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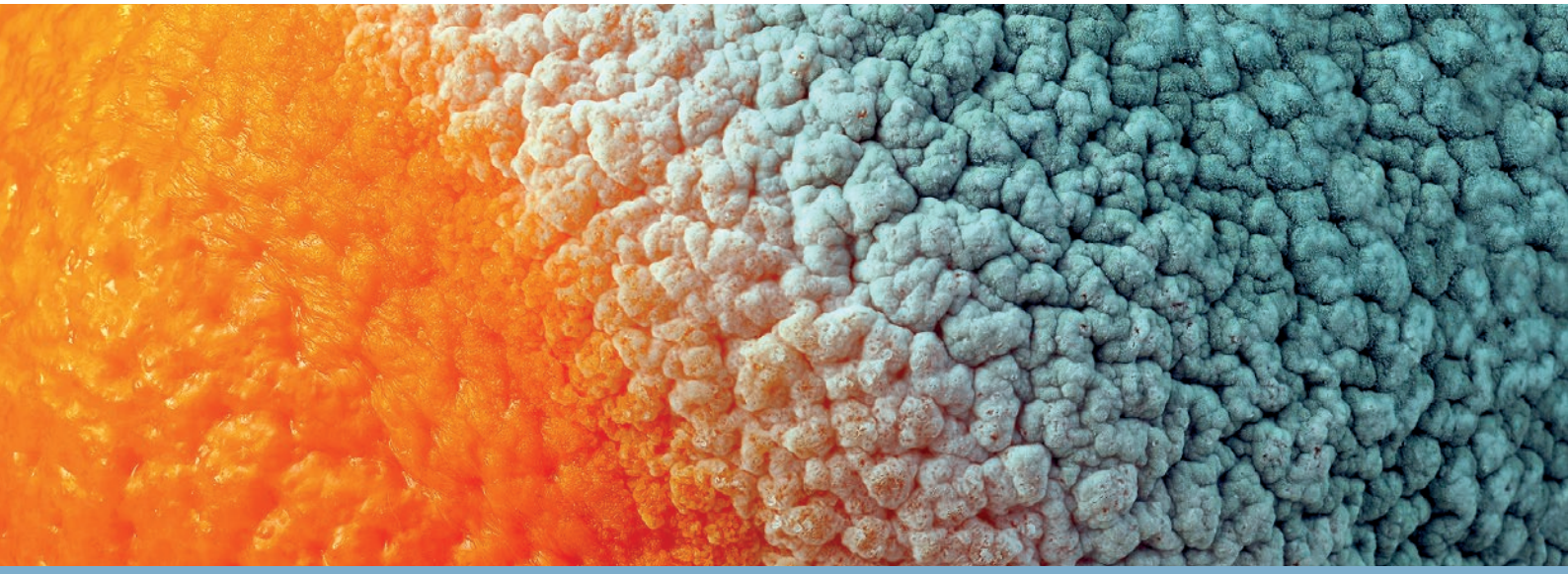

Max Rubner Conference 2018

# Fungi and Mycotoxins in Foods

October 8-10, 2018
Karlsruhe, Germany

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## Speaker Abstracts

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## Poster Abstracts

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# Speaker Abstracts

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# Mycotoxin Research at the MRI

**Rolf Geisen**
**Max Rubner-Institut, Department of Safety and Quality of Fruit and Vegetables**
**Karlsruhe, Germany**

The MRI conducts research in relation to aspects concerning food safety, food quality and nutrition with importance for the Federal Ministry of Food and Agriculture. In this respect, mycotoxins, which may occur in certain foods, are one issue in the range of topics concerning food safety. The tasks of the MRI are contributions to regulatory aspects as well as applied and basic research. Because the institutes of the MRI are organized according to the different food groups, questions concerning mycotoxin carry over to foods are treated at the Department of Safety and Quality of Milk and Fish Products as well as at the Department of Safety and Quality of Meat. Another important part of the mycotoxin work done at the MRI is the analysis of the occurrence of mycotoxins in the annual Germany-wide harvest of cereals to obtain a general overview about mycotoxin occurrence. These data are required by the ministry to be aware of changes in contamination risks. This work is carried out at the Department of Safety and Quality of Cereals. Lastly, the Department of Safety and Quality of Fruit and Vegetables is dealing with questions concerning the production and occurrence of mycotoxins in fruits and vegetables. Especially plant-type foods with a high water content are prone to fungal spoilage, and thus to mycotoxin production. The main toxins which are currently addressed at the MRI are the trichothecenes, the aflatoxins, ochratoxin, citrinin, patulin and the *Alternaria* toxins.

Applied research aspects treated at the MRI are measurements of carry over rates of aflatoxin from animal forage to cow´s milk, especially in high-yielding cows, the development and optimization of analytical methods as well as the development of measures to reduce mycotoxins in certain food commodities. Basic research aspects are genomics of mycotoxin producing fungi, analysis of molecular fungus-plant interactions, *in situ* transcription analysis of mycotoxin biosynthesis genes and the development of monitoring systems for *in situ* growth and mycotoxin biosynthesis.

The overall objectives of these approaches are the provision of methods and measures to increase and ensure food safety for the consumer as well as the provision of new data and concepts to support the ministry in questions concerning mycotoxin guidance and regulation.

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# Global mycotoxin problems, governmental regulations and MycoKey management in the food and feed chain

**Antonio F. Logrieco**
**Research National Council, Institute of Sciences of Food Production**
**Bari, Italy**

Mycotoxin occurrence in staple food commodities represents a great concern worldwide. These fungal metabolites constitute an important food safety hazard due to their demonstrated toxic, carcinogenic, teratogenic and mutagenic activity towards humans and animals. Mycotoxin contamination greatly affects global economy and international trade, accounting for billions of dollars lost every year all along the food and feed supply chain.

Mycotoxins are major food contaminants affecting global food security, especially in low and middle income countries. The management of good agricultural practices in the pre- harvest is a key issue for minimizing the risk of mycotoxin accumulation in the crops before the harvest. Such practices can involve crop rotation, tillage, proper fertilization and fungicide or biological control distribution, variety selection, timely planting and harvests. On the other hand, the reduction of mycotoxins along the food and feed chain is also highly depending from a correct post-harvest management that must aim firstly at the separation of the infected crop products from the healthy material. Therefore, the use of different tools such as manual sorting or optical sensors is also a crucial point for reducing the level of mycotoxin contamination of a given crop. Moreover, it is extremely important to prevent post-harvest contamination and develop practical and effective post-harvest procedures for mycotoxin reduction in the food supply chains and to provide alternative and safe use options for contaminated batches (e.g. by mycotoxin degrading/detoxifyying agents as enzymes, minerals, biopolymers and microorganisms).

An update review will be given on integrated management of pre-and post harvest practices aiming at the minimizing the risk of mycotoxin contamination along food and feed chains and main effective solutions, including the development of a MycoKey app, proposed and reached by EU project MycoKey (http://www.mycokey.eu/). In the MycoKey project a Mycotoxin Charter (http://charter.mycokey.eu/) was also launched with the aims to sharing the needs of a global harmonization regarding legislation and policies related to mycotoxins in order to optimize the efforts and minimize human and animal exposure worldwide with particular attention to poor and not regulated third countries.

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# The development and status of statutory regulations for mycotoxins in food and feed

**Hans P. van Egmond**
**Retired from RIKILT Wageningen University and Research**
**The Netherlands**

All nations have the right and the duty to protect their citizens and livestock from toxic substances in food and feed. Statutory regulations for mycotoxins have been established in food and animal feed in many countries since the late 1960's to safeguard humans and animals from the harmful effects that mycotoxins may cause. In Europe, and particularly in the EU, regulatory and scientific interest in mycotoxins has undergone a development in the last decades from autonomous national activity towards more EU-driven activity with a structural and network character.

Various factors may influence the decision-making process of setting limits for mycotoxins. These include information about the levels of human and animal exposure to mycotoxins and data about adverse effects, as well as the availability of methods for adequate sampling and analysis. Socio-economic factors such as commercial and trade interests and sufficiency of food supply have their impact as well. Weighing the various factors that play a role in the decision-making process to establish tolerable levels for mycotoxins is not a trivial process. Despite the dilemmas mycotoxin regulations have been established in many countries in the last half century, and newer and more detailed regulations are still being issued.

In the last decades various international enquiries were held on worldwide regulations and specific limits for mycotoxins. These were conducted by the National Institute for Public Health and the Environment (NL) for the Food and Agriculture Organization (FAO) of the United Nations, resulting in several FAO publications. Currently there are more than 100 countries worldwide with specific mycotoxin regulations for food and feed, while harmonised mycotoxin regulations exist in 5 larger regions. Worldwide, legal limits have been established for 13 mycotoxins and groups of mycotoxins: the naturally occurring aflatoxins, aflatoxin M1, agaric acid, deoxynivalenol, diacetoxyscirpenol, ergot alkaloids, fumonisins, ochratoxin A, patulin, phomopsins, sterigmatocystin, T-2 toxin and HT-2 toxin, and zearalenone.

In the presentation some insight will be provided in the way mycotoxin regulations and guidelines are achieved. A global overview will be given on specific limits and guidance values that exist for several major mycotoxins in food and feed in various parts of the world, with a more detailed focus on the EU mycotoxin regulatory situation. The question "Are mycotoxin regulations able to prevent health risks?" will also be discussed.

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# Mycotoxin control strategies: are they resilient enough under extreme environmental stresses?

**Naresh Magan**
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Staple food production is influenced by interacting abiotic and biotic factors. Under expected environmental stress in the next 25 years the resilience of key food production systems and the control of spoilage moulds and mycotoxins could be significantly compromised. There may be profound impacts on the food security agenda in terms of pre-harvest yield, and post-harvest losses. In the face of such environmental changes, the question arises as to whether the minimisation strategies for mycotoxigenic moulds and mycotoxins will have the necessary resilience.

While we are focussed on mycotoxins there are many associated impacts which are related and integral to the implementation of control strategies. For example, changes in pest diversity and reproduction may result in increased damage to staple crops, especially cereals, allowing increased infection by mycotoxigenic species and thus concomitant toxin contamination. While our focus is on mycotoxins, the calorific value of such contaminated staple commodities is also significantly impacted. Fungi, like many microorganisms, evolve rapidly to develop strategies for overcoming abiotic stress. We have some evidence that colonisation of maize by mycotoxigenic fungi such as *Aspergillus flavus* is resilient and not influenced by such climate-related abiotic factors, aflatoxin $B_1$ production may be increased.

We have used both RNAseq and phenotypic toxin production to evaluate these effects. It is also possible that control strategies would have to be changed to take account of possible acclimatisation by mycotoxigenic fungi under repeated exposure to extreme climate-related abiotic factors, which may enhance virulence and in some cases increase mycotoxin contamination. Acclimatisation aspects in the context of control strategies needs to be further examined. Strategies involving biocontrol of aflatoxins using atoxigenic strains have been very successful. The question arises as to whether the strains used will be resilient under climate-related environmental conditions or whether it will be necessary to modify the screening procedures or the formulations to ensure effective resilience for control of aflatoxins to be achieved in the future. Finally, in the context of climate-related abiotic stresses, the question arises as to whether legislation existing today may become out of step with the problems we face in the future. These aspects will be discussed in relation to the food security agenda and the implications that climate change-related scenarios may have on the resilience of control strategies for mycotoxins.

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# The ochratoxin A story in grapes and wine: Ecology, genomic and risk management

**Giancarlo Perrone, Massimo Ferrara, Michele Solfrizzo, Lucia Gambacorta, Filomena Epifani and Antonia Gallo**
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**Bari, Italy**

Ochratoxin A (OTA) is a potent pentaketide nephrotoxin diffusely distributed in food and feed products (grains, legumes, coffee, dried fruits, meats, beer and wine); it is also carcinogenic, neurotoxic, teratogenic and immunotoxic. This mycotoxin is produced by species of genus *Aspergillus* and *Penicillium*. OTA is the primarily mycotoxin risk in wine and dried vine fruits. Several studies focused on *Aspergillus* section *Nigri*, due to their role as causative agents of black rot of grapes, and subsequently as cause of ochratoxin A contamination. Nine different black *Aspergillus* species have been identified on grapes with different secondary metabolites profiles.

These species are often difficult to be identified by means of classical methods. The polyphasic approach used in our studies led to characterization of three new non toxigenic species occurring on grapes: *A. brasiliensis*, *A. ibericus* and *A. uvarum*. However, the main source of OTA contamination in grapes is *A. carbonarius*, followed by *A. niger* and *A. tubingensis*. This contamination is strongly related to climatic conditions, geographical regions (South Mediterranean climate is highly conducive), grape varieties, damage by insects, growing season (high susceptibility from early veraison to harvest, with a peak at ripening), and great variations may occur from one year to another.

Differently from other mycotoxins, the genes and the enzymatic stages involved in OTA biosynthesis pathway have remained unknown for long. In last years, genomics, transcriptomics and proteomics studies have provided new information to better define the molecular key steps of OTA biosynthesis. Genome sequencing of *A. carbonarius* led us to predict OTA cluster and to elucidate the key role of three genes (AcOTApks, AcOTAnrps and AcOTAhal) and the order of the enzymatic steps of the biosynthesis pathway. Other predicted genes in the cluster have been identified and analysed, such as a p450 monooxygenase and a transcription factor gene, likely involved in the structural and regulatory mechanisms of OTA production. Furthermore, transcriptomic analyses are in progress to study and clarify the complex genetic picture of the fungus during OTA biosynthesis at a deeper level. Interestingly, recent studies on climate change effects evidenced the influence of raising temperature and $CO_2$ levels on OTA production increase. Managing OTA contamination to reduce risks in grapes implies several strategies, such as implementations of good agricultural practices and risk maps, in association with the use of insecticides and fungicides when favourable climatic conditions occur. In addition, corrective actions can be adopted in wineries.

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# Bio-molecular diagnostics and high-throughput technology for surveillance of Fusarium Head Blight pathogens

**Tom Gräfenhan**
**University of Manitoba, Richardson Centre for Functional Foods and Nutraceuticals**
**Winnipeg, Manitoba, Canada**

Fusarium head blight (FHB) is an important disease of cereal grains that affects several segments of the grain industry worldwide, from production to processing and marketing. Losses from reduced yields are compounded by mycotoxin contamination and reduction in grain quality. Historically, mainly three *Fusarium* species *(F. graminearum, F. avenaceum and F. culmorum)* were associated with FHB infected wheat in western Canada. But over the last 20 years, *F. graminearum* has become the predominant species, which is known to produce a number of toxic trichothecenes including deoxynivalenol (DON), nivalenol (NIV) and calonectrin derivatives (NX).

These important phenotypic traits are also reflected by different genotypes of the pathogens. Agronomic practices and environmental conditions impose selective pressure on indigenous pathogen populations that are continuously adapting to changes of external factors. Shifts in the population structure of FHB pathogens cannot be detected or monitored by traditional agar plating and morphological observations. For this task, quantitative PCR (qPCR) methods have proven to be a powerful technology for the detection, identification and characterization of plant pathogens. Diagnostic tools utilizing species and/or trait specific qPCR assays allow high resolution strain typing of multiple target organisms simultaneously. However, sample preparation and limitations of proprietary platforms often pose bottle necks in the workflow, interfering with a more rapid characterization of *Fusarium* species and other pathogens.

For the surveillance and monitoring of FHB and other grain pathogens in Canada, we developed a high-throughput workflow including single kernel DNA extraction, bio-molecular assays and employing an open design qPCR system. The system consists of a quantitative real-time PCR unit and a multi-sample dispenser to rapidly change configuration of the nano-reaction chips allowing for more flexibility in the choice of diagnostic assays and for higher throughput. The newly developed workflow significantly increased our testing capacity, which comprises the analysis of more than three thousand harvest samples of cereal grains annually. Each crop year since 2014, 15,000–25,000 individual seeds of wheat, barley and oats were tested for common and quarantine plant pathogens as well as phenotypic traits such as trichothecene chemotype.

For grain producers and government authorities alike, results of the surveillance program on FHB and other fungal pathogens provide essential support to an integrated approach to on-farm risk management. In addition, open access to monitoring data and statistics allow the agrochemical industry to tailor more customized solutions to control regional FHB outbreaks and mycotoxin contamination in cereal crops. Our single-kernel workflow has also potential for assisting wheat and other cereal breeding programs with the selection of new lines for disease resistance.

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# Loop-mediated isothermal amplification (LAMP) assays for rapid and user-friendly diagnosis of mycotoxinogenic moulds in food sources

**Ludwig Niessen**
**Technische Universität München, Lehrstuhl für Technische Mikrobiologie**
**Freising, Germany**

The polymerase chain reaction (PCR) has become the Gold Standard for molecular diagnosis of microorganisms, including moulds. However, its demand for dedicated lab space, trained personnel and high-tech equipment as well as its proneness to inhibitory compounds present in sample materials has triggered the development of alternative technologies. LAMP makes use of a set of four primers binding to six binding sites within the target DNA and involves the use of a highly active thermophilic DNA polymerase with strong strand-displacement ability to amplify a portion of target DNA under isothermal conditions at 65 °C. The method is highly specific, rapid and does not need specific equipment. Moreover, crude DNA preparations or even total cells can be used as template.

Signal detection is by visual means, e.g. colour change of an indicator dye or occurrence of turbidity. During recent years, LAMP-based assays have been developed for the identification of various mycotoxinogenic moulds and for their detection in food and food raw materials. The LAMP method will be explained in detail and examples will be presented for the diagnosis of mycotoxin producers within *Aspergillus* and *Fusarium*.

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# Differentiation of toxigenic *Stachybotrys* isolates by MALDI-TOF MS

**Sebastian Ulrich, Christoph Gottschalk, Manfred Gareis**
**Chair of Food Safety, Veterinary Faculty, Ludwig-Maximilians-University of Munich**
**Oberschleissheim, Germany**

*Stachybotrys (S.)* spp. are omnipresent cellulolytic molds. Some species are highly toxic due to their ability to synthesize various secondary metabolites such as macrocyclic trichothecenes or hemolysins. Currently about 78 *Stachybotrys* spp. are described of which only two (*S. chartarum* Chemotyp S and *S. dichroa*) are known to produce the highly cytotoxic macrocyclic trichothecenes. These mycotoxins pose a serious health threat to humans and animals. The reliable identification of toxigenic *Stachybotrys* at species level is currently limited to genome-based identification and until recently, *Stachybotrys* spp. were claimed to be unmeasurable with MALDI-TOF MS techniques (Hettick et al., 2008). However, we could demonstrate that the differentiation of *Stachybotrys* strains by MALDI-TOF-MS is not only possible but also applicable for routine diagnostics (Ulrich et al., 2016).

Eleven reference strains of the American Type Culture Collection and the Technical University of Denmark were cultivated in triplicate (biological repetitions) for two days in malt extract broth. After washing the mycelia (1.5 ml) at first with 75 % ethanol, an additional washing step with dimethyl sulfoxide (10 %) was applied in order to remove unspecific low weight masses. Furthermore, mycelia were broken with roughened glass beads in formic acid (70 %) and acetonitrile. This optimized protein extraction protocol finally enabled the generation of MALDI-TOF MS reference mass spectra for eleven *Stachybotrys* species, among them the toxigenic *S. chartarum* chemotype. The method is in the meantime successfully adopted as important part in our routine work for identification of toxin producing *Stachybotrys* spp. isolated from animal feed samples such as hay and straw, but also from herbs and indoor/environmental samples.

The MALDI-TOF MS proved to be a fast and reliable method for identification and differentiation of *Stachybotrys* spp. but requires, as we have demonstrated, optimized pre-analytical processing steps.

Ulrich, S., Biermaier, B., Bader, O., Wolf, G., Straubinger, R. K., Didier, A., Sperner, B., Schwaiger, K., Gareis, M. & Gottschalk, C. (2016) Identification of Stachybotrys spp. by MALDI-TOF mass spectrometry. Anal Bioanal Chem 408, 7565-7581, DOI: 10.1007/s00216-016-9800-9

Hettick, J. M., Green, B. J., Buskirk, A. D., Kashon, M. L., Slaven, J. E., Janotka, E., Blachere, F. M., Schmechel, D. & Beezhold, D. H. (2008) Discrimination of Aspergillus isolates at the species and strain level by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry fingerprinting. Anal Biochem 380, 276-281

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# Impact of rainfall on *Fusarium* mycotoxins in wheat milling fractions

**Simon Edwards**
**Harper Adams University**
**Shropshire, UK**

Legislative limits for *Fusarium* mycotoxins decrease from unprocessed wheat to processed products. It is therefore essential for processors to understand the ability of their processes to reduce mycotoxins and factors that can influence these reductions. A previous observational study identified a seasonal difference in the distribution of deoxynivalenol (DON) but not zearalenone (ZON) within mill fractions. Rainfall is known to influence the production of these mycotoxins in wheat, but the effects of rainfall on their distribution within mill fractions is less understood. Field experiments were conducted to determine the impact of different watering regimes on the concentration of DON and ZON in harvested wheat grain and on their distribution in wheat mill fractions. Results indicated that pre-harvest rainfall is a requirement for ZON to be produced whereas DON appears to be in flux with increased grain moisture allowing further DON production and heavy rainfall causing leaching of DON from the grain.

For the mill fractions, repeated wetting and drying could cause movement of DON towards equilibrium across the mill fractions. Whereas, high levels of rainfall could cause a large reduction of DON in the grain, predominantly from the bran fraction, resulting in a proportional increase within white flour. ZON was detectable in fewer samples but results indicated it is less mobile within the grain.

It is important for processors to be aware of the variation of mycotoxin distribution within mill fractions and the drivers of this variation to ensure limits set for grain intake result in mill products within mycotoxin legislative limits.

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# Emerging and masked mycotoxins: Beyond traditionally determined food contaminants

**Franz Berthiller**
**University of Natural Resources and Life Sciences, Vienna**
**Tulln, Austria**

Mycotoxins can be defined as toxic secondary metabolites of molds. The term “emerging mycotoxins” is not so clearly defined though. One approach defines these compounds as “mycotoxins, which are neither routinely determined, nor legislatively regulated; however, the evidence of their incidence is rapidly increasing”.

The increasing findings of certain fungal metabolites might be explained by:
1. improved analytical techniques, which allow the concurrent determination of a large number of target analytes (e.g. multi-toxin methods);
2. “neglected” mycotoxins, which are not (yet) regulated (e.g. enniatins or moniliformin)
3. “surprising” finding of mycotoxins in parts of the world, in which they were not encountered beforE (e.g. due to climate change)
4. newly detected toxins (e.g. NX-toxins, or masked mycotoxins)

“Masked mycotoxins” are plant metabolites of mycotoxins. While the toxicological relevance of many of those compounds is still unknown, it has been clarified for the most important forms.

The above categories have in common that liquid chromatography – mass spectrometry (LC-MS) based methods play a crucial role in their discovery and in the determination of their occurrence. The rapid development of LC-MS methods allows to obtain a broad picture regarding fungal contamination of food. By far not all of the detected compounds are toxicologically relevant and therefore of little or no health concern to consumers. The presentation aims to give an overview on emerging mycotoxins and to illustrate some recent discoveries on their occurrence and toxicity.

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# Uptake and biotransformation of *Fusarium* mycotoxins in micropropagated *Triticum durum* Desf.

**Chiara Dall'Asta**
**Department of Food and Drug, University of Parma**
**Parma, Italy**

Plants employ different detoxification mechanisms to cope with the adverse effect of xenobiotics, such as mycotoxins. Multiple enzymatic pathways may lead in the formation of modified mycotoxins that, once entered the food and feed production chain, may significantly contribute to the overall toxic load related to mycotoxin exposure. Understanding the plant metabolism of mycotoxins and thus the toxicological role of resulting modified forms, is becoming increasingly important for risk assessment. While an increasing number of studies have been performed on deoxynivalenol and other Fusarium mycotoxins in soft wheat and barley, less is known about the biotransformation in durum wheat.

In vitro techniques represent a consolidated approach to investigate the metabolic fate of mycotoxins enabling the characterization of phase I and II biotransformation products. For this purpose, an in vitro model was set up to elucidate the uptake and metabolic fate of DON, ZEN, T2 and HT2 in durum wheat, using five wheat varieties with different level of resistance. In addition to plants, leaves and roots experiments were independently set up to study the organ- and tissue-biotransformation dependency.
Tissues and growing media have been analysed by HR-LC/MS to return the full profile of metabolites produced in plants. A large spectrum of phase I and phase II biotransformation metabolites were depicted, some of them never reported before. When possible in silico/in vitro approaches have been used for a preliminary evaluation of their toxicological relevance.

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# Modified forms of T2 and HT2-toxin: Identification, occurrence, intestinal metabolism

**Hans-Ulrich Humpf**
**Institute of Food Chemistry, Westfälische Wilhelms-Universität Münster**
**Münster, Germany**

T2 and HT2-toxin belong to the group of type A trichothecene mycotoxins and are mainly produced by *Fusarium sporotrichioides, F. poae and F. langsethiae*. They can be found in grain and grain products and consumption of contaminated food or feed can lead to toxic effects in humans and animals. For this reason the *European Food Safety Authority* has established a group tolerably daily intake (TDI) of 100 ng/kg body weight/day in 2011 which was amended in 2017 to 20 ng/kg body weight/day. Besides the parent mycotoxins modified forms came more and more into the focus during the last years. The term „modified mycotoxins“ summarizes in general all forms which differ in their chemical structure from the parent toxin. Such modified mycotoxins can be formed either during biotransformation by microorganisms, infested plants or the mammalian organism or by chemical reactions e.g. during food processing [1]. As modified mycotoxins are in most cases not detectable with routine analytical methods their occurrence might lead to an underestimation of the potential health risk of mycotoxins for humans or animals.

In this talk the structure elucidation of modified forms of T2 and HT2 formed either during food processing or during plant metabolism will be presented. Key question is especially in the case of mycotoxin conjugates whether they can be converted into the parent mycotoxin during digestion. To answer this question we developed different strategies for the synthesis of mg amounts of T2 and HT2 glucosides in both α and ß configuration as reference compounds [2]. The intestinal metabolism of the obtained glucosides was studied in the pig-cecum model. The results show that the parent toxins are released (and *metabolized*) during digestion contributing to the overall toxicity.

[1] Humpf, H.-U. et al. Modified mycotoxins: A new challenge? In: Encyclopedia of Food Chemistry, Elsevier, 2018.
[2] Schmidt, H.S. et al. Glucosylation of T-2 and HT-2 toxins using biotransformation and chemical synthesis: Preparation, stereo¬chemistry, and stability. Mycotoxin Res., 2018, doi.org/10.1007/s12550-018-0310-9.

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# Partnership to improve food security & food safety in developing countries: MYTOX-SOUTH

**S. De Saeger, A. Vidal and M. De Boevre**
**Ghent University, Faculty of Pharmaceutical Sciences,**
**Centre of Excellence in Mycotoxicology and Public Health**
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Food Safety takes a prominent role in the Food Security problem. Mycotoxins, toxic fungal secondary metabolites, are one of the main food safety threats in developing countries. Aflatoxins for instance cause liver cancer, while aflatoxins and fumonisins are related to stunting in African children (IARC, 2015). Co-occurrence of multiple mycotoxins in one crop as well as effects of climate change make this research field complex. The mycotoxin problem needs to be tackled in a multi-disciplinary way primarily focusing on prevention measures, but also mycotoxin analysis for monitoring and control purposes is definitely needed.

Moreover, in Africa and other developing countries, the possibilities for regular mycotoxin analysis are scarce to non-existent: analytical tests are expensive, there is a lack of expertise and training, there is insufficient technical support from companies selling analytical instruments and the focus is mainly put on aflatoxins, while other mycotoxins are being neglected.

To obtain a substantial mycotoxin reduction, a holistic approach is needed in which all different research fields of mycotoxicology, together with (international) stakeholders such as food industry and governments work together. MYTOX-SOUTH (http://mytoxsouth.org) is an intercontinental, multi-disciplinary partnership striving to improve food security and food safety through mitigation of mycotoxins at global level with the following long-term goals:

1. building human and infrastructural capacity through training of South partners,
2. bridging the gap between research and the development and
3. stimulate the environment for a fruitful public-private partnership to create a sustainable network.

This presentation will give examples of projects developed together with the South to study risk management strategies. Mainly, it will highlight the need for capacity development and how this can be practically achieved through training and education.

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# The Mycotoxin Menace in Sub-Saharan Africa

**Gordon S. Shephard**
**Institute for Biomedical and Microbial Biotechnology, Cape Peninsula University of Technology**
**Bellville, Cape Town, South Africa**

Dietary exposure to mycotoxins remains an important public health concern across the African continent, especially in sub-Saharan Africa, where climatic conditions are frequently favourable for the growth of toxigenic fungi. Geographically, the continent lies astride the equator and many agricultural areas lie in regions conducive to *Aspergillus flavus* and various *Fusarium* species. Although a wide range of mycotoxins has been detected in crops in sub-Saharan Africa, most attention has been drawn to aflatoxins and, to a lesser extent, fumonisins.

Much of African agriculture is conducted on a subsistence basis or by small holder farmers. Agricultural inputs can be poor and in many cases crop drying and on-farm storage are inadequate to prevent the growth of storage fungi and consequent increase in mycotoxin levels. Sorghum and millets were the traditional African cereal crops, but over the decades these have been supplanted by maize, which is more easily grown but susceptible to both aflatoxin and fumonisin contamination, either separately or together. In parts of West Africa and the Sahel, peanuts (groundnuts) are grown as an additional crop and problems with aflatoxin contamination are well documented.

The subsistence nature of many farmers means that few food choices are available and in many rural communities maize is consumed at daily levels of 400 g to 500 g per person. Health consequences of aflatoxin exposure have been found both in liver cancer and childhood stunting, as well human fatalities from aflatoxin poisoning (aflatoxicosis) in Kenya and Tanzania in East Africa due to consumption of contaminated maize. The issue is compounded by food insecurity, such that the only food available may be the contaminated crop and no alternatives can be obtained in the rural situation.

Unlike developed market economies, not all African countries have regulations concerning mycotoxins in food. Even where these exist, the challenges of surveillance and enforcement are many. A complicating factor is that small rural village markets and subsistence farmers with on-farm consumption are not covered by any regulations. It was in these areas that the devastating cases of aflatoxicosis emerged. These outbreaks highlight the need for vigilance in African settings and the formation of protocols to deal with these emergencies. Consideration needs to be given to appropriate fit-for-purpose analytical methods that could be used in field settings to identify aflatoxin contamination as the causative agent in outbreaks of liver disease of uncertain origin. Addressing all these challenges requires a multifaceted approach, partly tailored at the national level to address the supply chain, but also the introduction of community appropriate measures for small holder farmers. The Partnership for Aflatoxin Control in Africa (PACA) has received large-scale funding to address the aflatoxin problems, whereas the presence of co-occurring mycotoxins is infrequently acknowledged and remains mostly unaddressed.

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# Toxicity of mycotoxin mixtures

**Isabelle P. Oswald**
**INRA, Toxalim, Research Center in Food Toxicology**
**Toulouse, France**

Food is contaminated by multiple contaminants, mycotoxins being the most frequently occuring natural ones. Mycotoxins co-contamination is confirmed on the one hand by the co-occurrence of these toxins in food and feed stuff and on the other hand by co-exposure monitoring survey. The co-occurrence of mycotoxins in food and feed is explained by three different reasons: (i) most fungi are able to simultaneously produce several mycotoxins, (ii) commodities can be contaminated by several fungi simultaneously or in quick succession, and (iii) the complete diet comprised different commodities. In practice, the co-occurrence of mycotoxins represents the rule and not the exception. Besides mycotoxins, food can be contaminated with other contaminant such as heavy metals.

The toxicity of combinations of contaminant 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 contaminant have been carried out taking into account only one compound. A synergistic effect between trichothecenes mycotoxins was observed both for intestinal cytotoxicity and inflammatory response and the synergy was already seen at low doses. The combined exposure to DON and Cadmium was also studied in several human cell lines and interactions were specific to the target organ.

The importance of microbiota in intestinal health is gaining interest, with this aim the interaction between DON and microbiota was investigated. We demonstrated that DON exacerbated the intestinal DNA damages induced by Escherichia coli stains producing colibactin raising questions about the synergism between food contaminants and gut microbiota.

This demonstrated that mycotoxin cocktails can lead to synergistic interaction and that mycotoxin contamination should be taken in the global context of all food contaminants and the host intestinal microbiota.

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# Biomarker-based assessment of human exposure to citrinin

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Citrinin (CIT) is produced by several fungal species of the genera *Penicillium, Aspergillus*, and *Monascus*, and detected in various grains, foods, feeds and other commodities. CIT can exert nephrotoxicity in rodents and domestic animals, with pigs as most sensitive species. EFSA has set a human tolerable daily intake (TDI) of 0.2 µg/kg b.w. [1], yet noted that available CIT contamination data in food are inadequate for a dietary exposure assessment.

Since biomonitoring provides useful insights into exposure to mycotoxins, we developed a sensitive LC-MS/MS method for the detection of CIT and its metabolite dihydrocitronone (DH-CIT) in human fluids. It was applied to determine these biomarkers in urines of German adults, and revealed frequent exposure in this cohort [2], yet with average levels lower than those measured in urines from Bangladeshi cohorts [3].

But, estimating dietary mycotoxin intake based on urine biomonitoring data requires data on kinetics of CIT and its conversion to DH-CIT. Thus, we recently conducted a study in two volunteers who ingested CIT at doses below the TDI, and analysed the biomarker levels in urine and blood collected at timed intervals after dosing [4]. Blood plasma concentration-time profiles indicated a short half-life of CIT (median 9.4 h). Concentration-time profiles for the urinary analytes showed that CIT undergoes conversion to DH-CIT which is excreted along with parent compound. The cumulative excretion within 24 h of both analytes (sum of CIT and DH-CIT or 'total CIT') accounted for 40% (median value) of the ingested mycotoxin dose.

The new data on daily excretion for 'total CIT' now served to calculate a provisional daily CIT intake using published urine biomarker data, and compare the outcome to the TDI of 0.2 µg/kg b.w. set by EFSA [1]. Biomarker-based estimates for CIT intake in European cohorts were well below this TDI whereas CIT exposure of a Bangladeshi cohort (0.21±0.49 µg/kg b.w.) exceeded the TDI [4]. Furthermore, CIT and DH-CIT were analyzed repeatedly in morning urines of two male volunteers on their usual diet, to study biomarker variability over time (1-7 weeks) and exposure in persons with different food habits. Biomarker levels in the individuals' urines were rather constant, and indicated clear differences in their dietary CIT exposure. Their calculated CIT intake (based on the highest biomarker levels found) differed 3-fold, yet remained below the TDI for this nephrotoxic mycotoxin.

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# Aflatoxin: Food chain transfer from feed to milk

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Aflatoxins are formed by moulds of the genus *Aspergillus* under unfavourable conditions in feedstuffs. These mycotoxins are of great importance due to their high toxicity and strong carcinogenicity. Aflatoxin $M_1$ is a metabolite of aflatoxin $B_1$ formed in the liver that can be detected in milk when aflatoxin $B_1$ contaminated feed is used. In the WHO Global Burden of Disease (GBD) report, aflatoxin is one of the main issues. The intake of aflatoxins with food, even the regular intake of small amounts, causes serious health risks, which should be avoided at any rate. When looking at the aflatoxin problems in milk, efforts should focus on minimizing the exposure of dairy animals to aflatoxin containing feed.

After in 2013 aflatoxin contaminated feed was fed to dairy cows in Germany, some farms had to stop milk delivery and to discard the milk. Subsequent residue studies showed that the carry-over of aflatoxin $B_1$ from feed into milk was higher than the assumed rate of 1-2%. It seemed that high-yielding cows (> 30 kg milk yield/day) had a higher carry-over rate of approx. 6%, which is in coincidence with investigations from Israel with extremely high-yielding cows (> 11,000 kg/year).

The estimation of the transfer rate of aflatoxin $M_1$ is of major importance in order to determine the acceptable aflatoxin $B_1$ intake from feed. A maximum residue limit of 5 µg/kg in compound feed for dairy cattle, has been established for the EU (EU 574/2011). In the food sector, the maximum levels are set to achieve the following limits for aflatoxin $M_1$ in milk (EU Regulation 165/2010): Milk: 50 ng/kg; Infant milk formula and follow-on milk: 25 ng/kg; according to the German Contaminants Ordinance (KmV 2010), the value for dietary foods for babies or infants has been further reduced to 10 ng/kg. If the carry-over rate exceeds 1-2%, the maximum MRL of 50 ng/kg for milk, cannot be achieved by feeding compounds fulfilling the limit of 5 µg/kg.

Therefore, a carry-over experiment of aflatoxin $B_1$ into milk with high-yielding cows was performed at the experimental station of the MRI. The calculated carry-over was about 2% (range 1-3%), which is consistent with literature data (1 to 6%). These results show, that the current limit for feed (5 µg/kg AFB1) even in high-yield cows the residue level of 50 ng/kg AFM1 in milk can be kept.

In addition to the carry-over experiment part of the milk produced was processed into cheese (Edamer type) and yoghurt after reaching the plateau of aflatoxin $M_1$ excretion. For cheese the distribution of toxin in whey and curd was 75% and 25% respectively. During ripening no degradation was observed. The same was due for yoghurt.

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# Biocontrol of pathogen and toxicogenic fungi to reduce the entry of mycotoxins in the food and feed chains

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Plant pathogenic fungi *Aspergillus flavus*, and especies within the *Fusarium graminearum* complex infect seeds of the most important food and feed crops, including peanuts, maize, and wheat,. These fungal species produce aflatoxins,,and trichothecenes, specially deoxynivalenol, respectively, which threaten health and food security worldwide. Fusarium head blight (FHB) is a devastating disease that causes extensive yield and quality losses to wheat and other small cereal grains worldwide. Species within the *Fusarium graminearum* complex are the main pathogens associated with the disease, *F. graminearum* sensu stricto being the main pathogen in Argentina. Peanuts and maize are two important crops in Argentina economy and depending of the harvest season aflatoxin contamination can occur due to *Aspergillus* section *Flavi* infection. Different strategies including crop rotation, tillage practices, fungicide application and planting less susceptible cultivars are used in order to reduce the impact of pathogen and toxigenic fungi in the food and feed chains.

The development of fungicide resistance together with the rising of public concern on the risks associated with pesticides use led to the search for alternative environmentally friendly alternatives. Biocontrol offers an alternative tool that can be used in the frame of an integrated pest management to reduce the accumulation of mycotoxins in the food and feed chains.

This strategy is one of the most promising approach for preventing aflatoxin contamination in peanuts and maize at pre-harvest stage. Populations of native *Aspergillus flavus* non aflatoxin producers were evaluated based on phenotypic, physiological and genetic characteristics. Selected strains *A. flavus* nor aflatoxin neither cyclopiazonic acid producers were evaluated under field trials The efficacy of single and mixed inocula as potential biocontrol agents was evaluated through different harvest seasons. Reductions of aflatoxin contamination between 78.% and 89.% were observed in treated plots in comparison with the un-inoculated control plots. Two potential biocontrol agents *Bacillus velezensis* RC 218 and *Streptomyces albidoflavus* RC 87B were selected to evaluate their effectiveness to reduce *Fusarium* head blight severity and deoxynivalenol accumulation on both bread and durum wheat. Both *B. velezensis* RC 218 and S. *albidoflavus* RC 87B effectively reduced FHB incidence (up to 30%), severity (up to 25%) and deoxynivalenol accumulation (up to 51%) on durum wheat under field conditions.

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# Food safety management to control fungi and mycotoxins along the tropical food supply chain

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The concept of Food Safety Objective (FSO) is the maximum frequency and/or concentration of hazard in the food at the moment of consumption which gives an appropriate level of health protection. The concept of FSO was developed by the International Commission on Microbiological Specifications for Foods (ICMSF) as a way of understanding the effects of handling and processing on levels of microbial pathogens in foods. We have shown that this concept can also be applied to the formation and control of mycotoxins. This presentation provides a general overview of how the concept of FSO can be used to understand the increases and decreases in mycotoxin levels in foods, on the basis that international regulatory limits are equivalent to an FSO.

Examples on the ecology of the formation of some mycotoxins throughout the food supply chain are provided and control measures available to manage them in order to meet an FSO. Relevant mycotoxins such as aflatoxins, fumonisins and ochratoxin A in tropical food (maize, cocoa, coffee, brazil nuts and peanuts) are visualized using a novel graphical approach. For studying FSO, the important starting point is to know when a particular mycotoxin is produced. For example, *Fusarium* toxins are produced preharvest, ochratoxin A is produced postharvest and aflatoxins are produced both preharvest and postharvest. Different fungi produce different mycotoxins in different crops at different times.

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# Control of Mycotoxins in the Food Chain

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Mycotoxins are secondary metabolites of fungi with toxic effects on humans and animals, occurring frequently in many commodities, as cereals, nuts, fruits and products of animal origin. In many cases, mycotoxins are produced during crop growing, as a result of conducive environmental conditions. At this stage, their production may be reduced by adopting preventive good practices. At post-harvest, again if conducive conditions are present, mycotoxins may accumulate but, depending on the mycobiota, different fungi may develop and other mycotoxins may be produced, resulting in the contamination with different mycotoxins.

Food processing will also affect the mycotoxin content. Although most of the mycotoxin are resilient, it is well established that the final mycotoxin content may be reduced during processing. In this presentation, two case studies will be explored - aflatoxin M1 in dairy products, and ochratoxin A in wine – using the food safety objective approach.

The impact of climate change has been identified as an emerging issue for food and feed safety. The occurrence of mycotoxin in cereals due to climate change is expected to change, since *Aspergillus* species may find more suitable conditions for their development. Consequently, the level of aflatoxin contamination in maize for feed can increase. The shortage in good quality feeds can result in feed security problem and it may boost a food safety issue, due to the secretion of aflatoxin M1 in milk by dairy cattle, and its consequent carry-over to dairy products. When dairy products are manufactured from milk containing AFM1, the toxin is transmitted to the resulting products. In this food chain, safety objective as well as performance criteria should be considered.

The occurrence of OTA in wine is mainly due to the accumulation of OTA in grapes before the initial steps of processing. Most of the steps involved in winemaking will contribute to a decrease in OTA content, being the final carry-over of OTA the result of the combination of all stages in the wine chain. Changes in initial OTA content in grapes will determine changes in final OTA level in wine, unless mitigation steps are included. Again, climate change may affect current situations, and food safety objective should be closely monitored.

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# Technological Measures to Control Mycotoxin Concentration along the cereal chain

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The occurrence and dispersion of mycotoxins in crops and food are highly variable and depend on the nature of infection, the type of fungi, the terms during cultivation and at harvest time, the storage or transportation conditions and the diverse treatments during food production. Due to this variability, the technological measures influencing the mycotoxin concentration are of great diversity.

One example of cereal contamination interesting the public sphere is the major cause of sclerotia and resulting ergot alkaloid content in grain and grain based food. Since the risk evaluation of the European Food Safety Authority (EFSA) resulted in low tolerable intake levels, the whole German cereal production chain is prompted to minimize sclerotia and ergot alkaloid contamination at any step of grain handling. In addition, the Codex Alimentarius Commission, the intergovernmental body with over 180 members established by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), recently adopted an annex for minimization of sclerotia and ergot alkaloid contamination in the Code of Practice for the Prevention and Reduction of Food and Feed contamination (ISBN 978-92-5-107119-9).

Even when the recommendations to prevent and reduce mycotoxin contamination were strictly applied, it cannot be excluded that residues will remain in the final product. It is therefore essential to reveal production factors as appropriate for setting guideline values or maximum limits in cereal based food items. Furthermore, the official bodies monitoring food safety issues need to be able to resort to harmonised and validated methods for reliable detection and quantification of contaminants once maximum levels have been established. Additional rapid test systems enabling the contamination level are desirable at every step along the cereal production chain.

The presentation will address the before mentioned objectives based on examples, relevant literature and own investigations.

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# New Methods to Prevent Fungal Growth and Mycotoxin Biosynthesis in Foods

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A quarter of the world-wide crop is spoiled by filamentous fungi and their mycotoxins and weather extremes associated with the climate change lead to further deterioration of the situation. The ingestion of mycotoxins causes several health issues leading in the worst case to cancer in humans and animals. Common intervention strategies against mycotoxin producing fungi, such as the application of fungicides, may result in undesirable residues and in some cases to a stress induction of mycotoxin biosynthesis.

Moreover, development of fungicide resistances has greatly impacted pre- and postharvest fungal diseases. Hence there is the need to develop alternative strategies to reduce fungal infestation and thus mycotoxin contamination in the food chain. As alternatives, the use of light of specific wavelength, nanoparticles, supplements such as coumarin or mycoparasites such as *Trichoderma harzianum* have been proved for being effective and sustainable applications to substitute or complement classical antifungal strategies.

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# Poster Abstracts

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# Comparison of internal and external dietary mycotoxin exposures in five European populations: strategies for risk assessment

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In Europe, most mycotoxins that are known to contaminate food were assigned a non-zero maximum limit by the governing authorities. Consequently, practically the entire European population undergoes chronic, low-dose, variable dietary exposure to mycotoxins throughout their lives. In order to associate adverse health outcomes with dietary mycotoxin exposure, it is a requirement to accurately characterize the true extent to which mycotoxins are contaminating the diets of populations and of individuals. To this end, dietary analysis is considered a “gold standard” for estimating intake of not just nutrients, but food contaminants as well.

This external estimate of 38 food-borne mycotoxins was compared to internal measurements of the same plus 11 of their metabolized forms in the blood and urine, to verify if the calculated exposures correlate, or can be used to validate each other. National cohorts, subsets of the EFCOVAL study population, of approximately 60 individuals each from Belgium, the Czech Republic, France, the Netherlands, and Norway were analysed by LC-MS/MS. Mycotoxin exposure assessments are presented not only to characterize the issue of mycotoxin contamination in these countries, but also to help characterize each assessment method itself. The practical and logical strengths and weaknesses of each are presented, along with recommendations for further studies that may add substantial value to datasets acquired through these methods

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# Fungal Profile and Mycotoxin Contamination in Animal Feed in Urban and Peri-urban Zones of Bamako

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In animal production in Mali, food appears to be the major constraint. In fact, mycotoxin contamination of animal feed is common and widely spread in West Africa. Due to their ubiquity, mycotoxin producing moulds are capable of reducing the nutritional value of animal feed by elaborating several mycotoxins. Animal feed contaminated with mycotoxins has adverse effects on animal health and productivity. Also, mycotoxins may be carried over into meat and milk when animals are fed with contaminated feed. Samples of feed used for animal nutrition in Urban and Peri-urban Zones of Bamako were randomly collected and analyzed for fungal flora and natural incidence of mycotoxins. Ten mould genera were recovered, six of them known to be mycotoxigenic.

More than 11 species were determined. Fumonisins, deoxynivalenol and zearalenone were detected in all the samples, while Aflatoxins were not detected in samples from Massala. Thirty-six out of 36 samples were contaminated with zearalenone, 34 out of 36 were contaminated with Fumonisins and 26 out of 36 were contaminated with deoxynivalenol. Also, 7 out of 36 samples were contaminated with aflatoxins. This study indicates the need for continuous assessment of the mycological status of animal feed production, in order to ensure food safety.

# Modified Forms of Alternariol and Alternariol Monomethyl Ether and their Resorption and Metabolism in Human Caco-2-Cells

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Fungi of the genus Alternaria are ubiquitous and can contaminate a wide range of food and feed. Some Alternaria strains produce toxic secondary metabolites such as Alternariol (AOH) and Alternariol monomethyl ether (AME). Both mycotoxins have already been found in various foods including tomatoes, carrots, and cereals.

Similar to mammalian metabolism, contaminated plants are able to metabolize mycotoxins by altering their chemical structures, e.g. by conjugation with glucose. As a result, compounds are formed that are not detected in routine analysis. Due to the possible release of the parental toxin during digestion, it is important to elucidate the structures of the conjugated mycotoxins and to investigate their bioavailability.

Incubation of tobacco plant cell suspension cultures with AOH and AME resulted in metabolites, which were analyzed with 2D-NMR. For both toxins, AOH and AME, two glucosides and two malonylglucosides as well as one AOH diglucoside were unambiguously identified [1].

In order to assess the relevance of these so-called “modified” mycotoxins in intact plant tissues, tomatoes were infected with spores of Alternaria alternata. Besides small amounts of the already mentioned glucosides, sulfate conjugates were detected in large amounts, too. Subsequent studies showed that they were not formed by the tomato but by the mold itself. To study their metabolism in plants, the sulfate conjugates were incubated in tobacco suspension cells, resulting in the formation of three sulfoglucosides of AOH and one sulfoglucoside of AME. Their structures were unambiguously determined by using NMR. These metabolites were also found in A. alternata infected tomatoes. Mixed sulfate / glucoside diconjugates may also represent modified forms of other mycotoxins containing two or more hydroxyl groups [2].

Furthermore, first experiments with Caco-2 cells were performed. This system is a common in vitro model to study intestinal absorption as well as the metabolism of xenobiotic substances. Upon incubation of Caco-2 cells with glucosylated AOH and AME conjugates, a partial release of the parental toxins AOH and AME has already been demonstrated leading to an increase in the total exposure to the toxin.

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# Mycotoxin control as upcoming issue in legume production - Preliminary results

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Legumes are generally known as valuable plant protein sources. However, their production requires special expertise. In Germany, the Demonstration Network Pea and (Faba) Bean (DeMoNetErBo; funded by the BMEL) aims at advising farmers how to grow legumes efficiently and searches for ways of establishing feed and food value-added chains. On EU level, a similar approach for various kinds of legumes is funded in terms of the project LegValue. In both projects, University of Hamburg is analysing large sample sets and helps to develop criteria catalogues for quality parameters such as protein content, starch quality, tannins, fibres, etc. One very important quality aspect is the content of potentially adverse compounds including trypsin inhibitors, saponins and mycotoxins. Data on mycotoxin contamination in legumes is sparse, partly because only soy und peanuts fall under EU mycotoxin legislation and for other legumes no maximum levels have been set yet. Still, some studies suggest that zearalenone (ZEN) may contaminate soy products [1], while fumonisins have been reported for peas [2] and aflatoxins and ochratoxin A (OTA) can be formed as post-harvest contamination, e.g. on beans [3].

In order to systemize and ultimately help to control mycotoxin contamination, a large repository of authentic samples with known cultivars, regions, growing and storage conditions will be analysed. For this purpose, an analytical multi-mycotoxin method using tandem mass spectroscopy coupled to high performance liquid chromatography (LC-MS/MS), based on stable isotope dilution assay (SIDA), was developed and validated for grains and soy. The method is based on the draft of an upcoming CEN method and covers aflatoxins ($B_1$, $B_2$, $G_1$ and $G_2$), OTA, HT-2 toxin, T-2 toxin, fumonisins ($B_1$ and $B_2$), deoxynivalenol and ZEN quantitatively in the ppb range. It also includes a qualitative determination of various emerging mycotoxins such as enniatins, *Alternaria* toxins, further trichothecenes, modified forms of ZEN as well as phomopsins A and F. The latter hepatotoxic compounds are known to be formed primarily on lupins but also on other legumes such as beans and peas [4]. Samples from DemoNetErBo have been investigated exemplarily to get an overview on the situation of possible contaminations. While the legume dedicated projects will improve the acceptance and exploitability of the crop in general, the mycotoxin investigation will enhance the safety of legume based food and feed products in particular.

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# Occurrence of ochratoxin A in cereal-derived food products consumed in Kosovo

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Ochratoxin A (OTA) is one of the most prevalent mycotoxin contaminants of food crops. Among the agricultural products consequently contaminated by OTA are cereals. A survey was carried out during 2017–2018 in Kosovo, to assess food safety associated with the occurrence of OTA residues in different cereal food products. A total of 110 samples were collected, from domestic and imported products. Competitive enzyme-linked immunosorbent assay (ELISA) was used to measure the occurrence and concentration of OTA in the samples, and liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to confirm the presence of OTA in samples in which the toxin was detected by ELISA.

Overall, 12 % of the samples were contaminated with OTA. The frequency of OTA contamination was nine of 60 samples (15 %) in 2017, and four of 50 samples (8%) in 2018. OTA was detected in four wheat flour samples (9 %), five maize flour samples (15%) and in three rye flour sample (8%). The levels of OTA in the contaminated samples ranged from 0.26 to 1.95 µg $kg^{-1}$ in the four wheat samples, from 0.77 to 2.75 µg $kg^{-1}$ in the five maize samples and 0.77 to 1.31 µg $kg^{-1}$ in the three rye samples. None of the contaminated samples exceeded the maximum OTA levels of 3 µg $kg^{-1}$, set by the European Union Regulation and Kosovo Food Codex.

Presence of OTA in food products is considered to be serious problem for human health whereas this toxin (OTA) is recognized as possible carcinogenic to both human and animal health by International Agency for Research on Cancer. The results of this study suggest that cereal-derived products consumed in Kosovo presents no risk by human consumers exposure to OTA through their consumption. This is the first investigation about OTA in food commodities in our country, and needs continues monitoring and evaluation by the state Agencies.

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# Optimization of LC-MS/MS conditions for multi-mycotoxin analysis for mycotoxins biomarkers

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Mycotoxins have been ranked as an important chronic dietary risk factor. Their chemical structure, physical and chemical properties are widely varied. Usually, mycotoxins are present in biological fluids at low concentrations (ngmL-1). The analysis of suitable biomarkers of exposure can help with assessment of mycotoxin exposure. Thus, a sensitive method able to determine a wide range of mycotoxins and its metabolites is required.

The aim of this study was optimizing the LC-MS/MS conditions for analysis of about 30 toxins including deoxynivalenol (DON), zearalenone (ZEN), aflatoxin B1 and B2, T-2 toxin (T-2), HT-2 toxin (HT-2), fumonisin B1 (FB1), their metabolites and additionally: citrinin, nivalenol (NIV), fusarenon-X (FUS-X), diacetoxyscirpenol (DAS), sterigmatocystin, beauvericin (BEA) and enniatins (ENNs).

The mass spectrometer (QTrap 6500, Sciex) was operated in the multiple reaction monitoring mode (MRM) in positive and negative ionization mode, detecting the fragmentation of the mycotoxins molecular ions/ adducts. Ion source parameters were optimized. Several kinds of compositions of mobile phases, including organic modifiers (acetonitrile, methanol) and organic salts like ammonium acetate (1-20 mM) were tested. Four analytical columns: Luna: C18, C18 Omega Polar, C18 Omega PS and Phenyl-Hexyl (2 × 150 mm, 3 µm, Phenomenex) were tested. Different injection volumes, gradients, and flow rates were optimized to find suitable conditions of determination.

The most suitable mobile phase for ZEN determination consisted of acetonitrile - compared with methanol ten times higher signal intensity. The highest signal intensity and optimal peak shape for DON, T-2, and HT-2 were achieved with mobile phase with methanol - ten times higher in positive ionization mode compared with acetonitrile. Use of different columns resulted in similar signal intensity, chromatographic separation and peak shape for all analytes except fumonisins – their determination with Luna Phenyl-Hexyl resulted in very low S/N ratio and broad peaks. Ion source temperature was a crucial parameter to optimize for DON, NIV, FUS-X, DAS, ENNs, BEA, FB1, and FB2. Lower temperature: 250-350 °C (compared with 600 °C) resulted in about ten times higher signal intensity.

Finally, the following conditions were chosen: column - Luna Omega Polar (2 × 150 mm, 3 µm), mobile phase - 10mM ammonium acetate acidified with acetic acid and methanol, injection volume - 5 µL, flow rate - 600 µLmin-1, ESI-source temperature - 350 °C. Chromatographic run-time was 15 min. These LC-MS/MS conditions will be applied for analysis of mycotoxins biomarkers.

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