  
 Faculty of Agriculture, Shinshu University, Japan
- P69 *Development of a multimycotoxin method using stable isotope dilution assays to follow the fate of mycotoxins during the malting process*  
**Katharina Habler**<sup>1</sup>, K. Hofer<sup>2</sup>, C. Geissinger<sup>3</sup> and M. Rychlik<sup>1,4</sup>  
<sup>1</sup>Bioanalytik Weihenstephan, Zentralinstitut für Ernährungs- und Lebensmittelforschung (ZIEL), TUM, <sup>2</sup>Lehrstuhl für Phytopathologie, TUM, <sup>3</sup>Lehrstuhl für Brau- und Getränke-technologie, TUM and <sup>4</sup>Lehrstuhl für Analytische Lebensmittelchemie, TUM, Germany
- P70 *A sensitive UHPLC-MS/MS method for the analysis of ochratoxin A, fumonisins B1 and B2, zearalenone and its metabolites in meat and liver*  
**Vincent Hort**, C. Maleix, S. Dragacci, T. Guérin, F. Hommet and M. Nicolas  
 Pesticides and Marine Biotoxins Unit, Department of Chemical Contaminants in Food, Maisons-Alfort Laboratory for Food Safety, ANSES, France
- P71 *Determination of zearalenone in edible oils from Germany*  
 F. Wöhrlin, M. Koch, C. Fauhl-Hassek and **Sabine Kemmlein**  
 Department Safety in the Food Chain, Federal Institute for Risk Assessment (BfR), Germany

- P72 *New mycotoxin reference materials: crucial tools for quality assurance and food safety*  
**Robert Köppen**, K. Klein-Hartwig, J. Riedel and M. Koch  
 Federal Institute for Materials Research and Testing (BAM), Germany
- P73 *Rapid classification of mycotoxin contaminated food commodities by infrared spectroscopy and chemometrics*  
**Gregor Kos**<sup>1</sup>, D. McMullin<sup>2</sup>, M. Sieger<sup>3</sup>, T. Öner<sup>3</sup>, C. Zahradnik<sup>2</sup>, B. Mizaikoff<sup>3</sup> and R. Krska<sup>2</sup>  
<sup>1</sup>Department of Atmospheric and Oceanic Sciences, McGill University, Canada, <sup>2</sup>Department IFA-Tulln, BOKU Vienna, Austria and <sup>3</sup>Institute of Analytical and Bioanalytical Chemistry, University of Ulm, Germany
- P74 *Evaluation of next generation liquid chromatography-single quadrupole mass spectrometry for screening and quantitative analysis of multiple mycotoxin in foods*  
**Veronica M.T. Lattanzio**<sup>1</sup>, B. Ciasca<sup>1</sup>, S. Stead<sup>2</sup>, E. McCall<sup>2</sup>, S. Powers<sup>3</sup> and A. Visconti<sup>1</sup>  
<sup>1</sup>Institute of Sciences of Food Production, National Research Council of Italy, Italy, <sup>2</sup>Waters Corporation, UK and <sup>3</sup>Vicam, USA
- P75 *Indirect methods for the determination of conjugated forms of deoxynivalenol in cereals*  
**Alexandra Malachova**<sup>1</sup>, L. Stockova<sup>2</sup>, A. Wakker<sup>3</sup>, E. Varga<sup>1</sup>, H. Michlmayr<sup>4</sup>, R. Krska<sup>1</sup>, G. Adam<sup>4</sup> and F. Berthiller<sup>1</sup>  
<sup>1</sup>Christian Doppler Laboratory for Mycotoxin Metabolism and Center for Analytical Chemistry, Department IFA-Tulln, BOKU Vienna, Austria, <sup>2</sup>Crop Research Institute, Czech Republic, <sup>3</sup>Department of Bioanalysis, Laboratory of Food Analysis, Ghent University, Belgium and <sup>4</sup>Department of Applied Genetics and Cell Biology, BOKU Vienna, Austria
- P76 *Determination of T-2 and HT-2 toxins from maize by direct analysis in real time-mass spectrometry (DART-MS)*  
**Chris Maragos** and M. Busman  
 National Center for Agricultural Utilization Research Peoria, USDA-ARS, USA
- P77 *Applicability of two new immunoassays to the screening of mycotoxins in feed: biochip-based immunoassay for a multi-analytical approach and ELISA for the detection of aflatoxin B1*  
 M. Plotan, R. Devlin, **Julie Meneely**, J. Porter, R.I. McConnell and S.P. FitzGerald  
 Radox Food Diagnostics, UK
- P78 *Masked trichothecene mycotoxins: enzymatic synthesis and hydrolysis by  $\beta$ -glucosidases*  
**Herbert Michlmayr**<sup>1</sup>, A. Malachova<sup>2</sup>, P. Fruhmann<sup>3</sup>, E. Varga<sup>2</sup>, W. Schweiger<sup>4</sup>, C. Hametner<sup>3</sup>, G. Wiesenberger<sup>1</sup>, M. Lemmens<sup>4</sup>, F. Berthiller<sup>2</sup> and G. Adam<sup>1</sup>  
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- P79 *Validation of a method for the analysis of sterigmatocystin in cereals using immunoaffinity columns*  
 P. Brown, E. Marley and **Claire Milligan**  
 R-Biopharm Rhône Ltd., UK
- P80 *Analysis of various cheese samples for aflatoxin M1 using immunoaffinity columns*  
 P. Brown, E. Marley, J. Wilcox and **Claire Milligan**  
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- P81 *Validation of an automated system for analysis of mycotoxins in a range of samples using online immunoaffinity cartridges in conjunction with HPLC*  
 R. Remnev, E. Marley, C. Donnelly, E. Manning and **Claire Milligan**  
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- P82 *Simultaneous determination of mycotoxins using AOF MS-Prep® in conjunction with LC-MS/MS*  
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- P83 *The quantitation of mycotoxins in cereals using a simple sample extraction and LC-MS/MS using fast polarity switching and MRM scheduling*  
**Harald Möller-Santner**<sup>1</sup>, S. Kreppenhofer<sup>1</sup>, K. von Czapiewski<sup>1</sup> and A. Schreiber<sup>2</sup>  
<sup>1</sup>AB Sciex, Germany and <sup>2</sup>AB Sciex, Canada
- P84 *Highly sensitive and rapid simultaneous method for 45 mycotoxins in baby food samples by HPLC-MS/MS using fast polarity switching*  
**Stéphane Moreau**<sup>1</sup> and M. Levi<sup>2</sup>  
<sup>1</sup>Shimadzu Europe, Germany and <sup>2</sup>Shimadzu France SAS, France
- P85 *Detection of glucoside derivatives of Fusarium mycotoxins (masked mycotoxins) by high-resolution LC-Orbitrap MS*  
**Hiroyuki Nakagawa**<sup>1</sup>, Y. Matsuo<sup>1</sup>, K. Takahara<sup>2</sup>, Y. Sago<sup>1</sup>, M. Kushiro<sup>1</sup> and H. Nagashima<sup>1</sup>  
<sup>1</sup>National Food Research Institute, National Agriculture and Food Research Organization, (NARO) and <sup>2</sup>Thermo Fisher Scientific, Japan
- P86 *Development and validation of an LC-MS/MS method for detection of Fusarium mycotoxins and their masked forms in cereals: application on Finnish grains*  
J. Helkama<sup>1</sup>, **Alexis Nathanail**<sup>1</sup>, M. Jestoi<sup>1</sup>, E. Varga<sup>2</sup>, A. Malachova<sup>2</sup>, H. Michlmayr<sup>3</sup>, E. Sieviläinen<sup>1</sup>, G. Adam<sup>3</sup>, F. Berthiller<sup>2</sup> and K. Peltonen<sup>4</sup>  
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- P87 *A fast and specific non-competitive immunoassay for HT-2 toxin*  
H. Arola<sup>1</sup>, A. Tullila<sup>1</sup>, H. Kiljunen<sup>2</sup>, H. Siitari<sup>1</sup> and **Tarja Nevanen**<sup>1</sup>  
<sup>1</sup>VTT Technical Research Centre of Finland and <sup>2</sup>Verifin, Finnish Institute for Verification of the Chemical Weapons Convention (Verifin), Department of Chemistry, University of Helsinki, Finland
- P88 *Measuring existing and emerging mycotoxins in animal feed ingredients*  
**Michalina Oplatowska-Stachowiak**<sup>1</sup>, S.A. Haughey<sup>1</sup>, O. Chevallier<sup>1</sup>, G. Adam<sup>2</sup>, F. Berthiller<sup>3</sup>, R. Krska<sup>3</sup> and C.T. Elliott<sup>1</sup>  
<sup>1</sup>Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, UK, <sup>2</sup>Department of Applied Genetics and Cell Biology, BOKU Vienna, Austria and <sup>3</sup>Center for Analytical Chemistry and Christian Doppler Laboratory for Mycotoxin Metabolism, Department IFA-Tulln, BOKU Vienna, Austria
- P89 *A comparison of HPTLC, HPLC-UV, ELISA and LC-MS/MS methods for the determination of deoxynivalenol in livestock feed*  
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Veterinary Drugs and Biologics Division, Animal and Plant Quarantine Agency (QIA), Korea
- P90 *Direct analysis of aflatoxin M1 in raw not skimmed milk using B Zero Afla M1 ELISA kit*  
F. Diana, E. Bianco and **Lidija Persic**  
Tecna s.r.l., Italy
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- P92 *Selection and characterisation of aptamers for the rapid detection of aflatoxin in maize*  
**Viktoria Preiser**<sup>1</sup>, R.L. Mach<sup>1</sup>, R. Krska<sup>2</sup> and K. Brunner<sup>1</sup>  
<sup>1</sup>Institute of Chemical Engineering, Vienna University of Technology and <sup>2</sup>Department IFA-Tulln, BOKU Vienna, Austria
- P93 *Proficiency testing as a tool to evaluate performance of LC-MS/(MS) multi-mycotoxin methods in view of EU regulation*  
**Kees van Putten**<sup>1</sup>, H.P. van Egmond<sup>2</sup> and M. Solfrizzo<sup>3</sup>  
<sup>1</sup>Proficiency Testing Department, Ducares B.V., the Netherlands, <sup>2</sup>RIKILT Wageningen UR, the Netherlands and <sup>3</sup>Institute of Sciences of Food Production, National Research Council, Italy

- P94 *Innovative solutions for mycotoxin in-process control and monitoring within grain handling*  
**Mareike Reichel**, S. Staiger and S. Biselli  
 Eurofins WEJ Contaminants, Germany
- P95 *Tracing the metabolisation of T-2 and HT-2 toxin in barley by LC-HRMS based stable isotope assisted metabolite profiling*  
**Jaqueline Reiterer**<sup>1</sup>, C. Büschl<sup>1</sup>, E. Varga<sup>1,2</sup>, B. Kluger<sup>1</sup>, M. Sulyok<sup>1</sup>, I. Maloku<sup>3</sup>, F. Berthiller<sup>1,2</sup>, R. Schuhmacher<sup>1</sup> and M. Lemmens<sup>3</sup>  
<sup>1</sup>Center for Analytical Chemistry, <sup>2</sup>Christian Doppler Laboratory for Mycotoxin Metabolism and <sup>3</sup>Institute of Biotechnology in Plant Production, Department IFA-Tulln, BOKU Vienna, Austria
- P96 *Fusarium species fast identification and associated mycotoxins detection by MALDI-TOF*  
 J. Billard<sup>1</sup>, S. Heit<sup>1</sup>, P. Hart<sup>2</sup>, C. Paris<sup>3</sup>, R. Iloos<sup>4</sup>, O. Belgacem<sup>2</sup>, X. Framboisier<sup>1</sup> and **Emmanuel Rondags**<sup>1</sup>  
<sup>1</sup>Laboratoire Réactions et Génie des Procédés, CNRS, UMR 7274, Université de Lorraine, France, <sup>2</sup>Kratos Analytical, UK, <sup>3</sup>Ensaia, Laboratoire commun d'analyses, France and <sup>4</sup>Unité de Mycologie, Laboratoire de la Santé des Végétaux, ANSES, France
- P97 *Extraction of fumonisins using a water-based extraction method*  
**Alois Schiessl**<sup>1</sup>, B. Cvak<sup>1</sup> and M. Zheng<sup>2</sup>  
<sup>1</sup>Romer Labs Division Holding GmbH, Austria and <sup>2</sup>Romer Labs Singapore Pte. Ltd., Singapore
- P98 *Integration of DON extraction into a water-based multi-extraction method for mycotoxins in food and feed*  
**Alois Schiessl**<sup>1</sup>, B. Cvak<sup>1</sup> and M. Zheng<sup>2</sup>  
<sup>1</sup>Romer Labs Division Holding GmbH, Austria and <sup>2</sup>Romer Labs Singapore Pte. Ltd., Singapore
- P99 *Mid-infrared spectroscopy based on GaAs thin-film waveguide and quantum cascade laser technology as a tool for the detection of deoxynivalenol (DON) in maize extracts*  
**Markus Sieger**<sup>1</sup>, D. McMullin<sup>2</sup>, T. Oener<sup>1</sup>, G. Kos<sup>3</sup>, R. Krska<sup>2</sup> and B. Mizaikoff<sup>1</sup>  
<sup>1</sup>Institute of Analytical and Bioanalytical Chemistry, University of Ulm, Germany, <sup>2</sup>Department IFA-Tulln, BOKU Vienna, Austria and <sup>3</sup>Department of Atmospheric and Oceanic Sciences, McGill University, Canada
- P100 *A near infrared spectroscopy method for the rapid detection of aflatoxin B1 contamination in rice*  
 R. Putthang<sup>1</sup>, P. Sirisomboon<sup>2</sup> and **Cheewanun Dachoupan Sirisomboon**<sup>1</sup>  
<sup>1</sup>Department of Microbiology, Chulalongkorn University and <sup>2</sup>Curriculum of Agricultural Engineering, Department of Mechanical Engineering, King Mongkut 's Institute of Technology Ladkrabang, Thailand
- P101 *Environmentally friendly and cost-efficient analysis of aflatoxins in maize*  
**Victor Sobolev**  
 National Peanut Research laboratory, Agricultural Research Service, United States Department of Agriculture, USA
- P102 *Analytical method validation and monitoring of 12 mycotoxins in wheat grain, processed milling products, at a field study and at different points of Brazilian supply and commercial chains*  
 I.R. Pizzutti<sup>1</sup>, **Martien C. Spanjer**<sup>2</sup>, D.M. Souza<sup>1</sup>, J.M. Scholten<sup>3</sup>, A. de Kok<sup>3</sup>, J.V. Dias<sup>1</sup>, C.D. Cardoso<sup>1</sup>, C.W. Guterres<sup>4</sup>, J.S. Bruinsma<sup>4</sup>, R. Boss<sup>4</sup>, A.P.F. Valentini<sup>4</sup> and J.F. Wagner<sup>4</sup>  
<sup>1</sup>Center of Research and Analysis of Residues and Contaminants, Federal University of Santa Maria, Brazil, <sup>2</sup>Surveillance Development Department, Netherlands Food and Consumer Product Safety Authority Utrecht, the Netherlands, <sup>3</sup>Laboratory of Food and Feed Safety, Netherlands Food and Consumer Product Safety Authority, the Netherlands and <sup>4</sup>CCGL Technology-Central Cooperative of RS State, Brazil
- P103 *Rapid and simultaneously screening and quantitation of mycotoxins in different matrix using high resolution MS/MS instrument*  
**Jianru Stahl-Zeng**, S. Lock and D. Schleuder  
 AB Sciex, Germany

- P104 *Using ion mobility mass spectrometry and collision cross section areas to elucidate the  $\alpha$  and  $\beta$  epimeric forms of glycosylated T-2 toxin*  
**Sara Stead**<sup>1</sup>, M. McCullagh<sup>1</sup>, M. Busman<sup>2</sup>, S. McCormick<sup>2</sup>, D. Crich<sup>3</sup>, T. Kato<sup>3</sup>, V. Lattanzio<sup>4</sup> and C. Maragos<sup>2</sup>  
<sup>1</sup>Waters Corporation, UK, <sup>2</sup>National Center for Agricultural Utilization Research, USDA-ARS, USA, <sup>3</sup>Wayne State University, USA and <sup>4</sup>Institute of Sciences of Food Production, National Research Council of Italy, Italy
- P105 *In-house validation of a sample preparation method for analysis of fumonisin B1 in rice*  
M.H. Petrarca, E.A. Rossi and **Célia M. Sylos**  
Department of Food and Nutrition, Faculty of Pharmaceutical Sciences, São Paulo State University, Brazil
- P106 *Determination of the proportion of matrix-associated fumonisin B1 in different, animal feeding experiment-aided matrices after in vitro digestion*  
**Judit Szabó-Fodor**<sup>1</sup>, C. Dall'Asta<sup>2</sup>, C. Falavigna<sup>2</sup>, M. Kachlek<sup>1</sup>, A. Szabó<sup>1</sup> and M. Kovács<sup>1</sup>  
<sup>1</sup>MTA-KE Mycotoxins in the Food Chain Research Group, Faculty of Agricultural and Environmental Sciences, Kaposvár University, Hungary and <sup>2</sup>Department of Food Science, University of Parma, Italy
- P107 *Analysis of 12 mycotoxins in calves' milk replacer by means of UHPLC-MS/MS*  
**Els Van Pamel**<sup>1</sup>, G. Antonissen<sup>2,3</sup>, B. Valgaeren<sup>4</sup>, S. De Saeger<sup>5</sup>, S. Croubels<sup>3</sup>, and E. Daeseleire<sup>1</sup>  
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- P108 *NIR perspectives to detect Datura seeds and ergot bodies in cereals*  
**Philippe Vermeulen**, J.A. Fernández Pierna, P. Dardenne and V. Baeten  
Food and Feed Quality Unit, Valorisation of Agricultural Products Department, Walloon Agricultural Research Centre (CRA-W), Belgium
- P109 *Development and validation of an LC-MS/MS method for the simultaneous determination of type B trichothecenes*  
**Gorica Vuković**<sup>1</sup>, M. Starović<sup>2</sup>, V. Bursić<sup>3</sup>, S. Pavlović<sup>2</sup>, I. Jajić<sup>3</sup>, S. Krstović<sup>3</sup> and S. Starović<sup>2</sup>  
<sup>1</sup>Institute for Public Health of Belgrade, <sup>2</sup>Institute for Pesticides and Environmental Protection, and <sup>3</sup>Faculty of Agriculture, University of Novi Sad, Serbia
- P110 *Design, synthesis and efficacy evaluation of aflatoxin B1 analog template used for molecularly imprinted polymers production as possible high affinity and specificity sorptive material for aflatoxins*  
T.R. Yerramreddy, D. Davenport, J. Martinez, J. Withers and **Alexandros Yiannikouris**  
Center for Animal Nutrigenomics and Applied Animal Nutrition, Alltech, Inc., USA
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K.Y. Yeong, W.T. Ng and **Michael Zheng**  
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J.A. Torres Acosta<sup>1</sup>, U. Güldener<sup>2</sup>, E. Kunz-Vekiru<sup>3</sup>, C. Hametner<sup>4</sup>, C. Schmeitzl<sup>1</sup>, M. Münsterkötter<sup>2</sup>, R. Mitterbauer<sup>1</sup>, H. Bachmann<sup>1</sup>, J. Drexler<sup>1</sup>, G. Niederacher<sup>1</sup>, G. Wiesenberger<sup>1</sup>, A. Musilek<sup>5</sup>, D. Moll<sup>6</sup> and **Gerhard Adam**<sup>1</sup>  
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<sup>4</sup>Institute of Applied Synthetic Chemistry, Vienna University of Technology, Austria, <sup>5</sup>Institute of Atomic and Subatomic Physics, Vienna University of Technology, Austria and <sup>6</sup>BiomIn Research Center, Austria

- P113 *Creating novel approaches to mitigate aflatoxin risk in food and feed with lactic acid bacteria – mould growth inhibition and aflatoxin binding*  
**Sara H. Ahlberg**<sup>1,2</sup>, V. Joutsjoki<sup>1</sup> and H.J. Korhonen<sup>1</sup>  
<sup>1</sup>MTT Agrifood Research, Finland and <sup>2</sup>International Livestock Research Institute, Kenya
- P114 *Enzymatic hydrolysis of fumonisins: paving the way for a unique feed additive*  
**Markus Aleschko**<sup>1</sup>, D. Hartinger<sup>1</sup>, C. Kern<sup>1</sup>, P. Menczik<sup>1</sup>, A. Frank<sup>1</sup>, H. Schwartz-Zimmermann<sup>2</sup>, B. Grenier<sup>3</sup>, I.P. Oswald<sup>3</sup>, G. Schatzmayr<sup>1</sup> and W.-D. Moll<sup>1</sup>  
<sup>1</sup>BiomIn Research Center, Austria, <sup>2</sup>Christian Doppler Laboratory for Mycotoxin Metabolism, Department IFA-Tulln, BOKU Vienna, Austria and <sup>3</sup>INRA, Toxalim, France
- P115 *Efficacy of a mycotoxin deactivator (Unlike<sup>®</sup> Plus) to ameliorate the toxicity of a combination of mycotoxins in broiler chicks*  
T. Shannon<sup>1</sup>, D. R. Ledoux<sup>1</sup>, G. E. Rottinghaus<sup>1</sup>, D. Shaw<sup>1</sup>, **Olga Averkieva**<sup>2</sup> and E. Kobashigawa<sup>1</sup>  
<sup>1</sup>University of Missouri, USA and <sup>2</sup>Nutriad International, Belgium
- P116 *In vivo confirmation of broad spectrum efficacy of the mycotoxin binder Toxfin<sup>™</sup>*  
**Monique Baecke** and N. Tallorico  
Kemin Europa N.V., Belgium
- P117 *Characterisation of ergopeptine-hydrolase ErgA*  
**Verena Klingenbrunner**<sup>1</sup>, M. Thamhesl<sup>1</sup>, I. Schöner<sup>2</sup>, H. Schwartz-Zimmermann<sup>2</sup>, F. Berthiller<sup>2</sup>, G. Schatzmayr<sup>1</sup> and W.-D. Moll<sup>1</sup>  
<sup>1</sup>BiomIn Research Center and <sup>2</sup>Christian Doppler Laboratory for Mycotoxin Metabolism, Department IFA-Tulln, BOKU Vienna, Austria
- P118 *Use of a cell-wall deficient, selected lactic acid bacterium for the control of deoxynivalenol challenge in a commercial sow operation*  
J. Lorenz<sup>1</sup>, M.C. Christman<sup>2</sup> and **John A. Doerr**<sup>1</sup>  
Agrarian Marketing Corp., USA, MCC Statistical Consulting and Departments of Biology and Statistics, University of Florida, USA
- P119 *The anti-aflatoxigenic efficacy of Cynara cardunculus L. in sesame seeds (Sesamum indicum)*  
**Eleni Kollia**<sup>1</sup>, P. Markaki<sup>1</sup>, C. Proestos<sup>1</sup> and P. Zoumpoulakis<sup>2</sup>  
<sup>1</sup>Department of Food Chemistry, School of Chemistry, National and Kapodistrian University of Athens and <sup>2</sup>Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, Greece
- P120 *Reduction of the aflatoxins B1, B2, G1 and G2 in Italian piadina by isothiocyanates present in oriental and yellow mustard flours*  
**Fernando B. Luciano**<sup>1</sup>, L. Manyes<sup>2</sup>, J. Mañes<sup>2</sup> and G. Meca<sup>2</sup>  
<sup>1</sup>School of Agricultural Sciences and Veterinary Medicine, Pontifícia Universidade Católica do Paraná, Brazil and <sup>2</sup>Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Spain
- P121 *Antimicrobial devices containing allyl isothiocyanate to improve shelf life and safety of sliced mozzarella cheese*  
**Fernando B. Luciano**<sup>1</sup>, B.L. Tracz<sup>1</sup>, J. Mañes<sup>2</sup> and G. Meca<sup>2</sup>  
<sup>1</sup>School of Agricultural Sciences and Veterinary Medicine, Pontifícia Universidade Católica do Paraná, Brazil and <sup>2</sup>Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Spain
- P122 *Isolation and efficacy of biocontrol microorganisms from maize for inhibiting growth and fumonisin production by Fusarium verticillioides*  
N. I. Putra Samsudin, **Naresh Magan** and A. Medina-Vaya  
Applied Mycology Group, Cranfield Soil and AgriFood Institute, School of Energy, Environment and AgriFood, Cranfield University, UK

- P123 *Targeting Fusarium graminearum control via polyamine enzyme inhibitors and polyamine analogues*  
A. Crespo-Sempere, N. Estiarte, **Sonia Marín**, V. Sanchis and A.J. Ramos.  
Applied Mycology Unit, Food Technology Department, University of Lleida, Spain
- P124 *Biotransformation of deoxynivalenol to the metabolite de-epoxy-deoxynivalenol analysed in serum of pigs*  
**Sabine Masching**<sup>1</sup>, S. Schaumberger<sup>1</sup>, D. Schatzmayr<sup>2</sup> and G. Schatzmayr<sup>2</sup>  
<sup>1</sup>Biomin Holding and <sup>2</sup>Biomin Research Center, Austria
- P125 *Evaluation of the efficacy of three anti-mycotoxin additives on the toxicological effects of aflatoxins, fumonisins and T-2 toxin in broiler chickens and the determination of the in vitro coefficient of adsorption of aflatoxin B1*  
**Anna K. Oudshoorn** and Y.M. Han  
Nutreco Research & Development, the Netherlands
- P126 *Integrating ecological and molecular diagnostics data to prevent contamination of cured meats with ochratoxin A*  
**Alicia Rodríguez**<sup>1</sup>, Á. Medina<sup>1</sup>, J.J. Córdoba<sup>2</sup> and N. Magan<sup>1</sup>  
<sup>1</sup>Applied Mycology Group, Cranfield Soil and AgriFood Institute, Cranfield University, UK and <sup>2</sup>University of Extremadura, Spain
- P127 *Deoxynivalenol (DON) sulfonates: detoxification products and natural DON metabolites in rats*  
**Heidi E. Schwartz-Zimmermann**<sup>1</sup>, C. Hametner<sup>2</sup>, M. Paulick<sup>3</sup>, S. Dänicke<sup>3</sup>, S. Kersten<sup>3</sup>, V. Slavik<sup>1</sup>, D. Schatzmayr<sup>4</sup> and F. Berthiller<sup>1</sup>  
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- P128 *Deoxynivalenol degradation in crimped grain by ubiquitous silage bacteria*  
**Will Vevers**, J. Beal and R. Billington  
University of Plymouth, UK
- P129 *Reducing type B trichothecenes levels in maize by removal of fines*  
**Gorica Vuković**<sup>1</sup>, V. Bursić<sup>2</sup>, J. Kos<sup>3</sup>, R. Čolović<sup>3</sup>, D. Vukmirović<sup>1</sup> and J. Lević<sup>1</sup>  
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## Factors affecting toxin formation

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- P130 *RNA interference reduces aflatoxin accumulation by Aspergillus flavus in peanut seeds*  
**Renée S. Arias**, P.M. Dang and V.S. Sobolev  
National Peanut Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, USA
- P131 *Effect of drying treatments on fungal incidence and fumonisin contamination in maize kernels*  
P. Giorni<sup>1</sup>, C. Dall'Asta<sup>2</sup>, M. Cirlini<sup>2</sup>, G. Galaverna<sup>2</sup> and **Paola Battilani**<sup>1</sup>  
<sup>1</sup>Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore and <sup>2</sup>Dipartimento di Scienze degli Alimenti, Università di Parma, Italy
- P132 *Contamination of wheat grains with Fusarium mycotoxins, depending on variety and mill fraction*  
**Edyta Boguszevska** and G. Podolska  
Institute of Soil Science and Plant Cultivation, State Research Institute in Puławy, Poland
- P133 *The influence of maize dry milling on the content of free and masked fumonisins in model conditions*  
**Marcin Bryła**<sup>1,2</sup>, M. Roszko<sup>1</sup>, K. Szymczyk<sup>1</sup> and R. Jędrzejczak<sup>1</sup>  
<sup>1</sup>Department of Food Analysis, Institute of Agricultural and Food Biotechnology and <sup>2</sup>Faculty of Food Sciences, Warsaw University of Life Sciences, Poland

- P134 *Ochratoxin A in grapes: development of a prototype predictive model*  
**Marco Camardo Leggieri** and P. Battilani  
 Institute of Entomology and Plant Pathology, Università Cattolica Sacro Cuore, Italy
- P135 *In vitro study of the isoeopoxydon dehydrogenase gene expression in relation to the patulin production of *Penicillium expansum* on apple puree agar medium under different storage conditions*  
**Nikki De Clercq**<sup>1</sup>, G. Vlaemynek<sup>1</sup>, E. Van Pamel<sup>1</sup>, L. Herman<sup>1</sup>, F. Devlieghere<sup>2</sup>, B. De Meulenaer<sup>2</sup> and E. Van Coillie<sup>1</sup>  
<sup>1</sup>Technology and Food Science Unit, Institute for Agricultural and Fisheries Research (ILVO) and <sup>2</sup>Department of Food Safety and Food Quality, Ghent University, Belgium
- P136 *Checking for defective maize kernels to forecast mycotoxins contamination trends*  
 C. Brera, M.C. Magri, G. Trifirò, F. Debegnach, E. Gregori, G. Moracci and **Barbara De Santis**  
 GMO and Mycotoxin Unit, Department of Veterinary Public Health and Food Safety, Italian National Institute for Health, Italy
- P137 *A review on the impact of GM crops on their capacity to control mycotoxin content*  
**Barbara De Santis**<sup>1</sup>, M. De Giacomo<sup>1</sup>, R. Onori<sup>1</sup>, G. Kleter<sup>2</sup> and C. Brera<sup>1</sup>  
<sup>1</sup>GMO and Mycotoxin Unit, Department of Veterinary Public Health and Food Safety, Italian National Institute for Health, Italy and RIKILT Wageningen UR, the Netherlands
- P138 *Effect of pre-harvest rainfall on the concentration and distribution of deoxynivalenol and zearalenone in subsequent mill fractions of wheat*  
**Simon G. Edwards**, L.L. Kharbikar and E. T. Dickin  
 Harper Adams University, UK
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**Edson Ncube**<sup>1</sup>, B.C. Flett<sup>1,2</sup>, J. Van den Berg<sup>2</sup>, A. Erasmus<sup>1</sup> and A. Viljoen<sup>3</sup>  
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 C. Nobili<sup>1</sup>, G. Chitarrini<sup>2</sup>, F. Pinzari<sup>3</sup> and **Massimo Reverberi**<sup>4</sup>  
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- P154 *Lipid signals in the interaction between mycotoxigenic fungi and their hosts*  
 M. Reverberi<sup>1</sup>, **Valeria Scala**<sup>1</sup>, C. Dall'Asta<sup>2</sup>, M. Cirilini<sup>2</sup>, P. Giorni<sup>3</sup>, P. Battilani<sup>3</sup>, E. Camera<sup>4</sup>, M. Ludovici<sup>4</sup>, C. Nobili<sup>5</sup>, A.A. Fabbri<sup>1</sup> and C. Fanelli<sup>1</sup>  
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- P155 *Emerging mycotoxins on cereals in North Italy: the role of environmental and agronomic conditions on their occurrence*  
**Valentina Scarpino**<sup>1</sup>, F. Vanara<sup>1</sup>, M. Sulyok<sup>2</sup>, M. Blandino<sup>1</sup> and A. Reyneri<sup>1</sup>  
<sup>1</sup>Department of Agricultural, Forest and Food Sciences, University of Turin, Italy and <sup>2</sup>Center for Analytical Chemistry, Department IFA-Tulln, BOKU Vienna, Austria
- P156 *Biosynthesis and accumulation of fumonisins by diverse *F. proliferatum* strains in myceliar and liquid culture samples*  
**Łukasz Stępień**<sup>1</sup>, A. Waśkiewicz<sup>2</sup> and K. Wilman<sup>1</sup>  
<sup>1</sup>Department of Pathogen Genetics and Plant Resistance, Institute of Plant Genetics, Polish Academy of Sciences and <sup>2</sup>Department of Chemistry, Poznań University of Life Sciences, Poland
- P157 *Fumonisin content in maize elite inbred lines after inoculation with *Fusarium verticillioides**  
A. Waśkiewicz A.<sup>1</sup>, **Łukasz Stępień**<sup>2</sup> and E. Czembor<sup>3</sup>  
<sup>1</sup>Department of Chemistry, Poznań University of Life Sciences, <sup>2</sup>Institute of Plant Genetics, Polish Academy of Sciences and <sup>3</sup>Plant Breeding and Acclimatization Institute – NRI, Poland
- P158 *Varietal resistance in UK oats to HT-2 and T-2 producing *Fusarium langsethiae**  
**Tijana Stancic**<sup>1</sup>, A.A. Cowan<sup>2</sup>, C.J. Howarth<sup>2</sup> and S.G. Edwards<sup>1</sup>  
<sup>1</sup>Harper Adams University College and <sup>2</sup>IBERS, Aberystwyth University, UK
- P159 *Is height a susceptibility trait to *Fusarium langsethiae* infection on oats?*  
**Tijana Stancic**<sup>1</sup>, A.A. Cowan<sup>2</sup>, C.J. Howarth<sup>2</sup> and S.G. Edwards<sup>1</sup>  
<sup>1</sup>Harper Adams University College and <sup>2</sup>IBERS, Aberystwyth University, UK
- P160 *Reaction of winter triticale breeding lines to *Fusarium* head blight and accumulation of *Fusarium* metabolites in grain in two environments*  
**Halina Wiśniewska**<sup>1</sup>, T. Góral<sup>2</sup>, P. Ochodzki<sup>2</sup>, D. Walentyn-Góral<sup>2</sup>, I. Grzeszczak<sup>2</sup>, J. Bełtr<sup>1</sup>, Z. Banaszak<sup>3</sup>, M. Pojmaj<sup>3</sup>, D. Kurlito<sup>3</sup>, M. Konieczny<sup>3</sup>, G. Budzianowski<sup>4</sup>, A. Cicha<sup>4</sup>, K. Paizert<sup>4</sup> and H. Woś<sup>4</sup>  
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- P161 *Modelling the growth and mycotoxins production of *Aspergillus flavus* and *Aspergillus parasiticus* isolates in black pepper (*Piper nigrum* L.)*  
**Pratheeba Yogendrarajah**<sup>1,2</sup>, A. Vermeulen<sup>3</sup>, L. Jacxsens<sup>1,3</sup>, S. De Saeger<sup>2</sup>, B. De Meulenaer<sup>1</sup> and F. Devlieghere<sup>3</sup>  
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- P162 *RNAseq analysis of *Aspergillus flavus* transcriptome expressed during stressing growth conditions (oxidative stress and hypoxia) and consequential aflatoxin B1 synthesis*  
**Marco Zaccaria**<sup>1</sup>, M. Scarpari<sup>1</sup>, V. Scala<sup>1</sup>, S. Sanzani<sup>2</sup>, W. Sanseverino<sup>3</sup>, C. Fanelli<sup>1</sup>, A.A. Fabbri<sup>1</sup> and M. Reverberi<sup>1</sup>  
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## OCCURRENCE

### P1 – P32

#### P1

### **Mycotoxin contamination and occurrence of *Fusarium* ear rot on maize and *Fusarium* head blight on winter wheat in Kosovo**

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In Kosovo approximately 80,000-100,000 ha of wheat and maize are cultivated annually. Wheat is an important cereal crop in Kosovo and a major component of population food. After wheat, maize is the second most important cereal crop and a major component of animal feeds. The aim of the two-years-research work (2009 and 2010 on maize, 2010 and 2011 on winter wheat) was to analyse the mycotoxin contamination as well as the incidence and identity of the *Fusarium* species isolated from naturally infected wheat and maize kernels in Kosovo. Detection and quantification of the mycotoxins in maize and wheat was carried out by high performance liquid chromatography-tandem mass spectrometry. Maize samples found to be contaminated with deoxynivalenol (DON), DON-3-glucoside, 3-acetyl-DON, 15-acetyl-DON, zearalenone (ZEN), ZEN-14-sulfate, moniliformin (MON), fumonisin B1 (FB1) and fumonisin B2. On wheat samples, the following substances were detected: MON, DON, DON-3-glucoside, 3-acetyl-DON, 15-acetyl-DON, ZEN, FB1, nivalenol, enniatin A, enniatin A1, enniatin B, enniatin B1, enniatin B2 and culmorin. The results achieved through relevant field and laboratory analyses from naturally infected maize and wheat ears showed that the disease incidence of *Fusarium* ear rot (40%) and incidence of *Fusarium* head blight (32%) in Kosovo is high. The most frequently *Fusarium* spp. identified on maize kernels were *Fusarium subglutinans*, *F. verticillioides*/*F. proliferatum* and *F. graminearum*. On wheat kernels the most frequently *Fusarium* spp. identified was *F. graminearum*. Less frequently isolated species included *F. cerealis* and *F. avenaceum*. Still, to date the information on mycotoxin contamination on wheat and maize in Kosovo is very limited.

#### P2

### ***Aspergillus* and *Penicillium* toxins in chestnuts and derived products produced in Italy**

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Occurrence of aflatoxins, ochratoxin A, sterigmatocystin, ciclopiazonic acid, citrinin, roquefortine C and mycophenolic acid in fresh chestnuts and dried products was surveyed. A total of eighty-two samples were collected from retail outlets located in northern Italy. After specific extraction and purification through immunoaffinity or prepacked columns, mycotoxins were analysed using HPLC-FLD or HPLC-MS/MS. In fresh chestnuts, mycotoxins were rarely detected; on the other hand, widespread contaminations were found in dried products, particularly in chestnut flour. The incidence of aflatoxin B1 was 92.0 and 40.0% in chestnut flour (maximum value 58.6 µg/kg) and dried chestnuts, respectively; in chestnut flour, the percentage of samples exceeding the value of 2.0 µg/kg for aflatoxin B1 (maximum limit fixed by EC Regulation 165/2010 in dried fruits) was 24.0%. Chestnut flour was also often contaminated with ochratoxin A, citrinin, roquefortine C and mycophenolic acid, showing sometimes high values (particularly for mycophenolic acid); in 80% of samples, more of four mycotoxins were detected. To the best of our knowledge, in previous studies no data were reported about the occurrence of citrinin, roquefortine C and mycophenolic acid in chestnuts and derived products. These results showed that probably the contamination occurred in post-harvest, during exsiccation, storage and sorting; the high incidence and concentration found in chestnut flour are probably due to the practice of obtaining it by grinding the product coming from sieving of dried chestnuts.

### P3

## Occurrence of free and masked fumonisins in maize products available on the Polish market

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Fumonisins are secondary metabolites frequently found on maize infected by *Fusarium verticilloides* and *Fusarium proliferatum*. Fumonisins are regarded as hazardous mycotoxins that could induce negative health effects in mammals. This is particularly important in case of fumonisins classified as B type. Fumonisin B1 was classified by the International Agency for Research on Cancer to the 2B toxicity group. In recent years, it has been reported that fumonisins might occur in food products in so-called masked forms. These include fumonisins non-covalently interacting (hidden fumonisins) with food macromolecules such as starch or proteins. It has been reported that the concentration of hidden fumonisins in food products might be higher than the concentration of the free analogues. The aim of this work was to assess the concentrations of free and total fumonisins (B1, B2 and B3) in maize-based food products. The study framework covered 88 maize-based food products commercially available on the Polish market. Samples collected in 2013 included groats (n=15), starch concentrates (n=6), noodles (n=14), flour (n=20), maize flakes (n=19) and maize snacks (n=14). Ion trap mass spectrometer coupled to high performance liquid chromatograph was used for the purpose of this study. Isotope <sup>13</sup>C-labelled internal standards of B1, B2 and B3 fumonisins were used to assure method performance. Free fumonisins were separated and cleaned up using molecularly imprinted polymers (FumoZON AFFINIMIP; Polyintell, Val de Reuil, France). Total fumonisins concentration was determined after sample digestion in 2M KOH. The concentration of the masked fumonisins was calculated as a difference between the total and free fumonisins concentrations. Free fumonisins at concentrations above the limit of quantification (LOQ) were found in 50 samples (57%) with an average concentration of 390±676 µg/kg, 68 samples (77%) were classified as positive, with respect to the total fumonisins content (mean 574±1,177 µg/kg). In the studied group of maize products, the highest concentrations of both free and total fumonisins were observed in maize snacks. The average concentration of free fumonisins was 1,006±1,131 µg/kg, while total fumonisins concentration was 1,651±2,317 µg/kg. This group was also the most contaminated with fumonisins. The highest observed concentrations were 3,297 µg/kg (free ΣFBs) and 7,331 µg/kg (total ΣFBs). The lowest fumonisin concentrations were observed in maize starch concentrates. None of the testes concentrate samples had free fumonisins concentration above LOQ, while mean average total ΣFBs concentration was 82±42 µg/kg. The mean concentrations of hidden fumonisins varied among the tested food groups, and were in some samples higher than the concentrations of the free forms. **Acknowledgements.** This study was partly supported by the 2012/05/N/NZ9/01316 National Science Centre grant.

### P4

## Occurrence of citrinin in food and feed in Belgium

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Citrinin is a mycotoxin produced by several species of the genera *Aspergillus*, *Penicillium* and *Monascus* and occurs mainly in stored grains. Citrinin is nephrotoxic in animals and it has been implicated as a potential factor in human endemic Balkan nephropathy together with ochratoxin A, also a nephrotoxic mycotoxin produced by the same fungal species. The EFSA CONTAM Panel concluded that the impact of uncertainties on the risk assessment is large, and more data regarding the toxicity and the occurrence of citrinin in food and feed in Europe are needed to enable refinement of the risk assessment (EFSA, 2012). Recent results from our laboratory showed that citrinin or/and its metabolite dihydrocitrinone can be detected in 90 % of human urine samples (submitted and unpublished data) indicating the exposure to citrinin might be more important than has been assumed so far. This encouraged us to start a survey on the presence of citrinin in food and feed bought in Belgium. An LC-MS/MS method was established and validated for several matrices. The presence of citrinin in the food and feed samples will be reported.

## P5

### Aflatoxin M1 detection in Italian infant formula milk

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Food safety is a crucial topic that is a major concern both to consumers and authorities worldwide. A particular concern is primarily directed to some sensitive group of consumers that include infants or people who are affected by disease. The aim of this study was to assess the risk of aflatoxin M1 (AFM1) exposure by quantifying such mycotoxin in leading-brand infant formula milk products. Aflatoxins are toxic secondary metabolites produced by *Aspergillus flavus* and *A. parasiticus* fungal species. They are known to be the most potent natural toxicants of food origin. Aflatoxin M1 is the major metabolite of aflatoxin B1 (AFB1), as it is the product of the carryover of AFB1 when cows are fed with contaminated feed. As the diets of infants are mainly or exclusively based on milk, and taking into consideration baby body weight and the undeveloped infants' metabolism, the European Commission set a maximum level of AFM1 in infant milk and milk products of 0.025 µg/kg. It is a lower value compared with the maximum level in milk for adults (European Commission CE N.1881/2006). In this study, one-hundred and forty four infant formula milk samples were collected from the Italian market, including: follow-up formula (n=64, for infants from 6 months to 10 months), toddler formula (n=58 for infants from 10 months upwards) and 12 vaccine milk products. Among the samples, 60 were supplied as powder and 74 as liquid infant milk. With regard to AFM1 detection in milk and milk powder, the reference method UNI EN ISO 14501 was adopted with minor modifications. To this extent, the analytical method using immunoaffinity column clean-up and high-performance liquid chromatography (HPLC) with fluorimetric detection was preliminarily in-house validated, according to Eurachem guidelines. The obtained validation parameters (repeatability, reproducibility, accuracy) were compliant with the criteria as defined in the EU legislation (Commission Regulation (EC) No 401/2006). Limit of quantification (LOQ) was also calculated. In all cases, the obtained results fell below the maximum limit as set by Commission Regulation (EC) No 1881/2006. More specifically, as far as liquid milk is concerned, LOQ was 0.004 µg/kg, whereas for powder milk LOQ was 0.009 µg/kg. The results showed a lower AFM1 occurrence in liquid milk formula than in powder formula, ranging 0.004-0.011 µg/kg (mean value±SD, 0.008±0.002 µg/kg) and 0.009-0.028 µg/kg (mean value±SD, 0.018 ± 0.008 µg/kg), respectively. **Acknowledgements.** The study is supported by AIIPA (Associazione Italiana Prodotti Alimentari).

## P6

### Mycotoxin survey in plant and dairy waste streams for feed production: the NOSHAN approach

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Food processing activities in Europe produce large amounts of by-products and waste. The food loss and waste generated per capita in Europe has been estimated in the range 280-300 Kg/year; among whose, fruits (16.4%) and vegetables (25.8%) have the highest wastage rates. Such waste streams are only partially valorised at different value-added levels, although the main volumes are still managed as waste of environmental concern. The main focus of NOSHAN project (EU FP7, Grant Agreement 312140) is to investigate the process and technologies needed to use food waste for feed production at low cost, low energy consumption and with maximal valorisation of starting wastes materials. Nutritional value and functionality according to animal needs as well as safety and quality issues are addressed as main leading factors for the feed production using food derived wastes. The overall strategy for waste valorisation into feed and feed ingredients starts with the characterisation of plant and dairy waste streams according to their quality (i.e., bioactive compounds, nutritional factors) and safety (i.e., pathogens, mycotoxins) features. Selected materials undergo to technological treatments for stabilization and/or separation of components of interest before entering the bulk feed production. In consideration of the waste origin and of the water content of the material, toxinogenic mould growth may be supported, thus mycotoxin contamination should be monitored in raw materials and along the feed production chain to ensure the compliance of the final feed to the EU regulation. Mycotoxin occurrence in vegetable and dairy wastes was screened in 42 waste samples, considering both those compounds reasonably occurring in the original food as well as those potentially formed during waste storage. Aflatoxins and ochratoxin A were never detected at significant levels in the

considered samples. Concerning other mycotoxins, *Fusarium* toxins were found in spent grains, while patulin was found in apple and olive pomaces. The results clearly indicate that only those mycotoxins already occurring in the fresh products are found in the final waste. Further steps will include the monitoring of possible mycotoxin concentration/transformation along the bulk feed production chain.

#### P7

### **An Italian monitoring study for the evaluation of 'emerging mycotoxins', enniatins, beauvericin and citrinin profile in domestic wheat samples**

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Mycotoxins are toxic secondary metabolites produced under appropriate environmental conditions by certain fungi on a variety of crops. The contamination of foods and feeds with mycotoxins is of worldwide concern. In the European Union, maximum limits in food sector have been set for aflatoxins, ochratoxin A, patulin, thricothecenes and zearalenone. However, the most common grain-contaminating genera of fungi, *Fusarium* spp., *Penicillium* spp. and *Aspergillus* spp., are also capable of producing other toxic secondary metabolites, such as enniatins, beauvericin and citrinin, which are called 'emerging mycotoxins', since their interest is currently growing. A monitoring study to evaluate the presence of enniatins, beauvericin and citrinin in wheat was conducted on samples collected during 2012 and 2013 in Italy (Emilia Romagna, Marche, Latium, Tuscany, Molise, Sardinia, Sicily). A UPLC MS-MS multimycotoxin analytical method was preliminarily in house validated, according to Eurachem guidelines, for the identification and quantification of enniatins (A, A1, B, B1), beauvericin and citrinin. Limits of quantification (LOQ) were evaluated: 8 µg/kg for ENNs and BEA, 40 µg/kg for CIT. The monitoring performed showed a general increase in the levels of contamination for the harvest of 2013 in comparison with 2012 both for the average level of contamination (2012 ranges: ENNs 8-1492 µg/kg, beauvericin 45 µg/kg and citrinin <LOQ; 2013 ranges: ENNs 8-3,566 µg/kg, beauvericin 8-12 µg/kg and citrinin <LOQ) and the frequency of contamination (100% of positive in Emilia Romagna, Marche, Latium, Tuscany, Sardinia, 59% in Molise, none in Sicily). However, in both years and in all regions beauvericin and citrinin were virtually absent. The highest level of contamination for 2012 was recorded in Sardinia (ENNs 164 µg/kg), while in 2013 it was recorded in Tuscany with a high representativity of values for all the ENNs (highest ENNA 220 µg/kg, ENNA1 664 µg/kg, ENNB 3,566 µg/kg, ENNB1 1,019 µg/kg). In addition, in Tuscany, Sardinia and Molise 2012 samples it was found that ENNA was the most abundant representative of all enniatins, while enniatin B was the most abundant representative in the 2013 samples. These results represent a first systematic monitoring of these emerging mycotoxins in domestic wheat production. In addition, the results obtained from the project will be made available to the European Food Safety Authority, which received a mandate from the European Commission.

#### P8

### **Application of high resolution tandem mass spectrometry in analysis of mycotoxins, pesticides, and pyrrolizidine alkaloids in herbal-based dietary supplements**

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In recent years, herbal-based dietary supplements providing nutrients with beneficial effects on consumers' health have become an important part of the diet. However, the health benefits provided by biologically active components contained in herbs can be overshadowed by the presence of various contaminants which can pose a health risk for consumers. Besides of heavy metals and pesticide residues, also occurrence of natural contaminants such as mycotoxins and pyrrolizidine alkaloids has been detected in various commercial products. With regards to the complexity of these food matrices, analysis of contaminants present is a challenging task. Most of the existing studies describing the analysis of herbal-based dietary supplements are typically focused on one group of contaminants. In this study, we used employed newly developed multidetection analytical method for analysis of 323 pesticides, 55 mycotoxins, and 11 pyrrolizidine alkaloids using ultra-high performance liquid chromatography with mass spectrometric detection employing Q-Exactive™ high resolution tandem mass spectrometer. For sample preparation, QuEChERS-like extraction method was utilised. The performance characteristics of the presented method were critically assessed, and it was

concluded that the method fully complies the requirements of European Commission (SANCO/12571/2013). In the next phase, 90 popular brands of herbal dietary supplements (herbal teas, drops, tablets, etc.) purchased at the Czech market were analysed in order to estimate consumers' exposure to pesticides residues, mycotoxins, and pyrrolizidine alkaloids. Considerable differences of contamination between particular groups of samples analysed were found. In some samples, concentrations as thousands of µg/kg for both pesticides and mycotoxins and hundreds of µg/kg for pyrrolizidine alkaloids were found. When considering the average body weight of an adult person of 70 kg, the contamination of several samples even exceeded the values of acceptable daily intake (ADI) for pesticides and tolerable daily intake for mycotoxins (TDI). **Acknowledgements.** Supported by 'Operational Program Prague – Competitiveness' (CZ.2.16/3.1.00/22197) and 'National Program of Sustainability' (NPU I (LO) MSMT - 34870/2013).

## P9

### **Co-occurrence of aflatoxins and fumonisins in cereal mixtures marketed in Brazil**

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Cereal mixtures, basically constituted by soy extract, wheat fibre, rice flour, oatmeal, quinoa, beer yeast, flax seeds, maize meal, powdered guarana and cocoa, became popular among consumers looking for healthy products and are generally daily consumed. The simultaneous occurrence of two or more mycotoxins in food can potentiate the toxic effects on consumers. The aim of this study was to verify the co-occurrence of aflatoxins and fumonisins in cereal mixtures marketed in Brazil. Fifteen samples from different lots were acquired each month by internet and from supermarkets during seven months, adding up to 105 samples analysed. The unit sample was constituted for original package with minimum of 250 g. Extraction and clean-up of samples for aflatoxins and fumonisins determination were carried out using immunoaffinity columns. Identification and quantification of aflatoxin B1 (AFB1), AFG1, fumonisin B1 (FB1) and FB2 were performed by high performance liquid chromatography. The mean levels of mycotoxins found were 0.38±0.37 µg/kg for AFB1, 1.45±1.46 µg/kg for AFG1, 137.8±257.4 µg/kg for FB1 and 287.63±585.59 µg/kg for FB2. Results showed that five samples (4.76%) presented simultaneously AFB1 and FB1, one sample (0.95%) presented AFB1, AFG1, FB1 and FB2, three samples (2.6%) presented AFB1 and AFG1 and 14 samples (13.3%) presented FB1 and FB2. Although the samples had presented individual low levels of mycotoxins, it is known that the co-occurrence can potentiate the toxic effects in humans, thus it can represent risk to the consumers' health.

## P10

### ***Fusarium graminearum* species complex (FGSC) and *F. verticillioides* incidence in maize roots, crowns, stems and ears**

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The *Fusarium graminearum* species complex (FGSC) and *F. verticillioides* are two major pathogens of cereals. These pathogens cause *Fusarium* head blight on small grains as well as *Fusarium* and *Gibberella* ear, stalk, crown and root rot on maize. Both *F. verticillioides* and FGSC infections can produce secondary metabolites known as mycotoxins. *F. verticillioides* produce primarily fumonisins of which the most significant are the three analogues, FB1, FB2 and FB3. The FGSC produces the mycotoxins nivalenol (NIV), deoxynivalenol (DON) and zearalenone (ZEA). Maize plants were sampled from seven irrigated fields with visible *Fusarium* spp. disease symptoms. Entire plant samples (20 plants) were collected from three areas within each field, from adjacent areas of prematurely senesced and visibly green healthy looking plants. The amount of FGSC and *F. verticillioides* DNA within the roots, crown, stems (internode 1 and 2) and grain were determined. The

primer sets FgramB379 and FgramB411 as well as Fver356 and Fver412 were used to determine target DNA quantification of FGSC and *F. verticillioides* with qPCR, respectively. Mycotoxin analysis was performed on the grain using liquid chromatography-mass spectrometry. The concentration of FGSC DNA in the roots was low (<10 pg/μl) with only one field higher than 40 pg/μl whereas the concentration of *F. verticillioides* was on average higher and ranged from 2 pg/μl – 60 pg/μl. The crown and first two internodes had very high concentrations of FGSC DNA. The *F. verticillioides* DNA concentrations in the crown and first two internodes were all <500 pg/μl. The DNA concentrations of FGSC and *F. verticillioides* were fairly low in the grain, however, overall the DNA concentrations of *F. verticillioides* were higher than the FGSC DNA concentrations. Both the senesced and green visually healthy plants were infected with both FGSC and *F. verticillioides* and the related mycotoxin levels in the grain varied in significance. Thus, even though there were smaller areas of visible disease symptoms all the plants sampled were infected with FGSC and *F. verticillioides* and the grains were contaminated with mycotoxins. The results indicated that maize plants regardless of visual symptoms were at risk to contain undesirable secondary metabolites that may be harmful to human and animal health.

## P11

### ***Fusarium* mycotoxins in malting and brewing by-products**

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Recently, much research has been published on chemistry and biology of mycotoxin production by fungi that pose a serious threat to human and animal health worldwide. By-products formed during the malting and brewing process, such as germ, rootlets and spent yeast and grains, represent a nutritious and valuable feed for livestock. However, the above mentioned by-products can also be contaminated with mycotoxins that originate from grains and survive the unit operations applied during the malting and brewing process. It is therefore necessary to monitor and determine their type and quantity in order to ensure the total food chain safety along the principle 'from stable to table'. The aim of this work was to determine the types and quantities of *Fusarium* mycotoxins that can be transferred to animal feed under practical agronomical, malting and brewing process conditions. Two Croatian wheat varieties obtained from the Agricultural Institute in Osijek were used: OSK variety more susceptible to *Fusarium* infection, and Lucija, the less susceptible to *Fusarium*. Each variety was treated with the fungicide Prostaro to ensure realistic growing conditions. A standard malting procedure for wheat malt was implemented followed by degermination process and brewing. The starting wheat samples as well as by-products resulted from the malting and brewing process were analysed for mycotoxins, and deoxynivalenol (DON), 3-deoxynivalenol (3-ADON) and nivalenol (NIV) were determined by LC-MS/MS. Concentrations varied, depending on the sample. Germ/rootlets showed increased DON values of 510.38 μg/l and 818.45 μg/l for Lucija and OSK, respectively. Spent grains showed elevated DON value (1,177.86 μg/l) but only for Lucija variety. NIV values were 218.95 μg/l for Lucija and 347.36 μg/l for OSK. Spent yeast showed increased DON values for OSK variety (542.33 μg/l) but also for 3-ADON (1041.69 μg/l). Since these mycotoxins are greatly detrimental to animal health and economy, it is relevant to monitor their distribution in animal feed.

## P12

### **Worldwide mycotoxin occurrence: a ten year investigation**

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Since 2004, Biomin has conducted a yearly Mycotoxin Survey which provides an insight on the risks caused by the main mycotoxins found in agricultural commodities intended for animal feed. More than 26,000 samples from different countries around the globe have been evaluated for aflatoxins (Afla), zearalenone (ZEN), deoxynivalenol (DON), fumonisins (FUM) and ochratoxin A (OTA). Samples were analysed by high performance liquid chromatography (HPLC) and only single commodities by enzyme-linked immunosorbent assay (ELISA). Results were compared by taking into account the percentage of samples which lie above the EU guideline for aflatoxin B1 or the EC recommendation levels for all other toxins. The worldwide results depict the differences in mycotoxin problematic for each year. Zearalenone levels were particularly high in 2006, ochratoxins in 2007, aflatoxins in 2008, fumonisins in 2009 and 2010 is considered a DON-contamination peak year. The percentage of

samples with concentrations of DON above 900 ppb (EC recommendation for complementary and complete feedingstuffs for pigs) show fluctuations between a minimum of 9.4% in the first semester of 2014 and a maximum of 18.2% in 2010. Also the percentage of samples that lie above 100 ppb of zearalenone (EC recommendation for complementary and complete feedingstuffs for piglets and gilts) among the years shows high variations with a minimum of 9.5% in 2013 and a maximum of 22.4% in 2006. Results for total aflatoxins show that between 13.2% (in 2012) and 24% (in 2008) of the samples lie above the European maximum value of 5 ppb for aflatoxin B1 for complete feedingstuffs for dairy animals. A fumonisin contamination peak was observed in 2008, as 7.1% of samples contained fumonisins at concentrations above 5000 ppb (EC recommendation for complementary and complete feedingstuffs for pigs and horses). In 2007, OTA showed a peak contamination with 7.2% of all samples above the EC recommendation of 50 ppb OTA in complementary and complete feedingstuffs for pigs. The worldwide results highlight the different yearly mycotoxin problematic and underline the necessity of constant mycotoxins monitoring in feedstuffs for proper mycotoxin risk management.

### P13

#### **Occurrence of mycotoxins in a total diet study (TDS) for adults and children in the Netherlands**

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A total diet study (TDS) may be a valuable approach to assess the exposure of consumers to mycotoxins. Therefore, a TDS study was performed on the risk associated with mycotoxin intake in cooperation with the Dutch National Institute for Public Health and the Environment (RIVM) and the Netherlands Food and Consumer Product Safety Authority (NVWA). The average Dutch diet was divided into the main separate food categories, and a sample representative for each food category for both adults' and childrens' diets was prepared from various products proportionally to their intake within this food category. All products were prepared (cleaned, cut, boiled, baked, fried, etc.) as they are usually consumed. In total, more than 1300 individual food products were carefully processed to 88 homogenised samples. This work describes the results obtained on the 88 homogenised samples from the TDS study, which were analysed for 59 mycotoxins, including aflatoxins, ochratoxin A (OTA), trichothecenes, patulin, fumonisins, *Alternaria* toxins, ergot alkaloids and several emerging mycotoxins, such as enniatins and beauvericin. In general, the number of positive samples was low, and if positive the detected concentrations were low, close to the reporting limits for most of the cases. As expected, grains and grain-based products were positive for ergots alkaloids, enniatins (up to 58 µg/kg in rye- and maize-products), and deoxynivalenol (up to 116 µg/kg in breakfast cereals). OTA was detected in legumes, liquorice and coffee at low levels (~1 µg/kg). *Alternaria* toxins were the toxins that occurred for a wider variety of type of samples ranging from tomato sauce to nuts, cereals, chocolate or wine. Patulin was not detected in any of the samples, which included fruits and juices. After analyses, the analytical results will be combined with food consumption data of each of the food categories, which will allow the estimation of the dietary exposure to mycotoxins for the total Dutch population.

### P14

#### **Occurrence of *Alternaria* mycotoxins in food products in the Netherlands**

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The European Food Safety Authority (EFSA) published a Scientific Opinion on *Alternaria* mycotoxins in 2012, in which they concluded that *Alternaria* mycotoxins are of high concern for public health. The *Alternaria* mycotoxins alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA) and altertoxins (ATX) can induce harmful effects in animals, including carcinogenic and teratogenic effects. *Alternaria* mycotoxins can contaminate cereals, oilseeds and various fruits and vegetables such as apples, tomatoes, citrus fruits and olives. They can even grow at low temperatures, thus causing spoilage even during transport and storage. On 29 May 2012, the European Standing Committee advised to the Member states to collect data on the occurrence of the *Alternaria* mycotoxins: AOH, AME, TeA, tentoxin (TEN) and altenuene (ALT) in several food commodities. In

reply to this advice, a small survey (95 samples) was organized in the Netherlands. Its purpose was to screen the levels of *Alternaria* mycotoxins in fresh apples, tomatoes, citrus, figs, olives, sunflower seeds, cereals, apple juices and tomato sauces. The results revealed that AOH, AME, TeA, and TEN were detected in one or more food commodities, while ALT was not detected in any of the samples. TeA was found in a high number of samples (27%) and at high concentrations (up to 2,345 µg/kg). *Alternaria* mycotoxins occurred regularly in cereals, tomato sauces, figs, wine and sunflower seeds. Only incidental occurrence of the *Alternaria* mycotoxins was observed in fresh apples, citrus fruits, tomatoes and olives.

## P15

### Survey of UK retail food products for selected *Fusarium* mycotoxins including their masked forms

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A retail survey of UK food products was carried out for the presence of some *Fusarium* mycotoxins, including a number of masked mycotoxins. A total of 175 food samples were analysed and included flour, pasta, breakfast cereals, infant foods, beer and herbs and spices. An LC-MS/MS method using a Waters Acquity/Xevo TQS was developed for the determination of deoxynivalenol, deoxynivalenol-3-glucoside (DON-3-G), nivalenol, 3-acetyl deoxynivalenol, 15-acetyl deoxynivalenol, T-2 toxin, HT-2 toxin, zearalenone, zearalenone-14-glucoside, zearalenone-sulphate,  $\alpha$ -zearalenol,  $\alpha$ -zearalenol-14-glucoside,  $\beta$ -zearalenol, and  $\beta$ -zearalenol-14-glucoside. The newly developed LC conditions allowed full separation of 3 and 15-acetyl deoxynivalenol allowing these compounds to be quantified individually. No clean-up was used before the analysis, but C13 isotope-labelled standards for deoxynivalenol and zearalenone were used to assess matrix effects. The highest concentrations of deoxynivalenol were found in high fibre breakfast cereals, DON-3-G was also frequently detected in these products. The highest level of deoxynivalenol found was 375 µg/kg. This sample also contained 87 µg/kg deoxynivalenol-3-glucoside and 17.9 µg/kg zearalenone. Beer samples were frequently found to contain DON-3-G, often without the occurrence of deoxynivalenol. Analytical method performance was assessed by use of spiked samples and in-house reference samples. Lowest recovery values were observed for nivalenol and DON-3-G. Analytical QA was acceptable, in-house reference samples were within the acceptable range for all analyses. Spice samples were more problematic with large matrix effects observed, therefore an overspiked sample was analysed for each survey sample to assess recovery accurately. Zearalenone was the most frequently detected mycotoxin in spice samples, 8 samples contained zearalenone, the highest level was 46.5 µg/kg. Two samples contained zearalenone-14-glucoside and one contained  $\beta$ -zearalenol-14-glucoside at a low level. No sample exceeded any regulatory level and overall incidence of mycotoxins was low.

## P16

### Identification and quantification of mycotoxins in silages: an Irish national survey

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*Fusarium* and *Penicillium* have been identified in silages. These moulds are toxigenic and can produce secondary fungal metabolites, namely mycotoxins. Mycotoxins can induce a range of detrimental ailments in livestock including abortions, vomiting, lameness, immunosuppression, reduced feed intake and feed refusal. Monitoring feed for mycotoxins is requested by the European Food Safety Authority and this study includes all mycotoxins that are regulated in Commission Directive (EC) No 32/2002 and Recommendation (EC) No 576/2006. The objective of this study was to identify the mycotoxin challenge present in silages produced throughout Ireland. 150 farms were visited between December 2012 and March 2013. On each farm 2 core samples of silage were removed from either pit grass silages (n=88), pit maize silages (n=6) or baled grass silages (n=56) using a motorised coring device. For mycotoxin analysis, silage samples were dried at 40°C in a forced air circulation oven and milled (Retsch SM100) through a 1 mm pore sieve. Mycotoxin analysis was by a validated (Agilent 6460-1290) liquid chromatography tandem mass spectrometry (LC-



MS/MS) operating in both positive and negative electrospray ionisation modes. This 16-minute analytical run can detect 20 mycotoxins (aflatoxins A1, B1, A2 and B2, DON, HT-2, mycophenolic acid (MPA), roquefortines C and E, ochratoxin A, T-2, zearalenone, enniatins B, B1, A and A1, fumonisins B1 and B2, anadrastin A and beauvericin), and includes all the regulated mycotoxins concerned with feed. Mycotoxin data were analysed with PASW (SPSS 18) using ANOVA (GLM 1). Eight mycotoxins (roquefortine C, enniatin A, A1, B and B1, anadrastin A, beauvericin and MPA) were present within the silages. Although baled and pit silages had similar mycotoxin profiles, baled silages had a greater range of mycotoxin concentrations. The most abundant mycotoxin among the silages was MPA (baled), detected at 4,386 µg/kg DM. This is considered low compared to other European studies. MPA is considered an 'emerging risk' in feed by the EU Commission and data on its occurrence is being sought. In a national silage survey carried out to determine the mycotoxin challenge within silages produced in Ireland, eight mycotoxins (roquefortine C, enniatins A, A1, B and B1, anadrastin A, beauvericin and MPA) were detected. None of these mycotoxins exceeded EU legislation or recommendations.

## **P17**

### **The Black Sea region is a suitable area for mycotoxins production, with Ukraine as a prime example**

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The Black Sea region is one of the major areas of cereal production worldwide. Ukraine was worldwide the 7th maize producer and the 11th wheat producer in 2012 with a significant part that is exported. This makes the quality of cereals produced in Ukraine a value of interest in all cereals importer countries. To provide relevant information to the feed sector, Neovia researched 42 to 48 different mycotoxins (or metabolites) in 140 Ukrainian samples: 64 complete feeds for pigs (38) or poultry (26), 76 raw materials or under products (29 wheat, 22 maize, 9 barley, 8 soybean, 5 sunflower, 2 triticale, 1 sorghum). These analyses have been conducted by Laboce, a French public laboratory, using liquid chromatography coupled with a double mass spectrometry. A database of 6,117 values has been established. At a global level, it is interesting to see that complete feeds are usually more contaminated than raw materials. Indeed samples of complete feed contained a median contamination of 8 different mycotoxins, when it is 'only' 4.8 mycotoxins, per raw material sample. All feeds were contaminated by at least 2 mycotoxins and 12 molecules maximum, when 4/76 (5%) samples of raw materials were completely free of all mycotoxins researched. When it comes to detail, it can be observed that of the 100% contaminated feeds, 95% were contaminated by trichothecenes (95% by deoxynivalenol, 73% by T-2 and/or HT-2 toxins, 58% by 15-acetyl-deoxynivalenol, 27% by nivalenol). 73% contained tenuazonic acid. Fumonisins have been found in 72% of the feeds, zearalenones in 67% and ochratoxins in 63%. It was also observed that Ukrainian feeds seem to be highly contaminated by enniatins (10 out of 12 samples were contaminated) and beauvericin (3 out of 12). Other mycotoxins have also been found in these feeds, but at lower prevalence: 13% contained moniliformin, 5% ergot alkaloids, 2% aflatoxin and 2% citrinin. With regards to raw materials, all of them do not present the same mycotoxins risk. The protein rich raw materials (soybean, sunflower) are generally less contaminated with a median of 3 mycotoxins in them. On the contrary, maize seems to be the more problematic Ukrainian raw material: its median content is 6.5 different mycotoxins, with 13 maximum. In the middle of these 2 situations, other cereals (wheat, barley) are contaminated in median by 4 molecules. In conclusion, it appears clearly that raw materials produced in Ukraine are widely contaminated by mycotoxins, and mixing these raw materials afterwards leads to the feeds to become even more contaminated. Risks of using such raw materials locally, but also for importer countries, have to be taken seriously in order to avoid any health problem to farming animals.

## **P18**

### **Simple or multicontamination by mycotoxins in French wheat and maize**

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Mycotoxins analysis results are very frequently interpreted mycotoxin by mycotoxin, however it can be noted that the toxicity of a raw material can be strongly increased by the simultaneous presence of several mycotoxins. In collaboration with the Public French Reference Laboratory for mycotoxins analysis, Laboce, Neovia analysed 250 samples of French wheat and 69 samples of French maize

by using liquid chromatography coupled with mass spectrometry. In each sample, collected between July 2010 and December 2013, up to 43 different mycotoxins were tested. Within wheat samples, only 4% did not contain any mycotoxin, whereas 41% contained 1 to 2 different toxins, 33% contained 3 to 5, 18% had 6 to 9 mycotoxins, and 4% contained 10 mycotoxins or more. With regard to maize, 100% of the samples analysed were contaminated: 9% with 1 to 2 mycotoxins, 32% contained 3 to 5 mycotoxins, 52% had 6 to 9, and 6% contained 10 (or more) mycotoxins. Contaminations of French wheat occur mainly due to *Fusarium*. 88% of the samples analysed contained deoxynivalenol (DON, median 238 ppb, up to 4750 ppb), 61% contained nivalenol (median 20 ppb, up to 410 ppb), 20% T-2 or HT-2 toxin (30 ppb up to 1070 ppb) and 24% contained zearalenone (20 ppb up to 755 ppb), all produced by *Fusarium* spp. Other mycotoxins were found in high proportions in maize samples, like tenuazonic acid and ergot alkaloids (20 and 25%, 68 and 35 ppb of median concentration, respectively). Fumonisin was found in 6.5% of the samples with median concentration of 212 ppb, moniliformine in 4.8% at 169 ppb and ochratoxin A in 3.1% at 6.4 ppb. None of the samples contained aflatoxins. *Fusarium* contamination was even more predominant in French maize: 100% of the analysed samples contained DON (average 395 ppb, up to 10,600 ppb) and 99% contained DON metabolites like 15-acetyl-DON (75 ppb up to 6,210 ppb) or 3-acetyl-DON (up to 150 ppb). 86% of the samples also contained nivalenol (45 ppb up to 700 ppb) and 86% also had zearalenone (60 ppb up to 2070 ppb). Fumonisin was found in 62% of the samples (165 ppb up to 30,690 ppb), moniliformin in 10% (220 ppb), and tenuazonic acid in 14% (55 ppb). The high level of contaminations associated with important proportion of polycontaminations represent a real threat for animals. This study also demonstrates the extreme variability of mycotoxin contamination profiles, and also reveals the difficulty to predict their effects, specifically with potential additive or synergic effects. A special attention should be offered to analysis and interpretation of polycontaminations in raw materials in order to set the most efficient prevention plan against mycotoxicoses for livestock.

## P19

### Global mycotoxin survey in 2013 and 2014

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Mycotoxin occurrence in all kinds of commodities is a worldwide phenomenon. BIOMIN conducts an annual Mycotoxin Survey to raise awareness on the incidences of mycotoxins found in agricultural commodities intended for animal feed. The focus of this study is to evaluate the extent of mycotoxin contamination in various samples from different regions worldwide. From January 2013 to June 2014, a total of 6,169 samples sourced around the globe (2,757 from Europe) were analysed for aflatoxins (Afla), zearalenone (ZEN), deoxynivalenol (DON), fumonisins (FUM) and ochratoxin A (OTA). Samples were analysed using liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS, Spectrum 380®), high performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA). The latter method was only chosen for single commodities. All five major mycotoxins were highly prevalent as 82 % of all samples contained at least one of these mycotoxins. In 48 % of all animal feed and ingredient samples, more than one type of mycotoxin was found. Afla was present in 29 % of all samples, ZEN in 41 %, DON in 59 %, FUM in 58 % and OTA was detected in 23 % of all samples. The latest results showed once again that DON and FUM are the most prevalent groups of mycotoxins worldwide with an average contamination of 428 and 844 ppb, respectively. In total, 11 % of all the feed samples were above the EU guidance values for DON (900 µg/kg) in complementary and complete feedstuffs for pigs (EC, 2006). 22 % of all samples contained FUM at concentrations exceeding 1000 ppb. The mycotoxin survey results presented above indicate that these toxic substances are a serious concern in agricultural production. Multimycotoxin occurrences continue to be a global threat, with *Fusarium* mycotoxins being the major toxins found. An effective mycotoxin risk management program has to be applied to protect animals from the negative effects of mycotoxins.

## P20

### Mycotoxins and heavy metals in organic commercial cereal based foods sold in supermarkets in Greece

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Mycotoxins in crops and in processed foods thereof, are toxic and have adverse effects in human

health. Studies on the occurrence of mycotoxins in organic and conventional foods show that conclusive evidence is lacking on whether organic foods are less susceptible to mycotoxin contamination. Furthermore, investigation of the presence of heavy metals in crops and foods, have gained special attention due to their toxicity even at very low concentrations. The aim of the present work was to monitor the occurrence of mycotoxins and heavy metals in organic food products present in the Greek market. Thirty eight commercial organic food samples (of Greek and foreign origin) were collected directly from supermarkets in Thessaloniki, Greece between February and March 2014. Samples comprised different products of wheat, barley, oat, maize and rice (grains, flakes, flours, pasta, etc.), all marked with the BIO label. Samples were examined for the presence of four mycotoxins, i.e., aflatoxins (Afla), ochratoxin A (OTA), zearalenone (ZEA) and T-2 toxin (T-2) with enzyme-linked immunosorbent assay (ELISA), whereas heavy metals (Pb, Cd, Co, Ni, Cu, Fe, Mn and Zn) were determined with Optical Emission Spectrometer (Optima 2100DV, Perkin Elmer). Most of the tested samples were contaminated with Afla (97%) and ZEA (94%) at concentrations below the maximum levels adopted at Commission level, whereas OTA was found to be present in almost 45% of the analysed samples. No sample was contaminated with T2 toxin. Only one sample (whole oat meal) was found to be contaminated with OTA at a concentration exceeding the maximum level (4.86 µg/kg). In positive samples, Afla levels varied from 0.37 to 3.09 µg/kg, and ZEA levels ranged from 0.22 to 14.74 µg/kg. Levels of toxic elements Pb and Cd varied from 0.0089 to 0.2153 and 0.0042 to 0.1997 mg/kg, respectively. Only one sample (basmati rice) was contaminated with Pb above the maximum level set by Regulation EC No 1881/2006. Co and Ni were present at a concentration range 0.0077-0.1615 and 0.6460-23.8237 mg/kg. The average content of trace elements was in the following order: Cu< Mn<Zn<Fe. **Acknowledgements.** This work has been carried with the financial support of the European Social Fund and national resources through the Public Investment Program, Research Project ARCHIMEDES III, subproject ΟΠΣ 383572 'Monitoring mycotoxins and radioactivity level in cereal products of Greece'.

## P21

### **Screening and quantification of mycotoxins in various types of feed samples in northern Europe between October 2012 and June 2013**

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The survey was undertaken to investigate the prevalence of mycotoxins affecting various feed samples in Northern Europe. A total of ninety samples consisting of TMR, compound feeds, grass silage, maize silage, wheat and barley were received from UK and the Netherlands. The samples were screened for zearalenone (ZON), deoxynivalenol (DON), fumonisin B1, T-2 toxin, HT-2 toxin, aflatoxin B1 (AFB1), AFB2, AFG1, AFG2, ochratoxin A, sporidesmin A, fumonisin B2 and patulin using LC-MS/MS. Mycotoxins found to be present in the samples were DON, ZON, fumonisin B1, fumonisin B2, T-2 toxin, HT-2 toxin and AFB1. Of the total samples received for analysis, 70% were contaminated with mycotoxins. Among those 81.8 % maize, 12.5% grass silage, 75.0% wheat samples, 80.0% barley and 92.3% of TMR samples were contaminated with mycotoxins. All the compound feed samples contained one or more mycotoxin. Of the individual mycotoxins detected the most prevalent were DON and ZON at 76.2 % (48 samples) and 74.6% (47 samples), respectively. Samples containing fumonisin B1 and fumonisin B2 were 30.2 % (19 samples) and 22.2 % (14 samples), respectively. The number of samples found to contain T-2 and HT-2 toxin were 22.2% (14 samples) and 30.2 % (19 samples). Finally three samples were contaminated with AFB1 which equates to 4.8 % of the samples analysed. Most of the samples had more than one mycotoxin and that may exert additive or synergistic effects. For additive and synergistic effects, there are no set limits for mycotoxin levels. The total mycotoxin load (TML) for each sample was calculated and used to determine a relative low, medium or high risk factor. Low risk samples are considered to be in the range 0 to 200 ppb, medium risk 200 to 500 ppb and high risk samples those that contain more than 500 ppb total mycotoxins. The results showed: 64.4% of samples were considered to be 'low risk'; 22.2% of samples were considered to be 'medium risk'; and 13.3 % of samples were considered to be 'high risk'.

## P22

### Maize harvest 2013: 72 maize samples from Austria tested for more than 380 mycotoxins and secondary metabolites

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In an effort to further broaden the knowledge on mycotoxin occurrence and co-occurrence in feed in Austria, 72 maize samples were screened for more than 380 mycotoxins and other secondary metabolites. The maize samples collected from the harvest 2013 were analysed with a multimycotoxin LC-MS/MS method at IFA-Tulln according to Vishwanath *et al.* (2009). The analytical method has been transferred to a more sensitive mass spectrometer (QTrap® 5500) and extended to cover more than 380 metabolites (Malachova *et al.*, 2014; Streit *et al.*, 2013). Altogether, 128 different metabolites were found in the 72 samples. Between 16 and 84 metabolites were detected per single sample. 79 % of the samples were co-contaminated with 30-49 single mycotoxins and metabolites. The most frequent mycotoxins found in 100 % of samples were beauvericin, enniatin B/B1, aurofusarin, and culmorin followed by zearalenone (mean of positives 111 µg/kg; max. 1880 µg/kg), enniatin A1, moniliformin, and equisetin, all in 99 % of the samples. Deoxynivalenol (mean of positives 593 µg/kg; max. 3105 µg/kg) and nivalenol (mean of positives 27 µg/kg; max. 108 µg/kg) were detected in 96 % of the samples. Furthermore, 54 % of the samples were positive for HT-2 toxin (mean of positives 25 µg/kg; max. 216 µg/kg) and 31% for T-2 toxin (mean of positives 10 µg/kg; max. 44 µg/kg). Deoxynivalenol-3-glucoside always co-occurred with deoxynivalenol. More data on the metabolic fate, mode of action, toxicity and possible synergistic interaction of co-contaminating mycotoxins is needed, to allow a risk assessment associated with the presence of the mycotoxins and other secondary metabolites found in these samples. The results of the 72 maize samples from the Austrian harvest 2013, analysed with the multimycotoxin method based on LC-MS/MS, clearly showed that mycotoxin co-contamination is the rule.

## P23

### Multimycotoxin screening in Latin-American maize and soy samples

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Maize and soy are the most important feed ingredients for pig and poultry worldwide. Brazil, Argentina, Paraguay and Bolivia are the main exporting countries of maize and soy. A total of 51 samples (31 maize, 20 soy) from Brazil, 24 samples (9 maize, 15 soy) from Argentina, 20 samples (10 maize, 10 soy) from Paraguay and 6 soy samples from Bolivia were collected in June 2014 in the respective countries. The samples were screened for more than 380 mycotoxins and other secondary metabolites using liquid chromatography-mass spectrometry/mass Spectrometry (LC-MS/MS, Spectrum 380®). For the purpose of data analysis, limit of quantification (LOQ) level for each mycotoxin was adopted to determine positive samples. The aim of this study was to obtain information on the occurrence and contamination levels of multiple mycotoxins in maize and soy samples from Latin America. Each of the 52 samples of soy bean contained between 9 and 38 metabolites. The lowest average number of mycotoxins was found in Bolivian soy bean (14 metabolites/sample) and the highest average number was found in soy from Paraguay (29 metabolites/sample). The 50 different maize samples contained between 11 and 55 different metabolites with lowest number of metabolites found in maize from Paraguay (19 metabolites/sample) and highest number found in maize from Argentina (31 metabolites/sample). The most frequently occurring mycotoxins were beauvericin, and enniatins followed by kojic acid, emodin, fusarinolic acid, fumonisins, T-2 toxin and zearalenone. The observed median concentrations of the individual analytes were generally in the low µg/kg range with the exception of fumonisin B1. Out of more than 380 metabolites analysed, mycotoxins produced by *Fusarium* are still among the most frequently occurring ones. An important advantage of multimycotoxin analysis is the detection of normally undetected masked mycotoxins, as more than 50 % of e.g., deoxynivalenol, are thought to exist in commodities in a masked form. Performing multimycotoxin analysis also allows the evaluation of the occurrence of mycotoxins which are not commonly measured, such as beauvericin and enniatins. The effects of such mycotoxins on health and performance of humans and animals still need to be elucidated.

P24

## **Mycotoxins and other fungal metabolites in grain dust from Norwegian grain elevators and compound feed mills**

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Employees at grain elevators and compound feed mills are exposed to large amounts of grain dust during work, frequently leading to airway symptoms and asthma. Although the exposure to grain dust, microorganisms,  $\beta$ -1 $\rightarrow$ 3-glucans and endotoxins has been extensively studied, the focus on the mycotoxin content of grain dust has previously been limited to one or few mycotoxins. Our objective was therefore to screen settled grain dust from grain elevators and compound feed mills for fungal metabolites by LC-MS/MS and explore potential differences between work places within feed mills, seasons and climatic zones. Seventy fungal metabolites and two bacterial metabolites were detected. Trichothecenes, depsipeptides, ergot alkaloids, and other metabolites from *Fusarium*, *Claviceps*, *Alternaria*, *Penicillium*, *Aspergillus*, and other fungi were represented. The prevalence of individual metabolites was highly variable, and the concentration of each metabolite varied considerably between samples. The prevalence and concentration of most metabolites were higher in grain elevators compared to compound feed mills. Examinations of potential differences between seasons and climatic zones were inconclusive. All samples contained multiple mycotoxins, indicating a highly complex pattern of inhalational exposure. A mean exposure of 20 ng/m<sup>3</sup> of fungal metabolites was estimated, whereas a worst case scenario estimated as much as 10  $\mu$ g/m<sup>3</sup>. Although many of these compounds may be linked to toxicological and immunological effects through experimental or epidemiological studies, it still remains to be determined whether the detected concentrations implicate adverse health outcomes when inhaled.

P25

## **Study of black *Aspergillus* from Canadian vineyards and the associated risk of ochratoxin A and fumonisin contamination of wine**

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Ochratoxin A (OTA) is a mycotoxin that can be found in grain products, coffee, beer and wine. It is regulated in many products by the European Commission and recently Health Canada has proposed new regulated limits for OTA in cereals based on exposure assessments for Canadians. There is little reliable data on the presence of OTA and fumonisins in Canadian wine, but the relatively low levels found in Canadian grain have already increased costs and uncertainty for grain milling and food companies. We are conducting a three year comprehensive survey of the black *Aspergillus* from vineyards, to better understand their occurrence in Canada and the risk of OTA and fumonisin contamination of Canadian wine. In the first year of the study approximately 400 strains were isolated by plating either grape or soil samples collected from a number of sites in Canada's main wine producing area, the Niagara region of Ontario. The isolated strains were identified by DNA sequencing, the results show that four main species were found: *Aspergillus niger*, *A. uvarum*, *A. tubingensis* and *A. brasiliensis*. Interestingly, the main OTA producer on grapes in other parts of the world, *A. carbonarius* has not been isolated or detected by molecular methods (PCR and metagenomic analysis). *A. niger* is the only one of these species that is known to produce OTA and fumonisin. LC-MS/MS (Thermo Q-Exactive) analysis of our *A. niger* strains has revealed that a number produce FB2, FB4 and FB6 in trace amounts but none produced OTA. The secondary metabolite production by *A. uvarum* and *A. brasiliensis* is not well understood and is currently being further evaluated. From the first year of the survey it appears that *A. carbonarius* does not occur in Niagara region vineyards and the risk of OTA contamination of Canadian wine is very low.

P26

## Trichothecene mycotoxin levels in winter wheat harvested in 2013 from Ontario, Canada

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The objective of this study was to evaluate the level of mycotoxins in Ontario hard red and soft white winter wheat in 2013. Hard red winter wheat breeding lines from the cross 'Maxine' x 'FTHP Redeemer' and soft white winter wheat breeding lines from the cross 'Superior' x 'DB006W' were planted at three locations in southwestern Ontario, Canada in 2012. At maturity in 2013, the plots were harvested by a small plot combine and yield was reported at 14% moisture content. The harvested grain was sampled to determine deoxynivalenol (DON), 15-acetyl DON, 3-acetyl DON, nivalenol (NIV), T-2 and HT-2 toxins using a GC-MS system with a detection limit of 0.06, 0.05, 0.05, 0.12, 0.06 and 0.04 µg/g, respectively. *Fusarium* damaged kernels (FDK) level were measured using a SpecStar 2500-X near-infrared (NIR) analyser (Unity Scientific Inc.). Lower mean levels of DON were detected in hard red wheat than in soft white wheat at all locations (1.5 vs. 4.1 µg/g, 3.3 vs. 11.8 µg/g and 5.2 vs. 19.8 µg/g at Ridgetown, Inwood and Centralia, respectively). In addition to DON, 15-acetyl DON and HT-2 were detected in hard red wheat at all locations, while 15-acetyl DON, 3-acetyl DON, HT-2, T-2 and NIV were detected in soft white winter in one or two locations. Hard red line CA03-017 had the lowest DON level including the moderately resistant check 'AC Morley' at all locations (0.4, 1.2, and 1.1 µg/g at Ridgetown, Inwood and Centralia, respectively), suggesting a possible genetic tolerance to DON accumulation. In contrast, hard red line CA03-110 had the highest DON level across all locations (3.6, 4.3 and 8.4 µg/g at Ridgetown, Inwood, Centralia, respectively) and was significantly different from other lines ( $p < 0.05$ ). Soft white line DH1-28 had the lowest, while line DH1-45 had the highest DON level across all locations (1.6 vs. 7.9 µg/g, 6.0 vs. 17.0 µg/g and 10.0 vs. 18.0 µg/g at the Ridgetown, Inwood and Centralia, respectively). Significant negative correlation was detected between yield and DON accumulation and yield and FDK level across all locations ( $p < 0.05$ ). In general, relatively high levels of DON were detected in both classes of wheat, with some lines showing a level of tolerance. However, several times higher average levels of DON were detected in the soft white compared to the hard red winter wheat. The average DON level was higher than reported in previous years and we consider 2013 as *Fusarium* head blight epidemic year in Ontario.

P27

## Survey on patulin presence in apple juice

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Apple juice is considered as major source of patulin intake in human diet. Regarding its potential as a human carcinogen, patulin has been classified by IARC in group 3 (not classifiable as to its carcinogenicity to humans). The European legislation has established a maximum level of 50 µg/kg of this mycotoxin in apple juice. In this survey, patulin presence has been monitored in apple juice produced and commercialised in Serbia in 2014. In total, 30 samples were collected from supermarkets, all unique, in 1-2 litre packs, as available to the final consumers. Additionally, 10 samples of non-alcoholic drink containing 10-20% of apple juice were acquired. Analysis comprised ethyl acetate:hexane extraction, clean-up on C18 sorbent and quantification of patulin by HPLC with UV detection. Study of laboratory method performance preceded analysis of real samples, giving the following results: limit of quantification 1 µg/kg, recovery 85 and 70% for clear and cloudy juice, respectively, proficiency test z score 0.8. The survey has shown a widespread presence of patulin, with 80% of juice samples being positive and overall mean patulin level of 8.5 µg/kg. Among positive samples, 67% featured low level of patulin contamination (range 1-8.1 µg/kg, mean 3.6 µg/kg), 25% were in the range 10.1-21.4 µg/kg (mean 15.7 µg/kg), while two clear apple nectars had 37.1 and 65.4 µg/kg of patulin. As the most contaminated sample surpassed the maximum allowable level of patulin, analysis was repeated using molecularly imprinted polymer column for clean-up (Easy-MIP Patulin; R-Biopharm), and presence of patulin was confirmed. Regarding the fruit content, samples were equally distributed in groups with 100 and 50% fruit content. Cloudiness could influence patulin contamination of juice, but among collected samples vast majority (87%) were clear ones. Group of 100% apple juice showed 2-fold lower mean patulin concentration compared to 50% one. In the group of non-alcoholic drinks, patulin was present in 40% of samples, at the level of quantification. Patulin occurrence and level recorded in the present survey were higher in comparison with results obtained in survey

conducted on the Serbian market in 2013 (74% of positive samples, overall mean 5.8 µg/kg). The presence of patulin in food products can be regarded as an indicator of the quality of raw materials used, and preventive measures in juice production could be very effective in contamination reduction. Continuous collection of data is an important step in estimation of consumer exposure to contaminants in food.

## P28

### Study on patulin occurrence in fruit products for infants and children

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Patulin production is connected to the fungi belonging to *Penicillium*, *Aspergillus* and *Bysochlamys* species growing on fruits, especially apple. Therefore, presence of this mycotoxin in fruit products indicates use of unsound fruit. In order to limit exposure of infants and young children, maximum allowable level of patulin in infant food was established at 10 µg/kg. The aim of the study was to evaluate occurrence and level of patulin in apple-containing food for infants and children marketed in Serbia. The study included 54 samples of juice (21) and purée (33) for infants, as well as 38 samples of 0.2 litre pack juice with straw (usually intended for children). Samples were acquired from supermarkets in summer 2014, in a way to include variety of products and producers, content of one packet constituted a sample. Juice samples were classified as pure apple or mixed, and further according to fruit content (50/100%) and consistency (clear/cloudy). Analysis was based on ethyl-acetate:hexane extraction followed by solid-phase clean-up on C18 sorbent and HPLC-UV separation and detection. Laboratory method performance was characterised with LOQ 1µg/kg, mean recovery of 85 and 70% (clear and cloudy juice, respectively), precision in terms of relative standard deviation <5%. In infant food category, 43% of juice and 18% of homogenised purée featured patulin contamination, with overall mean levels of 1.9 and 0.8 µg/kg, respectively. Comparison of pure and mixed apple juice showed three-fold higher patulin incidence (83 vs. 27%) and four-fold higher mean level (4.1 vs. 1µg/kg) in pure apple juice. The sample with highest patulin content was clear apple juice with 100% fruit (8.3 µg/kg). Analysis of three additional samples of instant herbal tea for infants revealed patulin presence in one sample. To confirm the finding, sample was re-analysed using molecularly imprinted polymer column for clean-up (Easy-MIP Patulin; R-Biopharm) and patulin concentration of 26.2 µg/kg was determined, exceeding the maximum allowable level. In the group of juice with straw, an overall patulin incidence of 53%, mean of 2.6 and maximum of 16.4 µg/kg were observed. Patulin was detected in 54% of pure apple juice (mean 2.4 µg/kg) and 52% of mixed ones (mean 2.7 µg/kg). In both these groups 100% fruit content juice was characterised with higher patulin incidence and mean level compared to 50% one, and the same was shown for mixed cloudy juice vs. clear. A further monitoring of patulin level in food for infants and children should be conducted to control the health risk from this mycotoxin.

## P29

### Occurrence of mycotoxins and pesticides in products from organic farming

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In recent years, consumers' requirements for the information on the origin and overall quality of agricultural products they consume continue to increase. Organic products are grown under the conditions of organic farming which relies for example on the utilisation of specific crop rotation, fertilizers and natural pesticides. However, the absence of the application of synthetic pesticides could, under certain conditions, lead to more frequent occurrence of natural contaminants as mycotoxins. In this work, 38 cereal-based products from organic production were examined for the presence of 323 pesticide residues and 57 mycotoxins. For the isolation of target analytes, QuEChERS-based method was used. Multidetector analytical method based on ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) was enabled for separation and detection of target mycotoxins and pesticides. Mycotoxins with the highest incidence of detection were enniatins (11-79%), alternariol-methylether (AME, 45%), zearalenone (ZEA, 39%), and deoxynivalenol (DON, 26%). The most contaminated sample (maize cracker bread) contained 807 µg/kg of DON and 107 µg/kg of ZEA; these levels exceeded the maximum levels for

DON (500 µg/kg) and ZEA (100 µg/kg) given by Commission Regulation (EC) No 1126/2007. Concentrations of detected mycotoxins were in order of units to hundreds of µg/kg. Pesticide residues were detected in 5 samples (13%). Levels of pesticides residues were under 10 µg/kg, only one sample (maize grits) contained 34 µg/kg of chlorpyrifos-methyl. **Acknowledgements.** Supported by 'Operational Program Prague - Competitiveness' (CZ.2.16/3.1.00/22197), 'National Program of Sustainability' (NPU I (LO) MSMT - 34870/2013) and by the project QI111B154 of the Ministry of Agriculture of the Czech Republic.

### P30

#### **Occurrence of (masked) *Alternaria* toxins – a survey in foodstuffs commercially available on the Belgian market**

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The fungal genus *Alternaria* contains numerous species that can contaminate a wide variety of crops in the field and cause post-harvest decay of various fruits, grains and vegetables. In addition to causing economic losses, *Alternaria* spp. can form mycotoxins under certain conditions. Due to the possible risk for public health related to the presence of *Alternaria* toxins in food, the European Food Safety Authority stipulated that additional quantitative occurrence data are urgently needed to refine exposure assessment. Furthermore, *Alternaria* toxins can in line with other xenobiotics be partly metabolised, which may lead to the formation of conjugated metabolites in plants. These 'masked' mycotoxins are of human health concern as they may be capable to release their native precursors in the digestive tract of organisms. Therefore, a fast and sensitive UPLC-ESI<sup>±</sup>-MS/MS method for the determination of free (AOH, AME, ALT, TeA, TEN and ATX I) and conjugated (AOH- and AME-3-sulphate, AOH- and AME-3-glucoside) *Alternaria* toxins in multiple matrices, such as cereal products (rice, oat flakes), beer, fruit and vegetable juices (carrot, apple, grape juice), tomato products, lentils, sesame- and sunflower oils/seeds was developed and validated (in agreement with the criteria mentioned in Regulation 401/2006/EC; Commission Decision 2002/657/EC). The method, applying isotopically labelled internal standards, allowed for the simultaneous determination of 10 *Alternaria* toxins in a one-step chromatographic run (7 min). Minimal sample clean-up was carried out on the different matrices, making it possible to perform sampling, analysis and finally detection and quantification in less than 24h. Yet, low limits of quantification (<1-5 µg.kg<sup>-1</sup>) were obtained for all matrices. Subsequently, 250 samples of a variety of commercially available foodstuffs were analysed between February 2013 and September 2014. **Acknowledgements.** This study was funded by the Federal Public Service of Health, Food Chain Safety and Environment (Contract RF12/6261-ALTER).

### P31

#### **Mycotoxin survey: multimycotoxin analysis and distribution in feed commodities – an holistic approach to risk assessment strategies**

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The large number and structural diversity of mycotoxins has impeded rapid quantification using LC-MS/MS owing to varying toxin extraction efficiencies and interferences from feed and food matrices. We have successfully tackled these challenges by developing the 37+™ analysis. This novel method provides simultaneous and accurate quantification for more than 37 mycotoxins in feed in a cost-effective manner. This approach normalises losses during extraction and matrix suppression/enhancement by using labelled mycotoxins as surrogates and internal standards. In this analytical setting, four isotopologues were used to normalise the MS signals of known concentrations of 10 mycotoxin groups. During 2012 to 2014, more than 5000 feed samples were received from across the world and examined for mycotoxin using the 37+® analysis. The mycotoxins population followed a Gaussian distribution with measurable concentrations of mycotoxins detected in more than 99% of the samples (average = 8 different mycotoxins per sample) with only 14 samples containing no detectable



mycotoxins. Fumonisin closely followed by trichothecenes B were the most prevalent mycotoxins. Trichothecenes, ergot alkaloids and other toxins, such as *Aspergillus* and *Penicillium* toxins, found in stored feed accounted for 30% of the rest of mycotoxins found. For the first time, analysis of the distribution of *Penicillium* toxin as well as potential synergistic compounds such as fusaric acid has been made possible. Mycotoxin concentrations were further interpreted and normalised according to known species specific sensitivities. The latter were evaluated according to the principles of toxic equivalent factors used to performed risk assessment for PCBs, dioxins and furans and adapted to mycotoxins. This approach allowed evaluating the toxicological risk associated with levels of mycotoxins found in samples and normalised according to the impact of the distribution of the toxin for mixed animal species and can facilitate audits to monitor within time, changes in the mycotoxin distribution and consequent issues. In conclusion, the 37+™ analysis shows that the spectrum of mycotoxins that naturally contaminate feed commodities is exceedingly broad. For the first time, we are proposing a holistic strategy for accounting, reporting and performing a risk assessment for multiple mycotoxin contamination trends that were often neglected from other analytical approaches that only focused on a small number of contaminants analysed at once.

### P32

#### **Molecular quantification and genetic diversity of toxigenic *Fusarium* species in northern Europe and Asia**

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The highest DON and T-2/HT-2 toxin levels in northern Europe have been found in oats. About 12-24% of Finnish oat samples in 2012 contained >1750 ppb of DON. *Fusarium graminearum* is the most important DON producer in northern Europe and Asia and it has been replacing the closely related *F. culmorum* in northern Europe. The 3ADON chemotype of *F. graminearum* dominates in most northern areas, while the 15ADON chemotype of *F. graminearum* is dominating in Central and southern Europe. We suggest that the northern European population may be specialized more to oats than the southern European population. No clear correlation was found between *F. culmorum* DNA and DON levels. DNA levels of *F. graminearum* were in all cases in agreement with DON levels in 2011 and 2012, when DON was measured by GC-MS. When the RIDA®QUICK SCAN kit results (DON) were compared to DNA levels of *F. graminearum*, the variation was much higher. The homogenization of the oats flour by sieving seems to be connected to this variation. There was a significant correlation between the combined T-2 and HT-2 and the combined DNA levels of *F. langsethiae* and *F. sporotrichioides* in Finland in 2011 and 2012. New findings on the correlation between mycotoxins and DNA levels of *Fusarium* species in Finland are presented. There are not enough morphological characters to clearly separate all isolates of the European species *F. langsethiae* from the northern Asian species *F. sibiricum*, while the cosmopolitan species *F. sporotrichioides* can be easily identified based on morphological characters. The long TG repeat in the ribosomal IGS region is the only known DNA sequence that has been used to design a species-specific primer pair for identification of *F. sibiricum* isolates. Another way to identify *F. sibiricum* is to use a combination of a *F. sporotrichioides*-specific primer pairs, which give a positive signal both with *F. sporotrichioides* and *F. sibiricum* isolates, and *F. langsethiae*-specific primer pairs, which give a clear positive signal only with *F. langsethiae* isolates. *F. langsethiae* is a European species, while the main distribution of *F. sibiricum* is in northern Asia. The identification of the first a *F. sibiricum* isolate from Iran and the first *F. langsethiae* isolate from Siberia is confirmed by both molecular and morphological methods. A single isolate of *F. sibiricum* is also found in Norway. In conclusion, the actual distribution of *F. sibiricum* may be much larger than what is presently known.

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### P33

#### Negative effects of fumonisins in pigs and their effective counteraction

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The objective of the experiment was to evaluate the efficacy of a mycotoxin deactivation product in diminishing the toxic effects of fumonisins (FUM) added to piglet diets. Twenty four pigs were randomly divided into 4 treatments each with 6 replicates. The experiment lasted for 42 days. A negative control group of piglets received a diet without fumonisins contamination and the mycotoxin deactivation product. The positive control group received the same feed as the negative control group supplemented with 5 kg/ton of mycotoxin deactivation product (MDP). The third group of piglets received a diet contaminated with 50 mg/kg fumonisins. The fourth group received a diet contaminated with fumonisins (50 mg/kg) and 5 kg/ton of MDP. Evaluated parameters were: average daily weight gain; final body weight; feed conversion rate (FCR) and blood sphinganine/sphingosine ratio (Sa/So). The negative effect of fumonisins was observed in the daily weight gain of the pigs that received the contaminated diet. The addition of the mycotoxin deactivation product significantly improved ( $P \leq 0.05$ ) average daily weight gain when compared with animals offered the diet contaminated with fumonisins. Animals consuming diets contaminated with fumonisins showed lower final body weight and poorer FCR. Diets contaminated with fumonisins did not have any significant influence on blood Sa/So values. In conclusion, feeding contaminated diets to piglets resulted in a reduction of average daily weight gain (-10.9%), final body weight (-6.5%) and efficiency (FCR +5.5%). The addition of the mycotoxin deactivation product at 0.5% reduced the negative impact of toxic effects of fumonisins present in the contaminated feed.

### P34

#### *In vitro* effects of fumonisin B1 alone and combined with deoxynivalenol or $\beta$ -zearalenol on bovine granulosa cell proliferation and steroid production

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Fumonisin B1 (FB1) is a common maize contaminant frequently in co-occurrence with deoxynivalenol (DON) and zearalenone (ZEA). Potential reproductive effects of FB1 alone or in combination have been poorly investigated in animal species (Cortinovis *et al.*, 2014) and no information is available in cattle. Thus, the goal of this study was to determine if FB1 alone and combined with DON and/or  $\beta$ -zearalenol ( $\beta$ -ZEA), ZEA hydroxylated metabolite, can affect granulosa cell proliferation and steroid production. Ovaries from non-pregnant beef cows were collected from a local slaughterhouse and follicular fluid was aspirated from small follicles (1-5 mm) to isolate granulosa cells. Granulosa cells were cultured for 2 days in 10% foetal bovine serum followed by 2 days in serum-free medium containing 500 ng/ml of testosterone (as an oestradiol precursor), 30 ng/ml of FSH and 30 ng/ml of IGF-I with FB1 (0, 30, 100 ng/ml), DON (0 or 100 ng/ml) and  $\beta$ -ZEA (0 or 30 ng/ml). The results revealed that FB1 alone at all doses had no effect on granulosa cell numbers and steroidogenesis. In the presence of  $\beta$ -ZEA, FB1 at 30 ng/ml showed a stimulatory effect on granulosa cell numbers, whereas no significant interaction existed between FB1 and DON and between FB1,  $\beta$ -ZEA and DON. In regard to progesterone production, FB1 at 100 ng/ml showed a stimulatory effect in cells treated with  $\beta$ -ZEA, whereas no significant interaction was observed between FB1 and DON alone or with  $\beta$ -ZEA. Finally, FB1 (30 and 100 ng/ml) was found to enhance the inhibitory effect of  $\beta$ -ZEA on oestradiol production. In conclusion, the present study demonstrates that significant toxicological effects of FB1 are observed in co-exposure with  $\beta$ -ZEA in a bovine granulosa cell model.

### P35

## Effects of mycotoxin exposure on proliferation and apoptosis in the porcine intestinal mucosa

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Most fungi can produce several types of mycotoxins, and foodstuffs are often contaminated by more than one fungal species simultaneously. Therefore, humans and animals are generally exposed to multiple mycotoxins at the same time rather than one, yet studies on their combined toxicological effects are limited. The goal of this study was to compare the local effects of two commonly encountered trichothecene mycotoxins, deoxynivalenol (DON) and nivalenol (NIV) alone or combined, on the porcine intestinal mucosa. Two different pig samples were used: (i) from jejunal loops obtained by surgery on one part; and (ii) from animals after a 4-week dietary exposure. Seven piglets were used for loops: 5 pigs slaughtered to collect jejunum segments after 4 h following the injection of the loops and 2 pigs after 24 h. The mycotoxins were injected into the loops (12 loops/pig): control, DON, NIV and DON+NIV (1:1) at 0, 1, 3, and 10 µM. An *in-vivo* feeding trial was conducted on twenty-four piglets: a control diet-group, two DON diet-groups contaminated with either low-dose or high-dose DON, and a DON+NIV diet-group. Following the four-week trial, segments of jejunum were removed, prepared into histological sections. Both the loops and the *in vivo* jejunum segments were labelled with two immunohistochemical markers: Ki-67 for proliferation, and caspase-3 for apoptosis. In loops, the ratio proliferation/apoptosis in the jejunal mucosa was strongly decreased, by more than two-fold ( $p < 0.001$ ) in the mycotoxins-injected loops, 24 h after injection with DON or/and NIV (10 µM), compared to control loops. In the *in vivo* feeding trial, the proliferation of enterocytes was specifically analysed. Results suggested that mycotoxin contaminations at subclinical doses might inhibit proliferation of enterocytes, but no statistically significant difference was observed. Loop is a relevant and sensitive model to investigate *in situ* effects of co-occurring mycotoxins. However, it does not allow to model repeated exposures. There was no significant result from *in-vivo* feeding trial because of high individual variability.

### P36

## *In silico* in vitro approaches for zearalenone metabolism in human hepatocytes

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Knowledge about the conjugative metabolism of xenobiotics is crucial for estimating their toxic effects and intrinsic clearance in humans and livestock. Metabolic pathways investigations are commonly carried out by means of liver microsomes and/or hepatocytes, followed by mass-spectrometry based analysis. However, metabolite identification is still a time-consuming task, especially when the kinetic of formation of phase I and phase II derivatives has to be considered. In this context, advanced *in silico* techniques can successfully assist data processing, thus moving the bottleneck of metabolite structure elucidation from spectra interpretation to data acquisition. Recently, the MassMetaSite software has proved useful for automatic metabolite identification. MassMetaSite generates all possible phase I and phase II metabolites for a xenobiotic. Thus fragmentation pattern generated for each metabolite is used to drive the structural assignment from *in vitro* or *in vivo* experiments. This automatic procedure considerably speeds up metabolite identification in drugs and xenobiotics studies. To our knowledge, the MassMetaSite approach has not been previously applied to investigate the metabolism of micotoxines. As a proof of concept, we studied the liver phase I and phase II biotransformation of ZEN,  $\alpha$ - and  $\beta$ -ZEL and ZEN14G using MassMetaSite for automatic metabolite identification. The kinetic behaviour was monitored for 4 h in human hepatocytes. Glucuronide and sulphate derivatives were characterised and followed over the observation period, with results in agreement with those reported in the literature so far. Furthermore, novel desaturated metabolites of ZELs were identified and information about ZEN14G biotransformation in liver was obtained.

P37

### Masked forms of zearalenone mycotoxin and xenoestrogenic activity in MCF7-based assays: computational insight of mechanisms of action

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Zearalenone (ZEN) is a mycotoxin produced by several species of *Fusarium* fungus infecting small-grain cereals and maize worldwide. *In planta* it can be converted in the 14-O-glucoside form as a result of detoxifying mechanisms and it may be found in the edible parts of plants, thus being able to contaminate food and feed. In spite of this, few data on its toxicological activity are available so far. ZEN is a well known xenoestrogen together with its phase I metabolites  $\alpha$ - and  $\beta$ -ZEL, while the xenoestrogenic activity of ZEN-14-O-glucoside has never been investigated in cell-based assays. Very recently, we gained evidence of a possible estrogenic activity of ZEN14G in a MCF-7 based assay. Since it is commonly believed that conjugations may lead to a decreased toxicity, molecular mechanism of such activity has been studied using an *in silico* approach. The interaction between mycotoxins and cavities of human alpha oestrogen receptor has been investigated by using docking simulations. Computational analysis revealed that both conjugated forms are not able to exert agonistic activity. Such results are in agreement with experimental data stating the absence of binding up to 5.4  $\mu$ M. Accordingly, the possible reversion of the conjugated form to the aglycone was proposed, as a consequence of the cell metabolism. The conversion of ZEN14G to the parent compound and to other related xenoestrogenic metabolites has been monitored in MCF-7 cells up to 24 h. Intriguingly, the conjugated form rapidly decreased. After 24 hours, ZEN was found as main residual form into the cell. The reversion to aglycone is thus proposed as a possible explanation of the xenoestrogenic activity of ZEN14G in MCF-7 based assay.

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### Dietary exposure and microbial metabolism of DON and DON-3-glucoside in humans

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Deoxynivalenol (DON) and its masked metabolite DON-3-glucoside (D3G) are frequently detected in agricultural crops and occurrence is suggested to be linked to climate conditions during agricultural production. Dietary exposure to these mycotoxins has been demonstrated in humans and farm animals. The microbial degradation of mycotoxins has been reported by microbes derived from various ecosystems, but the activity of the human gut microbiota towards DON and D3G have not been fully studied. This study aims to assess the ability of human gut microbiota to degrade dietary D3G and DON and to estimate human dietary exposure using urinary DON as biomarker. Fresh faecal samples from 10 volunteers were prepared into slurries using M2 culture medium, spiked with DON or D3G and incubated anaerobically for up to 7 days. Mycotoxins were extracted into acetonitrile and detected using LC-MS/MS. Spot urine samples from 15 volunteers were collected in 2012 and again in 2013. Samples were cleaned through immunoaffinity columns and mycotoxins were detected using LC-MS/MS. Faecal microbiota of all 10 volunteers rapidly and efficiently cleaved D3G and released free DON. Two volunteers also possessed the microbial activity to transform DON to de-epoxy DON (DOM-1), a less toxic metabolite. These two volunteers also excreted DOM-1 in urine. Furthermore, we found that all 15 volunteers excreted detectable levels of DON in their urine. Average DON excretion was higher in 2013 following a year of high *Fusarium* prevalence in agricultural crops compared to 2012 with low *Fusarium* burden. Using urinary DON as biomarker for dietary exposure we estimate that the tolerable daily intake (TDI) for DON was exceeded in several volunteers in 2013, but not in 2012. This suggests that current maximum levels of DON permitted in food might not sufficiently protect consumers during years of high *Fusarium* prevalence. The findings of this study suggest that free DON is released from D3G in the human colon and could exert toxicity towards the colonic epithelium. Occasionally DON can be detoxified in the colon to DOM-1 and absorbed into systemic circulation. In one volunteer the proportion of urinary DOM-1 reached 16% of DON, suggesting a substantial protective effect against DON toxicity through microbial detoxification.

P39

### Effects of orally administered fumonisin B1, partially hydrolysed fumonisin B1, hydrolysed fumonisin B1 and N-(1-deoxy-D-fructos-1-yl) fumonisin B1 on the sphingolipid metabolism in rats

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Produced by a number of *Fusarium* species, fumonisins are a group of mycotoxins which show toxic effects particularly due to the alteration of the sphingolipid metabolism. The resulting change in the ratio of the sphingoid bases sphinganine (Sa) to sphingosine (So) is a biomarker for fumonisin exposure. The most frequently occurring fumonisin is fumonisin B1 (FB1); however, there additionally exist a wide range of fumonisin analogues, that may be generated by hydrolysis during gastrointestinal metabolism, e.g., partially hydrolysed fumonisin B1 (pHFB1a and pHFB1b) and hydrolysed fumonisin B1 (HFB1) or by food processing, e.g., N-(1-deoxy-D-fructos-1-yl) fumonisin B1 (NDF-FB1). The toxicological relevance of those compounds is still not completely known. Therefore, the aim of this work was to compare the sphingolipid metabolic effect of FB1, pHFB1, HFB1 and NDF-FB1 by investigating the Sa/So ratios in urine and kidney samples of rats. Hence, a feeding trial with male Sprague Dawley rats aged five weeks was conducted. Preparation of the experimental diets required production of the toxins and further purification, stability tests and inclusion of toxins (via spiked water) into a mixture of commercial rat feed and supplemented maize which were further used for cookie production. 20 male Sprague Dawley rats were allocated to 5 groups (n=4): four treatment groups FB1, pHFB1, HFB1 and NDF-FB1 (each containing 13.9 µmol of the respective toxin per kg) and a negative control group. After an acclimatisation period of one week, the animals received the respective diets and water *ad libitum* for 3 weeks. Urine and faeces samples were collected once a week by housing the rats individually in metabolic cages for 24 hours. On day 21, the rats were euthanised and kidney samples were taken. The Sa/So ratios in urine and kidney samples were determined by LC-MS/MS analysis. The average Sa/So ratio in urine samples of the FB1 group was above 5 on day 7, 14 and 21. In contrast, the average urinary Sa/So ratio of the other individual groups was approximately 0.2. Similarly, the only significant elevation of the Sa/So ratio in kidney samples occurred in the FB1 group. Consequently, in contrast to the parent FB1, the investigated fumonisin analogues pHFB1, HFB1 and NDF-FB1 do not disrupt the sphingolipid metabolism in rats based on the Sa/So ratios in urine and kidney samples.

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### Porcine, chicken, mouse, and fish derived cell lines as model systems to compare the cytotoxicity of DON and its metabolite after microbial transformation

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Deoxynivalenol (DON), a trichothecene produced by *Fusarium* species, is one of the most prevalent food- and feed-associated mycotoxins in cereals and cereal-derived products. *Genus novus species novus* BBSH 797 of the *Coriobacteriaceae* family, which was isolated of bovine rumen fluid, transforms DON into the less toxic metabolite de-epoxy-deoxynivalenol (DOM-1). For that reason, BBSH 797 is used as feed additive to counteract the toxicity of DON. The effect of DON and DOM-1 on cell proliferation was analysed for porcine and chicken peripheral blood mononuclear cells (BrdU test), IPEC-J2 cells (WST-1 assay (cytotoxicity), the rainbow trout cell line RTGill-W1 (Neutral Red assay (lysosomal activity) and Sulforhodamine B assay (protein synthesis)), and the murine macrophage cell line RAW 264.7 (WST-1 assay). Moreover, nitric oxide (NO) production, measured by Griess reaction, was determined by the latter. DOM-1 did not affect the viability of the tested cell lines at the added concentration (0.08-2.56 µM), whereas DON alone showed already a cytotoxic effect at the concentrations of ~ 0.642 µM. For the RAW 264.7 cell line, the addition of lipopolysaccharide (LPS) from *E. coli* O111:B4 (10 ng/ml) did not affect the cell performance either,

whereas DON decreased viability and NO production at 0.422  $\mu$ M. In contrast to DON, DOM-1 did not reduce the LPS-induced NO production (measured as % activity), not even at the highest concentration (26.9  $\mu$ M). The non-cytotoxic effect of DOM-1 cannot be explained by a decreased stability, as DOM-1 was retrieved in LC-MS/MS measurements, proving that DOM-1 was stable in the course of the experiment. Data therefore confirm the detoxifying abilities of *Genus novus species novus* BBSH 797 and support its use as a DON-detoxifying feed additive.

#### P41

### Immune response and urinary fumonisin B1 excretion in piglets fed low dietary levels of fumonisin

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The objective of this study was to investigate the immune response and the excretion of fumonisin B1 (FB1) in urine of piglets fed rations with low levels of FB1 for 28 days. Twenty-four piglets were randomly assigned into 4 experimental groups and given prepared rations containing 0, 2.0, 4.0 or 8.0 mg FB1/kg feed. Urine samples were collected on days 7, 14, 21 and 28 for determination of FB1 levels by liquid chromatography coupled to a tandem mass spectrometer (UPLC-MS/MS). On 3rd and 10th days of the experiment, piglets were immunised with ovalbumin and Stellamune® Mycoplasma vaccine. Blood samples were also collected at time intervals (14th, 21st and 28th days of the experiment) for determination of total (IgG; IgA) and specific immunoglobulins (IgG-anti-*Mycoplasma hyopneumoniae*) by enzyme-linked immunosorbent assay (ELISA). Mean levels of FB1 in urine of animals fed 2.0, 4.0 or 8.0 mg FB1/kg were 23 $\pm$ 6.6, 29 $\pm$ 10.4 and 47 $\pm$ 20.8 ng/ml, respectively. A progressive decrease in the urinary levels of FB1 was observed in all treatments during 28 days of exposure. Total IgG in plasma ranged from 6.6 $\pm$ 3.7 to 15.2 $\pm$ 2.7 mg/ml, 5.8 $\pm$ 2.6 to 16.9 $\pm$ 1.3 mg/ml, 7.2 $\pm$ 2.1 to 16.9 $\pm$ 0.7 mg/ml and 4.7 $\pm$ 3.6 to 16.5 $\pm$ 1 mg/ml in animals fed 0, 2.0, 4.0 8.0 mg FB1/kg feed, respectively. Compared to the control group, no differences ( $p>0.05$ ) were found between the IgG or IgA levels in piglets fed FB1. However, the IgG-anti-*M. hyopneumoniae* levels decreased ( $p<0.05$ ) in animals fed 4 mg (mean, 1.4 $\pm$ 2.0 arbitrary units (AI)) or 8 mg FB1/kg feed (mean, 2.9 $\pm$ 2.0 AI) at day 28, when compared to the control (mean, 9.9 $\pm$ 5.0 AI). Urinary FB1 was excreted linearly according to the toxin concentration in the feed, along with a decrease of levels in all the treatments over time. Results of this trial indicate that the excretion FB1 in urine of piglets decreases over 28 days of continuous exposure to low levels of FB1 in feed, although it was accompanied by immunosuppression effects during this period. **Acknowledgements.** Financial support: CAPES (Grant no. BEX 5780/13-6).

#### P42

### Exposure assessment of aflatoxin intake by determination of aflatoxin M1 in urine from residents of São Paulo, Brazil

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In the present study, aflatoxin M1 (AFM<sub>1</sub>) was determined in 114 urine samples from residents of Pirassununga, State of São Paulo, Brazil. Sampling procedures were performed four times in June 2011 (n=33), September 2011 (n=31), December 2011 (n=27) and March 2012 (n=23). The individual aflatoxin B1 (AFB1) and AFM1 ingestion by each volunteer was estimated by the analysis of bean, peanut, maize and milk products collected directly from home of residents by the time of urine collection, and a 24-hour dietary recall questionnaire. Analyses of AFB1 in bean, peanuts and maize products, and AFM1 in liquid or powder milk, cheese and yoghurt were performed by using immunoaffinity columns and high performance liquid chromatography (HPLC) with fluorescence detection (Shimadzu). For determination of AFM1 in urine samples, an ultra-performance liquid chromatograph (UPLC) system (Acquity I-Class, Waters) coupled to a Xevo TQ-S mass spectrometer (Waters) was used. Peanut products presented the highest AFB1 concentration in the range of 0.06 to

17.1 µg/kg (mean, 3.5±4.9 µg/kg) in 34% of samples analysed. Maize products had the lowest AFB1 levels, ranging from 0.27 to 7.7 µg/kg (mean, 1.0±2.0 µg/kg), but with the highest incidence (> 60%) and large variation of AFB1 concentration among sampling periods. AFM1 was quantified in 40% of fluid milk (range, 0.009 to 0.069 µg/l; mean, 0.03±0.02 µg/l), and in 30% of cheese samples (range, 0.091 to 0.30 µg/kg; mean, 0.16±0.12 µg/kg). Based on the occurrence levels of AFB1 and AFM1 in the foods analysed and the data from the 24-hour dietary recall questionnaire, the mean aflatoxin intake by volunteers varied between zero to 37.6 ng/kg body weight among the 4 sampling times. AFM1 was detected in 74 urine samples (65%), at levels ranging from 0.25 to 6.8 pg/ml (mean, 1.3±1.5 pg/ml). Although the aflatoxin levels in food products were low in all sampling periods, a positive correlation ( $r=0.48$ ;  $p=0.02$ ) was found between AFM1 concentrations in urine and the aflatoxin intake (AFB1 + AFM1) in the first sampling (June 2011). The low levels of AFM1 found in urine samples indicate low short-term exposure to aflatoxins. However, further investigations are needed to assess other sources in the diet for aflatoxin exposure in the population under study. **Acknowledgements.** Financial support: FAPESP (Grant no. 2010/20895-4).

#### P43

### Genotoxicity of aflatoxin precursors

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Aflatoxins are mainly produced by *Aspergillus flavus* and *A. parasiticus*. They are frequently found as contaminant of agricultural commodities, causing a wide range of toxic effects. Aflatoxin B1 (AFB1) is the most dangerous aflatoxin, demonstrating genotoxic carcinogenic effect and being classified by the IARC in Group 1 compound. Aflatoxins are produced from acetate and the biosynthetic pathway involves about 15 successive steps leading to different precursor molecules. Strategies to limit the exposure to aflatoxins include agricultural practices management, but also the use of pesticides or microorganisms that might block the AFB1 biosynthesis leading to the accumulation of aflatoxin precursors. The aim of this study was to compare the cytotoxicity and the genotoxicity of aflatoxins with their precursors. More specifically, we tested the aflatoxins: AFB1, AFG1, AFB2, AFG2; the early metabolic precursor norsolorinic acid (NOR), averantin (AVN) and versicolorin A (VERA); the late biosynthetic precursors sterigmatocystin (SC) and O-methylsterigmatocystin (OMSC). Aflatoxin M1 (AFM1), the main human metabolite of AFB1 and cyclopiazonic acid (CA) synthesised in parallel were also tested. The molecules were obtained commercially or purified from highly producing mutant strains of *A. parasiticus* and *A. nidulans*. The genotoxicity was assessed on three types of human cell lines (LS-174T from colon, HepG2 from liver and ACHN from Kidney) by quantifying of the phosphorylation of the histone H2AX (γH2AX), which reflects a global genotoxic insult, using the In-Cell Western technique. Our results indicated that the most genotoxic molecules on the three cell lines were in decreasing order ST, AFB1, AFG1 and VERA. AFM1 and OMST demonstrated genotoxic properties only on the LS-174T cell line. The other molecules tested (NOR, AVN, AFB2, AFG2) did not demonstrate any genotoxic potential. In conclusion, some metabolic precursors of aflatoxins (ST, VERA) demonstrated important genotoxicity towards human cell lines. This should be taken into account for the development of new strategies intended to reduce the aflatoxins exposure and for human risk assessment. **Acknowledgements.** Source of funding: ANR Aflafree, Agri Sud Ouest Innovation.

#### P44

### Deoxynivalenol exacerbates DNA damage in rats colonised by *Escherichia coli* producing the genotoxin colibactin

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The intestinal epithelium is the first barrier in contact with microorganisms and food contaminants. *Escherichia coli* are pioneer bacteria colonizing the gastro-intestinal tract of mammals within a few days after birth and are the predominant facultative anaerobic bacteria in adult microbiota. An increased number of newborns are colonized at birth with *E. coli* strains producing the hybrid peptide-polyketide genotoxin, named colibactin, that induces DNA-double strand breaks both *in vitro* in mammalian cell lines and *in vivo* in the intestine of neonates. Deoxynivalenol (DON) is a mycotoxin that contaminates human food chain frequently found in diet. Consumption of DON-contaminated food is associated with multiple side effects such as modulation of immune responses and impairment of the intestinal barrier function. The aim of this study was to analyse the consequences of a 'co-exposition' to genotoxic bacteria and the consumption of mycotoxin-contaminated diet on intestinal epithelium. We especially investigated the effect of this combination on DNA damage in intestinal epithelial cells. We first quantified the phosphorylation of H2AX histone, a marker of DNA-double strand breaks in the rat intestinal cell line, IEC-6 in contact with both DON and colibactin producing *E. coli*. DON had no direct genotoxic effect but DON exposure increased levels of genotoxicity in IEC-6 cells infected by genotoxic *E. coli* strain in a time- and dose-dependent manner. Newborn rats were then colonised at birth by genotoxic *E. coli* strain and after weaning were feed with DON-contaminated diet (2 or 10 ppm) for 4 weeks. At the end of experiment, DNA damages were detected by quantification of the phosphorylation of H2AX histone in nucleus. Whereas, only few or no DNA damage were observed in animals colonized by *E. coli* producing colibactin and in animals exposed to DON-contaminated diet. In contrast significant DNA damages were observed in intestinal epithelial cells of animals colonized by genotoxic *E. coli* strains and exposed to DON in a dose-dependent manner. DON is classified by IARC as 'not classifiable as to its carcinogenicity to humans' (Group 3). Our results indicate that this toxin is able to induce DNA damages in intestinal epithelial cells of adults colonised by a commensal genotoxic *E. coli* strain, raising some questions about the potential role of DON in carcinogenesis. **Acknowledgements.** Source of funding: ANR DON&Co.

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### **Masked DON and other DON metabolites do not exert intestinal toxicity: transcriptomic analysis of jejunal explants**

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Deoxynivalenol (DON) is a frequent mycotoxin in grains, produced by *Fusarium* fungi, which demonstrates multiple side effects such as modulation of immune responses, reduced feed intake and weight gain or impairment of the intestinal barrier function. Among animal species, pigs are the best model for humans and are very sensitive to DON. In wheat, DON can be conjugated to glucose to form DON-3-β-D-glucoside (D3G). Some bacteria isolated from digestive tracts or soil, are also able to de-epoxydise or epimerise DON to metabolites such as deepoxy-deoxynivalenol (DOM-1) or 3-epi-deoxynivalenol (epi-DON). The toxicity of these DON metabolites is poorly documented. The intestine is the first organ exposed to these molecules and so constitutes a relevant model. The aim of this study was to compare the intestinal toxicity of three DON metabolites (D3G, DOM-1 and epi-DON) with the one of DON. Intestinal explants from 6 pigs were treated with 10mM DON, D3G, DOM-1 or epi-DON for 4 h and transcriptomic analysis was performed using an 'Agilent Porcinet 60K'. Among the 34,775 probes expressed in array, 747 were differentially expressed in DON-treated explants when compared to Mock-treated ones. It represents 323 genes. It encompasses genes involved in various processes including inflammatory response, cell death and survival as well as hematological system development and function. Data obtained by microarray analysis were confirmed by real time qPCR, significant increase of the expression of the pro-inflammatory cytokines IL-1beta, IL-8, IL-17, TNF-alpha, IL-1alpha, IL-22, IL-12p40 are observed in DON treated explants. In contrast, no genes were differentially expressed between control and D3G, DOM or 3-epi-DON treated explants as demonstrated by DNA array and qPCR analysis. In conclusion, our data confirm the intestinal toxicity of DON and demonstrate that de-epoxydation, glucosylation or epimerisation of DON decrease the toxicity of this mycotoxin in this intestinal model.



P46

## Ochratoxin A induces micronucleus formation and inhibition of DNA repair pathway in human lymphocytes

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Contamination of food commodities by fungal toxins has attracted great interest because many of these mycotoxins are responsible for different diseases, including cancer and other chronic illnesses. Ochratoxin A (OTA) is a mycotoxin naturally present in food, and long-term exposure to food contaminated with low levels of OTA has been associated with renal cancer. In the present study, low doses of OTA (0.075, 0.15, 1.5, 5.0, and 15  $\mu\text{M}$ ) were assayed for determining the cytotoxicity, cytostaticity, and genotoxicity in human lymphocytes. The cytokinesis-blocked micronucleus (CBMN) assay, the comet assay and the DNA repair assay were used. Treatments with OTA were not cytotoxic but caused a cytostatic effect in human lymphocytes at 15  $\mu\text{M}$ . Micronucleus (MN) induction was observed at OTA concentrations of 1.5 and 5  $\mu\text{M}$ . OTA (0.075-5  $\mu\text{M}$ ) produced a slight increase in the percentage of DNA in the comets. In the repair assay, N-hydroxyurea (NHU) (10 mM) was used to detect non-repaired lesions produced by OTA (1.5 and 5  $\mu\text{M}$  OTA) after of 30 and 60 minutes. The OTA was capable of delay the repair capacity of the lymphocytes until 60 minutes. Our results indicate that OTA induces DNA stable damage at low doses that are neither cytotoxic nor cytostatic, and OTA delays DNA repair kinetics. **Acknowledgements.** The authors are grateful to the Spanish (Project AGL2011-24862) and Catalanian (XaRTA-Reference Network on Food Technology) Governments for their financial support. C.A. González-Arias thanks the Secretaria de Universitats i Recerca del Departament de Economia i Coneixement of the Generalitat de Catalunya for the pre-doctoral grant.

P47

## Risk ranking of mycotoxins for the Austrian population based on data from official control

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The method of risk ranking was applied for mycotoxins in food to identify data gaps in risk assessment. First, a hazard list has been set up due to existing information on toxicology. The list comprises aflatoxins B1, B2, G1, G2, M1, alternariol, alternariol monomethyl ether, deoxynivalenol (DON), 3-acetyl-DON, 15-acetyl-DON, ergot alkaloids, fumonisins B1, B2, nivalenol, ochratoxin A, patulin, T-2 toxin, HT-2 toxin and zearalenone. Risk ranking according to Mengelers, Geraets and Jeurissen (presented at Symposium SciCom2013 in Brussels, Belgium, 29 November 2013) is based on toxicity and exposure. The chronic toxicity of mycotoxins is scored according to their tolerable daily intakes from 0 to 10 (low to high potency). Five ranges of tolerable daily intakes of <0.1, 0.1-1, 1.1-10, 11-100 and >100  $\mu\text{g}/\text{kg}$  bw/day are given corresponding with TOX-scores 8, 6, 4, 2 and 0, respectively. The highest TOX-score of 10 is assigned to aflatoxins as genotoxic carcinogens. Based on Austrian occurrence and consumption data average and high exposures are estimated for all mycotoxins. About 6,500 official food samples were analysed for their mycotoxin concentrations within the period 2007 to 2013. The Austrian consumption data of the four population groups preschool children (3 to 6 years), school children (6 to 15 years), women and men (19 to 65 years) are used. The exposures are allocated to one of the eleven exposure groups corresponding to an EXPO-score from 0 to 10. Low intakes of up to 1 ng/kg bw/day are assigned to an EXPO-score of 0, intakes above 10,000 ng/kg bw/day to the highest EXPO-score of 10. Results are visualised in a risk matrix by plotting the TOX-score on the y-axis and the EXPO-score on the x-axis. The risk matrix is divided into three areas indicating different risk levels: green for low risk, orange for medium risk and red for high risk. For all population groups, DON represents the greatest risk among all mycotoxins at high consumption. The remaining mycotoxins mostly constitute a medium risk either owing to their toxicity or due to consumption pattern. Nivalenol and zearalenone pose the lowest risk especially at average consumption. Only a few samples have been analysed for ergot alkaloids, alternariol and alternariol

monomethyl ether so far. The risk assessments for these substances are therefore subject to the greatest uncertainty, which is also illustrated in the risk matrices.

**P48**

### **Assessment of consumer exposure to deoxynivalenol and its acetyl derivatives through consumption of cereal-based food from the Austrian market**

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From 2007 to 2013 cereal-based food was collected from the Austrian market and analysed for the *Fusarium* toxins deoxynivalenol (DON), its acetyl derivatives 3-acetyl-DON (3-ADON) and 15-acetyl-DON (15-ADON). In total 1890 food samples were analysed for their levels of DON. In 39% of the samples DON was determined in quantifiable concentrations ( $\geq 50 \mu\text{g}/\text{kg}$ ). The highest average DON level (mean:  $242 \mu\text{g}/\text{kg}$ ) was found in snacks. The highest concentration of DON of  $2110 \mu\text{g}/\text{kg}$  was measured in a sample of popcorn. 1746 food samples were also tested for 3-ADON and 15-ADON. The two derivatives occurred at a much lower frequency than DON. Thus, 3-ADON was determined in only two samples of breakfast cereals and 15-ADON in thirty one samples of maize products, wheat flour and beer. The estimated average intake of DON, 3-ADON and 15-ADON is  $1 \mu\text{g}/\text{kg}$  bw/day for preschool children,  $0.5 \mu\text{g}/\text{kg}$  bw/day for school children and  $0.4 \mu\text{g}/\text{kg}$  bw/day for women and men. DON contributed with more than 90% to the total exposure. Bread and pastries followed by pasta were the main contributing food groups. The estimated high intake of DON and its derivatives is  $0.9 \mu\text{g}/\text{kg}$  bw/day for school children,  $1 \mu\text{g}/\text{kg}$  bw/day for adults and  $2.3 \mu\text{g}/\text{kg}$  bw/day for pre-school children. Hence, a high consumption of cereal-based foods, such as bread and pastries, may lead to intakes above the maximum tolerable daily intake of  $1 \mu\text{g}/\text{kg}$  bw/day.

**P49**

### **Biomarker analysis in serum of pigs fed diets contaminated with fumonisins**

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Swine, besides horses and rabbits, is the species most affected by fumonisins. Intoxication with high doses of fumonisins leads to porcine pulmonary oedema. Even low contaminations of fumonisins predispose pigs to lung pneumonitis. Toxic hepatosis can occur in the presence of porcine pulmonary oedema but also without any visible effect on the lung. 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 Sa/So ratio can be used as a biomarker of effect to indicate fumonisin contamination. Aim of this study was to evaluate the effect of fumonisins and a counteracting agent in feed on the sphinganine/sphingosine-ratio (Sa/So) in serum of pigs. 48 weaning piglets with an average starting weight of 7.44 kg were allocated to four experimental groups of 12 animals each according to weight and gender. Control group received no FUM and no additive in the diet, the second group received 5 mg/kg FUM, the third group 5 mg/kg FUM and a fumonisin degrading enzyme (60 U/kg feed) and the fourth group only the fumonisin degrading enzyme (60 U/kg feed). All animals were ear tagged and individually weighed. In the course of the whole trial period all animals had free access to feed and water. Trial duration was 42 days. Besides performance parameters, serum samples were taken and analysed for biomarkers. In the course of the trial a negative impact of FUM on performance of the animals was observed. Sa/So-ratio, measured at day 42, was increased in the FUM group ( $p < 0.05$ ). By the addition of the fumonisin degrading enzyme levels of Sa/So were reduced to control levels again.

**P50**

### **Examination of the effect of deoxynivalenol (DON) and de-epoxy-deoxynivalenol (DOM-1) on intestinal, immune, and hepatic parameters**

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Deoxynivalenol (DON), a common feed contaminant, affects intestinal, immune, and hepatic

parameters. The intestinal bacterium, *Genus novus species novus* BBSH 797, is capable of transforming DON to the less toxic metabolite de-epoxy-deoxynivalenol (DOM-1) and therefore qualifies as a valuable feed additive. Intestinal epithelial cells present the primary target of DON following ingestion of contaminated feed. DON disrupts the intestinal epithelial barrier, allowing paracellular passage of undesired substances and pathogens. Furthermore, DON is known for its inhibitory effect on cellular protein synthesis and its induction of apoptosis. Thus, tissues with high protein turnover or quickly proliferating cells, such as immune cells, like peripheral blood mononuclear cells (PBMCs), are highly sensitive to DON. Finally, DON influences metabolic parameters such as albumin production in rat cells and human primary hepatocytes. Therefore, the effect of DON and DOM-1 on the barrier function of differentiated porcine intestinal epithelial cells (IPEC-J2), the proliferation of PBMCs and the albumin production of the human hepatocellular liver carcinoma cell line, HepG2, was determined. IPEC-J2 cells were differentiated in Transwell® membrane inserts and treated with DON or DOM-1 (both: 0.5-100 µM). Transepithelial electrical resistance (TEER, daily) and cytotoxicity (Neutral Red) was recorded over 72 h. DON significantly decreased TEER between 10-100 µM (24 h (IC<sub>50</sub>: 25.91 µM) and 48 h (IC<sub>50</sub>: 19.53 µM)) and 5-100 µM (72 h (IC<sub>50</sub>: 14.08 µM)). Viability was significantly decreased at 50 and 100 µM DON (IC<sub>50</sub>: 48.16 µM). DOM-1 did not affect permeability or viability of IPEC-2 cells. In parallel, immunorelevant effects of DON and DOM-1 were studied by examining proliferation (BrdU) of PBMCs isolated from porcine blood. DON decreased proliferation at concentrations of and exceeding 0.84 µM, whereas DOM-1 was over 400 times less anti-proliferative than DON. Finally, confluent HepG2 cells were treated with DON (0.21-215 µM) or DOM-1 (0.22-228 µM) for 24 h, after which albumin production (ELISA) and cytotoxicity (WST-1) was determined. DON (16.87-215 µM) reduced albumin production by a minimum of 50%, while DOM-1 had no effect. Additionally, while DON reduced viability, DOM-1 did not. Data confirm the detoxification mechanism of *Genus novus species novus* BBSH 797 and support its use as DON detoxifying feed additive.

## P51

### **N-deoxyfructosyl-fumonisin B1 may cause DNA damage in porcine mononuclear blood cells**

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Fumonisin, fungal toxic secondary metabolites produced mainly by *F. verticillioides* and *F. proliferatum* in maize, are relatively heat stable up to 100 °C, although it is known that food processing may induce chemical degradation via Maillard-type reactions at high temperature, forming mainly N-(1-deoxy-D-fructos-1-yl)-fumonisin B1 (NDF). Almost nothing is known about the toxicity of NDF so far. Very recently, the stability of NDF under gastrointestinal conditions was reported. Since it is supposed that the fumonisin B1 (FB1) primary amine group is responsible for most of its toxic activity, it could be reasonably hypothesised that N-derivatives exert a lower toxicity compared to their precursor. Accordingly, heating treatments of maize during food and feed production could be considered as a possible detoxification route. With regard to the physiopathologic action of FB1 on cells, the toxic responses are related to the inhibition of the ceramide synthetase activity and the subsequent imbalance in the cell lipid metabolism. The possible genotoxicity of fumonisins has been evaluated in recent years, and it was proposed that this toxin could produce genetic damage by means of an indirect mechanism involving the cellular oxidative stress. The aim of this work was to investigate the possible cytotoxicity and genotoxicity of NDF compared to FB1. In vitro MTT and Comet assays were performed using mononuclear cells (lymphocytes) isolated from healthy male pigs. In both cases for the treatment of cells 20, 50 and 100 µg/ml NDF or FB1 concentrations (respectively) were used and 24, 48 and 72-hour incubation times were applied. Based on the MTT results there is no statistical difference between the effects of two toxins on the cell viability. According to the comet assay results, there is significant difference between the distribution of the comet scores of the NDF and FB1 datasets (400 cells/toxin) and it seems that at identical toxin concentrations (100 µg/ml) NDF caused serious cell damage at a higher occurrence frequency after 72 h. Consequently, NDF is slightly more disruptive concerning DNA damage in lymphocytes, as compared to FB1. This is the first time that a possible toxic activity of N-derivatives of fumonisin B1 is reported.

P52

## Public health risk associated with the co-occurrence of mycotoxins in spices consumed in Sri Lanka

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A quantitative risk assessment of mycotoxins due to the consumption of chilli (*Capsicum annum* L.) and black pepper (*Piper nigrum* L.) was performed in Sri Lanka. A food frequency questionnaire was administered in order to collect consumption data of spices by households in the Northern (n=62) and Southern region (n=187). The mean chilli consumption in the North (12.11±4.72 g/head/day) was significantly higher (P<0.001) compared to the South (7.38±4.31 g/head/day). Consumption of pepper was lower (1.42±1.18 g/head/day) than chillies, and there were no significant differences between the two regions. Dietary exposure to aflatoxin B1 (AFB1) and ochratoxin A (OTA) due to chilli consumption based on the deterministic approach using the fixed mean concentration of the lower bound and various levels of consumption, ranged from 0-8.97 and 0.23-4.92 ng/kg bw/day in the North, while the respective values in the South were 0-5.53 and 0-3.03 ng/kg bw/day. The mean exposure to AFB1 (2.13-3.49 ng/kg bw/day) and only the very high percentiles for OTA (24.38-57.05 ng/kg bw/day) were found to exceed the tolerable daily intakes of the respective mycotoxins due to chilli consumption in both regions. Margin of exposure (MoE) estimations at the mean dietary exposure to AFB1 were remarkably lower for chillies (45-78) than for peppers (2,315-10,857). Moreover, the hepato cellular carcinoma (HCC) risk associated with the mean AFB1 exposure through chilli at the lower bound was estimated to be 0.046 and 0.028 HCC cases/year/100,000 based on the North and South consumption, respectively. Similarly, the annual estimated HCC cases per 100,000 due to black pepper consumption in the North were 0.0003 and 0.0002 based on the consumption in the South. Therefore, AFB1 exposure through chilli consumption should be considered as of high concern from a public health point of view in Sri Lanka. Exposure to other mycotoxins due to spices is very small or their risk could not be assessed.

P53

**Using stable isotope internal standard for the accurate quantification of cyclopiazonic acid with an HPLC-MS/MS method**

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Cyclopiazonic acid (CPA) is an indole tetramic acid mycotoxin with tremorgenic, neurochemical and mutagenic toxicity (Hayashi and Yoshizawa, 2005). It is produced by certain *Penicillium* and *Aspergillus* spp. (Dorner *et al.*, 1983), including two important industrial moulds for the production of fermented foods (*Penicillium camemberti* and *Aspergillus oryzae*) (Goto *et al.*, 1987). CPA is important as both single mycotoxin and co-contaminant, since many fungi can produce it simultaneously with other mycotoxins (Krska *et al.*, 2012); for example, many strains of *A. flavus* produce additionally to aflatoxin, CPA as major metabolite also (Dorner *et al.*, 1983); the formation conditions are similar for both of them, however it seems that CPA production favours higher temperature (Krska *et al.*, 2012). It is a natural contaminant in maize, peanuts, sunflower seeds, Kodo millet, cheeses and feedstuffs (Goto *et al.*, 1987; Krska *et al.*, 2012). Although, there is not any study about its mycotoxicosis for human, but its acutely toxicity was approved in several animal species like rats, swine, chicken, turkeys, guinea pigs, and dogs (Goto *et al.*, 1987; Krska *et al.*, 2012), which causes weight loss, diarrhoea, degeneration and necrosis of muscles and viscera, convulsion and death (Hayashi and Yoshizawa, 2005). By consuming contaminated feeds, the animals accumulate CPA in their muscles, milk and eggs and humans are exposed to CPA by ingestion them, as well as by direct consumption of contaminated agricultural products (Krska *et al.*, 2012). Therefore, it is important to have accurate analytical methods for the detection and quantification of CPA in foods and feeds. Several methods, based on immunoassay, capillary electrophoresis, thin-layer chromatography, high performance liquid chromatography and LC-ion trap electrospray MS/MS for the determination and purification of CPA, have been reported (Hayashi and Yoshizawa, 2005). We have developed and optimised an HPLC-MS/MS method for the detection and quantification of CPA in food and feed samples. To compensate the matrix effect in complex products and guaranty accurate quantification, a <sup>13</sup>C-labelled internal standard was used, which leads to enhance the method performance. The method was successfully applied to complex food matrixes like white mould cheeses.

P54

**Determination of deoxynivalenol and nivalenol in wheat and maize using immunoaffinity column cleanup and RPLC/UV**

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Deoxynivalenol (DON) and nivalenol (NIV) belong to a class of natural-occurring mycotoxins produced by *Fusarium* spp. DON and NIV are frequently detected mycotoxins in agricultural commodities worldwide. A method consisting of extraction, immunoaffinity column cleanup and reversed-phase liquid chromatographic (RPLC)/UV separation and quantitation was validated for the determination of DON and NIV in wheat and maize. 20 g test portion was extracted with 100 ml water. After blending and centrifugating for 3 min, respectively, the supernatant was filtered by a glass fibre filter, then passed through the immunoaffinity column containing antibodies specific for NIV and DON. The toxins bound on the immunoaffinity column were washed with water, and eluted with 0.5 ml methanol and 1.5 ml acetonitrile sequentially. The eluate (2.0 ml) was dried with nitrogen and redissolved in 0.5 ml mobile phase. The toxins were then subjected to RPLC/UV analysis. The accuracy and repeatability characteristics of the method were determined. Recoveries of NIV at 0.1~1.0 mg/kg spiked levels were from 72.8 to 110% for wheat and from 60.1 to 91.9 % for maize matrix. Recoveries of DON at 0.1~1.0 mg/kg spiked levels were from 70.0 to 100.4% for wheat and from 73.9 to 91.1 % for maize matrix. The relative standard deviation (RSDr) of NIV at 0.1~1.0 mg/kg spiked levels ranged from 1.5 to 14.7% for wheat and from 1.1 to 5.1% for maize matrix. The RSDr of DON at 0.1~1.0 mg/kg spiked levels ranged from 5.0 to 12.6% for wheat and from 3.0 to 4.7 % for maize matrix. The method was found to meet the AOAC method performance criteria for wheat and maize products. Results of analysis of processed foods are also presented.

**P55**

### **Multimycotoxins analysis using a unique solid phase extraction based on molecularly imprinted polymers**

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The development of liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/ MS) methods for the simultaneous detection and quantification of a broad spectrum of molecules has made easier the screening of a larger number of samples. In this study, we have developed a unique solid phase extraction (SPE) based on molecularly imprinted polymers (MIP) for multimycotoxins analysis according to regulations. MIPs are polymers with high affinity for some target molecules and good stability to aqueous or organic solvents as well as temperature. This study shows an efficient clean-up method for a broad family of mycotoxins, deoxynivalenol (and its derivatives), aflatoxins, ochratoxin A, fumonisins, zearalenone, HT-2 and T-2 toxins prior to LC-MS/MS detection. By this fast, robust and effective clean-up process, this method enables the analysis of multimycotoxins in cereals and different matrices according to regulations with high recovery yields.

**P56**

### **Determination of hidden fumonisins in maize using a phosphate buffer extraction**

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Fumonisin (FBs) can occur in maize as free forms (native, partially or totally hydrolysed), and as hidden FBs (interaction between FBs and matrix macro-constituents). The analytical methods to detect free FBs in maize and derived products usually involve extraction with aqueous methanol or acetonitrile; for hidden FBs, Dall'Asta *et al.* (2008, 2009) reported a method using alkaline hydrolysis (KOH 2M) and following CH<sub>3</sub>CN extraction. Recently, we published a simple phosphate buffer extraction for the determination of FBs in masa, maize and derived products (Pietri and Bertuzzi, 2012). The present work aimed at evaluating if this extraction is able to detect hidden FBs. For this purpose, six naturally contaminated maize samples, accurately milled and homogenised, were analysed using different extraction mixtures: CH<sub>3</sub>OH:H<sub>2</sub>O 3+1 (v/v), CH<sub>3</sub>CN:CH<sub>3</sub>OH:H<sub>2</sub>O 2.5+2.5+5 (v/v), phosphate buffer 0.4M, KOH 2M and following CH<sub>3</sub>CN extraction. Moreover, an aliquot of the extract obtained by CH<sub>3</sub>OH:H<sub>2</sub>O 3+1 and phosphate buffer 0.4M, was hydrolysed with KOH 2M. Three replicates for each extraction were carried out. After extraction, an aliquot of the extract was diluted and analysed by LC-MS/MS. For quantitative determination, certificate standards (Biopure) of FB1, FB2 and hydrolysed FB1 (HFB1) were used. Significant higher FB levels (both FB1 and FB2) were obtained using phosphate buffer in comparison with the organic aqueous mixtures (pattern: phosphate buffer 0.4M > CH<sub>3</sub>CN:CH<sub>3</sub>OH:H<sub>2</sub>O 2.5+2.5+5 = CH<sub>3</sub>OH:H<sub>2</sub>O 3+1); the FB1 concentrations obtained using phosphate buffer were similar to HFB1 concentrations found by KOH hydrolysis and following CH<sub>3</sub>CN extraction. Finally, the HFB1 data obtained by alkaline hydrolysis of the phosphate buffer solution were compared to FB1 data found by phosphate buffer extraction. The results were similar; in 4 samples, the HFB1 levels were slightly higher (between 3 and 8%) but no significant difference was found. These results confirmed the higher extraction efficacy of phosphate buffer in comparison with conventional extractions; moreover phosphate buffer can extract most of hidden FBs.

**P57**

### **Evaluation of the Puritox multimycotoxin purification column using LC-MS/MS**

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The LC/MS/MS has the advantage over traditional analytical techniques by allowing for multi mycotoxin analyses. This technology does have inherent limitations due to matrix effects resulting in signal suppression or enhancement of the target analytes. The removal of the sample matrix utilising a purification step prior to the analysis is one method that would help reduce this effect and allow for accurate quantification using an external calibration. An evaluation was performed on several matrices for the analysis of mycotoxins using the Puritox multimycotoxin purification column by LC/MS/MS. This

purification column is applicable for the most commonly found and regulated mycotoxins including aflatoxin B1, B2, G1 and G2, ochratoxin A, zearalenone, fumonisin B1, B2 and B3, and various Type A and B trichothecenes. This method uses a single acetonitrile/water extraction solvent and a rapid one step purification process prior to LC/MS/MS analysis. The removal of the sample matrix using this purification column reduces the problem of signal suppression and enhancement often seen with LC/MS/MS analysis.

**P58**

### **New electrochemical immunosensor kits for the determination of ochratoxin A, fumonisin B1 and deoxynivalenol in wine and cereal-based food**

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Ochratoxin A (OTA), fumonisin B1 (FB1) and deoxynivalenol (DON) are three of the most important mycotoxins due to their occurrence in a variety of foods, and high acute and chronic toxicity effects in human and animals. The International Agency for Research on Cancer has classified OTA and FB1 as Group 2B carcinogens (possibly carcinogenic in humans). FB1 (mainly in maize) and DON contamination are mostly found in cereals and cereal-based foods, while OTA can occur in cereals, wine, grape juice, dried wine fruit, cocoa, spices, meat products and coffee. To protect public health, the maximum legislative levels in the European Union (EU) and other countries are very restrictive. The maximum levels in the EU are in the range 0.5-10 µg/kg for OTA, 200-4,000 µg/kg for FB1, and 200-1,750 µg/kg for DON. We have developed electrochemical immunosensors for rapid, selective, and sensitive determination of OTA, FB1 and DON in concentrations lower than the EU maximum levels. After extraction from cereals and wine, analyses are carried out in approximately 60 min. Cross-reactivities between the different mycotoxins are very low (in the range 1.6-6%). The limits of detection (10% inhibition) are 0.29 µg/kg for OTA in cereals, 18 µg/l for OTA in wine, 6.0 µg/kg for FB1 in maize-based foods, 5.3 µg/kg for DON in cereals. The reproducibility of these electrochemical immunosensors is in the range 9-12% DSR. All these immunosensors were validated with certified reference materials and statistically compared successfully with official AOAC International high-performance liquid chromatographic methods. From this research, three electrochemical immunosensor kits have been developed and marketed for the determination of OTA, FB1 and DON in food. These commercial kits allow faster and more sensitive determination compared to other (e.g., ELISA) kits on the market. **Acknowledgements.** Ministry of Science and Innovation contract PTQ-10-03580, project IPT-2011-1766-010000, and Ministry of Education predoctoral grant AP2010-4609.

**P59**

### **New spectrophotometric immunosensor kits for the determination of ochratoxin A, fumonisin B1 and deoxynivalenol in wine and cereals**

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Ochratoxin A (OTA), fumonisin B1 (FB1) and deoxynivalenol (DON) are three of the most important mycotoxins occurring in a variety of foodstuffs, such as cereals, cereal products, dried wine fruits, roasted coffee, wines, grape juices and processed cereal-based foods for infants and young children. Owing to their high toxicity in human and animals, European Union (EU) legislation demands exhaustive analytical controls with the objective to protect the health of consumers by keeping these contaminants at toxicologically acceptable levels. The maximum levels in the EU are in the range 0.5-10 µg/kg for OTA, 200-4,000 µg/kg for FB1, and 200-1,750 µg/kg for DON. We have developed three new spectrophotometric immunosensors for these mycotoxins allowing analyses of extracts in approximately 50 min. The reproducibility is about 6-7% RSD. Sensitivities are: OTA, limit of detection (LOD) 0.52 and 0.16 µg/kg for cereals and wine, respectively, and EC50 0.15 ng/ml; FB1, LOD 6.0 µg/kg and EC50 0.15 ng/ml; DON, LOD 5.3 µg/kg and EC50 8.3 ng/ml. The LODs in food were calculated from the concentrations of the mycotoxins producing 10% inhibition against the antibody; EC50 relates to the incubation concentrations of the mycotoxins in the wells. All the immunosensors were statistically validated with certified reference materials by using official AOAC International high-performance liquid chromatographic methods. In all cases, the relative errors were less than 9%.

Three kits with reagents and consumables included are offered for the selective determination of OTA, FB1 and DON at concentrations lower than the legislative levels. Due to improved separation and procedure, these spectrophotometric immunosensors require less reagent volumes, have shorter incubation times, and show improved analytical performance compared to other commercial kits, e.g., ELISA. **Acknowledgements.** Ministry of Science and Innovation contract PTQ-10-03580, project IPT-2011-1766-010000, and Ministry of Education predoctoral grant AP2010-4609.

## P60

### Developments in multimycotoxin testing – MycoSpin™400 clean-up and stable <sup>13</sup>C-labelled internal standards improve accuracy and sensitivity in mycotoxin LC-MS/MS methods

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The need for multimycotoxin analyses is constantly rising. The technology of choice is LC-MS/MS and laboratories are using LC-MS/MS methods in their routine testing operation. Nevertheless, a problem with LC-MS/MS can be interferences from matrix components leading to differences in analyte ionisation. The application of fully <sup>13</sup>C-labelled internal standards will correct such mass signal intensities between various sample matrices and pure standard calibrants to ensure qualified analysis results. There are several advantages over alternatives like deuterated (<sup>2</sup>H) internal standards. Replacing <sup>12</sup>C by <sup>13</sup>C changes the total mass of the atom slightly, for deuterium the mass doubles, thus, <sup>2</sup>H-labelled mycotoxins might show retention time shifts, resulting in less accurate LC-MS/MS results. Nowadays, highly sensitive mycotoxin detection methods are demanded by EU legislation and subsequently by the mycotoxin testing food and feed safety market. To detect multiple mycotoxins at such low detection limits a sample clean-up step should be implemented in the LC-MS/MS method. A novel rapid multimycotoxin clean-up to reduce the matrix effect and thereafter increase the LC-MS/MS method sensitivity was developed by Romer Labs. The use of <sup>13</sup>C isotope-labelled internal standards in conjunction with the MycoSpin™ 400 multitoxin clean-up allows for a method which is applicable to analyse a wide variety of matrices, with no limitations by molecular mass, and a straightforward sample preparation. This poster will present a method set up for a multimycotoxin method to analyse multimycotoxins including aflatoxins (B1, B2, G1 and G2), fumonisins (B1, B2 and B3), diacetoxyscirpenol, T-2 toxin, HT-2 toxin, nivalenol, deoxynivalenol, 3- and 15-acetyldeoxynivalenol, ochratoxin A, fusarenon-X, and zearalenone in various food and feed samples. Therefore, the MycoSpin™ clean-up for a better LC-MS/MS sensitivity on complex sample matrices will be presented. Further the procedures of how to use <sup>13</sup>C-labelled internal standards within this method will be described. Moreover, the importance of applying internal standards in a multimethod will be illustrated. The overall recovery for mycotoxins with internal standard addition ranged from 72-120%. Recovery for mycotoxins without internal standard addition, in example fusarenon-X and 15-acetyl-deoxy-nivalenol could be as good as 100% for simple matrix, but also as low as 30% for complex matrix. The Limit of detection of the method ranged from 0.2-20 µg/kg, depending on the toxin.

## P61

### Determination of mycoestrogens in baby food

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Zearalenone (ZEA) is a mycotoxin produced as secondary metabolite by various *Fusarium* species. ZEA and its metabolites are endowed with an oestrogenic activity due to their capability for binding oestrogen receptors. ZEA toxicity is caused by direct contamination of crop (wheat, barley, rice, maize) and by the 'carry over' in animal tissues. In mammalian species, biotransformation of ZEA consists of phase I and phase II metabolism.  $\alpha$ -Zearalenol ( $\alpha$ -ZOL) and its stereoisomer  $\beta$ -zearalenol ( $\beta$ -ZOL) are the main phase I metabolites. As far as phase II metabolism is concerned, ZEA,  $\alpha$ -ZOL and  $\beta$ -ZOL are mainly conjugated with glucuronic acid. Because of ZEA,  $\alpha$ -ZOL and  $\beta$ -ZOL mycoestrogen effect, monitoring studies are required both to verify their occurrence in food and to assess the dietary exposure of the population to these mycotoxins, particularly in those vulnerable population groups such as infants and children. European Commission set a maximum level of ZEA in infant food of 20 µg/kg, lower than the maximum level for adults (European Commission (EC) No 1881/2006). No limits for  $\alpha$ -ZOL and  $\beta$ -ZOL have been established so far. The aim of this study was to



develop a method to identify and quantify ZEA,  $\alpha$ -ZOL and  $\beta$ -ZOL in infant food based on bovine meat. We referred and modified the Desmarchelier method (Desmarchelier *et al.*, 2014). A number of meat-based products were analysed with a glucuronidase procedure and QuEChERS pre-clean up. Samples were preliminarily mixed and incubated with a glucuronidase solution. After an initial extraction step, QuEChERS-Disque Waters® was used for pre-clean-up and the samples were later centrifuged. The resulting organic phase was defatted with hexane and the diluted extract was cleaned-up with EASI-EXTRACT® ZEARALENONE columns. Samples were analysed by HPLC-FL (column: ACE-Excel C18-amide, mobile phase: AcCN/H<sub>2</sub>O (2% CH<sub>3</sub>COOH) 53/47). The method was in-house validated and all performance parameters complied with EU guidelines (ZEA,  $\alpha$ -ZOL and  $\beta$ -ZOL recoveries were 83, 62 and 60%, respectively). A variety of samples were purchased in the Italian market and analysed for mycoestrogens.

## P62

### Challenges in the development of a water-based extraction method for screening of aflatoxins in food and feed

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Aflatoxins are metabolites of the fungi *Aspergillus flavus* and *Aspergillus parasiticus* and are toxic and carcinogenic. There are four principle types of aflatoxins: B1, B2, G1, G2. Of these, aflatoxin B1 is most widely distributed and exhibits the highest toxicity. It causes liver disease on animals and is a potential human carcinogen. Therefore, fast and easy methods, such as e.g., strip tests, have to be developed for screening of these toxins in food and feed. Extraction of aflatoxins from agricultural commodities is usually performed with organic solvents as methanol or acetonitrile, which can be harmful to untrained people and the environment at least as big amounts of solvent are used for sample extraction. The replacement of these substances by using water-based buffer systems was the most challenging part in developing such a test system. Our latest findings in implementing this new extraction method will be demonstrated within this presentation.

## P63

### Development of a water-based extraction method for zearalenone in maize

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The mycotoxin zearalenone is produced by *Fusarium graminearum* and most often it is found in maize. However, it also occurs in other important crops such as wheat, barley, sorghum and rye throughout the world. The major effects of zearalenone are oestrogenic and primarily involve the urogenital system. Existing and well known fast and easy screening methods as lateral flow devices (strip tests) or ELISA tests have to be enhanced and optimised steadily to monitor mycotoxins as zearalenone. Fast and simple extraction of these toxins from agricultural commodities is a crucial step in the development of such test systems. This is usually performed with organic solvents, such as e.g., methanol or acetonitrile, due to low solubility of zearalenone in aqueous solvents. If cereals are extracted on-site, untrained people are exposed to danger by using these substances and moreover they are harmful to the environment at least as big amounts of solvent are used for sample extraction. The reduction of organic solvent consumption or complete replacement is an issue of great interest and importance for the future. This poster will demonstrate the development of a water-based extraction method by employing a newly designed buffer system on an aqueous basis for the detection of zearalenone by using lateral flow devices.

## P64

### Quantitative analysis of 15 mycotoxins by liquid chromatography electrospray ionisation tandem mass spectrometry (LC-ESI-MS/MS)

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A quantitative LC-MS/MS multiresidue method for 15 mycotoxins in a broad range of matrices is presented. Currently, EC-regulated mycotoxins (aflatoxins B1, B2, G1, G2 and M1; fumonisins B1 and B2; ochratoxin A; deoxynivalenol and zearalenone, T-2 and HT-2 toxins) were surveyed as well as nivalenol, and 3- and 15-acetyldeoxynivalenol. Positive identification of mycotoxins by LC-MS/MS in samples was conducted according to the confirmation criteria defined in EU Commission Decision 2002/657/EC while quantification was performed by the isotopic dilution approach using fully <sup>13</sup>C-labeled mycotoxins as internal standard (IS). The extraction procedure was based on the QuEChERS (acronym of quick, easy, cheap, effective, rugged and safe) protocol, generally applicable to all analytes. In the particular case of AFLAs and OTA in baby-foods or in difficult matrices, the extract was submitted to a parallel IAC clean-up. The extraction procedure showed good performances for linearity ( $(r)^2 > 0.99$ ), recovery (70-120%) and precision (relative standard deviation for repeatability (RSD<sub>r</sub>) and intermediate reproducibility (RSD<sub>iR</sub>) < 20%), thus fulfilling the EU requirements. Details on sample clean-up and performance characteristics will be presented.

**P65**

### **Analysis of multiple mycotoxins by LC-MS/MS: in-depth analysis of column selectivity**

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Mycotoxins are toxic secondary metabolites produced by fungi, which can exist in food as a result of fungal infection of crops. Their strong resistance to decomposition and digestion cause mycotoxins to remain in the food chain. The analysis of mycotoxins in food and animal feed has been a challenge mainly due to the complexity of food matrices and desired low detection limits. In recent years, significant advances in the analytical techniques were applied to the detection of mycotoxins. There has been an increasing need for a method to detect multiple mycotoxins with a single sample preparation and analysis method. Previous research concentrated on an LC-MS/MS method for multimycotoxin analysis as mass spectrometry provides appropriate selectivity and sensitivity for detection. This study investigated the selectivity for over 15 common mycotoxins on a variety of solid-core HPLC columns with different stationary phase chemistries. The results of these analyses were evaluated for optimum resolution and selectivity. The separation of analytes from matrix was also important as often no sample cleanup is performed during analysis and matrix effects are highly probable. The choice of column chemistries will be presented with emphasis on overall method performance.

**P66**

### **Cost-effective methods for detection of deoxynivalenol in wheat at collector intake**

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The aim of this study was to estimate the cost-effectiveness of different methods for deoxynivalenol (DON) detection in wheat, being non-instrumental assays (dipsticks), instrumental chemical analytical methods, predictive models, and their combinations, at collector intake. To this end, a Monte Carlo model was developed that simulates the wheat supply chain from farm field to miller intake. Wheat batches were assumed to go through the chain and to be tested at intake at the collector. One of the six considered scenarios for detection could be used. At miller intake, all batches were tested using instrumental methods. Three different thresholds for DON concentration were considered above which the wheat batch was not accepted by the miller. These thresholds were: 500 µg/kg, 1,250 µg/kg and 1,750 µg/kg. The model then calculated the total costs for testing, for wheat batches that were not-accepted, and for the total amount of non-accepted wheat. Input data from the Netherlands were used. Model results showed that the predictive model for DON in wheat, either alone or in combination with either the dipstick method or the instrumental analytical method, or no testing was – on average – the most cost-effective testing method for the chain. But, the amount of rejected wheat and related costs could be very high. An instrumental method would result into the lowest amount of rejected wheat, but also into the highest costs for the entire chain. This model gives insights into cost-effective strategies for testing, and the division of costs over the different actors of the chain. The use of the model could lead to better appointing (the limited) resources for food safety control.

P67

## Metabolomic technology to decipher maize resistance to *Fusarium graminearum* infection and trichothecenes accumulation

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*Fusarium graminearum* is the main causal agent of *Gibberella* ear rot, an important fungal disease affecting maize. *Fusarium graminearum* also produces 'pre-harvest' mycotoxins, among which trichothecenes of B type (TCT B) are the most frequently encountered in. TCT B include deoxynivalenol (DON) and its acetylated forms (15-ADON and 3-ADON) along with nivalenol or NIV and its acetylated form fusarenone-X. Those toxins are extremely stable and represent a threat for human and animal health. Today, the actual problem with TCT B is not an acute exposure but more a prolonged daily exposure causing chronic toxicity. To protect consumers, the European Union has adopted regulations that set maximum contamination levels for mycotoxins including DON in foodstuffs. Genotypic selection is a promising tool to control the disease and TCTB contamination. Several meta-quantitative trait loci (meta-QTL) have been identified through molecular characterisation of *Fusarium* head blight resistance in wheat and barley (Somers *et al.*, 2003; Buerstmayer *et al.*, 2009) and of *Gibberella* ear rot in maize (Marin *et al.*, 2011). When considered together, the set of meta-QTL explain much of the *Gibberella* ear rot resistance, but taken individually QTL have small effects. Therefore their use for marker-assisted selection remains yet limited. An alternative is to identify biochemical markers linked to *Gibberella* ear rot tolerance. A biochemical profiling could actually be an efficient way to decipher plant-pathogen interactions and progress in screening resistant maize lines. The aim of this work is to focus on the metabolic profiling of *Fusarium graminearum* tolerance and toxin accumulation in kernels. The method we present in this review combines high resolution mass spectrometry and <sup>1</sup>H NMR, which efficiently enabled the determination of a large range of metabolites. This method was applied to kernels harvested at two developmental stages. Genotypes that exhibit differences in resistance were inoculated, or not, by a TCTB producing *Fusarium graminearum* strain through the silk channel. This type of analysis will increase our knowledge of tolerance mechanism in maize against *Fusarium graminearum*.

P68

## Determination of sterigmatocystin in grains using gas chromatography-mass spectrometry with on-column injector

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Sterigmatocystin (STC) is a mycotoxin produced by several species of *Aspergillus* and other fungi, particularly *A. versicolor*. STC is a precursor of carcinogenic aflatoxin B<sub>1</sub>, and exerts carcinogenic, teratogenic and mutagenic effect. A reliable GC-MS based analytical method using on-column injection has been developed and validated for the first time to determine STC in grains. In this method, STC was extracted with acetonitrile: water (84:16, v/v), and diluted the filtered extract with phosphate buffer before immunoaffinity column (IAC) clean-up. After elution and N<sub>2</sub> evaporation, the sample containing STC was re-dissolved into acetone and injected to the GC-MS system without derivatisation. Separation of STC was carried out by a capillary column (0.25 mm i.d. × 30 m, 0.25 μm) with a deactivated pre-column (0.53 mm i.d. × 0.6 m). Matrix effect was investigated in three different grain matrices (maize, wheat and rice), and slight matrix effect was observed after IAC clean-up. The calibration curve was linear in the range of 20–300 pg (as equivalents to 8–120 μg/kg in grains) with the coefficient of determination ( $r^2$ ) = 0.998. Repeatability of the measurements (relative standard deviation) was lower than 10%. The limit of detection (LOD) and quantification (LOQ) of the method were 6 pg (2.4 μg/kg in grains) and 20 pg (8 μg/kg), respectively. This developed method is sensitive, less expensive and could be useful to monitor STC contamination in grains.

P69

## Development of a multimycotoxin method using stable isotope dilution assays to follow the fate of mycotoxins during the malting process

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*Fusarium* head blight is assigned to a devastating fungal disease that has become of increasing importance in recent years. Similarly to other small grain crops the infection of barley causes enormous losses concerning yield, grade and end-use quality. Heavy infestation of brewing barley with *Fusarium* species can induce a severe mycotoxin contamination and can impair the solving as well as the malting and brewing properties. This disease is caused by various *Fusarium* species producing a wide range of toxins. Green house and field trials were conducted to compare the epidemiology of *F. culmorum*, *F. graminearum*, *F. avenaceum*, *F. tricinctum*, *F. langsethiae* and *F. sporotrichoides*. Besides malting quality factors (e.g., protein solubility, germination capacity, sorting) the metabolite spectrums of pure inoculated material was investigated. For the determination of potentially occurring mycotoxins including modified metabolites (e.g., deoxynivalenol-3-glucoside, deoxynivalenol (DON), acetylated DON derivatives, HT-2 toxin, T-2 toxin, enniatins, beauvericin, zearalenone) a multimycotoxin method was developed. The quantification of mycotoxins in barley and malt was carried out by stable isotope dilution assays using liquid chromatography-tandem mass spectrometry. As internal standards the previously synthesised <sup>13</sup>C- and <sup>15</sup>N-labeled analogs (e.g., <sup>13</sup>C<sub>2</sub>-acetylated DON, <sup>13</sup>C<sub>4</sub>-HT2-toxin, <sup>13</sup>C<sub>2</sub>-T2-toxin, <sup>15</sup>N<sub>3</sub>-enniatsins, <sup>15</sup>N<sub>3</sub>-beauvericin) were applied. The fate of *Fusarium* mycotoxins during the malting process was investigated by the established LC-MS/MS-multimethod. During malting, a difference in the amount of toxins before and after germination was observed. Mostly fungal growth was accompanied by the production of mycotoxins during the germination stage resulting in heavily loaded malt. The significant increases of some (modified) metabolites were noticeable and might result from the detoxification process in plants. This generated systematic information could clarify the interaction between *Fusarium* infection, symptoms and metabolites in the first important step of the brewing process.

P70

## A sensitive UHPLC-MS/MS method for the analysis of ochratoxin A, fumonisins B1 and B2, zearalenone and its metabolites in meat and liver

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In a report published in 2011 defining the main prospects of the organic food sector, the French Scientific Council for Organic Agriculture underlined that food safety was the prime motivation driving 95% of the consumers of organic food, although very little scientific data were available to support the assumption of a health benefit associated with organic products. The SOMEAT project (Safety of Organic MEAT) aims to provide scientific data to contribute to the debate on the presumed health benefit of organic meat products in regard to their possible chemical contaminants contents (mycotoxins, pesticides, organic pollutants, toxic metals and antibiotics) and the putative resulting toxicity potential for consumers. Within the frame of this project, the French National Reference Laboratory for Mycotoxins in Food of Animal Origin, has developed very sensitive methods to quantify low levels of fumonisins B1, B2, zearalenone and two of its metabolites in the muscle and liver of poultry and pork. Because mycotoxins are present at trace levels in these matrices, sensitivity was the key element of this development. The only way to attain such low levels was the use of immunoaffinity purification. Two different methods, using immunoaffinity clean-up columns were developed and isotope dilution was implemented to improve trueness. The first method was specific to ochratoxin A. Ethyl acetate extraction was followed by liquid-liquid purification and finally by immunoaffinity clean-up. For fumonisins, zearalenone and its two metabolites, the method consists of a single, and common, extraction by methanol/water followed by two distinct immunoaffinity clean-ups using columns specific for fumonisins and zearalenone respectively. The eluates of the purification columns were concentrated and then analysed by UHPLC-MS/MS. Limits of quantification are 0.1 µg/kg for ochratoxin A and 1 µg/kg for the other mycotoxins. The poster presents details of the methods and their performance criteria. **Acknowledgements.** This study is supported by the French National

## P71

### Determination of zearalenone in edible oils from Germany

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Zearalenone (ZEN) is a non-steroidal mycotoxin produced by several *Fusarium* species, which are commonly found on nearly every type of grain in Europe. In animal experiments, ZEN has low acute toxicity after either oral or interperitoneal administration, but it has been shown to be teratogenic, hepatotoxic, immunotoxic, genotoxic and carcinogenic. Because of its lipophilic properties and associated good solubility in oil, ZEN concentrations in edible oils are several times higher than in other cereal products in some occasions. However, by far the highest ZEN content has been measured in maize oil (up to 4,600 µg/kg). Notably levels of ZEN have also been reported in wheat germ and soy bean oils. In the European Union a maximum level for ZEN in refined maize oil of 400 µg/kg is currently in place. Actual data on the presence of ZEN in edible oils is scarce: an unsatisfactory situation in view of the fact that these products could form an important contribution to human ZEN exposure. For the present study two different analytical methods for the investigation of oil samples have been applied: Method 1 (Majerus *et al.*, 2009; the incorporation of the method into the Official Collection of Methods of Analysis according to § 64 of the German Food and Feed Code (LFGB) is currently under consideration; method draft accepted by CEN: mandate m/520 Project No. 3 'Foodstuff – Determination of zearalenone in vegetable oils including refined maize oil') is based on the dilution of the oil sample and subsequent liquid-liquid partitioning with a mixture of methanol and ammonium bicarbonate. This fast methodology showed however, for some samples, limitations in the quantification of ZEN due to matrix effects causing signal overlapping. For these samples, we used a method recently developed by the Federal Institute for Materials Research and Testing (BAM, Method 2 (Siegel *et al.*, 2010; Joint Research Project ZENOL 'Development and validation of an innovative analytical method for the selective determination of zearalenone in vegetable oils' funded by the Federal Ministry for Economic Affairs and Energy, BMWi, No. 01FS12033)). This selective method is based on solid phase extraction using hydrazine-functionalised particles in commercial SPE cartridges. After ZEN is covalently coupled to the solid phase by means of a hydrazone bond undesired matrix components can be removed very efficiently. Finally, ZEN is decoupled from the solid phase, leading to a highly purified extract. Sample extracts obtained by both methods were measured using HPLC-FLD. Beside the maize oil samples available on the German market today, also soy bean, sunflower and rapeseed oil samples as well as samples with challenging matrices such as hempseed and pumpkin seed oil have been investigated. We analysed refined and non-refined edible oils from organic and conventional production and also samples which were available only on-line. The results of more than 40 different edible oils will be presented and discussed.

## P72

### New mycotoxin reference materials: crucial tools for quality assurance and food safety

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Contaminations with moulds or fungi and their associated mycotoxins may occur during the whole production chain of a food product (i.e., 'from the field to the fork'). As the consumption of mycotoxin-contaminated products may induce acute and long-term chronic effects resulting in a teratogenic, carcinogenic, estrogenic, neurotoxic or immunosuppressive impact on human and animal health, determination and reduction of these compounds in food and feed is subject to the work of regulators, food business operators and researchers. Fungi of the genus *Fusarium* are the predominant mycotoxin producers in moderate climate zones. *Fusarium* toxins occur worldwide in a wide variety of foods, particularly in highly consumed cereals and cereal based products. The toxicologically – and hence also economically – most important *Fusarium* mycotoxins are zearalenone (ZEN) as well as the type A (T-2 and HT-2 toxin) and type B trichothecenes (e.g., deoxynivalenol (DON)). Driven by regulatory authorities, extensive consumer protection efforts were made by establishing fast and reliable analytical methods to determine the most common *Fusarium* toxins in cereals and derived

products. At the same time legally binding maximum levels were introduced for these matrices (Commission Regulation (EC) No 1881/2006; Commission Regulation (EC) No 1126/2007). While for DON and ZEN EU maximum levels are already in effect, new levels for T-2 and HT-2 toxin are currently under discussion. Recommendation 2013/165/EU on the presence of T-2 and HT-2 toxin in cereals and cereal products sets indicative levels for the sum of the T-2 and HT-2 toxins in food and feed, ranging from 15 µg/kg (infant food) to 2,000 µg/kg (oat milling products (husks)). To enforce the maximum levels and thus reduce consumer risks, strict controls of food and feed are of prime importance. For the sum of these reasons, certified (matrix) reference materials (CRMs) for *Fusarium* toxins are required. CRMs can contribute to aid in method validation and increase comparability and traceability in mycotoxin analysis. In the frame of the ERM® (European Reference Materials) initiative BAM has currently completed one CRM project on the field of mycotoxins (ERM®-BC720: T-2 and HT-2 toxins in oat flakes) and initialised a new one for ZEN in maize oil (candidate reference material ERM®-BC715), to close the current gap of available CRMs for mycotoxins in food. The preparation, characterisation and certification of the materials including homogeneity and stability studies based on ISO Guide 35 (Reference materials: General and statistical principles for certification; ISO/REMCO, 2006) will be outlined and discussed.

### P73

#### **Rapid classification of mycotoxin contaminated food commodities by infrared spectroscopy and chemometrics**

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The inherent limitations of current methods for mycotoxin determination in foodstuffs have necessitated the development of new analytical technologies. Infrared (IR) spectroscopic methods are among the most promising strategies for determining mycotoxin contamination in agricultural commodities or processed food products. IR-based methods are rapid and non-destructive techniques that require minimal technical training and sample preparation. Analysis is usually not labour intensive, and large quantities of chemicals are not required in comparison to existing sophisticated chromatographic techniques. These intrinsic qualities of IR-based methods render them an attractive option for high throughput analysis of foodstuffs on site. Fourier transform mid-infrared spectra (FTIR) were recorded for several food commodities including maize, wheat and peanuts utilising a portable spectrometer fitted with an attenuated total reflection (ATR) unit. Samples were either naturally infected or inoculated with *F. graminearum* or *F. verticilloides*. After drying, maize samples were ground and sieved whereas peanut and wheat samples were ground only. Reference mycotoxin (DON, fumonisins, aflatoxins) measurements for individual food commodity samples were made by LC-MSMS. Chemometric data analysis performed in Matlab included data pre-treatment and the selection of spectral windows corresponding to protein and carbohydrate regions of the spectrum, that are subject to alternation with fungal invasion. Principal component analysis (PCA) was utilised to identify clusters that were aligned with corresponding mycotoxin reference concentrations. Score plots of the two first principal components revealed clusters correlating the method of infection, high and low toxin content and invading fungal species, depending on the subset. Maize data sets, n=184 and subsets of, were successfully classified into clusters with <1000 µg/kg and >1000 µg/kg DON. Promising initial results were also obtained for peanuts, n= 40; aflatoxin concentration from <LOD to 914 ppb, and wheat samples, n=64; DON concentration from <LOD to 28,000 µg/kg. The presence of several varieties and fungal species in the sample set led to model destabilisation due to differences in protein and carbohydrate contents resulting in spectral differences unrelated to the degree of contamination. Current work focuses on the optimisation of the measurement procedure and the investigation of alternative classification methods such as discriminant analysis and neural networks.

### P74

#### **Evaluation of next generation liquid chromatography-single quadrupole mass spectrometry for screening and quantitative analysis of multiple mycotoxin in foods**

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Mycotoxin detection is of great importance in regulated environments such as food and animal feed analysis. Maximum permitted levels for the major mycotoxins, namely aflatoxins (AFB1, AFB2, AFG1, AFG2), ochratoxin A (OTA), fumonisins (FB1, FB2), deoxynivalenol (DON), and zearalenone (ZEA) are set by the European legislation (1881/2006/EC, 1126/2007/EC). Indicative maximum levels for the sum of T-2 (T-2) and HT-2 (HT-2) toxins have been recently issued in Recommendation 2013/165/EU. Although not regulated, attention is paid to the occurrence of nivalenol (NIV), another *Fusarium* toxin that frequently occurs in cereals also in combination with DON. Analytical methods for determination of major mycotoxins in food matrices need to be sensitive, selective and robust to provide accurate data when applied for monitoring, risk assessment, quality control and research. Most of the reference methods currently used for quality control purposes are based on immunoaffinity columns (IAC). However, due to the specificity of the antibodies used in these columns towards individual mycotoxins, different methods have been developed for a single mycotoxin or for closely related mycotoxins. In the last decade, the commercial availability of multimycotoxin IACs has opened new frontiers to the analysis of these contaminants in food control laboratories. A liquid chromatography/mass spectrometry (LC-MS) method was developed for the simultaneous determination of aflatoxins (B1, B2, G1, G2), OTA, fumonisins (B1 and B2), NIV, DON, ZEA, T-2 and HT-2 in cereal based foods. A double extraction approach, based on high speed blending with water followed by methanol was applied for the effective co-extraction of the 12 mycotoxins under investigation in 4 minutes. Multitoxin IACs (Mycotoxin1+™; Vicam) were used for cleanup of the extract. The simultaneous detection and quantification of the 12 mycotoxins was performed by LC-MS evaluating performances of a new mass detector based on single quadrupole technology (Acquity QDa Detector; Waters). Furthermore, mycotoxin fragmentation patterns obtained by in-source fragmentation were investigated to identify two characteristic masses per each mycotoxin. The resulting detection approach enabled to obtain quantitative and confirmatory information in a unique chromatographic run. Reliability of this approach was evaluated by establishing the repeatability of ion ratios (quantifier/qualifier ion) by repeated measurements in standard solution and matrix extracts. Method performances such as linearity range, recoveries from spiked samples, quantification limits were evaluated and proved the method to be suitable to assess with a single analysis, compliance of the selected food commodities with the EU maximum permitted or recommended levels for all regulated mycotoxins. The developed LC-MS detection approach could represent, in routine mycotoxin analysis, a cost-effective alternative tool to more sophisticated LC-MS/MS equipments. **Acknowledgements.** This work was carried out with the financial support of the Project MIUR – PON02\_00186\_3417512, 'New Strategies for Improvement of Food Safety: Prevention, Control, Correction' (S.I.Mi.S.A).

## P75

### Indirect methods for the determination of conjugated forms of deoxynivalenol in cereals

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Mycotoxins may occur not only in native forms, but also as so-called masked mycotoxins. Masked mycotoxins are formed *in planta* by linking small polar compounds to the mycotoxin molecule as a protective response to the xenobiotic toxin. The chemical modification leads to decreased toxicity for plants. Masked mycotoxins are then stored in vacuoles (extractable conjugated forms) or covalently or non-covalently bound to macromolecules (non-extractable bound forms). Extractable conjugated forms can be detected by appropriate analytical methods when their structure is known and analytical standards are available. Non-extractable bound mycotoxins cannot be extracted directly and have to be released from the matrix by chemical or enzymatic treatment prior to chemical analysis. The best known masked mycotoxin, deoxynivalenol-3- $\beta$ -D-glucoside (D3G), is formed in DON-contaminated wheat grains. Moreover, increase of D3G levels during malting and brewing was observed and also the presence of DON-diglucosides and DON-triglucosides in malt and beer was revealed. Recently, several other masked DONs were found to be formed in wheat (DON-S-cysteine, DON-S-cysteinyl-glycine, DON-glutathione). All those masked forms potentially might be cleaved to DON during food processing or hydrolysed in the digestive tract of mammals and thus contribute to the total dietary DON exposure. Indirect methods aim to determine the entire pool of masked mycotoxins in a sample by their conversion into the native toxin using chemical or enzymatic hydrolysis. This contribution aims

to provide a critical assessment of three indirect methods for total DON determination based on acidic hydrolysis using trichloroacetic acid, trifluoroacetic acid or trifluoromethanesulfonic acid. The stability/degradation of DON, D3G, 3-acetyl- and 15-acetyl-DON was assessed on spiked samples of wheat, maize and barley using an LC-MS/MS method which has been developed and validated for this purpose. Concerning potential enzymatic hydrolysis, the capability of several glucosidases of different classes to cleave D3G in wheat flour extract was tested. Judging from our experiments, acidic hydrolysis methods for the quantification of the total amount of DON in cereals are not recommended, while enzymatic methods show promising potential.

**P76**

### **Determination of T-2 and HT-2 toxins from maize by direct analysis in real time-mass spectrometry (DART-MS)**

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Ambient desorption ionisation techniques, such as laser desorption with electrospray ionisation assistance (ELDI), direct analysis in real time (DART) and desorption electrospray ionisation (DESI) have been developed as alternatives to traditional mass spectrometric-based methods. Such techniques allow the detection of analytes from surfaces under ambient conditions. A significant advantage of such technologies is the ability to detect analytes without the need to separate them from other sample components prior to desorption and ionization, potentially reducing or eliminating the need for time consuming sample preparation steps. In this work, DART ionisation coupled to a high resolution mass spectrometer (MS) was used for the rapid quantitative analysis of T-2 and HT-2 toxins extracted from maize. The DART technique relies upon excited-state helium atoms to produce reactive species that lead to analyte ionization. In the form of DART used here the beam of helium gas was directed onto a movable stage placed at a 90 degree angle to the inlet to the MS, into which the ions were drawn. Extracts of maize were placed onto paper disks upon the sample stage, and then positioned in front of the MS inlet. Sample preparation procedures and instrument parameters were optimised to obtain sensitive and accurate determination of the toxins. The lowest calibration levels (LCL) were 50 µg/kg for T-2 toxin and 300 µg/kg for HT-2 toxin. Quantitative analysis was performed with the use of matrix-matched standards employing <sup>13</sup>C-labeled internal standards for T-2 and HT-2 toxins. DART-MS of maize extracts spiked with T-2 toxin gave a linear response over the range of 50-1000 µg/kg. With the isotope dilution technique, good recoveries (89-117%) and repeatabilities (RSD 5.2-6.7%) were obtained at spiking levels of 100 and 1000 µg/kg. Similar results were obtained with HT-2 toxin, although the method was about 6-fold less sensitive for this metabolite. Trueness of the method was demonstrated by a good correlation with a certified reference material. Robustness of the method was demonstrated by analysis of an oat flour quality control material. These results indicate the potential for DART-MS to provide sensitive, convenient, quantification of mycotoxins from grains, and represent a step towards the goal of directly quantifying mycotoxins from sample surfaces.

**P77**

### **Applicability of two new immunoassays to the screening of mycotoxins in feed: biochip-based immunoassay for a multi-analytical approach and ELISA for the detection of aflatoxin B1**

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The determination of mycotoxins is important for animal feed safety as they are undesirable contaminants of cereals and cereal based products and can cause adverse health effects in animals. This study reports the applicability of two new immunoassays to the screening of mycotoxins in feed: a biochip-based immunoassay for the simultaneous detection of multiple mycotoxins (aflatoxins, ochratoxin A, zearalenone, fumonisins, trichothecenes, ergot alkaloids and paxilline) from a single feed sample and an ELISA for the screening of aflatoxin B1. Methodology: biochip-based immunoassay – simultaneous competitive chemiluminescent immunoassays, defining discrete test sites on the biochip surface and applied to the Evidence Investigator analyser, were employed; ELISA – the assay is a competitive ELISA with short incubation time (20 min in total). Samples were extracted from feed by liquid/liquid extraction. Biochip-based immunoassay results: aflatoxins (B1, B2, G1, G2), fumonisins (FB1, FB2, FB3), ochratoxin A, deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, diacetoxyscirpenol, HT-2 toxin, T-2 toxin, zearalenone and five of its



metabolites, nineteen ergot alkaloids and paxilline were detected. The detection limits were at and below the regulatory limits in feed (Directive 2002/32/EC, Commission Recommendation 2206/576/EC). ELISA results: the assay was standardised to aflatoxin B1 (cross-reactivity with aflatoxin B2 7%, with aflatoxin G1 18% and with aflatoxin G2 1%) and showed a detection limit of 1ppb. With both immunoassays, initial authentic feed sample comparisons (n=8) with LS-MS/MS showed 100% agreements for all analytes. The results indicate that these two new immunoassays are applicable to the screening of mycotoxins from feed: the simultaneous detection of a broad range of mycotoxins from a single feed sample with the biochip-based immunoassay and aflatoxin B1 with the ELISA.

#### P78

### Masked trichothecene mycotoxins: enzymatic synthesis and hydrolysis by $\beta$ -glucosidases

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Trichothecene class *Fusarium* mycotoxins, such as deoxynivalenol (DON, regulated in Europe), nivalenol (NIV, more prevalent in Asian countries), T-2 toxin and HT-2 toxin (occurring on wheat, barley and oat in Northern countries), can be inactivated *in planta* by formation of their glucoconjugates and escape routine detection. Therefore, such  $\beta$ -glucosides are considered masked mycotoxins. It is conceivable that current attempts to increase *Fusarium* resistance in wheat may increase the molar portions of trichothecene-glucosides. At present, there is insufficient information concerning bioavailability and toxicity of glucosylated mycotoxins. For example, DON-3- $\beta$ -D-glucoside (D3G) is resistant to acidic hydrolysis but can be cleaved by intestinal bacteria, thus reconstituting the toxin. For these reasons, it is vital to gain more information on occurrence and toxicological relevance of masked mycotoxins, a fact that is also increasingly recognised by regulative government bodies. Unfortunately, analytical standards for  $\beta$ -glucosides of NIV, T-2, HT-2 and acetylated DON derivatives such as 15-ADON are currently commercially unavailable. Therefore, it is crucial to obtain such compounds pure and in sufficient amounts required for analysis and toxicological risk assessment, for example in feeding trials. A biocatalytic strategy to produce several mycotoxin glucosides is presented. A family 1 UDP-glycosyltransferase was expressed in *Escherichia coli* and purified by affinity chromatography. Using UDP-glucose as co-substrate, the recombinant enzyme could completely glucosylate several mg of DON, NIV and HT-2 within short reaction times (<4 h) *in vitro*. The reaction products were purified by preparative HPLC and further analysed by NMR spectroscopy, confirming that in all cases the enzyme selectively catalyzed the formation of -3- $\beta$ -D-glucopyranosides. A different approach of coping with the masked mycotoxin problem is the hydrolysis by specific  $\beta$ -glucosidases and subsequent analysis of the aglycones. This may especially be of interest for the widely applied commercial DON assays that are based on antibody detection techniques. While most commercial enzymes tested were not suitable to convert D3G within acceptable time limits, we could identify a bacterial GH 3 type  $\beta$ -glucosidase with the capability to hydrolyze D3G even in complex matrices such as beer and cereal extracts in reasonably short times. This recombinant enzyme may therefore become a tool for convenient and rapid total DON analysis in cereal samples.

#### P79

### Validation of a method for the analysis of sterigmatocystin in cereals using immunoaffinity columns

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Sterigmatocystin is a precursor in the metabolic pathway for aflatoxin formation and like aflatoxin can be produced by several *Aspergillus* species. The main producer is *A. versicolor* which is ubiquitous in nature and has been found to grow on maize, bread, dried fruit, cheese, rice and fermented meat products. Although there are many reports on the occurrence of sterigmatocystin in various

commodities, many of the thin layer chromatography methods that were traditionally used lack adequate specificity. In March 2013, the European Food Safety Authority (EFSA) issued a tender for surveillance work on sterigmatocystin in a variety of grains including wheat, barley, rye, oats and rice intended for human consumption from 3 different European countries. R-Biopharm Rhône has developed an immunoaffinity column which selectively isolates and concentrates the mycotoxin from a range of cereal samples. The result is better clean up leading to improved chromatography and ultimately lower limits of detection.

## **P80**

### **Analysis of various cheese samples for aflatoxin M1 using immunoaffinity columns**

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Aflatoxins are naturally occurring mycotoxins that are produced by many species of *Aspergillus*. Aflatoxins are toxic and among the most carcinogenic substances known. After entering the body, aflatoxins may be metabolised and hydroxylated to produce aflatoxin M1. If cattle are fed with aflatoxin-contaminated feed this may result in aflatoxin M1 entering the food chain in dairy products like milk, cheese, yoghurt and baby food. Currently there is European legislation in place for aflatoxin in animal feed as well as various foods in order to reduce the risk of aflatoxin M1 being present in the human food chain. For aflatoxin M1, legislative levels are very low (0.05 ppb for milk and milk products and 0.025 ppb for infant formulae and dietary foods) therefore suitable methods are required for analysis of the toxin. R-Biopharm Rhône have developed Aflaprep M Wide immunoaffinity columns which are suitable for the analysis of aflatoxin M1 in a range of dairy products including cheese. Cheese is a diverse category of food, ranging from high moisture content cheeses such as Ricotta, soft cheeses like Brie to hard cheeses like Cheddar or Parmesan, which have low moisture contents. The recovery of trace levels of aflatoxin M1 from a complex cheese matrix can be challenging as the samples consists of essentially protein, fat, sugars and water, therefore it is important to thoroughly homogenise the cheese sample in a high organic solvent to water ratio in order to break down the sample. The Aflaprep® M Wide columns contain a gel suspension of monoclonal antibody which make the column highly specific and is essential in order to clean-up and isolate the toxin from the complex cheese samples. The sample is prepared as per the recommended method, taking into account the moisture content and passed through the column. Any toxin which is present in the sample is retained by the antibody within the gel suspension. The column is washed to remove unbound material and the toxin is then released by the antibody following elution with solvent, the eluate is collected and analysed by HPLC. All recoveries were between 82 to 93% demonstrating excellent clean-up and concentration of the toxin from the samples giving a much cleaner chromatogram and therefore providing more accurate and sensitive detection.

## **P81**

### **Validation of an automated system for analysis of mycotoxins in a range of samples using online immunoaffinity cartridges in conjunction with HPLC**

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R-Biopharm Rhône have developed a patented, online affinity cartridge, which can be used prior to HPLC. The cartridges are used together with the Symbiosis™ handling system and combine automated online sample application with quantitative analysis of mycotoxins. The affinity cartridge contains a monoclonal antibody that is specific for the mycotoxin of interest coupled to a hydrophilic polymer that can withstand high pressure. Using the Immunoprep® Online Aflatoxin cartridges recoveries ranged from 87 to 109% for cereals, 80 to 106% for dried fruits and 80 to 109% for peanuts. The Immunoprep® Online Ochratoxin cartridges gave recoveries ranging from 86 to 92% for cereals, 101% for coffee, 89% for dried fruit, 96% for wine and 84 to 107% for spices. Using the Immunoprep® Online cartridges, the sample application, washing and elution is performed online for up to a maximum of 12 samples before the cartridge is automatically removed and replaced with a new cartridge. The technology is a highly innovative automated analysis for the individual toxins and is highly specific, sensitive and rapid. A key advantage of this process is that during the LC run of one sample, the next sample is simultaneously passed through the affinity cartridge, reducing the time taken for subsequent sample clean-up to almost zero.

**P82**

## **Simultaneous determination of mycotoxins using AOF MS-Prep® in conjunction with LC-MS/MS**

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Due to increased demands on analysts and increasing international legislation more foods have to be tested for a number of mycotoxins. With the strong possibility of multiple mycotoxins present in the same commodity, it makes sense to determine more than one mycotoxin in a single analytical run. R-Biopharm Rhone has DZT MS-Prep® and AO ZON Prep® immunoaffinity columns in its portfolio and both products target and purify different combinations of mycotoxins present in cereals, cereal products and animal feed. R-Biopharm's newest addition to the multi immunoaffinity column range is AOF MS-Prep® which is suitable for the analysis of aflatoxins B1, B2, G1, G2, ochratoxin A, fumonisin B1 and B2 in maize and animal feed. Only one sample extraction is required for all toxins and recoveries and the limit of detection for these toxins were found to be greatly improved using AOF MS-Prep® in conjunction with LC-MS/MS. The immunoaffinity columns were found to exceed the minimum performance requirements set out by CEN demonstrating that the R-Biopharm Rhône methods can be adopted for use by ISO 17025 accredited laboratories. The method utilised normal standards instead of isotopic or matrix matched standards, saving time and money. When using AOF MS-Prep®, no issues were observed with ion suppression or matrix interference which, can often adversely impact identification and quantification of these mycotoxins.

**P83**

## **The quantitation of mycotoxins in cereals using a simple sample extraction and LC-MS/MS using fast polarity switching and MRM scheduling**

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Mycotoxins are produced by several strains of fungi both in the field, during storage, mixing and delivery of grain, human and animal food. Mycotoxins are known to be toxic and harm humans and animals as they are carcinogenic or otherwise cytotoxic and impair the immune system. Mycotoxins fall into several major classes and those which can affect the health of humans or animals include the aflatoxins, ochratoxins, *Fusarium* toxins, including fumonisins, zearalenone (ZON), trichothecenes, and ergot alkaloids. Regulations for mycotoxin contamination for some of the major classes have been set in different countries. In the European Union the mycotoxin limits were harmonized in the regulation for contaminants in foodstuffs. Traditionally mycotoxin analyses have been carried out using multiple methods, each method just suitable for one single mycotoxin or a group of chemically similar compounds, e.g., aflatoxins. This has been due to the wide range of polarities and physical properties of these compounds. Here we present a rapid, robust, sensitive and specific LC-MS/MS assay has been developed for the detection of several major classes of known toxic mycotoxins. The method uses a simple solvent extraction followed by a dilution and injection of extracts to achieve detection of mycotoxins below the regulatory requirements. Fast polarity switching and the Scheduled MRM™ algorithm were used with the AB Sciex Triple Quad™ 5500 system to cover all mycotoxins of interest and to detect them with the best sensitivity, accuracy, and reproducibility. The presented method has been tested on several cereal based samples and has been shown to be robust enough to detect these toxins below the required limits and met European legislation.

**P84**

## **Highly sensitive and rapid simultaneous method for 45 mycotoxins in baby food samples by HPLC-MS/MS using fast polarity switching**

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Mycotoxins are toxic metabolites produced by fungal moulds on food crops. For consumer food safety, quality control of food and beverages has to assay such contaminants. Depending on the potency of the mycotoxin and the use of the food, the maximum allowed level is defined by legislation. Baby food is particularly critical. For example, European Commission has fixed the maximum level of aflatoxin B1

and M1 to 0.1 and 0.025 µg/kg, respectively, in baby food or milk. Therefore, a sensitive method to assay mycotoxins in complex matrices is mandatory. In order to ensure productivity of laboratory performing such assays, a unique rapid method able to measure as much mycotoxins as possible independently of the sample origin is also needed. Sample preparation was performed by homogenisation followed by solid phase extraction using specific cartridges covering a large spectrum of mycotoxins. Extracts were then injected on an UHPLC column. Detection was performed by a triple quadrupole mass spectrometer in MRM mode. Fast polarity switching was used for simultaneous assay in positive and negative electrospray ionisation. The analysis time was less than 7 minutes to fully assay 32 prevalent mycotoxins. All studied mycotoxins were assayed in various samples: powdered milk, baby meals, cereals. Matrix effect was measured for each kind of sample to ensure that accuracy was acceptable despite the use of calibration standards prepared in solvent. Mobile phase selection was critical to give high sensitivity. Electrospray parameters (gas flows and temperatures) were cautiously optimised to find the optimal combination for the most critical mycotoxins. Since these parameters act in a synergistic way, a factorial design experiment is needed to find it. Manually testing all combinations in the chromatographic conditions is very time consuming. Therefore, new assistant software was used to generate all possible combinations and generate a rational batch analysis. In optimal conditions, a lower limit of quantification for aflatoxins and ochratoxin A at 0.01 and 0.025 µg/kg, respectively was reached. Other mycotoxins were quantifiable down to 0.5 µg/kg. This method easily fulfilled legal requirements for baby food control.

## P85

### Detection of glucoside derivatives of *Fusarium* mycotoxins (masked mycotoxins) by high-resolution LC-Orbitrap MS

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*Fusarium* fungi are plant pathogens infecting cereals known to cause disease called *Fusarium* head blight or scab. These pathogens infect ears and reduce grain yield and quality. Some of them also produce mycotoxins such as trichothecenes and zearalenone. For these *Fusarium* mycotoxins, several glucoside derivatives are reported and known as masked mycotoxins. Since the hydrolysis of these glucosides releasing their aglycons was reported, they are considered to present an additional potential risk for mycotoxins. Considering the structural similarity among *Fusarium* mycotoxins (especially trichothecenes) and their simultaneous prevalence in cereals, screening of new masked mycotoxins was performed. For the detection of masked mycotoxins, high resolution liquid chromatography-Orbitrap mass spectrometry (LC-Orbitrap MS) was used. Cereal samples (wheat or maize) naturally contaminated with trichothecenes were homogenised with acetonitrile/water (80:20, v/v) in the presence of acetic acid, and centrifuged. The supernatant was loaded on a solid-phase extraction (SPE) column, and the eluent was evaporated. The residue was dissolved in acetonitrile/water/acetic acid (5:94:1, v/v/v) and detection was performed with a LC-Orbitrap MS instrument (Exactive; Thermo Fisher Scientific). With the accurate mass and high-resolution (AM/HR) measurement, the detection of compounds whose chemical standards are not available was achieved. The identification was carried out on the basis of characteristic ions and fragmentation patterns observed with LC-Orbitrap MS. We detected masked mycotoxins derived from type B trichothecenes (fusarenon-X and nivalenol) in wheat grain that was artificially infected with *Fusarium* fungi. We further detected those derived from type A trichothecenes (T-2 toxin, HT-2 toxin, neosolaniol, di-acetoxyscirpenol, and mono-acetoxyscirpenol) in commercially available maize powder reference material. Although the absolute structures were not clarified except T-2 toxin-3-glucoside, glucosylation at the C-3 position in the trichothecene moiety seemed to be dominant based on the fragment profiles and concomitant detection of deoxynivalenol-3-glucoside (DON3Glc) in the identical samples. Our findings indicate that not only type B, but also type A trichothecenes are naturally glucosylated in plants such as wheat and maize. **Acknowledgements.** A part of this work was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (Research project ensuring food safety from farm to table MT-3218).

## P86

### Development and validation of an LC-MS/MS method for detection of *Fusarium* mycotoxins and their masked forms in cereals: application on Finnish grains

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In northern climates *Fusarium* is the most important fungal genus infecting wheat, barley and oats. The type A trichothecenes, T-2 toxin (T-2) and HT-2 toxin (HT-2), and the type-B, deoxynivalenol (DON) and nivalenol (NIV), as well as zearalenone (ZEN) are the most relevant to food safety *Fusarium* mycotoxins. Trichothecenes are associated with inhibition of DNA, RNA and protein synthesis, whereas ZEN causes oestrogenic effects. However, information on the long-term human and animal exposure to these compounds is scarce. In plants, mycotoxins are detoxified through conjugation with glucose, sulphate or amino acid moieties producing the so-called masked mycotoxins, e.g., DON-glucoside, HT-2-glucoside, ZEN-sulphate (Berthiller *et al.*, 2013). In the mammalian digestive tract the conjugated mycotoxins may hydrolyse back into their precursors with unknown toxicological implications. Therefore, more knowledge about the toxicological profile of masked mycotoxins is required to better understand their impact on health. Methods developed for routine analysis of native mycotoxins are not capable -as such- of detecting masked metabolites and thus, there is a need for rapid, reliable and robust methodology for sensitive and selective detection of these compounds. In this study, a quantitative analytical method for the simultaneous determination of DON, NIV, ZEN, T-2 and HT-2 and some of their masked metabolites in wheat, barley and oat flours was developed and validated using liquid chromatography-triple quadrupole-tandem mass spectrometry (LC-TQ-MS/MS). The 'dilute-and-shoot' sample preparation procedure was applied, employing extraction with acetonitrile:water:acetic acid (79:20:1, v/v/v). For oats, a hexane defatting step was implemented. Validation parameters included linearity, limit of detection, limit of quantification, specificity, selectivity, recovery, repeatability and inter-day precision. The developed method was used for the determination of masked mycotoxins as part of a nationwide survey of Finnish cereal grains. The cereals were harvested in several regions of Finland during 2013, and consisted of spring wheat (n=30), malting barley (n=20), feed barley (n=15) and oats (n=30). Thereby, the study provides for the first time survey data regarding masked mycotoxin occurrence in Finland.

## P87

### A fast and specific non-competitive immunoassay for HT-2 toxin

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Rapid methods for the detection of the mycotoxin contamination are needed for the efficient screening of high number of samples with fewer resources than required by the sophisticated analytical methods. Fast immunodiagnostic methods provide tools for *in situ* decision making preceding the further confirmatory analysis. Recombinant antibodies offer many advantages over the traditionally used poly- or monoclonal antibodies in immunoassays for mycotoxins. Recombinant antibody fragments can be isolated *in vitro* from the antibody gene libraries displayed on bacteriophages and produced cost-efficiently in large scale in *E. coli* bacteria. Here we demonstrate how recombinant antibodies enable a novel and very simple non-competitive immunoassay for HT-2 toxin. HT-2 and T-2 toxins are mycotoxins produced by *Fusarium* spp. We have developed recombinant antibodies against HT-2 and T-2 toxins from phage display antibody library containing ca. 10<sup>8</sup> different antibody clones from immunised source. The high-throughput selection and screening methods were used to increase the outcome. Depending on the binding properties, the most suitable antibodies were selected for different applications focussing on fast diagnostics and sample preparation. Antibody clone HT-2 (10) having 100% cross reactivity for HT-2 and T-2 toxins was used as a primary antibody in the development of HT-2 toxin specific anti-immunocomplex antibody from the VTT naïve antibody library. The best anti-immunocomplex clone (H5) was used as a Fab fragment or as an alkaline phosphatase enzyme fusion in homogenous and ELISA assays, respectively. In ELISA, the antibody-enzyme fusion simplifies the assay by reducing the steps required to complete the assay. Sample containing HT-2 toxin can be added together with the immunocomplex antibody-enzyme fusion to the wells, which are pre-coated by the anti-HT-2 toxin antibody. After one hour incubation and single washing step, the substrate can be added. In homogenous assays, the antibodies are labelled with FRET (fluorescence resonance energy transfer) compatible fluorescent labels. Labelled antibodies can be pre-dried to the

assay vials, e.g., microtiter plate wells, and read-out is ready in 5-10 minutes after sample application. The assay is highly specific for the HT-2 toxin only.

**P88**

### **Measuring existing and emerging mycotoxins in animal feed ingredients**

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There are more than 300 mycotoxins known to date with guidance or indicative limits in feed material in the EU in place only for aflatoxin B1, deoxynivalenol, zearalenone, ochratoxin A, fumonisin B1 and B2 and recently T-2 and HT-2. Thanks to the advanced analytical techniques more and more mycotoxins have been found to be present in feed material, including beauvericin, enniatins, ergot alkaloids and masked mycotoxins such as zearalenone-4-sulphate or deoxynivalenol-3-glucoside. The co-occurrence of regulated, emerging and masked mycotoxins raises additional safety questions due to the possible additive or synergistic effects. Funded by the Home-Grown Cereals Authority (HGCA) we have developed and validated UPLC-MS/MS method for the detection and quantification of 79 mycotoxins in distillers dried grains with solubles (DDGS) produced from wheat and maize. DDGS is a co-product of alcohol fermentation (beverage or biofuel production) and it is used as a feed material due to high content of proteins, fats and fibres. However, there is limited data on the safety of DDGS with regard to possible mycotoxin contamination. Therefore, the multimycotoxin method has been used to analyse approximately 100 samples (both wheat and maize DDGS) collected from the European biofuel manufacturing and feed industry. From 15 to 38 different mycotoxins, including regulated and emerging ones were found in each sample. Maize-based DDGS was mainly contaminated with enniatins, fumonisins, mycophenolic acid, fusaric acid, aflatoxins, alternariol, meleagrins, T-2 and ochratoxin A; while wheat-based DDGS with enniatins, deoxynivalenol, ergot alkaloids, fusaric acid, mycophenolic acid, emodin and ochratoxin A. The main mycotoxins found in DDGS will be used as targets for the development of a fast dipstick test for the field application and fast identification of the contaminated samples.

**P89**

### **A comparison of HPTLC, HPLC-UV, ELISA and LC-MS/MS methods for the determination of deoxynivalenol in livestock feed**

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The control of mycotoxins is a global challenge not only in human consumption but also in nutrition of livestock animals. The presence of mycotoxins in grains and livestock feed has been reported for decades and represents a worldwide problem for farmers. Mycotoxin contamination of livestock feed has become a major concern because it can cause acute or chronic mycotoxicosis in animals. *Fusarium* toxins, such as deoxynivalenol (DON) produced by *F. graminearum* and *F. culmorum*, is one of the most common contaminants of maize, wheat, barley, and livestock finished feedstuff. DON was characterised as a trichothecene and was given the name 'vomitoxin' because of its emetic effect on swine. In this study, a comparative study on the suitability of analysis methods, such as high performance thin layer chromatography (HPTLC), high performance liquid chromatography with UV detection (HPLC-UV), enzyme-linked immunosorbent assay (ELISA) and liquid chromatography with triple-quadrupole mass spectrometer (LC-MS/MS) for the determination of DON in livestock (pigs, poultry and cattle) feed was carried out. For over 90% of the analytes, recoveries were between 70 and 120% and precision (expressed as relative standard deviation) was mostly in the range of 5-10%. The limits of detection (LOD) were from <0.05 to 1 µg/kg in each of the analysis methods, which is sufficient for examination to verify compliance of products with legal tolerances. LC-MS/MS was the most sensitive method (LOD, 0.05 µg/kg) and HPTLC was the least sensitive method (LOD, 1 µg/kg). Overall, this study shows an excellent correlation between four independent methodologies and supports the validation of these technologies for assessment of contamination of DON in livestock feed. According to the obtained results, ELISA can be used as a reliable screening method, but the confirmation of positive results must be done by LC-MS/MS method.

## P90

### Direct analysis of aflatoxin M1 in raw not skimmed milk using B Zero Afla M1 ELISA kit

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Sample preparation is a key step conditioning the reliability of the analysis: the easier is the procedure, the lower is the possibility of error. Moreover, it affects the overall analytical time and cost, depending on the required instruments and accessories. Commercially available ELISA test kits for aflatoxin M1 in milk usually recommend to refrigerate the sample, and then to skim it by centrifugation. This is actually a dramatically easy preparation and it has recently reported that, when combined with a reliable screening tool, it guarantees better performances than instrumental analysis, for which immunoaffinity columns have to be used (Biancardi *et al.*, 2012). Nevertheless, centrifugation and defatting could represent a bottleneck especially in case of high throughput analysis or when fast results are needed. The aim of the present work was to investigate whether raw fat milk could be directly analysed by B Zero Afla M1, a new master-curve calibrated ELISA test kit for the quantitative detection of aflatoxin M1 in the range 10-200 ng/l. The assay was first validated onto reference materials to verify trueness and precision. 6 milk samples with assigned values ranging between 20 and 150 ng/l aflatoxin M1 were analysed. The mean recovery was  $98\pm 7\%$  ( $n=30$  determinations), the mean intra-assay coefficient of variation (CV) was 3% and the mean inter-assay CV was 8%. Then, 20 blank and low-contaminated milk samples ( $<20$  ng/l), with fat percentage ranging between 3.5 and 7%, were analysed both as skimmed and not skimmed. The mean relative signal (B/Bo) was  $86\pm 6$  and  $87\pm 6\%$  for defatted and native milk materials, respectively. Data showed normal distribution, and Student t test  $p$  value ( $>0.05$ ) confirmed that there were no statistical differences between the two populations. Similar results were obtained for six naturally incurred milk samples with aflatoxin M1 concentration ranging between 20 and 200 ng/l, and the correlation between skimmed and not skimmed samples was high ( $r^2=0.99$ ). This study showed that no different results are obtained as consequence of the presence of fat in milk samples, and therefore confirmed the possibility to avoid any sample manipulation before B Zero Afla M1 ELISA kit analysis. All the validation data indicate the high quality of B Zero Afla M1 ELISA kit, an innovative screening tool ensuring speed (only 30 min assay), ease of use, reduction of any additional source of error apart from assay implementation, and cost effectiveness, thanks to the absence of any calibrator apart the 'zero' to be run.

## P91

### A new lateral flow platform for the analysis of mycotoxins in cereals

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Regarding the demand for easy and quick mycotoxins analysis in cereals during raw materials acceptance, there is a new product line of versatile screening tools: Smart Strip Afla B1, Smart Strip Afla, Smart Strip DON, Smart Strip ZON and Smart Strip FUMO are easy-to-handle, rapid, lateral flow devices for the qualitative and quantitative detection of aflatoxin B1, total aflatoxins, deoxynivalenol, zearalenone and fumonisins. Smart Strip lateral flow kits are built as one-step assays with all reagents absorbed on the device itself, where no preincubation is required and which, apart from readers, do not require additional equipment. As sample preparation procedure is the same (except for deoxynivalenol), the same extract can be run on different strips and read together simultaneously, obtaining in a few minutes a multi-analytic profile of the sample. Two readers are available, both for either quantitative or qualitative results: Lab LFD Reader is a scanner connected to a PC able to read up to five different strips simultaneously; Smart Reader is a portable, stand-alone smart-phone based reader, suitable for outdoor analysis as well, able to read up to two strips simultaneously. The Smart Strip lateral flow platform meets all the European Commission Directive 2003/100/EC and the Recommendation 2006/576/EC requirements, as the LOQ for aflatoxin B1 is 5 ppb, for total aflatoxins 10 ppb, for deoxynivalenol 500 ppb and for zearalenone 100 ppb; for fumonisin, the work is still in progress. All the test kits are characterised by high specificity (in all case more than 95%). Smart Strip line is therefore a screening flexible platform to fit raw materials acceptance specific needs.

P92

## Selection and characterisation of aptamers for the rapid detection of aflatoxin in maize

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Aflatoxins are the main toxic secondary metabolites produced mainly by the two *Aspergillus* moulds *Aspergillus flavus* and *Aspergillus parasiticus*. These species are widespread in nature and can colonize many important agricultural crops such as cereal grains and legumes before harvest and during storage. Aflatoxins occur mainly in warm and humid regions, where high temperature and humidity are optimal for mould growth and toxin production. Aflatoxin B1 is considered the most common one in food and feed and also the most toxic from among the aflatoxins. To detect and quantify these mycotoxins, two strategies have been established until now: either analysis with high-end equipment such as HPLC-UV and LC-MS based methods, which are the most popular methods to analyse mycotoxins such as aflatoxins or rapid screening protocols with immunoassays such as ELISA and lateral flow devices (LFDs). Instrumental analysis offer excellent sensitivities but they frequently require skilled operators, extensive sample pre-treatment and expensive equipment. However, immunotests rely on antibodies which production and purification is expensive and elaborate. Furthermore antibodies are not stable under higher ambient temperatures and hence their shelf-life is rather short. In the recent years aptamers have been considered as a potential alternative to antibodies. These single stranded DNA oligonucleotides are analogous to antibodies in their range of target recognition and variety of application but they also possess several key advantages over their protein counterparts like greater specificity and affinity. Once the specific sequence of an aptamer is known it can be synthesised completely synthetically and the costs therefore are low. In this study, we describe the way towards aflatoxin binding aptamers. First the aflatoxin B1 was bound to magnetic beads which were incubated with a random library consisting of approximately  $10^{15}$  different sequences. These sequences were subsequently used for the iterative selection procedure named SELEX (systematic evolution of ligands by exponential enrichment). The conditions were varied in each of the four rounds to artificially evolve aflatoxin binding DNA oligonucleotides. To identify enriched sequences the DNA pool obtained after SELEX round two and four was sequenced using next-generation sequencing (NGS). This technique enables analysis of far greater numbers of aptamer sequences and enriched sequences can be identified much earlier in the selection process compared to standard bacterial cloning techniques. The five most enriched sequences were characterised and are now used for the development of LFDs and ELAA (enzyme-linked aptamer assay).

P93

## Proficiency testing as a tool to evaluate performance of LC-MS/(MS) multi-mycotoxin methods in view of EU regulation

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A proficiency test (PT) was organized for LC-MS/(MS) multimycotoxin methods for the determination of eleven mycotoxins (aflatoxins B1, B2, G1 and G2, ochratoxin A, deoxynivalenol, T-2 and HT-2 toxins, zearalenone, and fumonisins B1 and B2) in maize. This PT was earlier described by M. Solfrizzo *et al.* (2013) and De Girolamo *et al.* (2013). Although the different performance criteria for mycotoxins stated in the Commission Regulation (EC) No 519/2014 are based more or less on single-mycotoxin methods, the performance of the multimycotoxin methods was assessed by Ducares against the 'single-mycotoxin methods' requirements of the European Commission. The data assessment, with the requirements of the European Commission as the target standard deviation ( $\sigma$ ) values, was made on a naturally contaminated maize sample and a spiked maize sample. The z-score performance per mycotoxin was divided in three categories, namely  $|z| \leq 2$  satisfactory,  $2 < |z| \leq 3$  questionable,  $|z| > 3$  unsatisfactory. For the naturally contaminated maize sample the percentage of satisfactory z-score values ( $|z| \leq 2$ ) was for ten mycotoxins between 68 and 89%. For one mycotoxin (HT-2 toxin) a satisfactory performance of 97% was obtained. For the spiked maize sample the percentage of satisfactory z-score values ( $|z| \leq 2$ ) was between 43 and 94% for eight mycotoxins. For three mycotoxins a satisfactory performance was obtained of 100% (T-2 toxin), 97% (HT-2 toxin) and



97% (aflatoxin G2). The LC-MS/(MS) multimycotoxin methods do not meet the performance criteria for most of the mycotoxins in both maize samples in view of the Commission Regulation (EC) No 519/2014.

**P94**

### **Innovative solutions for mycotoxin in-process control and monitoring within grain handling**

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Occurrence of mycotoxins in grain will never be completely avoidable. Industrial self-control remains the best possibility to protect consumer's health and avoid cost-intensive rejections. Two main instruments are necessary to improve quality control on-site: suitable monitoring methods that give a comprehensive overview on contaminations in grain and help to assess risks, e.g., at the beginning of a harvest season and in-process mycotoxin control that enables rapid decisions, grain handling and storage management directly based on analytical results. To suit the workflow and requirements on-site, these tests must be rapid, cheap, easy to handle, and specific enough to detect at low legal limits. In praxis hardly any test fulfils these requirements. Furthermore, in grain industry, rapid on-site methods have to deliver representative results for large lots. Thereby, sampling remains the most critical step. To improve measurement certainty and shorten time of analyses, an innovative approach using grain dust samples was established. For on-site analysis, semi-automatic dust samplers were installed at grain handling and processing plants for in-process control. Dust samples were taken and directly analysed using adapted lateral flow tests. Previously developed and validated data models allowed calculating contaminations in the lot from concentrations determined in the dust. Measurement uncertainties could be reduced remarkably while reducing the measurement time by up to 80% compared to common praxis procedures. The fitness-for purpose of the new approach for in-process control of *Fusarium* toxins was shown for wheat and maize storage, silo management and release of shipments. To serve as good monitoring tool, multi-toxin screening methods have to cope with rising numbers of regulated and emerging toxins. Thereby, a main challenge is to find the best analytical compromise for fast, cost-efficient, but sensitive analysis of a maximum number of diverse molecules. A sensitive method based on HRMS for screening and quantification of various classes of toxins had been developed in a three step approach. Thereby, higher resolution and mass accuracy improved the simultaneous detection of >50 plant- and mycotoxins. The natural enhancement of mycotoxins in dust samples further improved the monitoring quality and enabled fast risk assessment at harvest or supplier evaluation. Sensitive monitoring methods and reliable in-process control based on dust sampling in combination with standard reference methods for verification may help to significantly improve mycotoxin control along the whole grain chain and therefore the implementation of EU regulations.

**P95**

### **Tracing the metabolisation of T-2 and HT-2 toxin in barley by LC-HRMS based stable isotope assisted metabolite profiling**

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T-2 and HT-2 toxins are secondary metabolites of various *Fusarium* species (*F. sporotrichioides*, *F. langsethiae* and *F. poae*) and belong to the group of type A trichothecenes. Especially in small grain cereals, barley as well, T-2 and HT-2 can be detected (Lattanzio *et al.*, 2012). The investigation of plant metabolism of mycotoxins is becoming increasingly important, because it might result in metabolites with similar or even higher toxicity (Berthiller *et al.*, 2013). In the present study, barley ears were inoculated with a 1:1 (v/v) mixture of <sup>12</sup>C-non-labelled and <sup>13</sup>C-fully-labelled toxin. Based on a recently developed metabolomics workflow (Kluger *et al.*, 2013), the samples were analysed by liquid chromatography-high resolution mass spectrometry (LC-HRMS). The software-program 'MetExtract' (Bueschl *et al.*, 2013) developed in our working group was used to extract toxin-related <sup>12</sup>C/<sup>13</sup>C corresponding peak pairs from the raw data. For the interpretation of T-2 and HT-2 metabolisation products, possible elemental formulas were calculated and further LC-HRMS/MS

measurements were carried out. This poster shows the results of this study and includes a discussion of the performance of the applied analytical strategy.

**P96**

### ***Fusarium* species fast identification and associated mycotoxins detection by MALDI-TOF**

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The barley-malt-beer chain is regularly affected by *Fusarium* contaminations leading to reduced yields, technological transformations concerns as well as safety issues. Due to the difficulty to predict the nature and levels of both mycological and mycotoxins contaminations in an evolving environment, a proper hygienic control of the whole chain is hard to achieve. Fast corrective actions based on phytosanitary and/or biological treatments as well as technological practices modifications are now required. In order to speed up the accurate adaptation of treatments and production processes, fast biological and mycotoxins analytical techniques are needed. In this context, the use of MALDI-TOF for the fast identification of *Fusarium* species encountered in the barley-malt-beer chain and for the detection of some associated mycotoxins is evaluated. Due to the typical concerns of the chain, this study focused on *graminearum* (generic cereal contamination), *langsethiae*, *poae* (T2 and HT2) and *tricinctum* (Enniatins) species of the *Fusarium* genus. These strains were isolated and identified by either IFBM or ANSES from field samples. Strains from the different species were cultivated in both liquid and solid media, in pure and mixed cultures for further MALDI-TOF analyses. A first step of this work consisted in the adaptation of the sample preparation conditions in order to get proper analytical signal as moulds are generally far more receptive to laser ionization than bacteria, due to different parietal composition. Then, pure culture samples have been analysed and the corresponding spectra treated to fill the Saramis database. Our results show that species level *Fusarium* identification is possible with the considered strains, even if the number of mass peaks is actually lower than the one obtained with bacteria. This identification can be achieved on at least 3 days liquid or 5 days solid cultures, with an analysis time of only some minutes, which speeds up the identification protocol. As far as mycotoxins detection is concerned, both enniatins, and T-2 and HT-2 toxins were accurately detected with the producing strains, whereas no detection took place with non-producing strains. However, mycotoxins detection was only achieved with solid media samples, which seems logical as solid state cultures correspond to the mycotoxin production physiological state. At last, our work showed that, without Saramis software adaptation, co-identification of *Fusarium* species in mixed cultures with up to 3 different species was achievable.

**P97**

### **Extraction of fumonisins using a water-based extraction method**

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The three most abundant fumonisins are fumonisin B1 (FB1), fumonisin B2 (FB2) and fumonisin B3 (FB3) and they have been declared as a potential human carcinogen by the International Agency for Research on Cancer. FB1 in particular has been also linked to leukoencephalomalacia and nephrotoxicity in animals. This group of toxins is produced by fungi growing in agricultural commodities and has to be tested for before grain is used for animal or human consumption. A fast and simple extraction method of grain, followed by a quick on-site screening method is necessarily needed. Extraction is usually performed using organic solvents which are harmful for human and the environment. To reduce solvents consumption, a water-based extraction method for fumonisins was developed recently. Complete replacement of organic solvents by using non-toxic aqueous buffer systems for extraction will be demonstrated by this poster.

P98

## Integration of DON extraction into a water-based multi-extraction method for mycotoxins in food and feed

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Approximately 25% of the worldwide cereal harvest is contaminated with naturally occurring toxins produced by fungi, called mycotoxins. Deoxynivalenol (DON), also called vomitoxin, occurs predominantly in grains as wheat, maize, barley, oats and rye. It is known as immunosuppressant and may cause kidney problems. Fast and simple extraction of this toxin from agricultural commodities can be done by using water. However, the integration of extraction of DON into a unique water-based method was the crucial step in development of such a multi-extraction method. One uniform extracts can further be used for rapid screening of other mycotoxins, such as aflatoxins, fumonisins, and zearalenone in grains by simple lateral flow device tests. This poster will demonstrate that extracts obtained by a unique, simultaneous, water-based method can further be used for detection of DON in cereals.

P99

## Mid-infrared spectroscopy based on GaAs thin-film waveguide and quantum cascade laser technology as a tool for the detection of deoxynivalenol (DON) in maize extracts

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Infrared-based (IR) analytical methods are rapid and non-destructive techniques that require minimal technical training and sample preparation. The MIR spectral region at 3-20  $\mu\text{m}$  provides fundamental vibrational and rotational fingerprint absorptions of organic molecules offering inherent molecular selectivity. This renders this spectral window attractive for optical sensing applications including the analysis of complex biological matrices such as food or feed. Quantum cascade lasers (QCLs) are among the most promising light source technologies for IR sensing applications due to their compact dimensions, long lifetime, and broad tunability when coupled with an external cavity (Charlton *et al.*, 2005, 2006; Young *et al.*, 2009; Wang *et al.*, 2012). The combination of highly sensitive thin-film GaAs/AlGaAs planar waveguides with broadly tunable QCLs (1925-885  $\text{cm}^{-1}$ ) was used to increase the sensitivity of conventional IR measurements of *Fusarium graminearum*-contaminated maize by attenuated total reflection (ATR). Maize samples were either naturally contaminated or inoculated with *F. graminearum* and the trichothecene deoxynivalenol (DON) was quantified by high resolution liquid chromatography tandem mass spectrometry (LC-MSMS). Ground maize samples were extracted with methanol and the extracts were subsequently analysed on GaAs waveguides. The spectral differences between samples with DON concentrations above and below the set EU regulatory limit for unprocessed maize were classified by developed chemometric models. In this study, extracts of DON contaminated maize were analysed by MIR spectroscopy using highly sensitive thin-film waveguides for the first time demonstrating their capability as novel food contaminant sensing devices. The inherent advantages of MIR sensing systems make them attractive options for high throughput, on-site, determination of fungal contamination in both solid and liquid foodstuffs. **Acknowledgements.** The research leading to these results has received funding from the European Union's Seventh Framework Programme managed by REA Research Executive Agency (<http://ec.europa.eu/rea>) (FP7/2007-2013) under grant agreement no. 314018 FP7-SME-2012-SME.

P100

## A near infrared spectroscopy method for the rapid detection of aflatoxin B1 contamination in rice

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Near-infrared spectroscopy (NIRS) is a powerful technique for characterising the chemical composition of materials, and to evaluate the quality and safety of food and agricultural products. The objective of this research was to apply NIRS, with a wavelength range between 950-1650 nm, for the rapid detection of aflatoxin B1 (AFB1) contamination in rice samples. Spectra were obtained on 105 rice samples, including 90 naturally AFB1- contaminated samples, and 15 artificially AFB1- contaminated samples, by reflection mode. Quantitative calibration models to detect AFB1 were developed using the original and pre-treated absorbance spectra in conjunction with partial least squares regression (PLSR) with prediction testing and full cross validation. The statistical model developed from the treated spectra (standard normal variate and detrending; SNVD) provided the best accuracy in prediction, with a correlation coefficient ( $r$ ) of 0.957, a root mean square error in cross validation (RMSECV) of 1.902  $\mu\text{g}/\text{kg}$  and a bias of -0.0040  $\mu\text{g}/\text{kg}$ . The results indicate that NIRS technique has great potential for the screening of AFB1 contamination in rice.

**P101**

### **Environmentally friendly and cost-efficient analysis of aflatoxins in maize**

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The extraction procedure adds a significant cost to the overall expense of aflatoxin analysis in agricultural commodities. An inexpensive and low-waste extraction method using a household espresso coffee maker was tested. This appliance was used for the high-temperature /high-pressure extraction of aflatoxins. The pump pressure of 18  $\text{kg}/\text{cm}^2$  and water temperature close to boiling point make this device suitable for rapid extraction. A representative ground maize sample was mixed with diatomaceous earth and an organic water-miscible solvent followed by extraction with hot water in an espresso coffee maker. The extract was then analysed for aflatoxins by HPLC. Recoveries of aflatoxins B1, B2, G1, and G2 from maize at 5  $\mu\text{g}/\text{kg}$  were: 80.3 $\pm$ 1.3, 89.1 $\pm$ 4.8, 86.4 $\pm$ 3.1, and 84.0 $\pm$ 4.6%, respectively.

**P102**

### **Analytical method validation and monitoring of 12 mycotoxins in wheat grain, processed milling products, at a field study and at different points of Brazilian supply and commercial chains**

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This study was aiming at validating an 'in house' multimycotoxin method for wheat matrix and its processed products, based on QuEChERS methodology, using UPLC-MS/MS detection. The 12 mycotoxins were divided in two different concentration level groups, according to their sensitivity in UPLC-MS/MS. The validation was performed by analyzing recovery samples at three different spike levels with seven replicates ( $n=7$ ) at each level. Linearity ( $r^2$ ) of calibration curves, accuracy (as % recovery), instrument limits of detection and method limits of quantification (LOD and LOQ), precision (RSD%), selectivity and matrix effects were determined for each individual mycotoxin. This method was applied to quantitatively determine the occurrence of mycotoxins in 476 samples of wheat from field trials, export and import grain types, grain processing fractions (flour and bran) and flour sold in supermarkets. Considering the detector responses of the 7 concentrations of calibration standards in solvent and in wheat matrix extract, the range of matrix effect (%) was from -26 to +9 %. Accuracy (as % recovery) and precision (% RSD) of the method were within the acceptable range (70-120%, resp. <20%) for all mycotoxins, at all 3 spike levels, except for T2- Toxin which could only be validated at the highest spike level (500  $\mu\text{g}/\text{kg}$ ). The method LOQ was 1  $\mu\text{g}/\text{kg}$  for aflatoxin B1, B2, G1, G2 and ochratoxin A, and 50  $\mu\text{g}/\text{kg}$  for fumonisin B1 and B2, deoxynivalenol, diacetoxyscirpenol, zearalenone and HT-2 toxin. Only for T-2 toxin the LOQ was 500  $\mu\text{g}/\text{kg}$ . All samples analysed were contaminated by at least one of the 12 mycotoxins, regardless of origin. In the field trial samples, factors such as

genotype, environment and fungicide treatment had influence on the concentrations of DON. Some samples of wheat grain for exportation showed contamination with DON and also FB1, FB2, besides the co-occurrence of ZEN and OTA. This is the first study reporting the presence of fumonisins in Brazilian wheat, as it was not reported before in the literature. In products of the milling process, redistribution of DON was observed, that is a decrease in concentration in wheat flour and increase in the bran fraction, compared with the initial concentration in the whole grain. This shows that this process is not effective for mycotoxins removal. Therefore, not surprisingly, DON and ZEN mycotoxins were found in flour samples purchased at supermarkets of Cruz Alta and Santa Maria cities, RS state in Brazil. **Acknowledgements.** ANVISA, MCTI, MAPA, CNPq, FINEP, NVWA, CCGL and UFSM are acknowledged for their support.

**P103**

### **Rapid and simultaneously screening and quantitation of mycotoxins in different matrix using high resolution MS/MS instrument**

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Mycotoxins are known to harm the health of humans and animals. They are known either as carcinogenic or otherwise cytotoxic and impair the immune system. Therefore, different countries have set regulations on mycotoxins. In the EU mycotoxin limits are harmonized in the regulation for contaminants in foodstuffs EC 1881/2006 of 19 December 2006 and the amended Regulation EC 1126/2007 of 28 September 2007. In July 2010 two new analytical methods for measuring aflatoxin B1 and zearalenone in infant food have been adopted as European benchmark (EN 15850, EN 15851). We will present the possibility to analyse different mycotoxins at a comparable detection level implemented into a high resolution Triple TOF screening/quantitation method. For our measurements, AB Sciex Triple TOF™ LC-MS/MS system was used. In one single LC-MS/MS run, multiple mycotoxins were detected; fast positive and negative switching was applied here. We will demonstrate using high resolution MS/MS mycotoxin library for screening and identification mycotoxins in different matrices. Detection limits of various mycotoxins were found to be comparable to the required values by EN standards. With this study we can demonstrate the possibility to analyse AFB1 and ZON at the defined infant food levels without sample concentration and implemented with a TripleTOF™ 5600 system screening method. Reproducibility in matrix was found to be lower than 10%. New software allows us to perform identification and quantitation in very rapid way. We can demonstrate that both reproducibility and Quantitation accuracy can be improved with no compromising on instrument sensitivity. The use of acquisition technique with positive/negative switching has enabled shorter run times without the loss in sensitivity. Due to its high resolution capabilities matrix effects can be further discriminated. Additionally quantitation and high resolution library confirmation can be achieved with just one single injection.

**P104**

### **Using ion mobility mass spectrometry and collision cross section areas to elucidate the $\alpha$ and $\beta$ epimeric forms of glycosylated T-2 toxin**

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Toxigenic fungi often grow on edible plants, thus contaminating food and feed with fungal metabolites. Plants can alter the chemical structure of mycotoxins as part of their defence against xenobiotics. The extractable conjugated or non-extractable bound mycotoxins formed remain present in the plant tissue but are currently neither routinely screened for in food nor regulated by legislation, thus they may be considered 'masked'. *Fusarium* species mycotoxins are prone to metabolism or binding by plants. Toxicological data are scarce, but several studies highlight the potential threat to consumer safety from these substances (Berthiller *et al.*, 2013). In particular, the possible hydrolysis of masked mycotoxins back to their toxic parents during mammalian digestion raises concerns. Masked mycotoxins may also elude conventional analysis because of modified physicochemical properties. All of these effects may lead to a potential underestimation or overestimation of the total mycotoxin

content of the sample. In this study, we report the use of high definition mass spectrometry (HDMS) as a powerful tool for the separation and characterisation of  $\alpha$  and  $\beta$  epimeric forms of glycosylated T-2 and related toxins. The  $\alpha$ -glycosylated T-2 standard was isolated from *Blastobotrys muscicola* cultures following exposure to T-2 and the  $\beta$  form produced *via* chemical synthesis. HDMS is a combination of high resolution mass spectrometry and high efficiency ion mobility separation. Ion mobility spectrometry (IMS) is a rapid orthogonal gas separation phase technique which allows another dimension of separation to be obtained within an UPLC timeframe. Compounds can be differentiated based on their size, shape and charge. In addition, both precursor ion and fragment ion information can be simultaneously acquired in a single injection in an HDMS experiment, referred to as HDMS<sup>E</sup>. HDMS<sup>E</sup> data not only provides additional peak capacity but also insights into the molecular characteristics of the analytes for example, the elucidation of different isomeric species and intra-molecular sites of protonation. The ion mobility data generated was used to calculate the collision cross section area (CSS) values within the data processing software (UNIFI Research Edition v.1.6.50) of 244.85 and 251.33 Angstroms for the  $\alpha$  and  $\beta$  T-2 glycosides, respectively. The combination of CCS, retention time, exact mass and fragmentation information provides a unique characteristic signature for the compounds. The individual CCS values derived for the  $\alpha$  and  $\beta$  epimers can be used to determine which epimeric form of the toxin is present in the sample and can serve as a valuable tool during toxicological and profiling studies.

## P105

### In-house validation of a sample preparation method for analysis of fumonisin B1 in rice

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A simple, rapid and cost-effective sample preparation method was validated for the determination of fumonisin B1 in polished parboiled rice. Polished parboiled rice is the product obtained from parboiling process, which the unpeeled rice is submerged in drinking water at a temperature above 58°C, followed by a partial or full gelatinisation of starch and then drying. The performance characteristics – selectivity, linearity, matrix effect, limits of detection and quantification, trueness and precision were evaluated, and the method was successfully applied to commercial samples. The method involves extraction with a 50% acetonitrile aqueous solution and glacial acetic acid, liquid-liquid partitioning with addition of anhydrous sodium sulphate and sodium chloride, followed by dispersive SPE clean-up with diatomaceous earth and anhydrous sodium sulphate. The final extract was analysed by HPLC-FLD after precolumn derivatisation with ortho-phthalaldehyde (OPA). The chromatograms obtained from fumonisin B1-free rice, rice spiked with fumonisin B1 standard, and rice naturally contaminated with the mycotoxin demonstrated the unequivocal separation of the fumonisin B1-OPA derivative from interferences, indicating the selectivity of the method. The assumptions that the regression residuals from the solvent and matrix-matched calibration curves are normally distributed, homoscedastic and independent were confirmed, and the high significance ( $p < 0.001$ ) of the regression and no significance of the lack of fit ( $p > 0.05$ ) indicated linearity in the range from 100 to 2,500  $\mu\text{g}/\text{kg}$ . Analysis of the matrix effects showed a significant difference ( $p < 0.05$ ) between the slopes obtained for the solvent and matrix-matched calibration curves using a *t* test, thus a matrix-matched calibration curve was used to calculate the mass fraction of fumonisin B1 in the samples. The limits of detection and quantification were 50 and 100  $\mu\text{g}/\text{kg}$ , respectively. The average recovery values were 96.3, 106.5 and 112% for spiked samples at 2,500, 1000 and 100  $\mu\text{g}/\text{kg}$ , respectively. Under the repeatability conditions, the relative standard deviation values were 5.5, 3.0 and 8.3%, and using the within-reproducibility conditions the values were 4.9, 2.0 and 2.7%, for spiked samples at 100, 1000 and 2,500  $\mu\text{g}/\text{kg}$ , respectively. To assess the adequacy of the method, ten different commercial brands were analysed. Fumonisin B1 was detected in two brands at levels of  $109.4 \pm 8.9$  and  $163.0 \pm 10.5$   $\mu\text{g}/\text{kg}$ , demonstrating that the proposed sample preparation method can be employed routinely for analysis of fumonisin B1 in polished parboiled rice. **Acknowledgements.** The authors are grateful to CNPq (National Council for Scientific and Technological Development) for financial support and for the master's scholarship awarded to the first author.

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## Determination of the proportion of matrix-associated fumonisin B1 in different, animal feeding experiment-aided matrices after *in vitro* digestion

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In this study, the occurrence of matrix-associated fumonisin B1 was analysed on two cereal substrates (maize and wheat), inoculated with *Fusarium verticillioides* (MRC 826) to determine the proportion of hidden FB1 produced along laboratory scale, artificial mycotoxin production (n=39, in all cases 2 replicates). Two parallel methods were applied: an *in vitro* human digestion sample pre-treatment and the routine extraction procedure, in both cases with subsequent LC-MS/MS analysis. It was found that all samples showed higher content of total fumonisin B1 after digestion, as compared to that of free fumonisin analysed only after extraction. The percentage of the hidden form in maize was 38.6% ( $\pm 18.5$ ), while for wheat it was 28.3% ( $\pm 17.8$ ), expressed as the proportion to total fumonisin B1, for the total dataset. The determination coefficient ( $r^2$ ) between extractable and total fumonisin B1 concentration for maize and wheat was 0.95 and 0.88, respectively. These results prove that the fungal culture used in several animal toxicological studies from the eighties does not differ in the proportion of hidden form from the natural (field derived) crop samples. The toxin exposure of the experimental animals determined by the routine fumonisin analysis was underestimated, generally by 40%, as bioaccessibility was not taken into consideration. This is crucial in interpretation (and maybe in the re-evaluation) of the results obtained in animal experiments.

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## Analysis of 12 mycotoxins in calves' milk replacer by means of UHPLC-MS/MS

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In Belgium, veal calves are predominantly fed liquid milk replacers, based on powder milk and vegetable protein sources such as soy, maize and wheat. These ingredients may imply a risk of mycotoxin contamination. The aim of this study was to develop a multi-mycotoxin ultra-high pressure liquid chromatographic-tandem mass spectrometric (UHPLC-MS/MS) method for the detection, quantification and identification of 12 mycotoxins in milk replacer. The mycotoxins included in this study were aflatoxins (AFB1 and B2), alternariol (AOH), alternariol monomethyl ether (AME), deoxynivalenol (DON), 3-acetyl-DON (3-Ac-DON), 15-acetyl-DON (15-Ac-DON), fumonisins (FB<sub>1</sub> and B<sub>2</sub>), ochratoxin A (OTA), T-2 toxin (T-2) and zearalenone (ZEN). <sup>13</sup>C<sub>15</sub>-DON, <sup>13</sup>C<sub>17</sub>-3-AcDON, <sup>13</sup>C<sub>24</sub>-T-2 and <sup>13</sup>C<sub>18</sub>-ZEN were used as internal standards. Gradient chromatographic separation was performed on an Acquity™ UHPLC (Waters) system equipped with an Acquity CSH Fluoro Phenyl column (1.7  $\mu$ m, 2.1 x 150 mm, Waters). The mobile phase consisted of a mixture of H<sub>2</sub>O + 0.3% acetic acid (A) and methanol (MeOH) + 0.3% acetic acid (B). Detection was performed with a Xevo TQ-S MS triple quadrupole system (Waters). The extraction procedure consisted of a first extraction step with MeOH followed by a second extraction step with acetonitrile/H<sub>2</sub>O/acetic acid (79/20/1). Specificity, linearity ( $R^2$ ), apparent recovery ( $R_A$ ), repeatability ( $RSD_r$ ), reproducibility ( $RSD_R$ ), limit of detection (LOD) and quantification (LOQ) were determined for the method developed. For the calculations of  $R_A$ ,  $RSD_r$  and  $RSD_R$  two concentration levels were considered. For all mycotoxins considered, the criteria of linearity ( $R^2 \geq 0.99$ ) and specificity were fulfilled. The average  $R_A$  (over the two concentration levels) varied between 85% and 107%, which is in agreement with the ranges stipulated in Commission Decision 2002/657/EC. Average  $RSD_r$  and  $RSD_R$  ranged between 0.9 and 10.5% and between 1.5 and 12.8%, respectively. The LOD values were within the range of 1-89  $\mu$ g/kg and 2-171  $\mu$ g/kg, respectively. The developed method was used to determine the degree of mycotoxin contamination in different samples of calves' milk replacer (Antonissen *et al.*, 2014).

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## NIR perspectives to detect *Datura* seeds and ergot bodies in cereals

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Since decades, near infrared (NIR) spectroscopy is widely used in the food and feed sectors to implement rapidly, inexpensive and efficient control tools for the quality of the products. NIR spectrum can be used to generate a fingerprint of a product. It can be affected by several factors as the presence of toxic substances which will modify the pattern of the spectrum. The aim of this work is then, to demonstrate, through two case studies, the performance of NIR technology to detect *Datura* seeds and ergot bodies in cereals. In the first study case, adequate sample presentation techniques are investigated to improve the performance of NIR methods. The recent high throughput sampling accessories allow the analysis of high number of samples by unit of time. This study aims to assess classical NIR instrument to detect in buckwheat grains, *Datura stramonium* seeds, a wild-growing plant found in crops and known for the high content in alkaloids. It was shown that classical NIR spectrometer using a specific sample presentation allowed analysing a higher number of reduced size subsamples by acquiring one spectrum by subsample. Moreover, simple chemometric tools, such as PCA, applied to the NIR spectra allowed discriminating between pure *Datura* seeds, buckwheat kernels and mixture of both. In the second study case, the *in situ* analysis is investigated using NIR hyperspectral imaging instrument which combines spectral and spatial dimension information. Up to several thousand spectra per sample can be collected. This study aims to assess NIR hyperspectral imaging to detect and quantify in cereals, ergot bodies, formed by the fungi *Claviceps purpurea*, which also contain alkaloids. It was shown that, using such imaging system, up to 50 kg of cereals can be analysed in 1 h and that the limit of ergot detection is 145 mg/kg, below the limit of 500 mg/kg fixed by the European Commission in 'intervention' cereals destined for humans. In conclusion, the high throughput sampling accessories and the hyperspectral imaging systems are adequate tools to bring the NIR technology in the field of the detection and quantification of contaminants. They allow scanning rapidly small sample fractions in order to lower the limit of the detection of the NIR techniques and to match the official control requirement. This approach can be used as control tool at the reception of grains in shipments and trucks.

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## Development and validation of an LC-MS/MS method for the simultaneous determination of type B trichothecenes

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Cereals and cereal-based food have often been found to be contaminated with mycotoxins deoxynivalenol (DON) after the infection of grain with the pathogenic fungus *Fusarium*. Both the pathogen and the infected plants can chemically modify DON, including acetylation, glucosidation and sulfation. DON metabolites are also known to be harmful to mammals. The failure to detect these could lead to significant underestimation of the toxic potential of a particular sample. An LC-MS/MS method was developed and validated for the simultaneous determination of zearalenone (ZEA), nivalenol (NIV), DON and their metabolites, including 3-acetyl-DON (3-ADON) and 15-acetyl-DON (15-ADON). Extraction was performed with acetonitrile/water. After filtration, the extract was cleaned-up using MycoSep push-through columns. The mobile phase consisted of a mixture of methanol and water with 5 mM ammonium formate; the flow was 0.5 ml/min in a gradient programme from 90% water to 5% water for 30 min. The method allowed the simultaneous determination of NIV, DON, 3-ADON, 15-ADON and ZEA in a one-step chromatographic run using an Agilent 6410B QQQ. The method was validated for several parameters, such as linearity, apparent recovery, limit of detection, limit of quantification, and precision. The limits of detection varied from 5.0 (ZEA) to 25.0 µg/kg (NIV), the limits of quantification varied from 20.0 µg/kg (ZEA), 50.0 µg/kg (DON) to 100 µg/kg (NIV). The results of the performance characteristics of the developed LC-MS/MS method were in good agreement with the criteria mentioned in Commission Regulation (EC) No 401/2006. **Acknowledgements.** The authors acknowledge the financial support of the Ministry of Education and Science, Republic of Serbia, project. ref. TR31018.



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## Design, synthesis and efficacy evaluation of aflatoxin B1 analog template used for molecularly imprinted polymers production as possible high affinity and specificity sorptive material for aflatoxins

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Aflatoxins are members of a large family of polyketide natural products known as mycotoxins. These toxins are produced by moulds of the *Aspergillus* sp., such as *Aspergillus flavus* or *A. parasiticus*, that contaminate a variety of feed and food materials and that can ultimately transfer in their native form or has metabolites in animal by-products such as milk, eggs or potentially meat. Aflatoxins represent a significant issue due to their high toxicity and carcinogenicity and regulatory levels are strictly enforced their occurrence in animal feeds and human food. In this context, there is a profound need to develop suitable devices for the specific isolation of aflatoxins and metabolites from animal by-products such as milk, both for diagnostic and mitigation purposes. Molecularly imprinted polymers (MIP's) are materials exhibiting molecular recognition of a target molecule. MIP's are synthesised in the presence of a template, a mimic to the targeted molecule, used as an imprint that is further washed away with suitable solvent after completion of the polymerization process, leaving a cavity in the polymer of the same stereochemistry, functionality and morphology to the template. When the MIP encounters the molecule of interest, the molecule is bound in the cavity with a receptor-like affinity. Two non-toxic aflatoxin B1 analogue templates (AFT-1 and AFT-2) were successfully synthesised by using a new and economically viable synthetic approach and further characterised by NMR and mass spectrometry. A pre-polymerization solution was prepared by mixing the aflatoxins analogue template (AFT-1 or AFT-2), styrene, 2-hydroxyethyl-methacrylate (HEMA), and ethylene glycol dimethacrylate (EGDMA) in a 1:3 v/v mixture of acetonitrile and toluene. Several template vs. monomers and monomers vs. cross-linkers ratios were used (1:2, 1:4, 1:7 and 1:5, 1:10, respectively) to produce gram quantities material. The solution was mixed thoroughly and kept for 1 hr at room temperature under nitrogen atmosphere. Then, the azo(bis)-isobutyronitrile (AIBN) initiator was added and slowly heated and maintained for 30 min at 60-65°C to precipitate the MIP microspheres. The template (AFT-1 or AFT-2) was removed from the MIP by continuous washing with toluene until complete disappearance of template in the washings as determined by HPLC-UV. Non-imprinted polymers (NIP's) were also prepared through the same procedure but in the absence of AFT-1 or AFT-2 analogue templates. Evaluation of the instant sorption properties of 4 preparations of MIP and NIP packed into a solid-phase extraction cartridge were evaluated toward aflatoxins dissolved in pH6.0 buffer and further eluted using a methanol solution. Sorption evaluation was measured using UPLC-MS/MS from collected eluents showing adsorption range between 50 and 100%. Adsorption kinetics were also evaluated according to time of incubation of a free flowing MIP and NIP materials interacting with 10 ml of a diluted solution of aflatoxin under agitation. Swelling, bleeding, and size fractionation properties of the MIPs were carefully investigated as well as prolonged or instant filtration properties as well as specificity of the material. Study of the adsorption efficiency of the MIPs and NIPs in more complex matrices is under investigation.

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## Development of a sensitive antibody against ergot alkaloids

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The ergot alkaloids are mycotoxins produced by several species of *Claviceps*, a genus of fungi that invades the female portion of the host plant and replaces the ovary with a mass of fungal tissue called sclerotium. Major ergot alkaloids include ergometrine, ergotamine, ergosine, ergocryptine, ergocristine and ergocornine. The ergot alkaloids are involved in either nervous or gangrenous syndromes in humans and animals that consume grains or grain products contaminated with the sclerotia of the fungus. Ergotism is one of the oldest known mycotoxicoses and is the result of consuming grain contaminated with sclerotia of *Claviceps* spp. In the food chain, it is known to infect more than 400 plant species, including some economically important cereal grains such as rye, wheat, triticale, barley, millet and oats. In 2012, the European Food Safety Authority (EFSA) carried out a study on ergot alkaloids and concluded that whilst the available data do not indicate a concern for

any population subgroup, the dietary exposure estimates relate to a limited number of food groups and a possible unknown contribution from other foods cannot be discounted. In light of this, a tolerable daily intake of 0.6 µg/kg body weight per day has been set. Hence, there is a need to screen the presence of ergot alkaloids contamination in food products. Immunoassays for ergot alkaloids analysis have been regarded as valuable screening methods to existing chromatographic techniques as they are less time consuming and do not employ expensive analytical equipments. Antibody against ergot alkaloids is a must in the development of the immunoassays. A sensitive rabbit polyclonal ergot antibody has been successfully developed. The sensitivity and specificity of the antibody were determined by a non-competitive direct enzyme-linked immunosorbent assay (ELISA) with Ergot-HRP conjugate as the marker. The antibody was developed by utilising a modified ergoline-based hapten coupled to bovine serum albumin (Ly-BSA) conjugate as the immunogen. The harvested antibody was sensitive being able to detect major ergot alkaloids especially ergometrine at parts per trillion (ppt) level.

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**Elucidation of the first step of the zearalenone detoxification pathway in *Trichosporon mycotoxinivorans***

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Zearalenone (ZEN), a potent oestrogenic mycotoxin produced by several *Fusarium* species, can be inactivated by the basidiomycete yeast *Trichosporon mycotoxinivorans*. ZEN is converted to a non-oestrogenic metabolite (Vekiru *et al.*, 2010), termed ZOM-1 (systematic name: (5S)-5-((2,4-dihydroxy-6-[(1E)-5-hydroxypent-1-en-1-yl]benzoyl)oxy)hexanoic acid). We proposed a two-step mechanism involving firstly a Baeyer-Villiger reaction leading to the formation of a hypothetical intermediate with a newly formed lactone bond, and secondly lactone opening by an esterase leading to the formation of ZOM-1. At this time the predicted intermediate could not be detected by LC-MS/MS in the cultures of wild-type *Trichosporon mycotoxinivorans* cells (Vekiru *et al.*, 2010). The gene encoding the first step of the proposed pathway could be identified by gamma- and fast-neutron irradiation of *Trichosporon mycotoxinivorans* in a TRIGA Mark II reactor, followed by screening for mutants unable to degrade ZEN, which were detected using a *Saccharomyces cerevisiae* oestrogen receptor bioassay. The determination of the genome sequences of the wild-type and mutant *Trichosporon mycotoxinivorans* strains and bioinformatical analysis revealed a large deletion containing a Baeyer-Villiger type monooxygenase candidate gene. Expression of a codon-optimised cDNA of this candidate gene in baker's yeast led to production of a metabolite of the mass of the expected hypothetical intermediate in ZEN treated transformants. After SPE pre-clean up and enrichment the metabolite was isolated by prep-HPLC and characterised by NMR. The biochemical properties of the new metabolite generated by the cloned Baeyer-Villiger monooxygenase will be reported.

**P113**

**Creating novel approaches to mitigate aflatoxin risk in food and feed with lactic acid bacteria – mould growth inhibition and aflatoxin binding**

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Aflatoxins, produced by *Aspergillus* fungi, are ubiquitous toxins and they can present a severe health risk to humans and animals if contaminated food and feed is consumed. Fungi live in the soil and on the surface of the crop and *Aspergillus* species are dominant in favourable conditions of maize cultivation areas. Climate change could threaten the production of safe food by promoting *Aspergillus* growth and aflatoxin production in food and feed. A novel biological approach using lactic acid bacteria (LAB) could reduce the health risks of aflatoxins through inhibiting mould growth, thus aflatoxin production and by binding existing aflatoxins. LAB are commonly used in fermented food production; they are also known to inhibit mould growth and interact with aflatoxins. LAB provide a potential novel approach to mitigate the mould growth and aflatoxin production in maize during storage and after food consumption. Mould growth inhibition by certain LAB strains may be caused by competition for resources between bacterial cells and fungi and/or production of antifungal compounds such as organic acids. Aflatoxin binding is more complex. Binding is a reversible reaction, which occurs on bacterial surfaces and involves interaction with carbohydrates, peptidoglycan and to some extent protein structures. Aflatoxin binding seems to be highly related to strain, matrix, temperature, pH, incubation time and related conditions. There are two different aspects of aflatoxin risk mitigation in this research. First is the fungal growth inhibition with LAB and second is aflatoxin binding from food

and feed with LAB. We have isolated 200 strains of bacteria from 21 different indigenous fermented dairy and cereal products prepared locally in different parts of Kenya. Firstly, these strains are being tested for their growth inhibition abilities against aflatoxin producing *Aspergillus* fungi in laboratory conditions. Secondly, the same strains are tested for their abilities to bind and retain aflatoxin M1 and B1. Later, these same effective strains will be tested in various food and feed matrices against *Aspergillus* growth and then the ones with most potential will be identified. This approach aims at providing a safe method of reducing aflatoxin absorption in human gastrointestinal tract after ingesting fermented maize or dairy products, which are contaminated with aflatoxins. Novel biological methods can have a role in preventing toxic effects of aflatoxins in food and feed. Exploitation of LAB is a good option for existing methods as LAB are generally recognised as safe. **Acknowledgements.** This research is done as part of FoodAfrica programme, which is a research, and development programme and the main funding agency being Finnish Ministry for Foreign Affairs. The research is partnering with MTT Agrifood Research Finland and International Livestock Research Institute.

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### **Enzymatic hydrolysis of fumonisins: paving the way for a unique feed additive**

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Fumonisin are predominantly produced by *Fusarium verticillioides* and *F. proliferatum* and are found worldwide as frequent contaminants of maize-based animal diets, with fumonisin B1 (FB1) as the most abundant representative. Fumonisin affect the immune system and intestinal integrity in animals even at low concentrations and cause various mycotoxicoses at higher concentrations, like porcine pulmonary oedema and equine leucoencephalomalacia. At molecular level, fumonisins inhibit ceramide synthase, a key enzyme in sphingolipid metabolism. The resulting imbalance of sphingolipids is responsible for the toxic effects, but at the same time can serve as a biomarker. Specific biological degradation of mycotoxins represents a promising alternative approach to chemical and physical detoxification strategies. Our technological goal is to provide enzymes as a feed additive that can degrade fumonisins in the gastrointestinal tract of animals. Starting from a soil sample, we identified the fumonisin degrading bacterium *Sphingopyxis* sp. MTA144. Whole genome sequencing revealed a cluster of eleven predicted genes associated with fumonisin degradation. Fumonisin esterase FumD was found to catalyze the first step in the FB1 breakdown pathway. It hydrolyses two tricarballic acid (TCA) side chains from the FB1 molecule, resulting in hydrolysed FB1 (HFB1). The FumD encoding gene was heterologously expressed and enzyme characteristics were determined to evaluate its suitability as a feed enzyme. To investigate kinetic parameters and specific enzyme activity, a LC-MS/MS approach based on TCA release during FB1 degradation was developed. We were able to directly prove efficacy of FumD to degrade FB1 *in vivo* by detecting formation of HFB1, and indirectly by restoring the sphingoid base ratio after supplementation of naturally fumonisin contaminated piglet feed with FumD. Importantly, by direct comparison of FB1 and HFB1 fed to piglets, we clearly showed that toxicity of HFB1 was much smaller or absent. Additional feeding trials with piglets were performed to determine the FumD dose required for complete *in vivo* fumonisin hydrolysis. Furthermore, first trials for broadening the species spectrum of FumD application were performed, and enzyme engineering was initiated to further improve enzyme properties. The production volume was successfully upscaled, fermentation as well as downstream processing protocols were optimised, and a process for drying and formulation of the enzyme was developed. As a result, FUMzyme<sup>®</sup> was released on Asian markets early in 2013 as the first and only feed enzyme for fumonisin biotransformation, followed by initiation of EU product registration. In conclusion, technologies for enzymatic gastrointestinal fumonisin degradation were successfully developed, and FUMzyme<sup>®</sup> represents the proof of principle for an alternative strategy to fight mycotoxins in animal nutrition.

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### **Efficacy of a mycotoxin deactivator (Unlike<sup>®</sup> Plus) to ameliorate the toxicity of a combination of mycotoxins in broiler chicks**

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The presence of mycotoxins in feeds and feedstuffs is an important concern for human and animal health. Many fungal species are capable of simultaneously producing several mycotoxins. Therefore, an individual grain source may contain more than one mycotoxin, or the incorporation of numerous grain sources, which are each contaminated with a different mycotoxin, into a single feed may result in feed that contains a number of different mycotoxins. The objective of this study was to evaluate the efficacy of the mycotoxin deactivator Unlike<sup>®</sup> Plus (UP) in ameliorating the toxic effects of a combination of mycotoxins (MM). One hundred fifty day-old straight run chicks were weighed, wing-banded, and assigned to chick batteries. A CRD design was used with 6 pens of 5 chicks assigned to each dietary treatment from hatch to day 21. Dietary treatments included: (i) basal diet (BD) containing no mycotoxins or UP; (ii) BD plus 0.50% UP; (iii) BD plus MM (1.00 mg/kg aflatoxin B1; 1.0 mg/kg ochratoxin A; 5 mg/kg fumonisin B1; 0.75 mg/kg T-2 toxin, and 0.5 mg/kg zearalenone); (iv) BD plus MM and 0.25% UP; and (v) BD plus MM and 0.50% UP. The BD was a maize soybean meal diet formulated to meet or exceed the nutritional requirements of growing chicks. Feed intake and body weight gain were significantly reduced ( $p<0.05$ ) in birds fed MM but UP at 0.25 and 0.50% improved the growth depression caused by MM. Relative liver weight was increased ( $p<0.05$ ) in birds fed MM but the addition of UP (0.25 and 0.50%) reduced the effects of MM. Relative kidney weight was also increased ( $p<0.05$ ) in birds fed MM, but 0.50% UP was able to reduce the increase in weight. Shank colour was reduced by MM ( $p<0.05$ ) but 0.50% UP was able to ameliorate the effect of MM on shank colour. Serum GGT was elevated ( $p<0.05$ ) in birds fed MM but UP addition (0.25 and 0.50%) to the MM diet returned values to control levels. Results indicate that UP was partially effective in ameliorating the toxic effects of MM in broilers, with the higher (0.50%) level being more effective.

**P116**

### ***In vivo* confirmation of broad spectrum efficacy of the mycotoxin binder Toxfin<sup>™</sup>**

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The contamination of animal feed with mycotoxins represents a worldwide and persistent problem. The most commonly mycotoxins that are occurring in animal feeds are aflatoxins, trichothecenes (e.g., T-2 toxin, HT-2 toxin, deoxynivalenol), ochratoxin A, fumonisins and zearalenone. Diverse negative effects as reduced production, poor reproductive performance and increased incidence of diseases are often observed. Therefore, mycotoxin control is essential to prevent economic losses, provide optimal animal health and performance and food safety. The use of a broad spectrum mycotoxin binder is a good practice to reduce the impact of mycotoxins on animal's health and performance. Kemin provides an innovative solution to combat the problem of mycotoxin contamination in feed with Toxfin<sup>™</sup>, a unique product with multi mycotoxin binding performance. Throughout the years several successful *in vivo* trials were performed with Toxfin. At first the binding efficacy of Toxfin to co-occurring mycotoxins was tested, feeding broilers diets contaminated with both *Fusarium* and *Aspergillus* mycotoxins. One hundred and fifty broilers of 24 days of age from a commercial farm were collected and housed in single cages and split in three groups: control (CN); mycotoxins (Mx); and mycotoxins+Toxfin (Mx+Tx). At the end of the experimental period, faeces were collected and analysed on the content of mycotoxins. In the control group, no mycotoxins were detected in the faeces, as expected, while mycotoxin excretion in the faeces was measured in the Mx and Mx+Tx groups. However, in the group fed the diets treated with Toxfin (Mx+Tx), a significantly higher excretion of respectively 26% AFB1 and 67% FB1 was measured compared to the Mx group. In another trial performed with Toxfin, the objective was to evaluate the effect of Toxfin in one-day old broilers fed mycotoxin contaminated diets during 35 days. The broilers were distributed among the 3 following groups: group 1, control (basal diet); group 2, control+mycotoxins (aflatoxins 250 ppb, ochratoxin A 250 ppb, citrinin 250 ppb, T-2 toxin 250 ppb); and group 3, control+mycotoxins+Toxfin (0.3%). In this trial, the efficacy of Toxfin was tested on several animal performance and health parameters by studying their performance, organ morphology, mycotoxin excretion and immunology. Adding Toxfin to the diets caused a significantly higher excretion of the mycotoxins and consequently, all previous described other parameters were improved by the inclusion of Toxfin in contaminated diets. The different *in vivo* trial results are showing that Toxfin is effective against a broad range of different mycotoxins.

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## Characterisation of ergopeptine-hydrolase ErgA

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Ergot alkaloids are mycotoxins mainly produced by the grain parasitic genus *Claviceps* and by *Neotyphodium* species in grasses. The use of contaminated grain and grasses as animal feed leads to major medical and economic problems in livestock production. Previously, we isolated an alpha/beta-hydrolase (ErgA) and an amidase (ErgB) from a bacterial strain, *Rhodococcus erythropolis* MTHt3, with ability to degrade ergopeptines to ergine and lysergic acid, respectively. With the long-term goal to provide ErgA as feed additive for gastrointestinal ergopeptine degradation, enzyme characteristics had to be determined. Temperature and pH dependence of ErgA activity were determined by analyzing ergotamine degradation in Teorell-Stenhagen buffer by HPLC-FLD measurement. For determination of temperature and pH optima, degradation experiments at temperatures from 10 to 50 °C or pH values from 3 to 12 were performed. Temperature and pH stability were analysed by pre-incubating ErgA at temperatures from 10 to 60 °C or pH values from 2 to 12 for one hour before applying it to degradation experiments, which were performed at 25 °C and pH 7. For evaluation of kinetic parameters, formation of metabolite 244-2, a stable metabolite of hydrolytic cleavage of ergotamine by ErgA, was analysed by LC-MS/MS measurement. The degradation experiment was performed in Tris-HCl buffer at 25 °C and pH 9, using substrate concentrations ranging from 0.8 to 34 µM. Experiments showed that ErgA had its optimal working conditions at 35 °C and pH 9. The enzyme was stable at temperatures between 10 and 35 °C and between pH 6 and 9. Pre-incubation of ErgA at 40 °C or higher as well as at pH values lower than pH 6 yielded a residual activity of less than 20%. Michaelis constant  $K_m$  was 0.57 µM,  $k_{cat}$  was 47.5 molecules/min and specific activity was 1.34 µmol/min/mg protein. With a view to application of ErgA as feed additive, the substrate affinity is of big advantage. Nevertheless, characteristics regarding pH and temperature have to be optimised in terms of stability during production and being active in the gastrointestinal tract of animals.

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## Use of a cell-wall deficient, selected lactic acid bacterium for the control of deoxynivalenol challenge in a commercial sow operation

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Mycotoxins effects on reproductive functions may have greater impact on commercial farms than the reductions in products (meat, milk, eggs) they may cause. Previously at these meetings (Doerr, 2008), we reviewed research involving use of a selected cell-wall deficient (L-form) lactic acid bacterium for the amelioration of multiple mycotoxin challenges in animals. Today, we report on the results of a commercial swine operation whose major toxin challenge was deoxynivalenol (DON; 850-2,200 ppb, n=21). The 750 sow farm had a conception rate of 82%; of those, 17% of sows lost the pregnancy at some point during gestation, both factors attributable, in part, to the consistent and continual mycotoxin challenge. A specialised L-form bacterium feed additive (Nutrisound Porcine®; Agrarian Marketing Corp.) was introduced after a 16 week pre-test data collection; the additive was included in ration for 16 weeks followed by a 16 week post-treatment data collection. Sows consuming the L-form additive for at least 8 weeks had significantly higher farrowing rates ( $p<0.05$ ) and highly significant lowered rates of lost pregnancies ( $p=0.0005$ ). Regression analysis showed that for each week of treatment there was a gain of approximately 0.5% in farrow rate and over the gestation period that regression was significant. By the end of trial, treated sows had delivered about 1.5 more pigs per litter than they did pre-treatment, and that same increase was sustained through weaning. For a farrow-to-wean farm, such an increase is very substantial. At the time of the trial, it was calculated that the economic gain to the farm in additional weaned pigs was over \$35,000 vs. a treatment cost of \$2,000. During the trial feeds (early gestation, late gestation, lactation) were sampled and submitted for analysis for DON. Results showed that the sow population was under almost continuous challenge by dietary DON exceeding 1000 ppb. The trial also demonstrated that the biological control of mycotoxin challenge offered by this specialized L-form bacterium is superior to clay-based binders, which generally, at best, offer 15-18% binding affinity for DON and other trichothecene mycotoxins and may be totally ineffective against other important *Fusarium* mycotoxins (e.g., zearalenone).

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## The anti-aflatoxigenic efficacy of *Cynara cardunculus* L. in sesame seeds (*Sesamum indicum*)

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Aflatoxins are a group of secondary metabolites produced by the species *Aspergillus flavus*, *A. parasiticus* and the rare *A. nomius*. Among these, aflatoxin B1 (AFB1) is the most naturally occurring compound of toxigenic isolates of *Aspergillus* species and the most dangerous contaminants of foods and feeds due to carcinogenic and mutagenic activity. Several studies have shown that sesame seeds (*Sesamum indicum*) are sensitive to aflatoxins producing fungal invasion and may therefore be contaminated with these mycotoxins, in particular AFB1. The aim of this study was to investigate the antifungal and anti-aflatoxigenic efficacy of *Cynara cardunculus* L. extracts in sesame seeds. The objectives of this study included the investigation of the antioxidant capacity of *C. cardunculus*, the inhibitory activity of *C. cardunculus* on mycelial growth of *A. parasiticus*, and AFB1 contents in spiked sesame seeds (*A. parasiticus* conidia) containing *C. cardunculus* extracts. The antifungal and anti-aflatoxigenic efficacy of *C. cardunculus* extracts was also investigated in yeast extract sucrose (YES) medium. All samples were incubated for 15 days at 30°C. Moreover, development and validation of a method for AFB1 determination in sesame seeds is described using a methanolic-aqueous extraction followed by immunoaffinity column clean-up and high pressure liquid chromatography with fluorescence detector. The recovery of the method was found to be 111.5% (RSD%=5.09), while the detection limit and quantification limit were 0.02 and 0.2 ng/g, respectively. The results showed that the total phenolic content of *C. cardunculus* heads (1.57 mg GAE/g fw) was higher than the total phenolic content of bracts (1.45 mg GAE/g fw) and stems (0.86 mg GAE/g fw). Head extracts displayed the highest DPPH scavenging activity (IC<sub>50</sub>, 0.91 mg/ml) as compared to bracts and stems. Heads of *Cynara cardunculus* also showed significant activity against *A. parasiticus* mycelial growth; the percentage of inhibition was found to be 42.1% (4 days of inoculation). Finally, *C. cardunculus* heads extracts showed complete inhibition of AFB1 both in sesame seeds (99.6%) and in YES medium (99.4%).

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## Reduction of the aflatoxins B1, B2, G1 and G2 in Italian piadina by isothiocyanates present in oriental and yellow mustard flours

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Aflatoxins (AFs) are mycotoxins produced mainly by the moulds *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. These mycotoxins are contaminants of many cereal grains, including wheat and wheat-based products. AFB1, the most abundant and toxic AF, is known to cause several species-specific adverse effects. Aflatoxins are considered hepatotoxic, teratogenic and mutagenic and are generally associated with hepatitis, haemorrhage, oedema, immunosuppression and hepatic carcinoma. Isothiocyanates (ITCs) are natural compounds produced by enzymatic cleavage of glucosinolates present in brassicas. They are potentially useful antimicrobial compounds for agrifood applications. In this study, Italian piadina (traditional flat bread made with wheat) was contaminated with *A. parasiticus* CECT 2981 and treated with gaseous ITCs generated from oriental or yellow mustard (0.1, 0.5 and 1 g flour), namely allyl isothiocyanate and p-hydroxybenzyl isothiocyanate, respectively. The antifungal activity of the ITCs towards *A. parasiticus* was evaluated as well as the production of AFs. The mustard-generated ITCs employed in this study inhibited the growth of *A. parasiticus*, reducing its mycelium size by 12.2 to 80.6%. The oriental mustard flour was more active, inhibiting the fungal growth by 68.5% at levels as low as 0.1 g, whereas 1 g of yellow mustard caused only a 30.1% reduction. The ITCs produced *in situ* also reduced AFs biosynthesis in Italian piadina. In particular, the use of oriental mustard flour reduced AFs content by 60.5 to 89.3%, whereas the reduction caused by yellow mustard flour ranged from 41.0 to 69.2%. In conclusion, the use of antimicrobial devices derived from oriental mustard flour that could generate allyl isothiocyanate would be appropriate to reduce the growth of *A. parasiticus* and the presence of AFs in Italian piadina.

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## Antimicrobial devices containing allyl isothiocyanate to improve shelf life and safety of sliced mozzarella cheese

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Sliced mozzarella cheese is very susceptible to mould contamination and visual fungal growth is a determinant factor for the shelf life of these products. *Penicillium* species are some of the most prevalent microorganisms found in sliced mozzarella, but other potentially dangerous fungi may grow in novelty products, such as mozzarella added with nuts or dried fruits. The objective of this study was to develop and test three antimicrobial devices containing allyl isothiocyanate (AIT) against the growth of *Penicillium digitatum* and *Aspergillus parasiticus* (aflatoxin B1 producer) inoculated in sliced mozzarella. Each slice of mozzarella (approx. 18 g) was inoculated with either fungus in 9 equidistant points with 10 µl of a solution containing 10<sup>7</sup> spores/ml. Slices of mozzarella were packaged in either a thermosealed plastic bag or a hermetically closed plastic tray. The packages received one of the following antimicrobial devices: (i) filter paper added with AIT; (ii) sticker containing AIT; or (iii) sachet containing mustard meal and water (for intrinsic production of AIT). AIT was added at different concentrations, namely 2, 4, 8 and 16 ppm. The quantity of mustard meal added to the sachet was calculated to provide the same AIT levels. Each treatment was composed of 9 repetitions. Treatments were compared to a control with no AIT added. The day when fungal growth was visually detectable was considered the end of the product's shelf life. Packages were inspected daily for 60 days. At the end of the experiment, samples containing *A. parasiticus* were used for aflatoxin quantification. Visual growth of *P. digitatum* for the control was observed after 19.2±1.4 days in bags and 26.6±2.1 days in trays, whereas *A. parasiticus* was visually detectable after 41.4±1.7 and 28.4±1.5 days in bags and trays, respectively. All AIT-treated cheese did not present any visual fungal growth for 60 days. However, AFB1 was present in the control (6.93±1.23 mg/kg) and in the groups treated with AIT at 2 ppm, paper filter with 0.030±0.004 mg/kg, sticker with 0.56±0.127 mg/kg, and meal with 2.015±0.049 mg/kg. These results showed that all three antimicrobial devices tested were able to significantly increase the shelf life of sliced mozzarella and reduce the production of AFB1 by *A. parasiticus*. Therefore, these devices could potentially be used by the food industry to enhance food safety and decrease the deterioration of these products.

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## Isolation and efficacy of biocontrol microorganisms from maize for inhibiting growth and fumonisin production by *Fusarium verticillioides*

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Microorganisms were isolated from maize grain and screened for efficacy against *Fusarium verticillioides* under different water activity ( $a_w$ ) x temperature conditions. A total of five bacteria and yeasts were screening *in vitro* on MEA/1/2 strength NA and maize meal agar. This showed that of the 5 strains the best gave 55% control of growth of *F. verticillioides*. This was a Gram-negative bacteria (BCA 5). Subsequent studies evaluated different ratios of the pathogen and the antagonist (100%, 75:25; 50:50, 25:75, 100% respectively) both on agar-based media and maize grain. The effect on fumonisin production under different  $a_w$  x temperature conditions were examined.

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## Targeting *Fusarium graminearum* control via polyamine enzyme inhibitors and polyamine analogues

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*Fusarium graminearum* has major impact in wheat cultivation as it not only reduces yield and seed quality but also constitutes a risk to public or animal health, owing to its ability to contaminate grains



with mycotoxins. Under high inoculum conditions, chemical fungicides are the main measure to control *F. graminearum*. However, resistance problems are emerging and control strategies based on new targets are needed. Polyamines are low molecular weight cations which have a key role in growth, development and differentiation. In this work, the possibility of using polyamine metabolism as a target to control *F. graminearum* has been assessed. It was found that the polyamine putrescine induces mycotoxin production, correlating with an over expression of *TRI5* and *TRI6* genes that are involved in deoxynivalenol (DON) biosynthesis and regulation, respectively. In addition, a homolog of the *Saccharomyces cerevisiae* *TPO4*, which is involved in the excretion of putrescine, was upregulated as the concentration of putrescine in the medium increased while *DUR3* and *SAM3* homologues, involved in putrescine uptake, were downregulated. When 2.5 mM D,L- $\alpha$ -difluoromethylornithine (DFMO), an inhibitor of the enzyme ornithine decarboxylase that catalyses the first and committed step in the polyamine synthesis, was added to the medium, DON production decreased from 3.2 to 0.06 ng/mm<sup>2</sup> of colony, while the growth was lowered by up to 70 per cent. Interestingly, an overexpression of the ornithine decarboxylase gene was observed when the *F. graminearum* culture was amended with DFMO. However, exogenous putrescine could overcome the effects of DFMO on growth and mycotoxin production. Because polyamines are regulated by its biosynthesis, degradation and transport, five polyamine transport inhibitors were also tested against *F. graminearum*. AMXT-1505 was able to completely inhibit *in vitro* both growth and DON production. When wheat spikes were treated with DFMO and subsequently inoculated with *F. graminearum* conidia, DFMO did not display any inhibitory effects on the fungus. Nevertheless, AMXT-1505 blocked *F. graminearum* growth in inoculated wheat spikes, reducing DON mycotoxin contamination from 76.87  $\mu$ g/g, present at the positive control, to 0.62  $\mu$ g/g. Therefore, the polyamine transport inhibitor AMXT-1505 may be useful as a target specific fungicide against *F. graminearum*. **Acknowledgements.** The authors are grateful to the Catalanian Government (XaRTA-Reference Network on Food Technology) for their financial support. N. Estiarté thanks the Secretaria de Universitats i Recerca del Departament de Economia i Coneixement of the Generalitat de Catalunya for the pre-doctoral grant.

**P124**

### **Biotransformation of deoxynivalenol to the metabolite de-epoxy-deoxynivalenol analysed in serum of pigs**

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Deoxynivalenol (DON) is one of several mycotoxins produced by various *Fusarium* species that frequently infect maize, wheat and other grains on the field or during storage. The main effects of long-term dietary exposure of animals to DON are weight gain suppression, anorexia and altered nutritional efficiency. Among all strategies to counteract DON in feed biotransformation to non-toxic products by the use of microbes or enzymes seems to be the most efficient approach. DON and its metabolites are important biomarkers to demonstrate the efficacy of DON deactivating products *in vivo*. The aim of this study was to proof the efficacy of a bacterial strain to detoxify DON into the non-toxic metabolite de-epoxy-deoxynivalenol (DOM-1) in the gastrointestinal tract of pigs. 24 animals were randomly assigned to three experimental groups [control, DON (2 mg/kg), DON (2 mg/kg) + bacterial strain (1.7 x 10<sup>8</sup> cfu/kg feed) according to weight, gender and overall conditions. DON source was naturally contaminated wheat. During the experimental period feed was supplied twice a day in the morning and in the evening. Serum samples of all animals in all groups were taken on day 1, 3 and 4. The blank serum sample was taken before feeding the experimental diets on day 1. Serum samples were analysed for DON and DOM-1 concentrations by LC-MS/MS method. There were no significant differences in blank serum samples between the three groups ( $p > 0.05$ ). Due to the presence of DON in the standard diet, small amounts of DON and DOM-1 produced by the native intestinal flora were also found in the blank samples. DON concentration in serum of the DON group was more than four times higher compared to the control and the DON+bacteria group ( $p < 0.05$ ). Addition of the bacterial strain converted DON into the non-toxic metabolite DOM-1 in the gastrointestinal tract. This resulted in a significant decrease of DON and a significant increase of DOM-1 in serum of the animals in this group ( $p < 0.05$ ). Biomarker analysis of pig serum samples demonstrated the efficacy of microbial biotransformation of DON *in vivo*.

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## Evaluation of the efficacy of three anti-mycotoxin additives on the toxicological effects of aflatoxins, fumonisins and T-2 toxin in broiler chickens and the determination of the *in vitro* coefficient of adsorption of aflatoxin B1

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The aflatoxin toxicity experiment was carried out with broiler chickens to evaluate the efficacy of three anti-mycotoxin additives (AMA): product A, product B and product XL. The study was conducted by Instituto SAMITEC (Brazil). A total of 300 one-day-old male chicks were used in five dietary treatments with six replicates each (10/pen). The treatments included: (1) control; (2) control + 1.4 ppm aflatoxin + 50 ppm fumonisins + 0.75 ppm T-2/HT-2; (3) treatment 2 + product A (0.2%); (4) treatment 2 + product B (0.2%); and (5) treatment 2 + product XL (0.2%). At day 21, the birds were slaughtered to determine the relative liver weight and total plasma proteins. Compared to the control animals, aflatoxin addition significantly and severely reduced 21-day body weight (-20.91%), feed intake (-17.15%), and total plasma proteins (-26.85%), while increasing the relative liver weight (+55.22%). However, addition of the three AMAs improved all the parameters above. Compared with birds with mycotoxins alone, the three AMAs increased 21-day body weight (+5.31% A, +7.70% B and +10.04% XL), feed intake (+2.39% A, +3.44% B and +8.40% XL), and plasma protein (+25.09% A, +9.36% B and +13.48% XL), while decreasing relative liver weight (-4.00% A, -9.05% B and -13.68% XL). In the *in vitro* study with artificial gastric juice, compared to the standard of aflatoxin B1 (1.0 mg/ml), the three products at 0.5% showed a coefficient of adsorption at pH 3.0 of 93.56, 98.98 and 99.85% respectively, and a coefficient of adsorption at pH 6.0 of 92.29, 99.59 and 99.73% respectively. It was concluded that product XL was the most efficacious in significantly decreasing the deleterious effects of the aflatoxins, fumonisins and T-2 toxin on broiler chickens.

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## Integrating ecological and molecular diagnostics data to prevent contamination of cured meats with ochratoxin A

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Moulds are commonly considered as undesirable in most food commodities since they may produce mycotoxins. One of the most important mycotoxins is ochratoxin A (OTA) which can have toxic effects on consumer health. Certain food commodities such as dry-cured ham and dry-fermented sausages are prone to be contaminated by OTA because of colonisation by OTA-producing *Penicillia* on the surface of the product during curing. The environmental conditions occurring throughout their processing and the high concentration of NaCl favours the presence of this type of toxigenic mould. The objective of this study was to examine the ionic water activities (from 0.97 to 0.84) on growth, *otapksPN* and *otanpsPN* gene expression and OTA production by *Penicillium nordicum* and *P. verrucosum* on ham- and cured sausage-based matrices. Matrices were inoculated with spore suspensions of the strain and incubated between 10 and 30°C (in dry-fermented sausages) for 12 days. Growth and OTA quantification was made every two days and, expression analysis every three days. Reverse transcription real time PCR (RT-qPCR) was used to examine effects of these two-way interactions on relative expression of key genes involved in OTA. The data sets allow us to link growth and phenotypic mycotoxin production with expression of key biosynthetic genes in the biosynthetic pathway for OTA production in order to avoid OTA. The molecular measurements were more sensitive than the analytical quantification of OTA. This suggests that the RT-qPCR gene expression of these two genes may be a good early indicator of potential contamination risk in dry-cured ham with OTA during the dry-cured ham and dry-fermented sausages ripening. The implications for these results will be discussed in the context of risk assessment in such products. **Acknowledgements.** Dr. A. Rodríguez would like to thank the Junta de Extremadura, Consejería de Empleo, Empresa e Innovación, and the Fondo Social Europeo (FSE) for a postdoctoral fellowship at Cranfield University (PO12016).

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## Deoxynivalenol (DON) sulfonates: detoxification products and natural DON metabolites in rats

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Inactivation of deoxynivalenol (DON) by treatment with sulfur reagents like sodium metabisulfite (SBS) or sodium sulfite is one of several post-harvest detoxification approaches. The reaction products are three DON-10-sulfonates (DONS 1, 2, 3) of different structure, stability and toxicity (Schwartz *et al.*, 2013, 2014). Devoid of the epoxide group and therefore non-toxic, DONS 1 is the most desired product. However, under classical treatment conditions, the initially formed reaction product is a ketone/hemiketal pair termed DONS 3, which is unstable at neutral to alkaline pH and converts back to DON under physiological conditions. Several factors including reagent concentration, type of sulfur reagent, moisture content, presence of propionic acid and storage time have been investigated, but future research is required to shift the formation from DONS 3 to DONS 1 and 2. Although the breakthrough in post-harvest reduction of DON by sulfur reagents has not yet been achieved, DON sulfonates are an upcoming research topic: DON sulfonate and de-epoxy-DON (DOM) sulfonate were reported as natural metabolites of DON in urine and faeces of rats and putative structures were proposed (Wan *et al.*, 2014). In order to verify this hypothesis, we analysed urine and faeces samples of a previously published rat trial where DON and DON-3-glucoside (D3G) had been administered by gavage and only 28 and 21% of the parent toxins had been recovered (Nagl *et al.*, 2012). By chemical synthesis, preparative isolation and NMR analysis of the expected compounds, DONS 2, D3GS 2 and DOMS 2, all characterised by hemiketal formation at C8, could be identified as main DON and D3G metabolites in rats, thereby contradicting the structures proposed in the literature. Currently, a method for quantitative determination of DON (conjugate) sulfonates and DOM sulfonates in urine and faeces of rats is developed and validated. This presentation will touch on the original identification of DONS as post-harvest detoxification products, comment on feeding trials with pigs administered DONS 1, 2 and 3 and present the most recent findings on the occurrence of DON sulfonate compounds as main DON metabolites in rats.

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## Deoxynivalenol degradation in crimped grain by ubiquitous silage bacteria

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Deoxynivalenol (DON) is a potent mycotoxin produced by *Fusarium culmorum* that contaminates a significant proportion of grain grown in temperate regions of the world. Grain testing regimes prevent serious human and animal toxicosis in developed nations, yet the cost implication of large-scale contamination on the producer can be significant. Grain with levels above 5mg/kg is rejected for animal feed; therefore a method of biological decontamination would be well received. Bacteria could be employed to fulfil such a task in a cost effective and safe process that could occur during conventional crimped grain fermentation and this idea forms the basis of the current investigation. This study utilises bacteria commonly associated with crimped fermented barley that were screened for using PCR-DGGE, metabolite characterisation by HPLC and directed selective media. Six species of omnipresent bacteria in silos where DON degradation originally took place were identified as being potential degraders of DON, of which two were able to reduce levels *in vitro* by 90% over two weeks in anaerobic minimal media and measured directly with HPLC. The lactic acid bacteria H1 and W1, along with other relevant ensiling bacteria were spiked into contaminated crimped grain (barley and wheat both naturally contaminated with DON by greenhouse inoculation with *Fusarium culmorum* spores, harvested and gamma irradiated) and incubated for four weeks in mini-silos to investigate the potential to use them in a biodegradation scenario. Silos were characterised in terms of organic acids, pH and viable counts on tryptone soya agar. Following IAC purification, the level of DON was not significantly reduced over the control for either wheat or barley suggesting an extended period of metabolic enrichment associated with a low nutrient media is necessary for biodegradation of DON.

Furthermore, the existence of a microbial assemblage able to degrade DON reveals important information on the fate of this resilient molecule in the crop debris/soil environment.

**P129**

### **Reducing type B trichothecenes levels in maize by removal of fines**

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Cereals and cereal-based food have often been found to be contaminated with the mycotoxin deoxynivalenol (DON) after infection of grain with the pathogenic fungus *Fusarium*. Both the pathogen and the infected plants can modify DON chemically, including acetylation, glucosidation and sulfation. DON metabolites are also known to be harmful to human and animal health. The failure to detect them could lead to significant underestimation of the toxic potential of a particular sample. Mycotoxin decontamination procedures can be divided in three different groups: chemical, biological and physical. Procedures from the first two groups are often not acceptable for lowering mycotoxin levels. For the purpose of physical cleaning of maize kernels a laboratory brusher has been developed. For the purpose of this experimental study, 3 samples of commercially available maize kernels were subjected to a brushing process. 100 g maize kernels were placed on the motionless screen of the brusher. The rotating part of the experimental device, the polypropylene bristle brush, was set to a high speed (>800 rpm). During the brushing of maize, dust and broken kernels were brushed out through the motionless screen. Before and after the brushing procedure, maize samples were taken and analysed for simultaneous determination of zearalenone, nivalenol, DON and its metabolites, including 3- and 15-acetyl-DON. By comparing control and brushed samples it could be noticed that removal of fines caused lowering of mycotoxin levels in all brushed samples. **Acknowledgements.** The authors acknowledge the financial support of the Ministry of Education and Science, Republic of Serbia, project ref. TR31018.

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### RNA interference reduces aflatoxin accumulation by *Aspergillus flavus* in peanut seeds

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Aflatoxins are among the most powerful carcinogens in nature. They are produced by the fungal pathogen *Aspergillus flavus* Link and other *Aspergillus* species. Aflatoxins accumulate in many crops, including rice, wheat, oats, pecans, pistachios, soybean, cassava, almonds, peanuts, beans, maize and cotton, posing a risk to food safety and causing economic losses. RNA interference (RNAi) signals can move from cell to cell in plants (Wang and Ding, 2010), and can also move systemically and translocate from the plant to pathogens or parasites in close contact with the plant, silencing genes inside them (Nowara *et al.*, 2010; Tinoco *et al.*, 2010, Tomilov *et al.*, 2008). We designed a multi-target RNA interference to silence genes in the aflatoxin synthesis pathway of *A. flavus*, used *Agrobacterium* to incorporate the binary vector into peanut (*Arachis hypogaea* L.) plants, and designed a method to test for aflatoxin accumulation in RNAi seeds. We determined aflatoxin B1 and B2 by quantification with ultra-high pressure liquid chromatography (UHPLC); between 82-100% reduction in aflatoxin B1 and 90-100% reduction in aflatoxin B2 for peanut seeds harbouring RNAi compared to the controls.

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### Effect of drying treatments on fungal incidence and fumonisin contamination in maize kernels

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The increase in fumonisin content in maize after drying was signalled by farmers and it was recently reported also in scientific papers. Because of the mandatory application of drying for a safe post-harvest management of maize, the physicochemical effects on kernels and their fumonisin content need to be investigated. Six maize hybrids collected in 2010 in Northern Italy were dried applying 2 different temperature x time combinations: 70°C x 24 h and 95°C x 9 h. Fungal incidence, kernel chemical composition (proteins, fat content, starch) and free and hidden forms of fumonisins (FBs) were investigated pre- and post-drying treatments. Fungal incidence was significantly reduced after thermal application and water activity decrease; both combinations of 'temperature x time' treatments were able to reduce more than 85% *Fusarium* spp. incidence, irrespective of hybrid considered. The dominant variation observed in fumonisin content in different hybrids was an increase, mainly in the free forms, therefore confirming farmer's claims, with few exceptions. Regarding chemical composition, fat content decreased 26 and 14% after grain exposure to 70°C x 24 h and 95°C x 9 h, respectively. Proteins and starch content resulted unvaried, but a possible retrogradation of starch, and in particular in amylose, has been supposed upon heating. This speculation was based on the analytical results obtained applying 2 different analytical methods that gave comparable results before and significantly different after the drying treatment. The entity of this supposed retrogradation differed between hybrids and the variation of detectable fumonisins after drying is apparently strongly related to these changes in starch structure. Although these are hypothesis and not conclusive remarks, they are very relevant in practice and they confirm the variation in detectable FBs occurring upon drying treatment. The topic needs further investigation to ascertain physicochemical changes in the matrix composition and their implication in fumonisin detectable amounts, possibly contributing to clarify the masking phenomena. **Acknowledgements.** This work was supported by FIRB-RBFR08JKHI, Italian Ministry of University and Research (MIUR).

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## Contamination of wheat grains with *Fusarium* mycotoxins, depending on variety and mill fraction

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The most important mycotoxins found in cereals during their growth in Poland are deoxynivalenol (DON), zearalenone (ZEA) and T-2/HT-2 toxin. All are produced by *Fusarium*. Contamination of cereal grains and cereal products with mycotoxins is one of the biggest problems of agriculture. The presence of mycotoxins is an important indicator of health hazard. Humans are mainly exposed to those harmful toxins when consuming food products such as flour products containing mycotoxins. Food made of wheat can be produced out of whole grains and wheat flour moreover bran is suitable for consumption as well. From the customer's point of view, it is interesting to assess the presence/absence and the content of mycotoxins in various mill fractions. Mycotoxins are mainly concentrated in the bran or can reach deeper into the grain, accumulate in the endosperm and thus can be found in flour. The investigation conducted at our institute aimed to assess the content of *Fusarium* mycotoxins in whole grain, flour and bran of various wheat cultivars belonging to *Triticum aestivum* ssp. *vulgare* - Bamberka, *Triticum spelta* - Rokosz, and *Triticum durum* - SMH 87. Grain samples of winter wheat cultivars collected in 2013 were analysed for contamination with deoxynivalenol (DON), zearalenone (ZEA), and T-2/HT-2 toxins by enzyme-linked immunosorbent assay (ELISA). Wheat was naturally infected by fungal pathogens producing mycotoxins. It was found that the grains were contaminated with the mycotoxins tested (DON, ZEA, and T-2/HT-2), where the highest contamination was for DON, followed by T2/TH2 toxin and the lowest for ZEA. It was concluded that the mycotoxin content was dependent both on the wheat species as well as the mill fraction. We found that *Triticum aestivum* ssp. *vulgare* - Bamberka had 2.9 times more DON accumulated in wholemeal, 6.2 times more in flour and 6.7 times more in bran compared to *Triticum spelta* - Rokosz. Similarly, the Bamberka variety exhibited higher contamination with T-2/HT-2 toxin compared to the Rokosz variety. However, ZEA was more abundant in *Triticum spelta* compared to *Triticum aestivum* ssp. *vulgare*. Our study shows that mycotoxins accumulate in each part of the kernel. Moreover, in general, Rokosz showed a lower level of mycotoxin accumulation compared to Bamberka. However, these interesting and promising results require further confirmation and validation using a larger number of wheat varieties.

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## The influence of maize dry milling on the content of free and masked fumonisins in model conditions

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Fumonisin (FBs) are among the most important mycotoxins that may contaminate maize. Masked fumonisins include hidden fumonisins that have been identified in raw maize. It is supposed that these compounds may non-covalently interact with macroconstituents of food ingredients (proteins, starch). The aim of the present work was to investigate how contents of free and masked fumonisins are going to change during milling process of maize, in model conditions. Milled grains were sieved to separate maize bran with germ from flour (about 17 and 83% of all milling products, respectively). High performance liquid chromatograph coupled to an ion trap Thermo-Finnigan LTQ mass spectrometer (Austin, TX, USA) was used to determine fumonisins in this study. The internal calibration technique was used. All FB1, FB2 and FB3 <sup>13</sup>C-labelled internal analytical standards were supplied by Biopure (Tulln, Austria). Free fumonisins were determined using FumoZON AFFINIMIP molecularly imprinted polymeric (MIPs) solid phase extraction cartridges supplied by Polyintell (Val de Reuil, France). Free and hidden fumonisins were released by means of chemical hydrolysis in 2M KOH and determined together with free (also hydrolysed) fumonisins. Concentrations of hidden fumonisins were calculated as the differences between total fumonisins (free+hidden) and free fumonisins. Both in maize and its milling products (flour, bran) concentrations of total fumonisins were significantly higher than concentrations of free fumonisins only ( $p \leq 0.05$ ). Levels of free fumonisins (free  $\Sigma$ FBs) found in maize grain, flour and bran were  $7,125 \pm 636$ ,  $5,101 \pm 564$  and  $12,061 \pm 777$   $\mu\text{g}/\text{kg}$ , respectively. Statistical evaluations using one way analysis of variance revealed significant differences in the concentrations

of free and total fumonisins in maize, maize flour and bran. The dry milling process of maize grain lowered the average concentration of free and hidden fumonisins in flour compared to maize grain by 28 and 20%, respectively. Calculated mean ratio hidden FBs/free FBs was 0,64 in maize grain and 0,72 in maize flour. On the other hand, the hidden FBs/free FBs ratio decreased from 0.64 in maize grain to 0.55 in maize bran, on the average. These observations can be explained by the difference between macroconstituents concentrations in various milling products that could non-covalently interact with fumonisins. **Acknowledgements.** This study was partly supported by the 2012/05/N/NZ9/01316 National Science Centre grant.

**P134**

### **Ochratoxin A in grapes: development of a prototype predictive model**

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*Aspergillus carbonarius* is considered the main responsible for ochratoxin A (OTA) contamination in grapes while *A. niger* and *A. tubingensis* were confirmed as dominant on berries. Ochratoxin A is a nephrotoxin and possible carcinogen fungal metabolite. The European Commission has set 2 µg/kg as maximum OTA content allowed in wine, must and grape juice being wine the second most important source of OTA in the human diet, after cereals. For these reasons, it is crucial for stakeholders in the grape-wine chain to predict OTA risk related to the geographic area and year. Thus, the aim of this study was to develop a mechanistic predictive model (OTA-grapes) based on the infection cycle of *A. carbonarius* on grapes. A relational diagram of *A. carbonarius* infection cycle was drawn. Data available in literature were used to develop mathematical functions for each step of the infection cycle (*A. carbonarius* sub-model), in relation to grape growth stages (grapes sub-model). Regarding sporulation and infection, few quantitative data were available and only limit conditions for their occurrence were established, while fungal germination, growth and OTA production were modelled based on temperature and water activity regimes. Regarding OTA production, wide variability was noticed between strains, i.e., different combinations of optimal temperature and incubation time to optimise the production, apparently not related to their geographic origin. Therefore, two different functions were developed to simulate the fungal community in vineyard. The model uses weather data (air temperature, relative humidity and rain) as input; the *A. carbonarius* sub-model was run from setting to ripening, growth stages predicted by the grapes sub-model, that uses as well meteorological data as input. A prototype model is now available; the next mandatory step regards the collection of a suitable set of vineyard data, including geo-referenced OTA contamination and related meteorological data to manage the validation, crucial step before OTA-grapes model delivery as support for stakeholders in the grape chain. **Acknowledgements.** Work supported by EC KBBE2007-222690-2 Mycored. Marco Camardo Leggieri carried out this work within the PhD School 'Agrisystem' of the Univeristà Cattolica del Sacro Cuore (Italy).

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### **In vitro study of the isoepoxydon dehydrogenase gene expression in relation to the patulin production of *Penicillium expansum* on apple puree agar medium under different storage conditions**

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*Penicillium expansum* is the main cause of blue mould rot, a serious post-harvest disease on apples worldwide. During long-term storage of apples, *P. expansum* produces the mycotoxin patulin, which may end up in apple-based products such as apple juice. Isoepoxydon dehydrogenase, encoded by the *idh* gene, is a key enzyme in the patulin biosynthesis pathway. In this study, a real-time PCR technique was developed to measure *idh* gene expression, in relation to patulin production and growth of *P. expansum* on apple puree agar medium (APAM), under different storage conditions. Three strains of *P. expansum* were one-point inoculated onto APAM plates. The plates were stored under conditions of: 20 °C – air (21% O<sub>2</sub>), 4 °C – air and 4 °C – ultra low oxygen (ULO; 3% O<sub>2</sub>). Samples were taken when colony diameters reached 0.5 and 2 cm. A real-time RT-PCR was developed to

quantify the gene of interest *idh* relative to three housekeeping genes *18S*, *β-tubulin* and *calmodulin*. Primers were constructed for all four genes. RNA was extracted from all samples and, after DNase treatment, converted to cDNA which, in turn, was used as a template for real-time PCR. The samples were analysed for their patulin content by HPLC-UV detection. Colonies at a growth stage of 0.5 cm produced patulin at 4 °C – ULO less than 50 µg/kg. At 4 °C and 20 °C – air, patulin production was in the range of LOD – 500 µg/kg (one strain < LOD) and 800-2,500 µg/kg, respectively. Patulin of 2 cm large colonies at 4 °C – ULO accumulated to a range of 10,000-30,000 µg/kg. Storage at 4 °C and 20 °C – air led to an increase of patulin to ranges of 20,000-60,000 µg/kg and 80,000-300,000 µg/kg, respectively. In order for patulin to be produced, the *idh* gene needs to be expressed. *P. expansum* colonies producing less than 50 µg/kg on APAM showed very low *idh* gene expression levels (<0.05 A.U.). All others grown at cold storage showed low expression profiles (0.70-2.55 A.U.), while at ambient storage high *idh* gene expression (1.80-7.34 A.U.) was observed. These results may be explained by a delayed metabolism under stress conditions (low temperature and/or oxygen). The developed real-time RT-PCR is a fast technique for quantifying *idh* gene expression of *P. expansum* in relation to its patulin production. The in-house patulin detection method was found to be as sensitive in detecting patulin as the developed real-time PCR in measuring *idh* gene expression.

### P136

#### Checking for defective maize kernels to forecast mycotoxins contamination trends

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Mycotoxins are chemical contaminants produced under specific biotic stressed conditions in a wide variety of food and feed commodities by secondary metabolism of fungi among which the most important are from the genus of *Aspergillus*, *Penicillium* and *Fusarium*. A wide range of crops is susceptible to mycotoxin contamination, posing a worldwide problem for human and animal health and an agricultural challenge due to the economical losses. To facilitate the segregation of contaminated crops during the very early stages of the agricultural chain, a two year study (2012-2013) was set to verify if a relationship between maize defective kernels and mycotoxins concentrations is statistically significant and a model may be carried out to forecast the latter only checking the former. Nine farms were involved in the study. Maize defective kernels (broken kernel, hurt kernels, damaged kernels, impure kernels and rotten kernels), mycotoxins (fumonisins, aflatoxin B1 and beauvericin) and humidity (before and after drying process) were provided and considered in the model. Two different sampling procedures were evaluated: one used by the farm (standard sample) and one following the Commission Regulation (EC) No 401/2006 (incremental sample). Forty-five samples (18 incremental and 27 standard, 2012) and forty-two samples (21 for each sampling methods, 2013) were collected. The main statistical methodology was based on correlation and stepwise multivariable regression with normal and bootstrap bias corrected confidence interval, separately for standard and incremental samples. Where more advanced methodology to reach the best forecast was needed, a multiple Box-Cox transformation and spline regression were used. Comparisons between years and sampling methods (standard or incremental) were carried out by parametric (Student's t, chi-squared) or non-parametric (Wilcoxon) tests. After checking the contamination in all the samples, the models highlighted goodness of fit. As regards fumonisins, impure kernels and/or rotten kernels were related with contamination values, for both years and both sampling procedures. As regards aflatoxins and beauvericin, the same results were achieved, excepting in standard samples 2012 and incremental samples 2013, respectively. In these cases the role played by humidity was essential. This study could be considered as a capable tool for farmers to forecast trend of contamination from the sole check of defective kernels.

### P137

#### A review on the impact of GM crops on their capacity to control mycotoxin content

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The Marlon project aimed to support post-market monitoring (PMM) of GM feed consumption by developing an open source epidemiological model. One of its main activities was to verify if, on the



basis of published scientific data, it is possible to define animal health indicators for monitoring four types of health effect (feed allergies, horizontal gene transfer, mycotoxin contamination and nutritionally altered crops). To achieve this, a desk study was performed to explore the potential risk scenarios. The case study focused on the altered levels of mycotoxins in GM crops is reported. The debate on the advantage of using GM crops for their capacity to control mycotoxin content is still open and needs to be ascertained in order to provide sound evidences of the potential benefits to be presented to the stakeholder community. The topic is of particular interest as the impact of mycotoxins on animal farms is an important issue and any solution devoted to reduce or minimize the concern is crucial for animal health, productivity and farm economy bringing advantage to both the agricultural and animal production system. However, whilst a trend is recognised, the scientific community has not produced undisputed results as regards the effect of GM crops on: (i) the development of a number of mycotoxins; (ii) the impact on the GM-based feed; and (iii) the impact on animal health. Various scientific studies and surveillance reports suggest that, under particular conditions of insect damage and (consequently) mould infestation to pest-insect-protected GM crops, the levels of certain mycotoxins may be lower than in conventional non-GM maize. While this would be a secondary effect of the insect resistance, impacts on livestock performance and health following consumption of feeds with altered mycotoxin levels can be envisaged and therefore the topic would lend itself for further exploration. While the report concludes that there is some evidence indicating lower fumonisin levels in insect-protected GM Bt crops, a more in-depth research will be needed to study the relationship between such crops and mycotoxin contamination, and the possible use of biomarkers for health impacts. Aflatoxins and fumonisins are considered to be the mycotoxins of interest for monitoring in dairy cattle and pigs, respectively, with the advantage that aflatoxins and their impacts on livestock can be monitored throughout the production chain.

**P138**

### **Effect of pre-harvest rainfall on the concentration and distribution of deoxynivalenol and zearalenone in subsequent mill fractions of wheat**

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*Fusarium graminearum*, the most important head blight pathogen of wheat produces the trichothecene mycotoxin, deoxynivalenol (DON) and an oestrogenic mycotoxin zearalenone (ZON) during infection. A field experiment was performed to identify the impact of pre-harvest rainfall on the concentration and distribution of DON and ZON in wheat mill fractions. Field plots of winter wheat (cv. Solstice) were inoculated with *F. graminearum*-infected oat grain at stem extension and mist irrigated at mid-flowering to encourage head blight infection. Plots were either; covered by a small polytunnel to mimic dry conditions, irrigated by sprinklers or left as non-irrigated uncovered control plots after the mid-milk growth stage. Plots were harvested when ripe (early harvest) and three weeks later (late harvest). Samples of grain were cleaned, conditioned to 16% moisture content and milled using a pilot scale Bühler mill. Bran and offal fractions were cleaned in a Bühler laboratory Impact Finisher. The resulting mill fractions were combined to form white flour, bran and offal. Each fraction was analysed for DON and ZON using Ridascreen ELISA assays. Results from this study indicated that: (i) pre-harvest irrigation in this experiment resulted in a large increase in ZON concentrations in harvested grain but did not alter the distribution within mill fractions, and (ii) pre-harvest irrigation in this experiment resulted in a small increase in DON concentrations in harvested grain but significantly altered the distribution within mill fractions with an increased proportion of DON in white flour. As well as knowing the concentration of mycotoxins within wheat grains, it is also important for processors to know the impact of processing on these mycotoxins so they can predict the concentration of mycotoxins in finished products. This study shows that for DON the impact of milling is variable dependent on the degree of pre-harvest rainfall.

**P139**

### **A model for risk-based monitoring of mycotoxins in feed ingredients**

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Quality control of animal feed ingredients should be based on the risk for feed and food safety. Therefore, a calculation model was developed to predict the contribution of individual feed ingredients

to the risk for human and animal health related to specific contaminants. The model was based on the general approach: risk = exposure x severity. The model was applied to deoxynivalenol (DON) and aflatoxin B1 (AFB1). Model input includes the total annual production of compound feed for the major farm animal species (cattle, pigs, poultry), the total annual usage of locally produced and imported feed ingredients, and the country of origin. For each feed ingredient and country of origin combination, the risk of contamination is estimated (scale 0.01-1) for each mycotoxin based on trends in available analytical data and expert judgement including risk factors as climate, production process, quality control, etc. Finally, for each mycotoxin two toxicity factors, for animal health and human respectively are estimated for each animal category (e.g., broilers, laying hens, etc.) based on expert judgement of the direct toxicity for the target animal and the indirect risk for human health via consumption of the animal products. Least cost feed formulation was used to compose representative diets based on available ingredients for the major animal categories. This provided the quantitative distribution of the total annual use of each ingredient among different animal species and categories. Subsequently, the total risk of each feed ingredient and country of origin combination for a specific mycotoxin was calculated based on the volume, the use of the feed ingredients for different target animals, the toxicity factors for animal and human health and the potential contamination for the country of origin. The model provides the relative contribution of each feed ingredient and country of origin combination to the total risk of a specific mycotoxin in animal feed. Details can be provided, for example regarding risk for animal and human health. At present the model comprises information on AFB1 and DON. It can be further optimised and easily extended to other mycotoxins and feed ingredients, provided that information regarding their toxicity for target animals and human health and the risk of contamination in countries of origin can be obtained. The quality of the model predictions largely depends on the quality of the input data. In conclusion, the presented model is a helpful tool to optimally allocate resources for (official) safety control of animal feed ingredients.

#### **P140**

### **Influence of pearling process on levels of deoxynivalenol and deoxynivalenol-3-glucoside in wheat**

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Deoxynivalenol (DON) is a well known mycotoxin mainly produced by *Fusarium graminearum* and *F. culmorum*. It can be found as a major contaminant in cereals, worldwide. It has been demonstrated that plants are able to convert DON into conjugated forms, among those deoxynivalenol-3-glucoside (DON3G) has been found to be the main DON metabolite in wheat and it is normally referred as masked mycotoxin. Among cereal-based foods, masked mycotoxins occur at higher levels in whole-grains or fibre-enriched products. Sometimes, a decrease in mycotoxin content may occur upon food processing (i.e., milling, fermentation, cooking steps), although treatments can also lead to the cleavage of masked forms as well as to further transformation steps. In particular, it was shown an increase of DON level in wheat samples after processing due to the activity of some enzymes that are able to hydrolyse DON3G to release DON. The aim of the present study was to determine the occurrence of DON and DON3G in grain samples at various degrees of pearling. For this purpose, different samples were pearled incrementally in a laboratory abrasive-type grain testing mill. A total of seven samples were obtained from each batch, representing 5% of the initial grain weight: the whole unprocessed wheat, the 0-5%, 5-10%, 10-15%, 15-20%, 20-25% and the residual 75% of the unprocessed kernels. DON and DON3G content of each fraction was quantified using an UHPLC-MS/MS method. Data showed that DON contamination decreased from the outer to the inner layers. The different fraction underwent to enzymatic treatments with laminarinase,  $\alpha$ -amylase, cellulase, protease and xylanase to check the possible release of DON and DON3G from fibres and cell walls. Furthermore, the samples underwent to a simulated digestion assay to check the possible release of an additional DON amount after ingestion.

#### **P141**

### **Fate of mycotoxins along industrial biscuits and rusks processing, with deoxynivalenol and ochratoxin A as main targets**

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Mycotoxins are toxic natural secondary metabolites produced by fungi on agricultural commodities which occurrence cannot be avoided into finished processed foods. Food processing, such as sorting, cleaning, milling and thermal processes, can potentially alter mycotoxins: mechanical or thermal energy may cause, in fact, transformation and/or degradation. The aim of this work is the study of the contemporary fate of free and masked mycotoxins in bakery processing, with particular emphasis on deoxynivalenol (DON) and ochratoxin A (OTA), along two different industrial technologies related to biscuits and rusks production. In particular, starting from naturally contaminated bran, we studied how DON and OTA concentration can be influenced by modifying ingredients and operative conditions: sugar, milk, cocoa, pH, yeast, enzyme content, fermentation-baking-toasting time/temperature. The experiments were performed using statistical Design of Experiment (DoE) schemes to synergistically explore the relationship between the analytical responses and all the above indicated independent variables, looking for an optimisation of the process through selected combined production trials between pilot plants and industrial lines. Samples were collected during transformation from raw-material to final product considering all the relevant steps: dough, fermented dough, baked and toasted products; they were then stored at -20 °C and subsequently analysed with an LC-MS/MS multimycotoxin method to: (i) determine the simultaneous possible presence and changes of different mycotoxins; and (ii) focus particularly on the evolution of OTA, DON and its conjugates (mainly deoxynivalenol-3-glucoside and 3/15-acetyldeoxynivalenol). The obtained models show a good fitting, robustness and prediction capability, suggesting the most significant ingredients and parameters, which can concretely contribute to the minimization of the free forms level in the final food product.

**P142**

### **Understanding and managing the relationship between insect damage and mycotoxin accumulation in transgenic grain maize**

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Maize production frequently sustains significant yield and grain quality losses due to mycotoxin contamination. In addition, ear-feeding insect damage is becoming increasingly important as the Western bean cutworm (*Striacosta albicosta*; WBC) has recently expanded its range into Ontario and is not well controlled with currently available technologies. An LC-ESI-MS/MS method was validated for detection of 20 *Fusarium* mycotoxins. The effectiveness of 5 transgenic Bt-maize events for control of WBC damage was studied in 2010-12 at Ridgeway, ON, in field plots (5 row x 4 m x 0.76 m x 4 reps, RCBD). One WBC egg mass was pinned to the central maize plant of each plot in 2010 and 2011 and 12 ears per plot were infested with one 3rd instar WBC larva per ear at the R3 stage in 2012. The most effective control of WBC damage was provided by hybrids expressing Cry1Ab x Vip3A over all years. Reduced WBC damage resulted in a significant reduction of toxins related to *Fusarium verticillioides*/*F. proliferatum* (fumonisins and moniliformin). The toxins of *F. graminearum* (deoxynivalenol (DON) and related compounds) which are traditionally associated with *Fusarium* ear rot in Ontario were not reduced. Regression was used to characterise the relationship between WBC and mycotoxin accumulation in harvested grain. Damage was significantly associated with fumonisins accumulation in every year of this study. Conversely, DON was not associated with WBC damage. The relationship between insect feeding damage to maize ears, ear rot infection and mycotoxin accumulation in grain maize was studied in non-Bt maize, planted in mid-June, inoculated with 1 ml of 2.5x10<sup>5</sup> conidia of *F. graminearum* at silk emergence and initial silk browning, followed by irrigation with overhead misting until the grain dent stage (1 row x 4 m x 0.76 m x 4 reps, RCBD). WBC damage was simulated at different stages of maize cob development with 3 levels of severity. Damage during silking (R1) did not contribute significantly to the final toxin content in harvested grain. Higher *F. graminearum* toxins were found when damage was inflicted at milking (R3) and dent (R5) stages and only zearalenone was significantly accumulated when damage was inflicted at latter stages (R6). Damage at the blister stage (R2) caused higher accumulation of *F. verticillioides*/*F. proliferatum* toxins. Fumonisins also significantly accumulated at milk stage (R3). Toxins produced by *F. avenaceum* and *F. sporotrichioides* were found when damage was done at latter stages of maize cob development. The interaction between all these toxins, the respective fungi and the implications on management practices require further study.

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## Feasibility study on predictive model of aflatoxin B1 via using versicolorin A as a bio-indicator

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Predictive mycotoxins reports are extremely important to storage crops. However, a practical model is still a challenge. Versicolorin A (Ver A), an important precursor of aflatoxin B1 (AFB1), is used as a bio-indicator to forecast AFB1 contamination. At first, the influence of storage temperature, water activity and levels of Ver A on AFB1 production was investigated. Eight maize samples contaminated with various levels of moulds (cultured from aflatoxin-positive maize) were stored under slightly wet condition at 18 and 28°C for 36 days, separately.  $a_w$  and AFB1 at every 3-days and Ver A at the start were detected. Results showed that  $a_w$  regarding significant starting of AFB1-producing was at 0.76 and 0.80 at 18 and 28°C, respectively. Maize with a starting level of VerA of 100±3 ppb could be dangerous. When maize had a higher level of Ver A, high levels of AFB1 were found after storage under wet conditions. Maize with a low level of VerA (<100±3 ppb) at the start would be safe (AFB1 < 2 ppb) after storage in wet environment. Subsequently, 100 maize samples ( $a_w$  not higher than 0.65) were collected from farmers in China in early 2013. The whole period of storage (Guangzhou, China, air condition) was monitored. The levels of Ver A and AFB1 were detected at the start and every three-months of storage. Results showed: (i) Ver A at the start appeared to be significant correlated with AFB1 after storage for 3, 6, and 9 months (under Guangzhou air condition); and (ii) the logarithm of Ver A in March showed a linear relationship with the logarithm of AFB1 in June, September and December. These results suggest that through a collection of enough data of Ver A and AFB1 with the monitoring of  $a_w$ , it is possible to predict near future contamination levels of aflatoxins of maize (under certain storage conditions) via the calculated model of Ver A levels prior to storage.

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## Climate change factors and *Aspergillus flavus*: effects on gene expression, growth and aflatoxin production in vitro and in maize

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There is a significant interest in the impact that climate change factors may have on mycotoxigenic fungi. We have examined both *in vitro* and *in situ* on maize grain the impact that three way interactions between water availability, temperature and elevated CO<sub>2</sub> has on the relative expression of a structural and regulatory gene involved in the biosynthesis of aflatoxin B1 (AFB1), growth and phenotypic AFB1 production. Thus the detailed objectives of this study were to obtain scientific data on the impact that interactions between water stress (water activity,  $a_w$ ; 0.97, 0.95, 0.92), temperature (34, 37°C) and CO<sub>2</sub> exposure (350, 650, 1000 ppm) may have on the growth, gene expression of biosynthetic genes (*afID*, *afIR*), and AFB1 production by a type strain of *A.flavus* on a conducive medium and on maize grain. The study showed that while  $a_w$  affected growth there was no statistically significant effect of temperature or CO<sub>2</sub> exposure. The effect of these interacting factors on *afID* and *afIR* gene expression showed that at 34°C there was maximum relative expression of the *afID* under the control conditions (34°C, 350 ppm) with a decrease in expression with elevated CO<sub>2</sub> and water stress. For *afIR* expression at 34°C, there was a significant increase in expression, but only at 0.92  $a_w$  and 650 ppm CO<sub>2</sub>. However, at 37°C, there was a significant increase in expression of both *afID* and *afIR* at 0.95 and 0.92  $a_w$  and 650 and 1000 ppm CO<sub>2</sub>. There was an associated increase in AFB1 in these treatments. In contrast at 34°C there were no significant differences for interacting treatments. Similar results were obtained when *A.flavus* was inoculated in stored maize grain. This is the first study to examine these three-way interacting climatic factors on growth and mycotoxin production by a strain of *A.flavus*. This provides data which is necessary to help predict the real impacts of climate change on mycotoxigenic fungi.

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## Kinetics of ochratoxin A, deoxynivalenol and its conjugates during baking

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Cereal products are the main source of exposure to ochratoxin A (OTA) and deoxynivalenol (DON). Processing of cereals at high temperature may affect mycotoxin content. However, the reported extent of OTA and DON reduction during thermal food processing seems to be quite variable and dependent on the processing conditions applied. In addition, the co-occurrence of conjugated DON forms has been documented in wheat, especially deoxynivalenol-3-glucoside (DON-3-glucoside), 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON). The fate of DON-3-glucoside 3-ADON, 15-ADON and de-epoxy-deoxynivalenol (DOM-1) behaviour has hardly ever been studied before. The current study aimed to investigate OTA, DON and DON conjugates (DON-3-glucoside, 3-ADON, 15-ADON and DOM-1) stability during baking in a small size model bakery product, small enough to avoid temperature gradients in it. Temperature (from 140 to 200°C), time (from 0 to 40 min) and initial mycotoxin concentration were assayed as factors. It was shown that OTA and DON can be highly reduced by baking. Both temperature and time were two essential factors in the reduction of OTA and DON due to baking. Thus, the mycotoxin reduction increased with the time and while low reductions at 140°C were detected after 40 min, reductions over 70% were detected for the same time at 200°C. OTA is a little bit more stable than DON through the baking treatment, with lower percentages of reduction at the same conditions. Interestingly, DON-3-glucoside under mild baking conditions increased (>300%). On the other hand, it was rapidly inactivated under harsh conditions, for instance 20 min at 180°C or 15 min at 200°C. 3-ADON content decreased with the heat treatment, whereas DOM-1 increased. Finally, inactivation/release kinetics were studied for OTA, DON, DON-3-glucoside and DOM-1 within the temperature range of 140-200°C. First order kinetics were applied and thermal constant rates (k) were calculated. The temperature effects and differences in stability between analysed mycotoxins in activation/release were analysed by using Arrhenius equation with a good regression coefficient. The use of the developed kinetics can be a useful tool for the control of OTA, DON and DON conjugates levels in the bakery industry. In conclusion, the different levels of reduction/increase observed depending on the baking conditions could explain the differences in previous reports. Moreover, the size of the product may be a key factor which affects the reduction of the mycotoxins due to heat treatments and may also explain previous contradictory results. **Acknowledgements.** The authors are grateful to the Spanish (Project AGL2011-24862) and Catalanian (XaRTA-Reference Network on Food Technology) Governments for their financial support. A. Vidal thanks the Spanish Government (Ministry of Education) for the pre-doctoral grant.

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## Metabolic fate of the *Fusarium* mycotoxins T-2 and HT-2 in wheat

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T-2 toxin (T-2) and HT-2 toxin (HT-2) are *Fusarium* mycotoxins classified as type A trichothecenes. They can inhibit RNA, DNA and protein synthesis causing haematotoxicity and immunotoxicity. Cereal grains and grain-derived foods are the main contributors to T-2/HT-2 exposure. Based on existing toxicological and occurrence data, the European Food Safety Authority (EFSA) proposed a tolerable daily intake (TDI) of 100 ng/kg bw for the sum of T-2 and HT-2. To date, the metabolism of T-2 and HT-2 in several mammalian species has been investigated with a significant number of metabolites characterised. However, very limited information exists regarding the metabolic fate of these mycotoxins in plants, where formation of masked mycotoxins might occur. In this study, wheat plants of the variety *Remus* (susceptible to *Fusarium* head blight) were treated with a mixture of either uniformly (U)-<sup>13</sup>C<sub>24</sub>-labelled T-2 and non-labelled T-2, U-<sup>13</sup>C<sub>22</sub>-labelled HT-2 and non-labelled HT-2 or with acetonitrile:water (50:50, v/v) as control. At time-point zero, the appropriate inoculation solution (T-2, HT-2 or control) was injected to each of two adjoining spikelets of flowering wheat ears. This

process was repeated to the spikelets located in the row directly above the treated ones also at time points 48, 96, 120 and 144 h, subsequent to the first treatments. Sampling was performed 24 h after the last inoculation and wheat ears were shock-frozen in liquid nitrogen and stored at -80°C until analysis. The wheat ears were finely ground, extracted and analysed with a liquid chromatography-quadrupole time-of-flight mass spectrometer (LC-QTOF-MS) system in full scan mode. An updated version of the MetExtract software (Bueschl *et al.*, 2012, 2014) was used to extract MS peak pairs belonging to possible T-2 and HT-2 metabolites. Annotation was based on the accurate mass measurements, as well as LC-MS/MS spectra. According to our findings, HT-2 is putatively biotransformed into the previously reported HT-2-glucoside, HT-2-di-glucoside, in addition to a number of other metabolites e.g., HT-2-malonyl-glucoside. HT-2 was also found to be acetylated into T-2 in minor extent probably by the function of wheat acyltransferases. Interestingly, HT-2 was found to be a major metabolite of the *in planta* detoxification of T-2 as in the case of mammals. Several novel metabolites of T-2 are also reported here.

#### P147

### Insecticide applications indirectly reduce *Fusarium* ear rot and fumonisin production in maize

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Lepidopteran stem borers are associated with increased incidences of *Fusarium* ear rot (FER) in maize grain. This study investigated the effect of benfuracarb and beta-cyfluthrin insecticide application on FER and fumonisin production. Field trials were planted using a randomised complete block design. Inoculation treatments were: *F. verticillioides* inoculation and *B. fusca* infestation, *B. fusca* infestation and an uninoculated/uninfested control. Each of these was then treated with no insecticide, beta-cyfluthrin and benfuracarb. *Busseola fusca* larvae were dispensed into the whorl of each plant six weeks after emergence and a *F. verticillioides* spore suspension was inoculated through the silks. All primary ears harvested at physiological maturity were visually rated for FER, stem borer damage determined by measuring tunnel length (cm) and total fumonisin (B1, B2 and B3) quantified using HPLC. Fumonisin-producing *Fusarium* species (target DNA) were quantified using qPCR. An ANOVA and linear regression analysis were performed on the data. Results indicated that FER incidence was significantly reduced when benfuracarb and beta-cyfluthrin were applied on the *B. fusca*-infested plots but not on the *F. verticillioides* and *B. fusca* inoculated plots. Fumonisin levels were not significantly reduced in the *B. fusca* infested plots but benfuracarb significantly reduced fumonisin levels where *F. verticillioides* and *B. fusca* inoculations were carried out, however, beta-cyfluthrin had no significant effect. There were no significant reductions in *B. fusca* damage on the ears irrespective of inoculation treatment when treated with either insecticide. There was a weak positive correlation ( $r=0.23$ ;  $p<0.05$ ;  $n=144$ ) between fumonisin production and *B. fusca* damage. This suggests that ear damage is possibly one of the factors that result in increased fumonisin production. *Busseola fusca* infestation alone did not result in increased target DNA in maize ears and target DNA was affected by seasonal effects, suggesting environmental conditions play a major role in infection and ramification of fumonisin-producing *Fusarium* species in maize kernels. This study indicated that *F. verticillioides* and *B. fusca* inoculations as well as *B. fusca* infestation alone increased FER, but only the *F. verticillioides* and *B. fusca* inoculations significantly increased fumonisin production relative to the non-inoculated control. Therefore, the application of insecticides to control *B. fusca* indirectly reduces FER (when plants were inoculated with *B. fusca* only) and benfuracarb reduced fumonisin production (where plants were inoculated with both *F. verticillioides* and *B. fusca*). Insecticide use could potentially reduce FER and fumonisin production in resource-poor subsistence farming systems.

#### P148

### *Claviceps purpurea* and alkaloid in cereals: variability relationship and pattern

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*Claviceps purpurea* is able to infect numerous of cereal species and grasses in Europe and worldwide. The pathogen forms a sclerotium also named ergot, which overgrows the developing grain. The sclerotium is a mass of mycelium that contains alkaloids. In 2006, the European Commission recommended to perform monitoring on the presence of ergot alkaloids and determine, whenever

possible, simultaneously the sclerotia content in the sample to be able to improve the knowledge on the relation between the content of sclerotia and the level of individual ergot alkaloids. Therefore, we collected over 1000 field samples of common wheat, durum wheat, triticale, barley, rye and oat. Those field samples were collected on 2012 and 2013 harvests (in 2013, common wheat and durum wheat were harvested in collaboration with FranceAgriMer). 337 samples contained ergot bodies. Therefore, alkaloid contents were analysed on those samples. Ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, ergocornine and their corresponding epimers (-inine forms) were analysed by liquid chromatography-tandem mass spectrometry. For each sample, alkaloid content is reduced to 1g ergot bodies. Total average alkaloid content is 0.32% varying from 57 to 36,385 µg/g ergot bodies, which means from 0.006 to 3.6% of ergot bodies weight. The ratio between -ine and -inine forms is 2.6:1. Ergotamine, ergocristine, ergosine and their corresponding epimers represent 72% of total alkaloids. The mean of total alkaloids content of *C. purpurea* is 3,201 µg/g. A variance analysis applied to harvest year and host plant showed that 2012 and 2013 harvest were similar, and the host plant did not have influence on the alkaloid contents. A variance analysis on data applied to harvest year, host plant and ergot content has led to the identification of critical factors involved in alkaloids content: host plant ( $p=0.50$ ) and harvest year ( $p=0.65$ ) did not influence alkaloid contents. Alkaloid contents depend on ergot content ( $p<2e-16$ ). Taking this in consideration, we developed a first alkaloid content predictive model based on a linear regression with the backward selection method applied to ergot content. This model explains 75% of the variability of alkaloid contents. Alkaloid contents in grain samples depend first on ergot contents. In relation to toxicological studies, this model can be a first tool for defining critical ergot content under which an analysis of alkaloids is not necessary. In a second time, this model could help grain stores target fields where risk level is high and adjust grain cleaning and analysis strategies.

**P149**

### **Contamination of wheat grain with *Fusarium* mycotoxins dependent on cultivar and the location of cultivation**

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The most important mycotoxins found to contaminate cereals during their growth under weather conditions found in the area of Poland during plant vegetation are produced by *Fusarium* fungi. Mycotoxin contamination is highly dependent on environmental factors, such as humidity, temperature and rainfall. The aim of the present research was to assess the influence of the location and cultivar on *Fusarium* mycotoxins deoxynivalenol (DON), zearalenone (ZEA) and T-2/HT-2 toxin contamination of winter wheat grains. The experiments were conducted in the years 2009, 2010 and 2011 at 5 different locations situated in different regions of the country. 10 different winter wheat cultivars were included. Wheat was harvested in full maturity phase. To analyse the levels of contamination with DON, ZEA and T-2/HT-2 toxins, an enzyme-linked immunosorbent assay (Veratox; Neogen) was implemented. The weather conditions during wheat maturation in 2009 and 2010 years were not favourable for mycotoxin formation. In none out of 5 locations or in any of the 10 cultivars the detected levels of mycotoxin contamination exceeded the maximum allowed level. In 2011, the level of mycotoxins was much higher compared to previous years and dependent on the location of plantation. The highest contamination with DON was detected in southeast Poland (Czesławice, 580 ppb). However, T-2/HT-2 was most abundant in northeast Poland (Krzyżewo, 24.6 ppb). Furthermore, the cultivar had influence on the severity of mycotoxin contamination. In 2011, cultivars Ostroga, Markiza, Muszelka and Tonacja grown in southeast location (Czesławice) showed accumulation of ZEA that was higher than the maximum allowed level. The accumulation of DON and T-2/HT-2 did not exceed the maximum allowed level, however, the level of mycotoxins was cultivar-dependent. While searching for relationship between morphological features and the amount of mycotoxins accumulated in the grains, it was found that the height of the plants, the structure of the glume and thickness of the culm highly correlated with mycotoxin accumulation. Shorter cultivars, which have a thin culm, were highly contaminated. We have shown that not only the geographical location but also features of particular cultivars influence mycotoxin accumulation. While cultivating wheat for food production, it is important to consider different levels of susceptibility of various cultivars to mycotoxin accumulation.

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## The impact of harvest time and storage conditions of organic winter wheat grain on aflatoxins and ochratoxin A production

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Aflatoxins (AFs) and ochratoxin A (OTA) are the major mycotoxins produced by *Aspergillus* and *Penicillium*. They contaminate cereals grain and its products during storage. The aim of this study was to develop a method for storing grain that would reduce contamination of grains with storage mycotoxins. Organic winter wheat grain was placed in a brick warehouse and a wooden barn. Two different warehouses were used to determine the influence of storage conditions on the occurrence of mycotoxins in grains. Stored grain was checked before harvest and then every two weeks during storage for fungi and content of mycotoxins. Winter wheat grains were contaminated with AFs and OTA at all periods of storage. It was found that the better conditions for storing grain were in the brick warehouse rather than in the wooden barn. Storage mycotoxins were detected in winter wheat grain directly after harvest, which indicated the possibility of contamination of the tested grain with fungi of the genus *Penicillium* and *Aspergillus* while still in the field before harvest. This might be related to the long period of keeping a fully mature grain in the field to achieve a low moisture content. During that time, weather conditions could be favourable for the development of storage fungi on the ears and then in the grain.

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## The influence of 1-methylcyclopropene on *Alternaria alternata* fungal growth and mycotoxin production in tomatoes

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Post-harvest decay of tomatoes is a problem of great concern to producers, which causes serious economic losses. *Alternaria alternata* is known to be one of the pathogens responsible of tomato decay. Ripeness of the fruit affects the susceptibility to infection, being more vulnerable as the fruit ripens. Thus, it is necessary to control the maturity state of fruits to prevent their decay. Ethylene production directly affects ripening and senescence processes. In this sense, ethylene antagonists, such as 1-methylcyclopropene (1-MCP), are increasingly used in food industry to control the maturity of fruits, maintaining the quality of fruits and vegetables. Its action consists on the delaying of the natural ripening process. There are many studies focused on the effect of 1-MCP on the quality parameters of tomatoes. However, little is known about the effect of 1-MCP on fungal pathogens of tomato. In this work, we have analysed the effect of 1-MCP on three different varieties of tomato inoculated with *A. alternata*. We studied fungal development, production of the mycotoxins alternariol (AOH), alternariol monomethyl ether (AME) and tentoxin (TEN), and gene expression of two genes related to the production of AOH and AME, i.e., a polyketide synthase (*pksJ*) and a transcriptional regulator (*altR*). Tomatoes were kindly supplied by producers of the area without any post-harvest treatment. The fruits were disinfected and afterwards inoculated with  $10^5$  conidia/ml of *A. alternata*. Food industry storage conditions were simulated. Following treatment with 1-MCP (0.6 µl/l for 24 h), tomatoes were stored for 7 days at 10°C and the product lifetime was simulated with an additional storage of 7 days at 20°C. Results suggest that 1-MCP tends to increase fungal growth compared to the control. However, this effect depends on the tomato variety. Additionally, 1-MCP has a tendency to decrease mycotoxin production but, as in the case of growth infection, the variety has a large influence. Regarding gene expression, there were no differences between the profile of tomatoes treated with 1-MCP and untreated. Since 1-MCP seems to increase *Alternaria* lesion size in some varieties, it would be recommendable to study more carefully this disadvantage with each tomato variety. **Acknowledgements.** The authors are grateful to the Catalan Government (XaRTA-Reference Network on Food Technology) for their financial support. N. Estiarté thanks the Secretaria de Universitats i Recerca del Departament de Economia i Coneixement of the Generalitat de Catalunya for the predoctoral grant.



P152

## **Influence of antioxidant profile of two buckwheat species on *Aspergillus flavus* growth and aflatoxin production on achenes**

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The promotion of minor grain crops would be desirable, since their potential use in the expanding market of functional and healthy ingredients suggests good prospects in the near future, respecting consumers' needs and preferences while minimizing the related impact on the environment, in accordance with the topic 'Food & Healthy Diet' reported in Horizon 2020. In the last decades, in fact, the supply of carbohydrates to the European food system has relied mainly on a few major crops meanwhile other grain crops, because of their lower yield potential, gradually have been confined to play a marginal role. Among these, buckwheat (*Fagopyrum* spp.), a 'pseudo-cereal', appears worth considering especially in the production of healthy foods since its flour, derived from achenes, is enriched in bioactive compounds and, due to the lack of gluten, may be used for coeliac diet composition. Within buckwheat species, *F. tataricum* achenes possess a large amount – higher than the common buckwheat *F. esculentum* – of rutin, a flavonoid known for its antioxidant activity. Ongoing climate change favours plant susceptibility to the attack by pathogenic (often mycotoxigenic) fungi with a consequent, dangerous, increase of mycotoxins into previously unexploited feed- and foodstuff. In particular, *Aspergillus flavus*, under suitable environmental conditions as those currently occurring in Italy may produce aflatoxin B1 (AFB1), the most carcinogenic compound of fungal origin being classified as IARC group 1. The composition of achenes, especially the antioxidants, deeply affects the ability of several mycotoxigenic fungi to exploit the host and to produce toxins. In this study, the viable achenes of two buckwheat species, *F. tataricum* (var. Golden) and *F. esculentum* (var. Aelita) have been inoculated with an AFB1 producer fungus (*A. flavus* NRRL 3357) to analyse their own performances against fungal invasion and toxin contamination. Notably, we sought for the existence of a correlation between the tococls/flavonoids amount (constitutive and infection-induced) in the buckwheat achenes and *A. flavus* ability to grow and produce toxin during achene infection. Results suggest that achenes of *F. tataricum*, the best producer of antioxidant compounds in this study, are less susceptible to *A. flavus* infection and consequently but not proportionally to mycotoxin contamination with respect to *F. esculentum*. Moreover, rutin-derived quercetin appears more efficient in inhibiting aflatoxin biosynthesis compared to the parent compound. All these are reasons to promote the cultivation and use of minor grain crops to develop safe and healthy novel food ingredients and to assure these crops may result in grower rewards.

P153

## **Effect of ionic water activities and temperatures on growth and ochratoxin A production by a strain of *P. verrucosum* on a dry-cured sausage-based medium**

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Dry-cured sausages are an important group of foods produced and consumed in Europe and exported world-wide. The quality and microbiological safety of these products is critical for the economic success of this industry. In recent years many studies have become evident that environmental conditions during the ripening/curing process including the salt content are conducive to surface growth of a mycobiota population in this specialized ecological niche. In fact, some mould species, including *Penicillium verrucosum*, can contaminate the dry-cured sausages with ochratoxin A (OTA). The uncontrolled mould colonisation and OTA production in these industries require studies and urgent action to minimise its entry into this food chain for producers, distributors and consumers. The objective of this work was to obtain scientific data on the impact that interaction between ionic water stress (water activity,  $a_w$ ; 0.97, 0.94, 0.90, 0.87 and 0.84) and temperature (30, 25, 20, 15 and 10°C) may have on the growth and OTA production by a *P. verrucosum* strain isolated from dry-cured meat

products on a dry-cured sausage-based medium over a period of 12 days. This study showed that some intra-strain differences were observed. Under extreme water stress conditions ( $a_w$  0.84) *P. verrucosum* did not grow at any of the temperatures evaluated. The *P. verrucosum* was more tolerant at moderate ionic water  $a_w$  conditions ( $a_w$  0.94 and 0.90) and high temperature values (20 and 25°C). In contrast, the pattern of production of OTA was very different from that for growth. Higher amounts of OTA were produced when more freely available water ( $a_w$  0.97) are present at any temperature tested. Statistical analyses showed that  $a_w$  and temperature were significant factors for growth and OTA production by *P. verrucosum*. These results are discussed in the context of the effect of these ecological factors on the relative importance of this toxigenic species in contaminating dry-cured sausages with this toxin. **Acknowledgements.** Dr. A. Rodríguez would like to thank the Junta de Extremadura, Consejería de Empleo, Empresa e Innovación, and the Fondo Social Europeo (FSE) for a postdoctoral fellowship at Cranfield University (PO12016).

**P154**

### **Lipid signals in the interaction between mycotoxigenic fungi and their hosts**

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Lipid molecules are gaining momentum as signals exchanged by interacting organisms during pathogenic and/or symbiotic deals. Some class of lipids, especially those over-represented in the interaction interfaces actively drive the fate of the interactions. Notably, some lipid-based compounds may reprogram the transcriptome of both pathogen and host, leading to defence responses such as programmed cell death in plants or mycotoxin synthesis in the pathogen. In relation to this, host-cuticle components, such as sphingolipids, and oxidised fatty acids, such as oxylipins, may contribute to drive host-pathogen interactions. According to available studies, the plant sphingolipids are involved in signalling pathways that promote hypersensitive response and associated programmed cell death in plants whilst some phyto-oxylipins may affect the production of secondary metabolites in pathogenic fungi. Our recent studies demonstrate that these lipid-related compounds play a pivotal role in modulating mycotoxin synthesis in *Aspergillus flavus*, *Fusarium graminearum* and *F. verticillioides* during their interaction with respective hosts. In relation to this, we set up a whole method, i.e., from extraction up to discriminant chemometric analysis, based on the use of reverse-genetic, LC-TOF, LC-MS/MS and bioinformatics approaches. The method provides 2 steps, an untargeted and a targeted lipid compound mining procedures. The use of this method into the previously indicated biological systems allowed us to pinpoint specific lipid markers of plant susceptibility/tolerance to mycotoxigenic fungi.

**P155**

### **Emerging mycotoxins on cereals in North Italy: the role of environmental and agronomic conditions on their occurrence**

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Nowadays, it is becoming increasingly necessary to collect occurrence data of the so-called 'emerging' mycotoxins in the most important cereal areas of the European Union to correctly consider the exposure and the risk assessment. To obtain major information about the incidence of these mycotoxins, a first investigation has been conducted to individuate the agronomic and environmental conditions affecting emerging mycotoxin contamination in cereals cultivated in North Italy. For this purpose, a monitoring was carried out on maize from 4 regions (Piedmont, Lombardy, Emilia-Romagna and Veneto) during the period 2012-2013. In each year, about fifty representative maize samples have been collected at different sites of the investigated regions. Moreover, in the 2011-2013 period, a first investigation on wheat only from Piedmont Region was carried out. Maize and wheat samples were analysed by an LC-MS/MS based multi-mycotoxin method. Applying this method, 15 of the most abundant mycotoxins were detected in maize samples: fumonisins (FUMs), moniliformin (MON), fusaproliferin (FUS), fusaric acid (FA), bikaverin (BIK), beauvericin (BEA), equisetin, aflatoxins

(AFs), ochratoxins, aurofusarin, deoxynivalenol (DON), deoxynivalenol-3-glucoside (DON-3G), zearalenone (ZEA), culmorin and butenolide. Moreover, 17 mycotoxins were also detected in wheat samples: enniatins (ENNs), aurofusarin, MON, tentoxin, equisetin, DON, DON-3G, culmorin, BIK, BEA, 3-acetyldeoxynivalenol (3-ADON), nivalenol (NIV), ZEA, decalonectrin, butenolide, alternariol (AOH) and alternariol methyl ether (AME). On average, the relative percentage of presence of the different mycotoxins detected in maize samples ranged from 60 to 80% for FUMs, 5 to 10% for MON, 5 to 12% for FUS, 2.7 to 3% for FA, 2 to 3% for BIK, and 1 to 2% for BEA. The other mycotoxins were present <1%, with remarkable and hazardous AFs contamination values in 2012, in Emilia-Romagna (on average, 106 µg/kg), Veneto (on average, 45 µg/kg) and Lombardy (on average, 18 µg/kg), during a growing season characterised by high temperatures and water stress. Moreover, the results underline the crucial role of ECB (*Ostrinia nubilalis*) on the diffusion of several emerging mycotoxins, such as MON, FUS, FA as well as FUMs. On the other hand, in wheat samples from the Piedmont region the most abundant mycotoxins were DON and culmorin, with a higher average contamination in the growing season with the highest *Fusarium* head blight (FHB) pressure (2011-2012). Moreover, the results obtained for wheat samples showed that the fungicides application in all experiments reduced significantly also ENNs, aurofusarin, MON, tentoxin and equisetin content. In conclusion, the first results obtained in the current study remark the crucial role of cultural practices and environmental conditions on the diffusion and possible control of the emerging mycotoxins.

## P156

### **Biosynthesis and accumulation of fumonisins by diverse *F. proliferatum* strains in myceliar and liquid culture samples**

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*Fusarium proliferatum* is a polyphagous plant pathogen common all over the world. It is capable of infecting numerous crop plants and contaminating their tissues with a wide range of mycotoxins, of which group B fumonisins, beauvericin and moniliformin are regarded to be the most important. Fumonisins have been identified in diverse plant materials, including maize, rice, asparagus, peas, garlic, figs and pineapple, just to mention the few crop plant species. Previous studies have shown that strains of *F. proliferatum* originating from different hosts, based on the genomic sequences analysis were discriminated into robust groups, highly correlated with the host plant species. Consequently, their mycotoxigenic abilities have been examined by *in vitro* culturing of over 30 strains originating from more than 10 plant species in sterile rice cultures for 14 days and analysing the final accumulation of FBs. It can be concluded that collection strains, frequently maintained on artificial media for a long period of time, synthesise small amounts of FBs compared to the *F. proliferatum* strains freshly isolated from environmental samples. For the most efficient producers, the maximum FB1+FB2+FB3 amounts exceeded 2 mg per g of dry weight and only five of the fresh strains produced less FBs than 1 mg/g. Of the collection strains, only two strains out of thirteen produced more FBs than 0.5 mg/g. Furthermore, thirteen chosen strains have been cultured on liquid medium to evaluate the kinetics of FBs biosynthesis and the influence of five host plant extracts on fungal biomass production and mycotoxin biosynthesis. Extracts were prepared from pineapple, asparagus, maize, garlic and peas, and mycotoxin levels were measured after 14 days in liquid media and fungal mycelia. Cultures were separated by centrifugation, freeze-dried and dry weight of all cultures was measured and compared to the control culture with no extract added. In most of the cases, the mycelia contained much higher FBs levels than the respective media, implying that the mycotoxin may be bound by the pathogen to lower its effect on the fungus. **Acknowledgements.** This research was supported from the Polish National Science Centre Project 2011/01/B/NZ8/00162.

## P157

### **Fumonisins content in maize elite inbred lines after inoculation with *Fusarium verticillioides***

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Poland is the fifth European maize producer with an increasing cultivation area of this crop. Ear rots caused by *Fusarium* pathogens are important diseases affecting yield and causing grain contamination with mycotoxins. The country has variable weather conditions with significant differences between geographical regions and years. Because of this, *Fusarium verticillioides* and *F. graminearum* are two main ear rotting species commonly associated with maize kernels and their occurrence frequencies and prevalence depend on temporary environmental conditions. The aim of this study was to determine the variation for ear rots resistance and fumonisins contamination caused by *Fusarium verticillioides* among broad base early, mid-early and late groups of maize elite inbred lines. Plant materials belonged to different genetic categories, such as flint, dent, Lancaster, IDT and SSS types. For the field experiment, the RCBD (randomised complete block design) model was used. About 25 plants of maize inbred lines were grown in one row in three replicates. Each single ear of plants tested was inoculated with conidia suspensions 10-12 days after silking time during the 2011-2012 seasons and the disease symptoms were scored later. After the harvest, ears were separated into kernel and stalk (rachis) fractions and subjected to *Fusarium* species isolation and identification as well as mycotoxin quantification. Based on the obtained results, the group of the most resistant and most susceptible genotypes were taken to determine fumonisins content in the kernel samples and rachis using an HPLC method coupled to a fluorescence detector (FLD). All analysis were confirmed by LC-MS/MS. *F. verticillioides* was found as a major pathogen present in the samples tested but accompanied by *F. proliferatum* and *F. temperatum* identified occasionally as naturally infecting agents. Fumonisin contents varied greatly among the tested lines and also when rachis and kernel fractions were compared. Interestingly, natural infection (control plants) and artificial infection resulted in different mycotoxin accumulation patterns for rachis and kernel fractions.

#### **P158**

### **Varietal resistance in UK oats to HT-2 and T-2 producing *Fusarium langsethiae***

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In 2013, the European Commission published the recommendation to monitor the combined concentration of *Fusarium* mycotoxins, HT-2 and T-2 (HT2+T2) in food and feed that should be revised in 2015. These two toxins are the most potent trichothecene A mycotoxins with tolerably daily intake of 0.1 µg/kg bw/day. They can be produced by several *Fusarium* species, with *Fusarium langsethiae* identified as the main producer on UK oats. In studies across the UK between 2002 and 2008, around 16% of oat samples collected at harvest exceeded the indicative level of 1000 µg/kg HT2+T2 for unprocessed oats intended for human consumption. From 2005-2011, oats from over 20 winter and more than 14 spring national recommended list variety trials were analysed for the presence of HT2+T2 mycotoxins. All winter variety trials had higher levels of HT2+T2 compared to the spring variety trials. Much is known about the impact of agronomy on deoxynivalenol (DON) accumulation in wheat, however, this has not been clearly identified for HT2+T2 on oats. It is not clear whether the difference observed between winter and spring varieties is due to agronomic (i.e., drilling date) or genetic difference. To test the hypothesis that the difference observed was not due to agronomy, six spring and six winter varieties were drilled together in randomised block experiments at three locations in the UK in autumn and spring of two consecutive seasons (2012 and in 2013). Grain samples were collected after harvest and quantified for HT2+T2. Results indicate that in field experiments where high levels of HT2+T2 were detected, some varieties of winter oats had higher concentrations of HT2+T2 compared to other winter and spring varieties regardless of drilling date.

#### **P159**

### **Is height a susceptibility trait to *Fusarium langsethiae* infection on oats?**

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HT-2 and T-2 (HT2+T2) are the most potent trichothecene A mycotoxins. They are produced by several *Fusarium* species, with *Fusarium langsethiae* identified as the major producer on UK oats. In studies across the UK between 2002 and 2008, around 16% of oat samples collected at harvest exceeded the indicative level from the European Commission of 1000 µg/kg HT2+T2 for unprocessed oats intended for human consumption. Dwarf varieties of oats are short-strawed compared to conventional varieties. The mycotoxin concentration of dwarf varieties tends to be higher than

conventional varieties although the relationship between plant height and mycotoxin levels is not consistent. Differences may be due directly to the morphological trait of crop height or maybe due to genetic linkage. To test the hypothesis that the difference observed was not due to the morphological trait of crop height an experiment was conducted with two varieties whose height was additionally modified by plant growth regulator (PGR) treatments. Two winter oat varieties (Gerald and Balado) were drilled together in factorial designed experiment (variety x PGR dose) at one site in the UK in autumn of two consecutive seasons (2013 and 2014). Grain samples were collected after harvest and quantified for HT2+T2 and fungal biomass of the HT2+T2 producing *Fusarium langsethiae*. Preliminary results suggest that height might not be the predominant factor for the susceptibility of oats to HT2+T2 producing *Fusarium langsethiae*.

**P160**

### **Reaction of winter triticale breeding lines to *Fusarium* head blight and accumulation of *Fusarium* metabolites in grain in two environments**

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Resistance to *Fusarium* head blight of 75 winter triticale lines and 3 cultivars (Fredro, Borwo and Tomko) was evaluated. Triticale was sown in field experiments at two locations, Cerekwica near Poznań and Radzików near Warsaw. The experiment was established as a complete block design with two replication in 1 m<sup>2</sup> plots with a row spacing of 12.5 cm. At flowering, triticale heads were inoculated with three *Fusarium culmorum* isolates. FHB index was scored. The height of plants was evaluated. After the harvest, the percentage of *Fusarium* damaged kernels was assessed. Grain was analysed for the concentration of deoxynivalenol (DON), 3AcDON, 15AcDON and nivalenol using a gas chromatography technique. The Concentration of zearalenon was analysed with AgraQuant® ZON test kit. The average FHB indexes were differential at both locations and amounted to 20.1% in Cerekwica (range, 5.5-56.8%), and 12.4% in Radzików (range, 4.3-28.0%). The mean FHB index for the two locations was 16.3% (range, 7.6-35.4%). The heads of some genotypes were severely infected with yellow rust. This hindered FHB assessment and made correlation of FHB index at two locations insignificant. The height of plants varied greatly between locations; average in Radzików was 120.2 cm and less in Cerekwica (88.8 cm) where the soil is poor (sandy clay). Plant height did not correlated with FHB index in Radzików and weakly negative correlated in Cerekwica ( $r = -0.333$ ). For both environments, there was a significant but weak negative relationship ( $r = -0.235$ ). **Acknowledgements.** Research was supported by the Ministry of Agriculture and Rural Development, project HOR hn 801-8/14.

**P161**

### **Modelling the growth and mycotoxins production of *Aspergillus flavus* and *Aspergillus parasiticus* isolates in black pepper (*Piper nigrum* L.)**

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The main objective of the study was to characterise the growth and production of various mycotoxins by the isolates of *Aspergillus flavus* and *A. parasiticus* in black pepper whole corns and to develop predictive kinetic models describing the growth rate as a function of temperature and water activity ( $a_w$ ). The growth and mycotoxins production of two *A. flavus* and one *A. parasiticus* strain were studied following a full-factorial design at three temperatures (22, 30 and 37°C) and seven  $a_w$  (0.826-0.984) levels. The colony growth rate ( $\mu_{max}$ , mm/day) expressed as an increase in colony radius (mm) per unit of time and lag phase ( $\lambda$ , days) were estimated using the linear regression of the growth curves. The growth rate was modelled as a function of  $a_w$  using Ratkowsky, Rosso cardinal and Gibson models at different temperatures. Combined effect of temperature and  $a_w$  on growth rate was assessed using the

general polynomial, extended Gibson, Arrhenius Davey and multifactorial cardinal models. The selected *A. parasiticus* and *A. flavus* isolates followed, in general, a lag-linear growth curve in black pepper with few exceptions mainly under marginal conditions. Optimum growth was observed at 30 or 37°C depending on the isolate. The *A. parasiticus* isolate failed to grow only at the two very extreme growth conditions. The Rosso model using the square root of  $\mu_{max}$  ( $r^2=0.75-0.99$ ; root mean square error (RMSE) 0.13-0.43) and general polynomial model ( $r^2=0.89-0.94$ ; RMSE 0.18-0.26) gave the best fitting to the experimental data. The estimated minimal  $a_w$  was around 0.62-0.84 and optimal  $a_w$  varied from 0.88 to 0.95 depending on the type of the strain. Mycotoxins (aflatoxins B1, B2, G1, G2, sterigmatocystin and O-methyl sterigmatocystin) were analysed using LC-MS/MS. High variability in mycotoxins production restricted the modelling of mycotoxins production in black pepper. However, these predictive growth models could be used in prevention of mould growth which could eventually control the accumulation of mycotoxins in black pepper.

**P162**

### **RNAseq analysis of *Aspergillus flavus* transcriptome expressed during stressing growth conditions (oxidative stress and hypoxia) and consequential aflatoxin B1 synthesis**

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*Aspergillus flavus* is a cosmopolitan, saprotrophic and pathogenic fungus with a diverse host range, although it is mainly associated with cereal grains, legumes, and tree nuts. *A. flavus* is able to grow on several substrates exploiting its distinctive, enormous production of conidia. Infections may occur in the field, but often show no symptoms until post-harvest storage and/or transport. In fact, it is during the post-harvest phase that ideal conditions for growth usually take place. Furthermore, many *A. flavus* strains produce significant quantities of aflatoxins, in particular aflatoxin B1, which is regarded as the most carcinogenic metabolite synthesised in nature. Several environmental conditions are responsible for inducing aflatoxin synthesis, but oxidative stress in particular has been identified as the main event capable of causing a major boost in toxin production. To better understand how the different conditions may influence aflatoxin biosynthesis and the metabolic patterns responsible, we used RNA-Seq technology to profile the *A. flavus* transcriptome under different stressing events. Oxidative stress has been induced by addition of menadione 0.1mM to the culture medium. We also investigated transcriptome expression during hypoxia, as hypoxic stress is a condition that a fungus thriving on a substrate usually undergoes. Twelve cDNA libraries were prepared according to the Illumina protocol representing the transcriptome of *A. flavus* during hypoxic and normoxic environment, and separately during presence or absence of oxidative stress. All the experiments were conducted at three different time points (24, 48 and 96 h). Sequencing produced 9-19 million reads (50 bp each) per library, 94% of which was above the quality score 30. The raw sequence data were processed, filtered and normalised using the Illumina pipeline to generate fast-q files. All reads were mapped to *A. flavus* coding sequences using commercially available software to calculate the RPKM (reads per kilobase of transcript per million mapped reads) for each clustered gene. Multiple gene lists were generated corresponding to different RPKM-based comparisons between stress conditions and sampling times, ultimately enabling the identification of new and known differentially expressed genes. Protein interaction networks were then built. Fifteen genes were selected for differential expression validation by real-time PCR. In conclusion, with the high resolution and sensitivity afforded by RNA-Seq, we were able to get remarkable insight into the regulatory processes of aflatoxin biosynthesis in *A. flavus*.

**ADDENDUM TO POSTERS**  
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**Co-contamination of ochratoxin A, citrinin, trichothecenes and zearalenone in raw materials and feed**

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Mycotoxins mainly produced by species of the *Aspergillus*, *Penicillium* or *Fusarium* genus may occur in all kinds of feed and feed raw materials. Contamination by toxins is very common and co-contamination is frequently observed. The aim of the study was determination of the level of citrinin (CIT) contamination of selected grains (oats, barley, wheat, triticale and maize) and feed samples contaminated with ochratoxin A (OTA). Additionally, main *Fusarium* toxins, i.e., deoxynivalenol (DON), nivalenol (NIV), T-2, HT-2 and zearalenone (ZEN) were determined. The extraction of OTA and CIT was done on immunoaffinity columns Ochraprep (R-Biopharm) and CitriTest HPLC (Vicam), respectively. OTA and CIT were analysed with HPLC-FLD (Lachrom Elite; Merck-Hitachi). Trichothecenes and ZEN were determined by applying Bond Elut<sup>®</sup> mycotoxin columns and LC-MS/MS. The selected samples were contaminated with OTA in the range of 0.66-15.7, 0-86.3, 0.4-88.0 and 0.48-1.91 ppb for small grains, maize, feed and DDGS samples, respectively. Citrinin was detected in 25% of the grain samples, 16.7% of the maize samples and 6.9% of the feed samples. CIT was not found in DDGS. All the feed, DDGS and maize grain samples were contaminated with *Fusarium* mycotoxins. The highest amount of CIT (recently revised by EFSA) was detected in oat (116 ppb) and maize (325 ppb) grain. The research proved co-contamination of mycotoxins in feed, small grain, maize and DDGS samples. The results are summarised in the following Table:

Samples	Mycotoxin level (ppb)	OTA	CIT	DON	NIV	T-2	HT-2	ZEN
Feed (n=58)	positive mean	n=58 5.04	n=4 2.64	n=58 550	n=58 40,0	n=58 20.0	n=58 35.7	n=58 37.9
Maize grain (n=6)	positive mean	n=5 22.5	n=1 54.2	n=6 932	n=6 28.3	n=6 27.8	n = 6 37.3	n=6 94.4
Small grain (n=12)*	positive mean	n=12 8.5	n=3 11.2	n=11 238	n=11 248	n=10 18.0	n=11 55.4	n=10 8.18
DDGS (n=5)	positive mean	n=5 1.12	n=0 -	n=5 541	n=5 33.2	n=5 7.58	n=5 29.4	n=5 66.3

\*n=11 for DON, NIV, T-2, HT-2 and ZEN.

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**Mycotoxin situation in Nigeria**

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Following the outbreak of aflatoxicosis and the enormous economic loss in the poultry industry of Britain in 1961, the federal government of Nigeria initiated screening studies to determine the extent of aflatoxin contamination of groundnut and groundnut products in order to protect export trade. Since then, toxigenic fungi and mycotoxins have been found in various foods and feedstuffs in many regions of Nigeria. Mycotoxigenic fungi belonging to not less than forty-five fungal genera and about twenty different mycotoxins have been detected in Nigerian foods and foodstuffs, and more are continually being discovered. The predominant species are *Aspergillus*, *Fusarium* and *Penicillium*. About 150,000 new HCC-liver cancer cases reported annually in the world may be attributable to aflatoxin exposure. Nigeria has experienced high aflatoxin exposure levels in humans, especially in the northern Guinea and derived savannas and also reported the highest estimated number of cases of hepatocellular carcinoma attributable to aflatoxins in the whole world. A country-wide assessment was thus

commissioned by the Meridian Institute in 2013 supporting the Partnership for Aflatoxin Control in Africa (PACA) in collaboration with the mycotoxicology society of Nigeria (Mycotoxson), a not-for-profit organisation founded in 2006 and the first and only formalised mycotoxicology society in Africa. This country assessment was organised around the three pillars of PACA, i.e., agriculture and food security, trade and health that are adversely affected by aflatoxins. The identified crops of concern were maize and sorghum. At the national level, aflatoxin contamination in maize and groundnuts was estimated to be 7,761 liver cancer cases resulting in a total burden of 100,965 disability-adjusted life years (DALYs). At a prevalence rate of 20 µg/kg, the monetised burden of aflatoxin contamination was between \$112 and 942 million, which was about 0.5% of Nigeria's gross domestic product in 2010; thus, the impact of aflatoxin contamination was more likely to be felt in the health sector. Further work by local researchers showed that the cancer incidence attributable to dietary aflatoxins was between 25-9,120%. This is due to a lack of aflatoxin control in the agriculture sector and the lack of enforcement of aflatoxin standards in the domestic market. Furthermore, no premium is paid for aflatoxin-free commodities. Therefore, on 29 April 2013, Mycotoxson urged the UN to set aside a day to be known as 'mycotoxin day' due to the dangers posed by mycotoxins to food safety and public health.



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