PROGRAMME & REGISTRATION

THE INTERNATIONAL
NETWORKING CONFERENCE FOR
THE FOOD AND FEED INDUSTRY

THE World Mycotoxin Forum

THE THIRD CONFERENCE

10-11 November 2005

GRAND HOTEL HUIS TER DUIN
NOORDWIJK AAN ZEE
THE NETHERLANDS

FINAL PROGRAMME
&
ABSTRACTS OF LECTURES AND POSTERS

THE
World Mycotoxin Forum®
THE THIRD CONFERENCE

The international
networking conference for
the food and feed industry

10–11 November 2005
Noordwijk aan Zee, the Netherlands

the World Mycotoxin Forum®

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Dr. Gordon Shephard	Medical Research Council, South Africa
Dr. Angelo Visconti	Institute of Sciences of Food Production (ISPA CNR), Italy

Welcome at the third conference of World Mycotoxin Forum®!

Dear participant,

The **World Mycotoxin Forum® –** the international networking conference for the food and feed industry – is the leading international meeting series on mycotoxins of interest to the food and feed industry.

The main objectives of the **World Mycotoxin Forum®** are:

- to provide a unique platform for the food and feed industry, 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; and
- to discuss strategies for prevention and control of mycotoxin contamination ensuring the safety of food and feed supply.

We are pleased to present you the lecture and poster abstracts of the third conference of the **World Mycotoxin Forum®**. As a comprehensive overview the third conference of the **World Mycotoxin Forum®** offers an excellent way to network and to share ideas, providing a reference source for anyone involved in this field. Invited speakers only will present their contributions to the conference in plenary meetings, parallel sessions and discussions. Poster sessions, spotlight presentations, and the concurrent instrument/manufacturers exhibition are an integral part of this conference. They provide the opportunity for intensive informal discussion.

The following key issues will be addressed at the third conference of the **World Mycotoxin Forum®**:

- regulatory issues and international developments;
- progress on major mycotoxins and emerging problems;
- mycotoxin prevention: what's on the horizon;
- trends in mycotoxin analysis; and
- mycotoxins in the feed chain.

The **World Mycotoxin Forum®** invites you to take part in the discussions with participants from different disciplines and to meet business relations in your area. We wish you an active and fruitful meeting!

On behalf of the Advisory Committee,

Daniel Barug

the World Mycotoxin Forum®

Forum Secretariat

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Key to the abstracts of lectures and posters:

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

PROGRAMME

Thursday 10 November 2005

08.30 **Opening of the third conference of the World Mycotoxin Forum®**
Hans P. van Egmond, M.Sc.
National Institute of Public Health and the Environment (RIVM), the Netherlands

Plenary meeting
Regulatory issues and international developments

08.45 **Keynote presentation: food issues**
Mycotoxins in the context of food risks and nutrition issues
Dr. Gordon S. Shephard
Medical Research Council, PROMEC Unit, South Africa

09.30 **Keynote presentation: feed issues**
Moulds and mycotoxins as undesirable substances in animal feed
Prof.dr. Johanna Fink-Gremmels
Utrecht University, Faculty of Veterinary Medicine, Division of Pharmacology, Pharmacy and Toxicology, the Netherlands

10.15 **Networking break: coffee and tea**

10.45 *Decision-making process and overview of recent and future EU legislation on mycotoxins in feed and food*
Frans Verstraete
The European Commission, Health and Consumer Protection Directorate-General (DG-SANCO), Brussels, Belgium

11.15 *Chinese approach towards international regulations*
Dr. Xiumei Liu
Ministry of Health, Chinese Centre for Disease Control and Prevention, National Institute of Nutrition and Food Safety, China

11.45 *The impact of mycotoxin legislation on world trade*
Dr. Felicia Wu
University of Pittsburgh, Graduate School of Public Health, USA

12.15 *Research and regulatory priorities in the US*
Dr. Jane F. Robens
US Department of Agriculture, Agricultural Research Service (USDA-ARS), National Program Leader Food Safety and Health, USA

12.45 *Launch of the International Society for Mycotoxicology*
Dr. Angelo Visconti, Institute of Sciences of Food Production (ISPA CNR), Italy
The International Society for Mycotoxicology (ISM) is being founded in 2005 and will be formally launched at the third conference of the **World Mycotoxin Forum®**. The objectives of the Society are to promote research on mycotoxins and toxigenic fungi thereby leading to prevention and reduction in exposure to mycotoxins, enhanced food safety and a greater public awareness of this area.

13.00 **Buffet lunch and exhibition**

Thursday 10 November 2005

Parallel session 1
Progress on major mycotoxins and emerging problems

Chair: Dr. Angelo Visconti
Institute of Sciences of Food Production (ISPA CNR), Italy

14.00 *EFSA scientific opinions on mycotoxins in feed*
Prof.dr. John Gilbert
Central Science Laboratory, UK

14.30 *Occurrence of Fusarium mycotoxins in feed*
Prof.dr. Manfred Gareis
Federal Research Centre for Nutrition and Food (BFEL), Institute for Microbiology and Toxicology, Germany

15.00 *Aerosol mycotoxins in indoor air: animal and human health effects*
Dr. Eeva-Liisa Hintikka
Finnish Institute of Occupational Health, Finland

15.30 **Networking break: coffee and tea**

16.00 *Emerging issues in Central Europe – would mycotoxins be a threat?*
Prof.dr. Diana Bánáti
Central Food Research Institute, Hungary

16.30 *Emerging issues in Southern Europe: the Italian case of aflatoxins*
Prof.dr. Gianfranco Piva
Università Cattolica Sacro del Cuore, Institute of Food Science and Nutrition (ISAN), Italy

17.00 *Overview of the MYCO-GLOBE specific support action activities*
Dr. Ranajit Bandyopadhyay
International Institute of Tropical Agriculture (IITA), Nigeria

17.30 – 18.10 **Spotlight presentations theatre 1**
A special presentation facility for sponsors and exhibitors to promote their products in more detail.

17.30 *Mycotoxin analysis - from screening to confirmation: the ochratoxin A case*
Dr. Ronald Niemeijer
R-Biopharm, Germany

17.50 *New developments and applications for mycotoxin detection*
Dr. Cor Arts
Euro-Diagnostica, the Netherlands

18.10 End of spotlight presentations theatre 1

18.10 – 19.00 **Poster presentations at the Scientific Café**

Thursday 10 November 2005

Parallel session 2
Mycotoxin prevention: what's on the horizon?

Chair: Dr. Alain Pittet
Nestlé Research Center, Switzerland

14.00 *Structural and functional genomics of the aflatoxigenic Aspergillus flavus*
Dr. Deepak Bhatnagar
US Department of Agriculture, Agricultural Research Service, Southern Regional Research Center (USDA-ARS-SRRC), USA

14.30 *Proactive identification of emerging mycotoxins: a holistic approach*
Dr. Marcel Mengelers
The Food and Consumer Product Safety Authority (VWA), the Netherlands

15.00 *Biocompetitive exclusion of toxigenic fungi*
Dr. Peter J. Cotty
University of Arizona, Department of Plant Sciences, USA

15.30 **Networking break: coffee and tea**

16.00 *The use of GMOs as a prevention strategy for mycotoxin formation*
Dr. Bruce G. Hammond
Monsanto, USA

16.30 *Applications of forecasting deoxynivalenol in wheat using DONcast for grain producers, the grain industry and regulators*
Prof.dr. Arthur W. Schaafsma
University of Guelph, Ridgetown College, Canada

17.00 *Technologies for the reduction of mycotoxin contents in wheat*
Dr. Urs Dübendorfer
Bühler, Switzerland

17.30 – 18.00 **Spotlight presentations theatre 2**
A special presentation facility for sponsors and exhibitors to promote their products in more detail.

17.30 *Quantitative determination of aflatoxin in grain from 0 to 25 ppb using a lateral flow assay and reader*
Dr. Steven J. Saul, Charm Sciences, Inc, USA

17.50 *A new fluorescence based test kit for rapid and quantitative determination of deoxynivalenol in grains*
Dr. Zaneta Kubus, IFA-Tulln, Austria

18.00 End of spotlight presentations theatre 2

18.00 – 19.00 **Poster presentations at the Scientific Café**

Friday 11 November 2005

Parallel session 3
Trends in mycotoxin analysis

Chair: Ton van Osenbruggen
TNO Quality of Life, the Netherlands

09.00 **Extensive lecture**
Mycotoxin analysis: an overview of classical, rapid and emerging technologies
Prof.dr. Rudi Krska
IFA-Tulln, Center for Analytical Chemistry, Austria

09.45 *Multi-mycotoxin analysis by LC-MS/MS in a single sample extract*
Dr. Martien Spanjer
The Food and Consumer Product Safety Authority (VWA), the Netherlands

10.15 *Immunoaffinity clean-up/fluorescence detection methods for mycotoxins*
Dr. Michelangelo Pascale
Institute of Sciences of Food Production (ISPA CNR), Italy

10.45 **Networking break: coffee and tea**

11.15 *Standardisation activities in mycotoxin methodology*
Marcel de Vreeze, M.Sc.
NEN, the Netherlands

11.45 *How to handle measurement uncertainty?*
Dr. Joerg Stroka
EC-DG JRC Institute for Reference Materials and Measurements (IRMM), Belgium

12.15 – 12.55
Spotlight presentations theatre 3
A special presentation facility for sponsors and exhibitors to promote their products in more detail.

12.15 *Immunoaffinity column technology - multiple analyte columns with HPLC and LC/MS: a view toward the future of mycotoxin testing*
Dr. Patricia Jackson
VICAM, USA

12.35 *Ultra performance LC-MSMS for improved analysis of mycotoxins*
Dr. Sandra Rontree
Waters Corp., the Netherlands

12.55 End of spotlight presentations theatre 3

13.00 **Buffet lunch and exhibition**

Friday 11 November 2005

Parallel session 4
Mycotoxins in the feed chain

Chair: Manfred J.C. Hessing
Nutreco, the Netherlands

08.45 *The potential carry-over of various mycotoxins into animal products*
Dr. Sven Dänicke
Federal Agricultural Research Centre (FAL), Institute of Animal Nutrition, Germany

09.15 *Current concepts in the etiology and prevention of mycotoxicosis in livestock and poultry*
Prof.dr. Trevor K. Smith
University of Guelph, Department of Animal and Poultry Science, Canada

09.45 *The potential of dietary mycotoxin sequestering agents*
Prof.dr. Timothy D. Phillips
Texas A&M University, College of Veterinary Medicine & Biomedical Sciences, USA

10.15 Adsorptive chemical properties of yeast cell wall based product toward several mycotoxins
Dr. Alexandros Yiannikouris
National Centre of Scientific Research (CNRS), Group of Bio-inorganic Analytical Chemistry, France

10.45 **Networking break: coffee and tea**

11.15 *Detoxification of mycotoxins by biotransformation*
Dr. Gerd Schatzmayr
Biomin, Austria

11.45 *Mycotoxin feed issues in Latin America*
Dr. Magda Carvajal
Universidad Nacional Autónoma de México, Instituto de Biología, Mexico

12.15 – 12.45
Spotlight presentations theatre 4
A special presentation facility for sponsors and exhibitors to promote their products in more detail.

12.15 *The success and failures of mycotoxin adsorbents in basic research and field applications*
Dr. Karl Dawson
Alltech, USA

12.35 *Mycofix® Plus - three combined strategies guarantee success in mycotoxin control*
Dr. Diane Schatzmayr
Biomin, Austria

12.45 End of spotlight presentations theatre 4

13.00 **Buffet lunch and exhibition**

Friday 11 November 2005

14.00-16.00 **Brief presentations and panel discussion**

Moderators
Hans P. van Egmond, M.Sc.
National Institute of Public Health and the Environment (RIVM), the Netherlands

Jan Willem van der Kamp, M.Sc.
International Association for Cereal Science and Technology (ICC), Vienna, Austria

Panel members

Dr. Deepak Bhatnagar	US Department of Agriculture, Agricultural Research Service, Southern Regional Research Center (USDA-ARS-SRRC), USA
Denis Ketabi	CAP Industries, France
Frans Verstraete	The European Commission, Health and Consumer Protection Directorate-General (DG-SANCO), Brussels, Belgium
Prof.dr. Chris Wild	University of Leeds, Centre for Epidemiology and Biostatistics, Molecular Epidemiology Unit, UK
Dr. Felicia Wu	University of Pittsburgh, Graduate School of Public Health, USA

Purpose and organisation of the panel discussion
What we need to address in the future to bring uniformity in trade and identify the reality of the mycotoxin problem? How do we generate the resources to try to solve the mycotoxin problem as a whole? The purpose of this session is to discuss debatable issues of mycotoxins in worldwide regulations, economics and health.

During the first part of the discussion the panellists will give brief presentations (10-15 minutes each) on:

- Worldwide regulations
  Are they working? Being implemented? Are they too stringent? Are some nations demanding too much of other nations?
- Economic impact of mycotoxins
  What is real? Direct and indirect costs? Are we underestimating?
- Health hazards
  Are mycotoxins true health hazards? There are so many varied reports worldwide, claiming all sorts of things. We need to sort the real ones and the common ones out.

During the second part of the discussion questions from the participants will be answered.

16.00 Highlights and conference closing

Jan Willem van der Kamp, M.Sc.
International Association for Cereal Science and Technology (ICC), Vienna, Austria

16.30 End of the third conference of the World Mycotoxin Forum®

LECTURES

Mycotoxins in the context of food risks and nutrition issues

Gordon S. Shephard
Medical Research Council, PROMEC Unit, South Africa

The consumption of food, essential for the continuance of life, carries with it significant risks over a broad range of categories extending from the dietary imbalances leading to the epidemic of obesity in many areas of the world, the food insecurity and consequent poor nutrition in many developing countries to the presence of a wide range of contaminants of universal concern. The significance of these contaminants to human health varies depending on whether acute or chronic effects are being considered. For the former, microbiological contamination and consequent food poisoning ranks as the primary concern in all societies. However, based on scientific risk analyses, mycotoxins may be considered as a major concern for chronic health effects. It is widely recognised that whereas over 300 fungal mycotoxins are known to exist, there are five agriculturally important toxins (aflatoxin, fumonisin, ochratoxin A, deoxynivalenol and zearalenone) whose known or suspected effects on human health is of a nature to warrant significant attention. These mycotoxins are natural contaminants formed in the field or in storage and their presence in cereal and other crops can be greatly reduced, but not totally eliminated.

Concern over chronic carcinogenicity has led the International Agency for Research on Cancer (IARC) to evaluate these mycotoxins and classify aflatoxin B_1 and its natural mixtures as Group 1 (carcinogenic to humans) and fumonisin B_1 and ochratoxin A as Group 2B (possibly carcinogenic to humans). Although the carcinogenic effects of mycotoxins have long received prominence, more recent concerns have arisen over growth retardation and childhood stunting (aflatoxins), immunosuppression (aflatoxins and deoxynivalenol), neural tube defects (fumonisins) and possible endocrine disruption (zearalenone).

Concerns over food-borne toxins are increasingly being addressed by science-based risk analysis, which incorporates risk assessment, risk management and risk communication. Risk assessments, with their components of hazard identification, hazard characterisation, exposure assessment and risk characterisation, have been performed for mycotoxins by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The exposure assessments and hence risk characterisations are clearly influenced by the type and quantities of food consumed by individual populations or groups within a population. The health risks posed to populations in the developed world are generally orders of magnitude lower than those posed by contaminated diets in the developing world. Given the dietary variety available and consumed, the market competition for quality food, the presence and ability to enforce legislative regulations and the possibilities for management posed by food processing industries, the populations of the developed world are mostly well protected. Recently published surveys based on food baskets in northern Europe have confirmed this low exposure in the general population. Much the opposite is true in the developing world, where a lack of dietary variation, lack of financial means to purchase alternative produce and the absence or unenforceable nature of regulations imply great risks from food contamination. This situation is exacerbated in subsistence farming communities with limited choices and resources, as evidenced in 2004 by the deaths of over 100 people in rural Kenya due to acute aflatoxicosis.

The 2002 WHO World Health Report listed global threats to human health. The divide between developing and developed world can clearly be seen in that five of the top ten risks

(underweight, unsafe sex, iron deficiency, indoor smoke from solid fuels and unsafe water, sanitation and hygiene) apply predominantly to the former, while the other five threats (high blood pressure, tobacco consumption, alcohol consumption, high cholesterol and obesity) can be seen as developed world problems, which also begin to manifest in developing world populations as socio-economic conditions improve. In the listed human health threats faced by developing countries, mycotoxins are not specifically mentioned. However, they may be seen to play a modulating role in many of these factors. Aflatoxin exposure in weaning foods has been linked to childhood underweight and stunting in west Africa, while the immunological suppression associated with aflatoxin could play a vital role in many of the diseases (e.g., AIDS, gastric diseases from unsafe water, respiratory tract infections from indoor fuels) associated with these health threats. The modulating effect of aflatoxin in cases of zinc, iron and vitamin A deficiency in human health is not clear, but evidence from animal nutrition would suggest it could be significant.

Changing agricultural patterns across the developing world can also bring about changes to mycotoxin exposure and nutritional patterns. In Africa, the indigenous cereal sorghum, which is less susceptible to mycotoxin contamination, is rapidly being replaced by non-indigenous maize as a dietary staple. At the same time, the introduction of biotechnology interventions such as genetically modified maize to reduce mycotoxin exposure is subject to considerable problems.

It may be concluded that whereas current or impending measures greatly protect general populations in developed countries, special population groups such the young, the old, the pregnant and the infirm, may need careful consideration. However, across the developing world, significant improvements in food safety are urgently needed. It is to be hoped that the drive to achieve the UN Millennium Development Goals will concomitantly reduce food risks from mycotoxins.

Moulds and mycotoxins as undesirable substances in animal feed

Johanna Fink-Gremmels
Utrecht University, Faculty of Veterinary Medicine, Division Pharmacology, Pharmacy and Toxicology, the Netherlands

The history of mycotoxins as contaminants of animal feeds reflects the recent trends in animal farming. Originally directed towards local markets, professional animal husbandry has emerged into a competitive global business in which basic feed components, by-products as well as the ultimately obtained animal-derived products are subjects of international trade. The first prominent mycotoxicosis reported in the UK in the 1960s, resulting from groundnuts imported from Brazil, still marks the increasing awareness of animal health professionals that the quality of feed materials plays a pivotal role in animal health management and cost-effective production.

For many years, mycotoxins in animal feeds were primarily assessed with regard to potential health hazards for the consumer, resulting from carry-over of mycotoxins or their toxic metabolites into animal-derived products. Many countries established for example maximum tolerance levels for aflatoxin M_1 in dairy milk in the 1970s and 1980s. The second toxin of public health concern was ochratoxin A (OTA), which due to its long biological half-life, forms undesirable residues in bloods serum, kidneys and liver of, for example, fatting pigs, resulting in prescribed maximum tissue levels in the Scandinavian countries. Quantitative exposure assessment revealed, however, that the contribution of animal- derived products to overall human exposure to OTA is generally small (with the exception of children that might be high consumers of certain regional specialities), and hence no further measures to define maximum tolerance levels for feed material were taken at the European level. This applies also for other mycotoxins, as carry-over rates into edible products of animals are too low to form a public health risk. Hence, the group of aflatoxins remained the only class of mycotoxins for which a harmonised European feed legislation was established.

Largely unnoticed by the general public, many countries with an intensive animal production, however, established so called guidance levels for the feed industry in consideration of the detrimental effects of mycotoxins on animal productivity. The background of these guidance levels often remains obscure. While some countries based their guidance levels on clinical reports in the veterinary literature, others propagated a zero tolerance for undesirable substances including mycotoxins in animal feeds. As the latter approach creates trade barriers, transparency regarding the assessment of feed quality parameters was requested. A quantitative risk assessment of the impact of mycotoxins on animal health and productivity, faces a number of intrinsic obstacles:

Interactions with feed components and co-exposure

Mycotoxins are not a homogenous group of toxins, and their chemical diversity explains why any monolithic assessment of the adverse effects is not justified. The evaluation of adverse effects has to be individualised, summarising the toxicological characteristics of a defined chemical, i.e. an individual toxin. The available toxicological data in target animal species, however, are in most cases too incomplete to allow a dose-response assessment for relevant toxicological endpoints. For example, the protein content of the diet as well as the individual liver function explains inter-individual and inter-species differences in the tolerance towards of individual aflatoxins and in the rates of carry over of aflatoxin M_1 into dairy milk. Numerous plant ingredients (as well as other contaminants) modulate the hepatic CYP 450 activity, the enzyme family that is responsible for the formation of the aflatoxin-epoxides, representing the ultimate toxic principle of aflatoxins. Animal rations are composed of

commercially acquired mixed feeds or concentrates, supplemented to a varying degree with on-farm produced materials, the latter escaping any quality control measures as exemplified by the unexpected outbreak of aflatoxicosis (and increased aflatoxin M_1 concentration in milk) in Italy in 2003. Finally, the tolerance to the mycoestrogen zearalenone (ZEN) remains a matter of controversial debate, as different age groups respond different to toxin exposure. Hence at least in pigs, the degree of adverse effects varies with time point of exposure. As in the living animal, ZEN is converted into the more potent alpha-zearalenol, maturity of the liver, the major organ performing this biotransformation step, as well as co-exposure to other hormonally active compounds determines the outcome of toxin exposure.

Taken together the current practice in animal nutrition of farm animals results inevitable in the exposure to various toxins, also because of the fact that most fungal species produce more than one toxin at the same time.

Interaction with infectious agents

Deoxynivalenol (DON) is commonly measured as marker of exposure to trichothecenes. The no-effect level for DON has been derived form studies measuring feed intake and weight gain in mice, and (in consideration of their higher sensitivity towards DON) in pigs. Recently, this approach is challenged by the increasing insight into the molecular mechanisms of DON as modulator of cellular signal transduction pathways. Evidence suggests an interaction between DON and bacterial diseases, resulting in a lower tolerance of individual animals towards DON and an increasing susceptibility towards infectious agents following DON exposure. Moreover, practical experience indicated that DON impairs humeral immunity, leading to disease outbreaks in animals, which had been vaccinated according to standard protocols. Immune suppression had been described for many toxins, including the trichothecenes, aflatoxins, fumonisins and ochratoxins.

Conclusion

Mycotoxins have currently to be considered as unavoidable contaminants of many feed materials in all parts of the world, not only in tropical or subtropical regions. The degree of contamination is influenced by the local climate, but also by the current agricultural practice during pre-harvest plant production, harvesting, and post-harvest measures to prevent fungal contamination. Animal health professionals have to consider the adverse health effects of feed contaminants when establishing the diagnosis in cases of reproduction disorders, reduced weight gain and low performance on a farm. For the animal health industry it remains a challenge to quantify the adverse effects of (moderate) toxin concentrations, and to develop intervention strategies at the farm level.

Decision-making process and overview of recent EU legislation on mycotoxins in food and feed

Frans Verstraete
The European Commission, Health and Consumer Protection Directorate (DG-SANCO), Brussels, Belgium

The EU harmonisation of legislation on contaminants, including mycotoxins, in food fulfils two essential objectives: the protection of public health as major objective, but also to ensure the proper functioning of the internal EU-market.

Council Regulation (EEC) No. 315/93 of 8 February 1993 laying down Community procedures for contaminants in food is the framework for the Community action on contaminants, including mycotoxins, in food. This Regulation does not apply to contaminants, which are the subject of more specific Community rules such as pesticide residues, veterinary drug residues, etc. The framework Regulation provides:

- that food containing a contaminant in an amount, which is unacceptable from the public health viewpoint, shall not be placed on the market;
- that contaminant levels shall be kept as low as can reasonably be achieved by following good practices at all stages of the production chain (ALARA);
- that, in order to protect public health, maximum levels for specific contaminants shall be established where necessary (by comitology);
- for mandatory consultation of a scientific body (EFSA) for all provisions which may have an effect upon public health;

A scientific risk assessment comprises a hazard identification, toxicological evaluation and risk characterisation. A tolerable intake is the level of intake at which no harmful effects are expected to occur. In cases of genotoxic compounds, no safe level can be identified and therefore no tolerable intake can be set.

Through the exposure assessment the foods/food groups contributing significantly to the exposure are determined. The human exposure is assessed against the tolerable intake in order to define the measures to protect public health. Based on the provisions and principles laid down in this framework Regulation, maximum levels for the following mycotoxins have been established at EU level: aflatoxin B_1, aflatoxin total (B_1, B_2, G_1 and G_2), aflatoxin M_1, ochratoxin A, patulin and the *Fusarium* toxins deoxynivalenol, zearalenone, fumonisin B_1+B_2 and T-2 and HT-2 toxin.

Besides the establishment of maximum levels, detailed rules for the sampling and methods of analysis for the official control have also been established. And codes of practice have been elaborated (patulin) or are under elaboration (*Fusarium* toxins) to ensure a high level of consumer protection.

As the consequence of frequent findings of high levels of aflatoxins in some products originating from some third countries, specific safeguard measures have been taken imposing special conditions as regards the import of these products.

Directive 2002/32/EC of 7 May 2002 of the European Parliament and of the Council on undesirable substances in animal feed is the framework for the Community action on undesirable substances, including mycotoxins, in feed. This framework Directive provides:

- that products intended for animal feed may enter for use in the Community from third countries, be put into circulation and/or used in the Community only if they are sound, genuine and of merchantable quality and therefore when correctly used do not represent any danger to human health, animal health or to the environment or could adversely affect livestock production;

- that, in order to protect animal and public health and the environment, maximum levels for specific undesirable substances shall be established where necessary (by comitology);
- for mandatory consultation of a scientific body (EFSA) for all provisions which may have an effect upon public health; and
- that products intended for animal feed containing levels of an undesirable substance, which exceed the established maximum level may not be mixed for dilution purposes with the same, or other, products intended for animal feed and may not be used for the production of compound feed.

Based on the provisions and principles laid down in this framework Directive, maximum levels for aflatoxin B_1 and rye ergot have been established at EU level. Guidance values are under discussion for ochratoxin A, and the *Fusarium* toxins deoxynivalenol, zearalenone and fumonisin B_1+B_2 in cereals and cereal products intended for animal feeding and for compound feedingstuffs (for sensitive animal species). Furthermore discussions are ongoing to replace the current provisions on rye ergot (sclerotia) by specific maximum levels for some ergot alkaloids.

In the presentation, particular attention will be paid to the regulatory framework for mycotoxins in food and feed, the procedure for setting maximum levels (decision-making process) and the specific provisions on mycotoxins in food and feed.

Chinese approach towards international regulations

Xiumei Liu
Ministry of Health, Chinese Center for Disease Control and Prevention,
National Institute of Nutrition and Food Safety, China

National food hygiene standards system

There are four hundred and forty-two food hygiene standards in China. Four hundred and twenty-four are National Standards and eighteen are issued by the Ministry of Health. They include food contaminants, tolerance levels for mycotoxins and microbial pathogens, pesticide residues, control of food additives, and nutrition strengthening. So far, the tolerance limits for aflatoxin B_1 in various foods, aflatoxin M_1 in milk and milk products, patulin in fruits products, and deoxynivalenol (DON) in wheat and corn were issued.

Method development

High performance liquid chromatography methods are used for determining total aflatoxins in foods. Aflatoxin B_1, B_2, G_1 and G_2 can be completely separated simultaneously, the detection limits being 0.10μg/kg, 0.025μg/kg, 0.10μg/kg and 0.025μg/kg, respectively. The recovery rates of different spiked levels for aflatoxin B_1, B_2, G_1 and G_2 were more than 80%, and the coefficients of variance were less than 3.0%. Aflatoxins were detected in 70.27% of the corn samples with an average level of 36.51μg/kg, the highest level being 1098.36μg/kg. 14.86% Of the corn exceeded China's tolerance limit for aflatoxins. In peanuts, the aflatoxin detection rate was 24.24%, with an average level of 0.27μg/kg and a highest level of 437.09μg/kg. 3.03% Of the peanuts exceeded China's and Codex tolerance limits. All rice, walnut and pine nut samples met China's tolerance limits for aflatoxin B_1. Both the HPLC and McAb-ELISA method for detecting fumonisin B in food are reported, the detection limits being 12 μg/kg and 10 ng/ml, respectively. Furthermore, the method for determining sphinganine (Sa) and sphingosine (So) in human urine by HPLC, which can measure low concentrations of Sa in human urine, was successfully established. Patulin was determined by reversed-phase HPLC using a Supelco C_{18} column and an ultraviolet detector set at 275nm. The recovery for patulin at 20-100 μg/l level is 73.07-82.70%. The method offers a good separation of patulin from other constituents, especially from 5-hydroxymethyl-2-furaldehyde (HMF), a common contaminant.

Research

Molecular biological studies of *Fusarium moniliforme* and fumonisins-producing strains were carried out. Two pairs of type specific primers for *F. moniliforme,* type I and type II, Fu5/4, Fu3/2, and one pair of genus-specific primer for *Fusarium* genus*,* Fu3/F4, were designed based on 18S, 5.8S, 28S rDNA and internal transcribed spacer (ITS) sequence of fungi. Three pairs of PCR primers, P1/P2, P3/P4 and Fum5F21/ Fum5R, were designed based on the polyketide synthase gene involved in fumonisins biosynthesis. The specific PCR technique for detecting *F. moniliforme* strains and fumonisin-producing *F. moniliforme* strains were developed. The fumonisin B biosynthase gene was detected from all 32 isolates, which was in accordance with fumonisin determination by HPLC. The rDNA and ITS sequence of the fumonisin-producing *F. moniliforme* isolates in China and 5 typical strains of ATCC were analysed. The FumrDNA probe was useful for identifying different types of *Fusarium verticillioides* isolates from different food samples and studying gene polymorphism of the strains, although it was not able to differentiate fumonisin-producing strains from non fumonisin-producing strains. The FumPKSFB probe could absolutely identify fumonisin-producing strains and is suited for studying the toxigenicity and gene polymorphism of fumonisin-producing strains isolated from different geographical areas and different food samples.

The impact of mycotoxin legislation on world trade

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To protect human and animal health, a number of nations worldwide have adopted standards for foodborne mycotoxins. These standards are far from being harmonised, however, though harmonised standards would be crucial for settling trade disagreements between food-importing and food-exporting nations. In fact, the 2003 Council for Agricultural Science and Technology (CAST) Mycotoxin Report lists development of uniform foodborne mycotoxin regulations as one 21st-century goal [1]. Such uniform regulations should balance two important issues: the costs of meeting such regulations, and the health benefits that result from the regulations. The purpose of this study is to move policymakers toward that balance through an integrated assessment of risks and economics, with the goal of informing development of mycotoxin regulations that meet the standard of reasonable certainty of no harm.

Mycotoxins are considered unavoidable contaminants in foods in that best-available technologies cannot yet eliminate their presence in pre-harvest or post-harvest crops. As mycotoxins can be toxic or carcinogenic to humans, many nations have established regulatory standards on permissible mycotoxin levels in food. Thus, aside from health risks, mycotoxin contamination can also reduce the price paid for crops or cause widescale market rejection.

Losses from mycotoxins in the US and other industrial nations are typically associated with these economic losses as opposed to illnesses or deaths from the toxins. In less developed countries, however, the economic and health impacts of mycotoxins are far more severe. There, many individuals are not only malnourished but also chronically exposed to high mycotoxin levels in their diet [2], resulting in deaths from severe toxicoses to various cancers to diseases of malnutrition.

Globalisation of food trade has further exacerbated mycotoxin-related losses in two important ways. First, strict mycotoxin standards imposed by importing nations mean that less developed countries are likely to export their best-quality foods while keeping contaminated foods domestically, resulting in higher risk of mycotoxin exposure in those nations. Second, even the best-quality foods produced in these nations may be rejected for export at more strict standards, meaning millions of dollars in losses. Such a dilemma has led United Nations Secretary-General Kofi Annan to comment, "… the European Union regulation on aflatoxins costs Africa $670 million each year in exports of cereals, dried fruit and nuts. And what does it achieve? It may possibly save the life of one citizen of the European Union every two years… Surely a more reasonable balance can be found".

This study attempts to move international policy decisions toward that "more reasonable balance" by providing an integrated assessment that can aid policy makers in creating safe and feasible mycotoxin standards. Analysis is conducted of the risks and costs associated with two important classes of fungal mycotoxins: fumonisins in corn, and aflatoxins in peanuts.

Fumonisins are produced by *Fusarium verticillioides* (formerly *F. moniliforme), Fusarium proliferatum*, and some related species [3]. Studies have linked consumption of fumonisin-contaminated grain with elevated human oesophageal cancer incidence in Africa, Asia, and Latin America [4]. As fumonisin B_1 reduces the uptake of folate in different cell lines, fumonisin consumption has been implicated in connection with neural tube defects in human babies [4,5].

The US Food and Drug Administration (FDA) has set guidelines to industry for levels of fumonisin acceptable in human food at 2 mg/kg [6]. At the moment, very few regulations exist in other nations regarding acceptable fumonisin levels. The 56th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2001 has, however, recommended a provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg bodyweight per day [7]. In 2002, the European Commission proposed a standard of 0.5 parts per million (mg/kg) in grain (Food and Drug Administration/Joint Institute for Food Safety and Applied Nutrition International Workshop on Mycotoxins) [8]; thus far, no such limit has come into legislation.

Aflatoxins are mainly produced by *Aspergillus flavus*. Aflatoxins are the most potent chemical liver carcinogens known. The combination of aflatoxin with hepatitis B and C, prevalent in China and sub-Saharan Africa, is synergistic, raising more than tenfold the risk of liver cancer compared with either exposure alone [2]. Aflatoxins are also associated with stunting in children [9] and possibly immune system disorders [10].

FDA's action level for total aflatoxin in food is 20 µg/kg [11]. Many other nations have established maximum tolerated levels of aflatoxin; some of these levels are shown in Table 1. Notably, the European Commission has set a total aflatoxin standard of 4 µg/kg in food, considerably more precautionary than any national or international standards currently existing. Table 1 shows that these maximum tolerated levels vary greatly between countries, requiring harmonisation to remove the extreme variability in standards.

Table 1. National maximum tolerated levels for aflatoxins in human food. A more complete list can be found in the CAST Mycotoxin Report [1].

Nation	**Total aflatoxin standard in human food** (µg/kg)
Australia	5
China	20
European Union (EU), harmonised	4
Guatemala	20
India	30
Ireland	30
Kenya	20
Taiwan	50

The empirical economic model presented in this study shows that moving from a harmonised fumonisin standard in corn of 2 mg/kg to 0.5 mg/kg would result in an increased worldwide annual market loss of over $200 million through rejected corn; with the USA, China, and Argentina bearing the brunt of the economic burden. Likewise, moving from a harmonised aflatoxin standard in peanuts of 20 µg/kg to 4 µg/kg would result in an increased worldwide annual market loss of about $350 million through rejected peanuts. Again, the USA, China, and Argentina would bear the brunt of the economic burden, with China and sub-Saharan African nations losing 90% of their peanut export market.

The main beneficiaries of stricter mycotoxin standards should be the food importers; however, the resulting health benefit in these cases described above is negligible. A JECFA study has found that where hepatitis B and C incidence are low, reducing aflatoxin in food from 20 µg/kg to 10 µg/kg would reduce the risk of mortality by 2 in 1 billion annually: undetectable by epidemiological standards [12]. Thus, nations that would benefit most from

more stringent mycotoxin standards are those that are net importers of corn and peanuts, and have high prevalence of hepatitis B and C. As mentioned earlier, the top importers of corn worldwide are Japan, Korea, Mexico, Egypt, Canada, and Taiwan; and the top importers of peanuts are the European Union, Indonesia, Japan, and Canada. With the possible exception of Taiwan, all these nations have low hepatitis B and C incidences. Conversely, the nations with high hepatitis prevalence – China and sub-Saharan Africa – may increase the risk to their populations by attempting to export their best-quality crop and keeping the most contaminated food to be consumed domestically.

The integrated assessment presented in this study shows how addressing a broader set of health, economic, and risk questions can better inform policy decision-making, particularly when arriving at internationally acceptable standards for trade. If foodborne mycotoxin regulations were based solely on direct health effects, important questions of the economic feasibility of meeting excessively strict standards could be ignored, with potentially disastrous consequences for less developed food-exporting countries.

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Research and regulatory priorities in the US

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Research and regulatory priorities are linked very tightly. The regulatory agencies regulate what is recognised to be harmful by the scientific community and the public; in most cases this is determined by research, carried out following an observed toxicity. As an example we all know that aflatoxin was first recognised following importation of Brazilian ground nuts to Great Britain in the 1960s. The toxicity to turkey poults was easily observed but research was required to identify the causative mould *Aspergillus flavus* and afterwards, the identification of the proximate causative substance, aflatoxin. Because aflatoxin is very widespread, and because this mycotoxin is because this mycotoxin is easily identified by its characteristic fluorescence, it was soon found in other food commodities, particularly corn, cottonseed and tree nuts in addition to peanuts. Later, regulatory agencies guidance for fumonisin in corn followed its isolation by South African scientists in native corn based foods as they were searching for the cause of the high incidence of oesophageal cancer in native peoples. The association of fumonisin in corn with 'corn stalk' disease in horses was quickly established where the association with the causative fungus *Fusarium moniliforme* had already been known.

Today, both regulatory agencies and research must deal with observations of toxicity, or at least reduced weight gain, in swine associated with their consumption of deoxynivalenol (DON). This leads to the question of what this observation means for humans who may consume DON in their food, and research is called upon to provide the answers. Regulatory agencies need specific information regarding the adverse effects of the specific causative toxin or group of closely related toxins which may occur in animals, or better yet in humans, both acutely and following low dose chronic administration. These agencies need to know the amounts, which are responsible for the various toxicities, so they can carry out risk assessments, and establish appropriate action levels or issue appropriate guidance to the industries. The regulatory agencies need methods to determine the amounts, which may be present in specific affected commodities in order to take appropriate action to prevent contaminated commodities from reaching consumers. Most importantly, research must seek answers beyond the bare bones needs of regulation; for if we are to truly provide a safe food supply to consumers, we must have the appropriate knowledge to pass on to producers to enable them to grow crops free of the toxins.

Mycotoxin research priorities today encompass genomics of the fungi both descriptive and functional, technology to breed resistant crops, competitive exclusion, ecological relationships of the fungus and the toxin, both within and in the greater environment surrounding the crop, agronomic practices, mechanism of action of the toxin in animals, appropriate sampling and detection methodology for regulatory agencies and the producing industries. ARS will also begin to look at the incidence and amounts of mycotoxins in foods for susceptible populations, e.g., baby food. Advances in these areas offer the promise of the ability to produce food where mycotoxins do not constitute either a health or trade problem. ARS is going back in some cases to examine older studies for involvement of specific mycotoxins in chronic unresolved and/or periodic toxicity problems such as decreased eggshell quality. Detection methods included non-destructive testing using specific spectral based methods. Ultimately ARS wants to be able to detect mycotoxins in the field prior to harvest and/or remove mycotoxin contamination from moving streams of large volume commodities such as is common practice today with high value nut crops. A new area for mycotoxin research is the Homeland Security to provide the methods needed to detect mycotoxins in unusual commodities where they are not found naturally but may have been place in a bioterrorism event.

EFSA scientific opinions on mycotoxins in feed

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EFSA provides risk assessment advice to Health and Consumer Protection Directorate-General (DG-SANCO) through a system of scientific panels. Each panel comprises a chair plus a maximum of 20 experts who are appointed through open competition and are selected in an individual capacity. The panel on 'Contaminants in the food chain (CONTAM Panel)' deals with questions on contaminants in food and feed, associated areas and undesirable substances such as natural toxicants, mycotoxins and residues on non-authorised substances not covered by another Panel. All questions regarding mycotoxins in food and feed are therefore dealt with by this panel and the advice is published in the form of an Opinion. The process is very formalised and detailed questions are posed by EFSA and a timetable for responding is prescribed. Usually significant specialist work is required to draft an opinion and this is undertaken by a working group with members co-opted from outside the panel but chaired by a panel member and with others from the panel contributing to the working group.

Since the inception of EFSA in 2002 there have been six requests from DG-SANCO for opinions on mycotoxins in animal feed. The requests have covered aflatoxin B_1, deoxynivalenol, zearalenone, ochratoxin A, ergot alkaloids, and fumonisins and the panel opinions were published in February, June, July and September 2004, and April and June 2005, respectively. Apart from aflatoxin B_1 where the question was related to whether existing limits for aflatoxin B_1 offered adequate protection in terms of aflatoxin M_1 excretion into milk, the other questions had a similar format, in each case the terms of reference requesting:

- Consideration of the toxic exposure levels (daily exposure) of the mycotoxin in question for different animal species of relevance above which signs of toxicity can be observed.
- The level of transfer/carry over of the mycotoxin in question from feed to products of animal origin which might result in unacceptable levels of the mycotoxin in products of animal origin.
- Identification of feed materials which could be considered as sources of contamination by the mycotoxin and characterisation of distribution levels of contamination.
- Assessment of the contribution of the different identified feed materials as sources of contamination by the mycotoxin in question to overall exposure of different animal species, to their impact on animal health and to the contamination of foods of animal origin.

As a general rule the terms of reference which accompany requests to EFSA also ask that the opinion identifies eventual gaps in available data which need to be filled in order to complete the evaluation. Research needs are usually also identified by the panel.

Some general observations can be made about the six opinions on mycotoxins in animal feed that have been generated by the CONTAM panel. Mostly the toxicology was well described and the most sensitive species could be identified although in some instances for example for farmed fish and some minor species, information was lacking. Generally the information as to which components of feed were likely to be contaminated with the various mycotoxins was well known, but the occurrence data specifically on animal feed was generally lacking. Most estimates of levels of potential contamination have been based on levels determined in cereals either known to be intended for human consumption or cereals where the intended use has not been known. There was therefore found to be very little surveillance data available specifically on animal feeds as finished products (e.g., compound feed) nor were analytical methods available that had been validated specifically for feedingstuffs. Notwithstanding these difficulties the six opinions were all published to schedule and provided appropriate advice to DG-SANCO. A summary of the salient points in these opinions will be presented.

Occurrence of *Fusarium* mycotoxins in food and assessment of dietary intake

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In a European scientific cooperation (SCOOP) task pooled data on the occurrence (frequency and levels) of *Fusarium* mycotoxins in food were collected and combined with food consumption data of the Member States in order to calculate the dietary intake of these toxins by European consumers. The data are used to assist the Commission in developing EU legislation to increase protection of consumers. This SCOOP task 3.2.10 [1] was carried out between 2001 and 2003, and was separated in the three sub-tasks trichothecene mycotoxins, zearalenone and fumonisins. The scientific committee for food has earlier expressed scientific opinions on deoxynivalenol, nivalenol, T-2 and HT-2, zearalenone and fumonisins [2]. Within this project thirteen countries were asked to provide information on the exposure of the population to *Fusarium* toxins in their country. Twelve countries provided data on trichothecenes, 9 countries each on zearalenone and fumonisins.

Table 1. Overview on *Fusarium* toxin occurrence data submitted by the participating countries.

***Fusarium* toxin**	**Countries**	**Number of samples**	**Positive samples**
Type B trichothecenes			
Deoxynivalenol	11	11,022	57%
Nivalenol	7	4,166	16%
3-Acetyldeoxynivalenol	6	3,721	8%
15-Acetyldeoxynivalenol	3	1,954	20%
Fusarenon X	3	1,872	10%
Type A trichothecenes			
T-2 toxin	8	3,490	20%
HT-2 Toxin	6	3,032	14%
T-2 Triol	2	1,389	6%
Neosolaniol	2	1,323	1%
Diacetoxyscirpenol	3	1,886	4%
Monoacetoxyscirpenol	1	853	1%
Verrucarol	1	121	0%
Zearalenone	9	5,018	32%
Fumonisins			
Fumonisin B_1	9	3,863	46%
Fumonisin B_2	6	1,010	42%
Fumonisin B_3	1	239	36%
	Sum	**44,959**	

Table 1 summarises the information structured by country, mycotoxins, number of samples analysed and percentage of positive samples. The database covers altogether 16 *Fusarium* mycotoxins and 44,959 analyses. Positive samples ranged from 0% (verrucarol) to 57% (deoxynivalenol) of all samples.

Table 2 presents a summary of food categories most frequently contaminated with *Fusarium* mycotoxins. Cereals ranking first, among them corn and wheat showed the highest level of contamination with *Fusarium* mycotoxins.

Table 2. Summary of food groups most frequently contaminated with *Fusarium* mycotoxins.

***Fusarium* toxin**	**Main food items/food groups contaminated (percentage of positive samples)**
Type B trichothecenes	
Deoxynivalenol	corn (89%), wheat* (61%)
Nivalenol	corn (35%), oats (21%), wheat*(14%)
3-Acetyldeoxynivalenol	corn (27%), wheat*(8%)
Type A trichothecenes	
T-2 Toxin	corn (28%), wheat (21%), oats (21%)
HT-2 Toxin	oats (41%), corn (24%), rye** (17%)
Zearalenone	corn (79%), corn milling fractions (51%), corn based products (53%); wheat (30%), wheat milling fraction (24%), wheat based products (11%); baby food (23%)
Fumonisins	
Fumonisin B_1	corn (66%), corn flour (79%), corn based products (31%), corn flakes (46%); wheat (79%)
Fumonisin B_2	corn (51%)

* wheat and wheat flour
** rye and rye flour

Consumption data for the three subtasks trichothecenes, zearalenone and fumonisins were provided from 11, 9 and 7 participating countries, respectively. Various methodologies were used in the Member States to calculate the food consumption for all population and/or specific groups of consumers (details see parts A-C of the report [1]). Dietary intakes were calculated for trichothecenes, zearalenone and fumonisins from 12, 9 and 7 countries, respectively. An overview of the calculations of mean dietary intakes and the exploitation of the TDI-values is given in Table 3. The calculated average dietary intake values for the most *Fusarium* toxins were found to be considerably below the (t)-TDI-values. Higher intakes and a transgression of the (t)-TDI values were observed for the group of infants and children. Intakes higher than the TDI were noted for the sum of T-2 toxin and HT-2 toxin.

Table 3. Range of average dietary intakes* calculated as percentage of the TDI-values.

Mycotoxin	**TDI** (µg/kg bw/day)	**Population**	**Adults**	**Infants**
Deoxynivalenol	1	0.8% - 33.8%	14.4% - 46.1%	11.3% - 95.9%
Nivalenol**	0.7	4.2% - 11.1%	0.8% - 8.2%	3.7% - 22.6%
T-2 + HT-2 toxin**	0.06	18.3% - 250%	61.7% - 171.7%	26.7% - 563.3%
Zearalenone**	0.2	13.4%	5.3% -14.5%	3% - 27.5%
Fumonisins $B_1 + B_2$	2	0.8% - 13.2%	0.1% - 14.1%	22.3%

* mean food consumption and mean occurrence data
** temporary TDI

Conclusion

The results of this task demonstrate that *Fusarium* mycotoxins are widely distributed in the food chain in the EU. The main sources of intake are products made from cereals, in particular wheat and corn. While the dietary intakes of *Fusarium* toxins are often less than the TDIs for the respective toxin for the entire population and adults, they are close or even exceed in some cases the TDIs for risk groups like infants and children.

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Aerosol mycotoxins in indoor air: animal and human health effects

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Mycotoxins are "natural products produced by fungi that evoke a toxic response when introduced in low concentrations to higher vertebrates by a natural route" [1]. This definition applies well to aerosol mycotoxins, i.e. toxic fungal metabolites in the air. In recent years, interest has increased concerning inhalation exposure to mycotoxins first of all in water damaged houses with mould growth but also in workplaces with high mould exposure. The principal mycotoxins that contaminate food and feed are aflatoxis, trichothecenes, ochratoxin A and fumonisins. Aflatoxins, DON and ochratoxin A may have some importance as aerosol mycotoxins in agriculture, while *Stachybotrys* toxins have a dominant role in indoor problems of water damaged houses.

Mycotoxins in air samples and dust

Mycotoxins have been reported in air or in dust samples mainly from agricultural environments where people have to handle dusty materials and the fungal concentrations are high.

Aflatoxin concentrations between 12 and 150 ng/g airborne dust have been detected in the air near combine harvesters, corn dumping stations and grain elevators [2]. During laboratory shelling and pilot handling operations, an aflatoxin level of 7.6 ng/m³ has been detected in airborne dust from highly *Aspergillus flavus*-contaminated lots of peanuts [3]. The airborne concentration of aflatoxin B_1 in dust collected during harvest and grain unloading ranged from 0.04 to 92 ng/m³. Higher aflatoxin B_1 levels (5-421 ng/m³) were found in the airborne dust samples collected from enclosed animal feeding buildings and during bin cleaning (124-4849 ng/m³) [4]. Airborne aflatoxin in rice and maize processing plants in India in the samples of respirable dust varied from 12 to 29 pg/m³. In a maize processing plant the loading/unloading area and the oil mill showed aflatoxin amounts of 800-816 pg/m³ only in the respirable dust samples [5].

Several trichothecenes T-2 toxin, diacetoxyscirpenol, roridine A and T-2 tetraol were found in dust samples of ventilation systems in office buildings in the Montreal urban area [6]. Low levels of deoxynivalenol (DON), 3 and 20 ng/m³, were found in two samples collected during grain milling on a farm in Finland. The dust samples for mycotoxin analyses were collected with a high volume sampler at an airflow rate of 833 L/min., the volume of the sample being 16-50 m³ [7].

Ochratoxin A at concentrations of 306-1500 ppb was found in dust samples collected from the heating ducts of a problem household [8] and 0.2-70 µg/kg in settled dust samples from cowsheds [9]. Several *Fusarium* toxins and ochratoxin A were found in grain dust samples during threshing of wheat [10].

Health effects of aerosol mycotoxins on animals

The early veterinary papers on *Stachybotrys* contamination of hay and other rough forage [11,12] often mention that the lungs of animals that have been eating mould contaminated feed have been affected. The total mycotoxin intake may consist of toxins received in forage or feed via the digestive route and in inhaled air through the respiratory route.

In a local epidemic caused by *Stachybotrys* contaminated wheat straw on sheep [13] marked

haemorrhages were found in the respiratory organs and the straw was found to contain up to 8.3 million *Stachybotrys* spores per gram of ground straw. In an outbreak suspected to be caused by *Stachybotrys* contaminated straw in equine population [14] 216 mules, donkeys or horses out of the 242 exposed animals died.

Health effects of aerosol mycotoxins on man

Farm workers handling *Stachybotrys* contaminated straw of hay belong to the risk group for human *Stachybotrys* toxicosis. The disease called “pulmonary mycotoxicosis“ was introduced by Emanuel and co-workers [15] reporting an acute febrile illness, with marked inflammatory reaction in lung tissue in farm workers who inhaled massive quantities of airborne fungal propagules when cleaning their silos. The often-quoted paper by Croft et al. [16] and the hospital reports by Etzel et al. [17] and Dearborn et al. [18] are case reports on possible airborne *Stachybotrys* toxicosis.

Animal experiments on inhalation of mycotoxins

Experimental inhalation of mycotoxins or mycotoxin containing spores has been studied in animals with several mycotoxins using different techniques. Inhalation of pure T-2 toxin was at least 10 times more toxic than systemic administration to mice [19]. Mycotoxins associated with common indoor moulds may affect cellular production of pro-and anti-inflammatory cytokines [20,21].

Conclusions

At present analysing for mycotoxins in agricultural or indoor environments is difficult and measurements of airborne mycotoxins generally require use of high-volume samplers in combination with sensitive chemical or immunological methods for analysis. Risk-assessment on the inhalation of mycotoxins can not be made based on the analysis of deposited dust or on the presence of mycotoxins in crude building materials [22]. Therefore, with the development of more efficient methods for sampling and analysis, air sampling will help us better understand the health effects of animals and man exposed to airborne mycotoxins.

Assessment of exposure-related risks to humans or animals would require us to estimate the effects and concentrations of all airborne fungal and bacterial propagules and emissions, taking into account possible synergistic effects.

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Emerging issues in Central Europe – would mycotoxins be a threat?

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Outbreaks of foodborne illnesses have occurred over the past worldwide. Nevertheless, recently they have made consumers increasingly aware of the naturally occurring threats to food safety all over Europe. We have to be aware that food is not only an agricultural and trade commodity but also an essential emotional, political and public health issue. Consumers make usually an emotional choice based on their cultural, educational and social background. Once we are aware of the expectations of consumers, we have to ensure that food producers have the responsibility for food safety nevertheless in effective and efficient co-ordination of the food safety functions of all governmental and non-governmental agencies will share responsibilities for food. The harmonisation of food legislation was the first and most important step to establish the framework and to enhance the activities of the participants of the food chain. The most important principles of national food legislation are in line with that of the EU and international expert bodies. The protection of consumer health is a fundamental principle of our legislation. Besides harmonisation of legislation proper enforcement ought to be emphasised. The importance of risk analysis should be further emphasised and responsibilities to perform risk assessment and risk management ought to be declared as well. The importance of risk communication need to be understood to avoid confusion and misunderstanding experienced during the "paprika scandal" in Hungary last year and consumers should be informed on a regular basis. All problems arising continuously, sometimes unexpectedly posing risks for all of us, for the society, for consumers all over should be and could be solved by co-operation of those in the food chain. Relevant organisations emphasised the importance of traceability of all food, feed and their ingredients and recommended maximum transparency in the operation of risk analysis.

Regional similarities and differences in mycotoxin contamination and possible sources (e.g., cereals, paprika) of infection in the region will be discussed. Detection and enforcement activities will be compared in the presentation with reference to the "paprika scandal" attracting attention Europe-wide.

The social demand for safe foods of excellent quality not presenting health risks is continuously rising. Despite of the social and technical development there is more and more reasonably worry about the foods this century. Are our foods safe? What hazards should be taken into consideration? What risks are there? What to do at social and economical level to prevent health problems in our societies? How to benefit from our resources available most efficiently to achieve the best or avoid the worst?

Emerging issues in Southern Europe: the Italian case of aflatoxins

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In the past centuries, similarly to many populations in Central Europe, the Italian population suffered from ergotism, mainly in rye-growing areas. In Florence during the 18th century, poor quality, moth-contaminated grain, was considered to be the cause of a number of health problems affecting people from all social classes. The Accademia dei Georgofili, founded in Florence in 1754, set as one of its priorities the promotion of potato growing and consumption, in order to replace and supplement cereals in bread making and help reduce the above mentioned problems.

Based on currently available evidence, the most important and relatively recent epidemic-like manifestation in which mycotoxins were probably involved was pellagra, which affected thousands of people in Northern Italy in the 1800s. Symptoms described included gastrointestinal disease, dermatitis and severe central nervous system (CNS) disorders, which were so severe that they lead to the setting up of a network of psychiatric hospitals. In order to prevent, medical doctors paid special attention to corn quality, which was often quite poor. Maize was grown without irrigation and therefore was frequently affected by water deficiency-related stress. Long-cycle varieties were used, which matured in late autumn, and harvesting and drying operations were not carried out in good conditions. Grain was often severely damaged during harvesting and inadequately dried in the sun; therefore, storage was not safe and corn usually developed moulds. Because of relevant health problems related to maize consumption, the Italian Ministry of Agriculture passed a law in 1883, "*aimed at controlling the causes of pellagra by prohibiting the sale, distribution and grinding of corn (Zea mais) whenever found to be immature, rotten or damaged"*. This was probably one of the first legislative measures aimed at mycotoxin control in cereals.

The first identification of a mycotoxin, as a fungal metabolite related to dangerous effects on animals, was in 1960 when aflatoxin was described. Since the early 1960's we have observed (Northern Italy) a number of important cases of mycotoxicosis in animal production: turkey mortality after eating imported aflatoxin B_1 (AFB_1) contaminated peanut meals (late 1960s); severe hyperestrogenism and rejection of food in pigs fed with zearalenone (ZEA) and deoxynivalenol (DON) containing corn; tail necrosis in steers due to toxin T-2 contaminated corn silage; leukoencephalomalacia (LEM) lesions in horses fed with fumonisin B_1 containing corn and various cases of toxicosis in pigs and poultry caused by cereals containing trichothecenes or ochratoxin A (OTA). Except for one case due to aflatoxin contaminated imported peanut meal, all major episodes were caused by contamination of cereals grown in Italy.

Surveys on maize kernels before or at harvesting in different years showed fusaria as the dominant fungi, mainly *Fusarium verticillioides* and *F. graminearum* in rainy and cool years, with sporadic isolation of aspergilli. Consequently, fumonisins are normally detected in maize at units of ppm level. DON is normally lower than 1 ppm; based on data over the last ten years, only in 1996 were more than 2 ppm measured as the mean content of samples collected in Northern Italy.

In 2003 a new problem broke out in Italy: aflatoxin in home produced maize and consequently in milk. The summer was extremely hot and dry, which was confirmed by meteorological data for the area. Just to give one example, considering 3 years (1996, 1999

and 2003) and 3 sites in Northern Italy, in the Eastern, Central and Western area, the mean temperature during the summer was around 19°C in the first year, around 21°C in the second and around 23°C in 2003. Total rainfall was around 235 mm in 1996 and 1999, and less then half that in 2003 (98 mm). Maize plants were drought stressed and there was a widespread reduction in crop cycle length, with rapid drying and anticipated ripening. As a consequence, farmers harvested grain with a lower humidity than usual and frequently it was stored without any drying or cleaning. Based on knowledge available, *Aspergillus flavus* should have been very competitive in this situation with plants stressed due to dry and hot weather. The ears were harvested with low grain humidity and grain was stored without pre-processing. This hypothesis was confirmed by analysis in September of samples from different maize-growing areas in Northern Italy. A survey of 110 samples, initially planned to monitor the occurrence of fumonisin, showed a 75% positive sample for AFB_1 with a mean and a maximum value of 4.4 and 154.5 ppb, respectively. Farmers were alerted to pay attention to aflatoxins as well as fumonisins, but things were not taken seriously until the first detection of aflatoxin M_1 (AFM_1) in milk.

The feeding of dairy cows with contaminated corn led to severe widespread contamination of milk. The problem was immediately identified by manufacturers of milk for human consumption and by sanitary inspectors. In Lombardy (Northern Italy) alone, over a period of 20 days in early October 2003, 4,321 inspections were carried out on samples of milk from different farms (n=2061) or dairy factories (n=808) and over 33% were above the threshold of 0.05 µg/kg of AFM_1 established by Regulation 466/2001. Several thousand tons of milk found to be above the legal limit were destroyed. Farmers were not allowed to put their milk on the market in the days immediately after a positive test until their milk was found, in a later inspection, to be back at the acceptable level. Systematic analysis of corn and raw materials used in dairy cow feeds and a well-run information campaign rapidly reduced the problem. In fact, based on evidence collected by Lombardy Region inspectors, the percentage of samples over the value of 0.05 µg/kg of AFM_1 dropped to 2.5% in dairy farms and to 18.5% in farms in the period November 2003 – January 2004. These data match our laboratory data (over 800 samples) that found 14% of milk samples over the threshold, over the period October 2003 – February 2004, with a peak of 27% in October. Action suggested and adopted by farmers included analysis of corn grain and elimination from the diet, if found with contamination above 5 µg/kg. Based on the results of our tests over the period October 2003-February 2004, corn grain samples for animal feed showing values above 5 and 20 µg/kg were found to be 43 % and 22%, respectively. Corn mash and especially silage showed limited contamination.

To comply with milk production parameters, daily intake of AFB_1 had to be measured. The effectiveness of a value of 5 µg/kg, established for feed, depends on intake, daily production, average stage of lactation of the herd and contamination of other components of the diet (ex. corn silage or mash). Other measures involved careful corn cleaning to remove broken kernels and dust. These fractions were found to be more contaminated, so much so that, thanks to the above procedures, batch contamination was considerably reduced by up to 60-70%. Diet supplementation with clay-based sequestering substances gave good results, with a reduction of AFM_1 levels in milk by up to 50%, when adequate administration procedures were followed (clay added to contaminated corn meal). Administration of sequestering substances directly in the feed led to less reliable results. Doubts have been raised on long-term use of sequestering substances in the diet, due to possible alteration of milk properties needed for cheese making (hard cheese, products made with crude milk which require long-term maturation, such as Grana Padano or Parmigiano Reggiano). These doubts have not been confirmed and we are currently conducting a trial on this matter.

In the areas with higher corn contamination, it was necessary to define acceptable levels of AFM_1 for certain types of cheese. Regulation 466/2001 established an AFM_1 threshold for milk of 0.05 µg/kg. For dairy products, only processing techniques are to be taken into

account. Milk AFM_1 concentration factor in hard cheeses (such as Grana) had to be determined, in order to define a maximum temporary limit of 0.45 µg/kg for this category of cheese (Ministry of health).

In 2004 and 2005, different climatic conditions and compliance with guidelines by farmers led to a dramatic reduction of the problem. Nevertheless, some cases of kernel contamination above the legal limit were also detected in these 2 years. They were not expected because of not particularly dry or hot weather conditions. A point to be considered is surely the increase of overwintering inoculum of *A. flavus* due to its relevant presence in 2003. Then, contaminated samples were mainly associated with the short growth cycle of the hybrids, with early harvesting around mid August, and the absence of irrigation. Probably, periods of drought, even if short, affected these crops causing favourable conditions for *A. flavus*.

A big Italian research project, AFLARID (aflatoxin reduction in milk), has been funded by the Ministry of Agricultural Policy. Different aspects of the milk and dairy production chain are being considered, in order to identify suitable and appropriate action and procedures. An alert system based on meteorological data collection and elaboration and the development of a predictive model for *A. flavus*, is central to the project.

Overview of the MYCO-GLOBE specific support action activities in developing countries

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"Integration of mycotoxin and toxigenic fungi research for food safety in global system" is the title of the MYCO-GLOBE Specific Support Action project that aims to disseminate the outcomes of a wide range of European research projects in the area of mycotoxins and toxigenic fungi. Information dissemination is carried out by supporting, stimulating and facilitating the participation of and co-operation with countries that have signed bilateral scientific and technological co-operation agreement with the EU. In particular, the MYCO-GLOBE project (http://mycoglobe.ispa.cnr.it) continues to:

- create a consortium of international experts in the area of mycotoxins and toxigenic fungi;
- identify priorities of common interest by sharing results of the European research with the major international networks involved in the field of mycotoxins and toxigenic fungi;
- evaluate research policy in the Specific Support Action area by conferences focused on advanced research tools; and
- improve co-operation in the Specific Support Action area by transfer of knowledge to developing countries (mainly in Africa) and sharing information with advanced countries (USA and Australia) by bilateral workshops and visits.

The MYCO-GLOBE project is divided into six work packages, one of which deals with an international conference to disseminate information on mycotoxins in Africa where mycotoxin exposure is pervasive among local population. The objectives of the conference, held in Accra, Ghana during 13 to 16 September 2005, were to:

- identify key mycotoxin constraints of major crops in the tropical Africa and the Mediterranean;
- explore challenges and opportunities to mitigate the pervasive influence of mycotoxins on public health and international trade;
- disseminate information on health and trade-related issues; and
- sensitise policy makers and opinion-leaders on the urgency of reducing mycotoxin problem.

Information on detection methods, institutional arrangements and policies associated with public health and trade were shared during the conference. The goal of the conference was to recommend priority actions in the areas of technical, institutional and policy options for improving public health and trade through management of mycotoxins from "field to fork". The various technical sessions included:

- Overview of health and trade issues, with five presentations – one each from South Africa, Kenya, USA, UK, and Belgium.
- Mycotoxin contamination and toxigenic fungi in Africa and the Mediterranean basin, with six presentations – one each from Ghana, Botswana, Turkey, Italy, USA and the Netherlands.
- Detection methods and mycotoxin management of key commodities in Africa & Europe, with eight presentations – two from Italy and one each from the Netherlands, India, Benin, France and UK.
- Approaches to mycotoxin management, with five presentations – three from USA and one each from Nigeria and Benin.
- Institutional issues in mycotoxin management, with five presentations – one each from the Netherlands, Ghana, Taiwan, USA and Nigeria.

- International programs on mycotoxins, with four presentations – one each from UK, Italy, USA and Mexico.
- Nominal group discussion, with eight working groups discussing one or more of the five topics in two sessions:
  - How can health issues related to mycotoxin exposure in Africa be mitigated?
  - Identify approaches you recommend to foster and integrate agriculture, nutrition and health research for mycotoxin management in Africa.
  - Identify institutional innovations needed for African farmers to produce and market safe food both domestically and internationally.
  - Identify appropriate interventions and approaches for mycotoxin management in Africa.
  - Identify biological questions that need answers to enable African farmers to produce mycotoxin-safe crops.
- Plenary session, with presentation of reports from group discussions, concluding remarks from a FAO representative and closing address by the Deputy Minister of Environment and Science, Ghana.

The conference program and abstracts of papers are available at the conference website: http://www.iita.org/mycotoxinconf/ABSTRACT_Final.pdf. Additionally, the participants presented 27 posters in two posters sessions. CABI Publishing, UK will publish an edited book based on the presentations made at the conference.

The conference attracted 109 participants from 28 countries in Africa, Europe, Asia, North America and South America. The countries represented were Argentina, Belgium, Benin, Botswana, Burkina Faso, Cameroon, Democratic Republic of Congo, France, Ghana, India, Italy, Kenya, Malawi, Mali, Mexico, New Zealand, The Netherlands, Nigeria, Norway, South Africa, Taiwan, Tanzania, Togo, Turkey, Uganda, UK, USA and Zambia. Participants included scientists, parliamentarians, heads of institutions, policymakers, trade-related specialists and health specialists from government and non-government organisations and the private sector. The MYCO-GLOBE project was the primary sponsor of the conference. Other co-sponsors included the Food and the Agriculture Organization of the United Nations, International Sorghum and Millets Program and Peanut Collaborative Research Support Program of USAID, and the African Agriculture Technology Foundation. The organisation of the conference was the responsibility of the International Institute of Tropical Agriculture (IITA), Council of Scientific and Industrial Research (CSIR) of Ghana, and Institute of Sciences of Food Production (ISPA CNR) of Italy.

The future activities of the MYCO-GLOBE project includes organisation of the following:
- *Fusarium* identification laboratory workshop, June 2006, Bari, Italy. Co-sponsored by Kansas State University.
- Training workshop on analytical methods for the detection of mycotoxins in food systems, June 2006, Bari, Italy.
- International conference on advances on genomics, biodiversity and rapid system for detection of toxigenic fungi and mycotoxins, September 2006, Bari, Italy.
- International conference on mycotoxin management and detection methods, March 2006, Cordoba, Argentina.

Selected participants from developing countries will be encouraged to participate in these future workshops and conferences to further integrate developing country mycotoxin professionals into the worldwide community of mycotoxin researchers. Through the MYCO-GLOBE activities in developing countries, there would be improved awareness of the impact of mycotoxins on health of local population, methods of mycotoxin management, and the importance of standards and quality assurance procedures in developing countries for improving exports to the EU.

Structural and functional genomics of the aflatoxigenic *Aspergillus flavus*

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Genomic studies on several *Aspergillus* and *Fusarium* fungal species are underway in various labs in different parts of the world. Among these are structural and functional genomics of the toxin producing species *Fusarium graminearum* (trichothecene producer), *F. verticillioides* (fumonisin producer), and *Aspergillus flavus* (aflatoxin producer). Our project continues to identify, through Expressed Sequence Tag (EST) technology, the complex gene array involved in fungal biology. The Institute for Genomic Research sequenced 26,000 clones and identified 7218 unique ESTs; 30 percent of these had related sequences in the GenBank database. The fully annotated EST data set was released to the public by Gene Index constructed at TIGR (http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=a_flavus). DNA microarrays have been constructed at TIGR for functional studies of *A. flavus* biology. The high density and high quality microarray of 6684 short amplicons representing 5002 unique gene elements include the 31 known aflatoxin cluster genes. Gene expression profiling experiments by microarrays have been used by our group and others to successfully identify differentially-expressed genes associated with aflatoxin production in *A. flavus* and *A. parasiticus* grown under different nutritional and environmental conditions. We are using near-isogenic developmental and secondary metabolic mutant of *A. flavus* and *A. parasiticus* in the proposed microarray studies that will not require shifts in growth media. This will allow us to more accurately select specific subsets of differentially-expressed genes that are involved in aflatoxin production and fungal development. A national project at the North Carolina State University (with collaboration of SRRC) to sequence the entire *A. flavus* genome (at TIGR) was funded and is nearing completion with a 5X depth of coverage (79 scaffolds; scaffold size ranging from 4.5 Mb to 1.0 kb). The 36.3 Mb *A. flavus* genome sequence data are available to the collaborative laboratories and have recently been also made available to the public at the TIGR web site (http://www.tigr.org) and at the NC State website (http://www.aspergillusflavus.org). Environmental influences on the fungus, ecological/ evolutionary significance of *A. flavus* propagation, fungal virulence, and aflatoxin formation as manifested by changes in gene expression profiles and global signal transduction within the fungus can be rapidly analysed using this genomic information.

Proactive identification of emerging mycotoxins: a holistic approach

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The increasing complexity of feed and food production systems, increased globalisation of trade, market introduction of novel foods and application of new food processing technologies may lead to unforeseen, (re-)emerging hazards with a negative impact on human health, environment and economy. There is a need for a systematic approach to identify emerging hazards associated with feed and food production, like emerging mycotoxins, in a global setting. The European Commission has also acknowledged the necessity of an improved identification of emerging risks in order to maintain a high level of protection of human life and health [1]. A system for the proactive identification of emerging hazards may in the long term be a useful preventive instrument. From 2004 onwards the Dutch Food and Consumer Product Safety Authority (VWA) has led two European projects in order to develop a scientific approach for the identification of emerging risks.

Approach

Traditionally, the identification and assessment of food related risks are achieved by using expertise from within the feed and food supply chain. However, due to recent developments in globalisation, food technology, climate changes, political and social developments, consumer behaviour and perception, it becomes necessary to look at information available outside the food supply chain. Exploring only the food chain for the identification of emerging risks may be a too narrow approach and may in specific cases only identify a problem when food safety is already threatened. Therefore, the following hypothesis has been postulated: the information necessary for the identification of emerging risks is likely to be drawn from a combination of knowledge both from inside as well as from outside the food supply chain (i.e. covering the fork to farm/fisheries chain and its host environment). A more proactive attitude towards emerging risks requires an investigation of various fields of interest inside and outside the food supply chain. Within these fields of interest (so-called influential sectors) several factors may be important with respect to the identification of emerging risks. Substantial changes in these so-called critical factors could lead to the emergence of new risks.

Our holistic approach was developed in 2004 in a project (called PERIAPT) co-sponsored by the European Commission within the Sixth Framework Programme for Research and Technological Development (FP6) “Integrating and Strengthening the European Research Area (ERA-NET)”. The vision envisaged was tested in an international two-day workshop held in Bonn last year [2]. Subsequently, a Dutch focus group of experts on mycotoxins studied the applicability of the approach in more detail.

In 2005 the holistic concept is further explored in a project carried out for the European Food Safety Authority (EFSA). During the first phase of this project (called EMRISK) a retrospective assessment of five food incidents from the past was carried out in order to identify important sources that may provide information on signals, so-called indicators, that indicate (directly or indirectly) the (possibility of) occurrence of an emerging hazard. One of the case studies was a major incident (with casualties) related to aflatoxicosis that took place in Kenya in 2004.

Results

Host environment analysis
During the first phase of the PERIAPT project an analysis of the host environment of the feed and food supply chain was carried out. This led to the identification of eight influential sectors and several critical factors within each sector (at least 3 factors per sector). This host environment model was presented at an international workshop held in Bonn last year. A stakeholder's panel with different scientific and managerial expertise was recruited from academia, research centres, food industries, regulatory agencies (regional, national and EU), international governmental organisations, media, and consumer organisations across Europe and the United States. They were informed of the envisaged holistic vision and were challenged to comment on our approach. The vast majority of the stakeholder's panel agreed that a holistic approach could be used to develop a system for the proactive identification of emerging risks. Profound discussion led to a slight adjustment of the proposed host environment and 9 influential sectors and 29 critical factors were identified and prioritised by using an expert choice system.
Another outcome of the workshop was the recommendation to form a focus group on a certain group of hazards, i.c. mycotoxins, to test the applicability of the holistic vision in more detail.

Focus group mycotoxins
The focus group on mycotoxins (organised by RIVM) consisted of Dutch scientists working in different fields of research related to mycotoxins (mycologists, biologists, plant pathologists, toxicologists, analytical chemists, etc.). The focus group concluded that the holistic approach was a feasible approach and that separate analyses of the food supply chain and the host environment for identifying indicators of mycotoxin-related emerging risks was not considered as being appropriate. Overall, emphasis for identifying indicators is placed on conditions for fungal growth and conditions for mycotoxin production rather than on mycotoxins as such.

Aflatoxicosis in Kenya
During the first phase of the EMRISK project a retrospective assessment of five food incidents from the past was carried out. One of these case studies (carried out by FAO/ESNS) was the occurrence of acute aflatoxicosis in eastern Kenya in mid 2004.
Although several critical factors have been identified in the host environment analysis they are still rather comprehensive items of the influential sectors. Therefore, it was proposed to identify in each case study several indicators and to identify for each indicator an important source that may provide information on the indicator. Finally, the sources were analysed on aspects like quality and accessibility and, whenever possible, the most important sources of information were identified.

The identified indicators for acute aflatoxin contamination were attributed to 8 out of 9 influential sectors. The main indicators contributing to the mycotoxin contamination included: (i) the level of food availability in the population; (ii the lack of application of good practices; (iii) changing (and atypical) weather patterns; and (iv) the immuno-compromised status of the population. The sources of information related to these indicators were, respectively: (i) FAO GIEWS and FIVIMS; (ii) farm and post-harvest production records; (iii) meteorological databanks; and (iv) public health records and media coverage.

All of the information should be accessible free of charge, but not all information is easily accessible. The reliability of information also varies, depending on the nature of the information. Quantifiable information is generally more reliable, while qualitative data is less reliable.

Conclusions

- Taking all indicators into account it was concluded that apart from information on technical aspects, information on human behaviour (e.g., non-compliance) is also very important.
- Essentially, the holistic approach is considered a feasible approach.
- Furthermore, a system for the proactive identification of emerging hazards must take several indicators simultaneously into account. Consequently, it was suggested that the contribution (of the change) of the indicators should somehow be weighted.
- It could be argued that such a system may be able to identify an emerging hazard, but that it does not quantify the associated risk.

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Biocompetitive exclusion of toxigenic fungi

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Aflatoxins are highly toxic cancer causing fungal metabolites known to cause immune-system suppression, growth retardation, cancer, and death in both humans and domestic animals. Aflatoxin contamination of crops is caused by members of *Aspergillus* section Flavi and *A. flavus* is the most common cause of contamination on most crops worldwide. For over three decades, research directed at developing methods to improve management of aflatoxin contamination has increased our knowledge of the biology, genetics, ecology, physiology and evolution of aflatoxin producing fungi. One result of this knowledge has been the development of strategies utilising strains of *Aspergillus flavus* that do not produce aflatoxins to reduce contamination of crops through competitive exclusion of aflatoxin producers. These strains are called either non-toxigenic or atoxigenic. Over a decade has passed since the first patent outlining use of such strains was granted and several different attempts to utilise various *A. flavus* strains have progressed to commercial use. Currently two *A. flavus* strains have registrations with the US Environmental Protection Agency as biological pesticides for the management of aflatoxin-producing fungi. In commercial practice, atoxigenic strains of *A. flavus* are applied during crop development on a fungal food source that fuels initial reproduction and dispersal of the biocontrol agent. The atoxigenic strains compete with aflatoxin producers both for resources associated with crop development and during crop colonisation. In the process, atoxigenic strains both displace aflatoxin producers and directly interfere with the contamination process. Although applications cause significant shifts in the composition of *A. flavus* strains associated with crops and an associated reduction in crop aflatoxin content, overall crop infection by *A. flavus* and the quantity of *A. flavus* propagules on the crop at harvest do not differ between treated and untreated crops.

Aflatoxin contaminated cottonseed was both the first target for which atoxigenic biocontrol strains of *A. flavus* were developed and the first crop for which an atoxigenic strain pesticide registration was granted. Indeed commercial fields of cotton have been treated in Arizona since 1996 with over 50,000 hectares treated to date. In 2005, treatments were made to commercial fields in Arizona, Texas, and California. Interest in managing aflatoxins in cottonseed stems from the premium paid by dairies for cottonseed that meet dairy-feed aflatoxin limits dictated by regulations. However, use of atoxigenic strains in cotton is not just favoured because of single season influences on cottonseed but also because of long-term influences atoxigenic strain applications have on communities of *A. flavus* resident in agricultural soils. Properly timed atoxigenic strain applications result in displacement of aflatoxin producers from the crop environment. Induced changes to the structure of fungal communities resident in agricultural areas persist in soils, to varying extents, through complex crop rotations and over multiple years. Thus, applications increase incidences of the applied atoxigenic strains and decrease aflatoxin producers in the environment over multiple years. Treatments have been particularly effective against the highly toxigenic S strain of *A. flavus*. Long-term influences of atoxigenic strain use have led to efforts to develop area-wide aflatoxin management programs based on atoxigenic strains. Positive influences on community composition are frequently achieved in fields adjacent to and/or nearby treated fields. However, just as the atoxigenics move to neighbouring fields, so do aflatoxin-producing strains move from nearby untreated fields into treated fields, eroding long-term benefits. Thus, under certain circumstances, greatest potential for atoxigenic strain use is thought to be in area-wide management programs. Area-wide management programs may allow long-term and area-wide reductions in the average aflatoxin-producing potential of fungal communities and thereby reduce aflatoxins throughout the environment and in all crops produced. It is in this type of long-term, area-wide management that atoxigenic strain

use may be most controversial and yet have the greatest potential benefits.

The complex economics of aflatoxin contamination limits the speed with which management based on atoxigenic strains is adopted. However, persistence of atoxigenic strain influences may allow favourable economics when single season benefits would not. Indeed, atoxigenic strain material may be made inexpensively using any readily available substrate that supports atoxigenic strain sporulation. Thus, atoxigenic strain technology may have the potential to be adapted to poor regions with few resources. In Arizona, atoxigenic strain material is produced on wheat seed in a facility run by the Arizona Cotton Research and Protection Council, a grower run organisation, and provided to farmers for five dollars per acre. This grower run production facility may serve as a model for delivery of a variety of microbes useful in agriculture.

The species *Aspergillus flavus* is divided into vegetative compatibility groups (VCGs) by a genetic system that limits gene flow between genetically dissimilar individuals. Thus *A. flavus* exists in complex communities composed of numerous VCGs that vary in many characteristics including aflatoxin-producing ability. Soils are typically occupied by many VCGs, even within a single gram, and crops are infected by many VCGs simultaneously. The infecting fungi interact and low aflatoxin producers modulate toxin production by highly toxigenic VCGs. This is one basis for the activity of atoxigenic strains. For this type of biological control, the biological control agent must have ecological competence and be able to increase in an epidemic manner when activity is needed. In the future, optimal use of atoxigenic strains may result from selecting strains, from the vast diversity of *A. flavus*, which have competitive advantage in target locations, crop rotations, and soils. Indeed application of multiple VCGs adapted to the specific circumstance may provide the best displacement and also result in a more diverse modified fungal community. Increases in the diversity of the modified community may result in improved resistance to reestablishment of aflatoxin producers in treated areas.

Qualitative modification of fungal communities resident in agricultural areas through the selection and application of favourable fungal strains has only recently begun. Extensive room exists for improvement of many aspects including strain selection, formulation, and application strategy. There is considerable need for creative research to explore the extent to which such approaches may be applied to reduce the exposure of humans and domestic animals to mycotoxins.

The use of GMOs as a prevention strategy for mycotoxin formation

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Mycotoxins are toxic metabolites of fungi that contaminate grain/seed and produce a variety of adverse health effects when consumed. The economic impact of mycotoxin contamination of food crops has been estimated to range from 0.5 to 1.5 billion dollars/year in the United States alone [1]. In other world areas, the economic impact of mycotoxin contamination has not been well studied, but is expected to be significant. As a consequence, scientists around the world are actively engaged in finding ways to reduce mycotoxin contamination of food crops. Biotechnology is providing tools for researchers to understand the complex dynamics involved in fungal infection and production of mycotoxins. The fungal synthetic pathways for mycotoxins such as aflatoxin, fumonisin and deoxynivalenol (DON) are complex, involving multiple enzymatic steps. Biotechnology has helped to identify the genes involved in mycotoxin synthesis. Gene-knockout fungal strains are being used to determine key enzymatic steps in mycotoxin production [2,3]. The regulation of genes involved in mycotoxin production has been investigated using RNA silencing targeted against mycotoxin biosynthetic pathway-activating transcription factors [4]. All of these research efforts may help to identify critical control points in mycotoxin production that could provide research leads for future mycotoxin reduction. Some have proposed introducing genes into plants that produce enzymes to degrade mycotoxins [5]. Others are using biotechnology to help identify natural resistance factors to fungal infection that are present in plants including maize [6]. Once identified, the coding sequences for these resistance factors may be introduced into susceptible varieties of maize to see if they improve protection against fungal infection.

Biotechnology is also helping to develop healthier maize plants that can withstand environmental stress factors that increase susceptibility to fungal infection in the field. These factors include insect damage, heat and drought stress, nitrogen deficiency and genetic susceptibility [7,8]. Insect feeding injures corn kernels, creating ports of entry for fungi that produce ear rot and mycotoxins. Protection of corn kernels against insect feeding can reduce fungal infection and mycotoxin contamination. This has been demonstrated with Bt maize (YieldGard Cornborer®, registered trademark of Monsanto Technology, LLC), which produces the Cry1Ab protein that controls corn borers throughout the growing season. Cry proteins are the active ingredients of Bt microbial insecticides. They have been safely used on agricultural crops around the world for 40 years because their insecticidal mode of action is highly specific against target lepidopteran insect pests and does not affect non-target organisms [9,10].

Scientists who first evaluated Bt corn in field tests in the United States (Iowa) found less damage from corn borers and lower ear rot and fumonisin levels in the grain [11]. These initial observations were followed by more extensive field trials conducted across the United States and in other countries (France, Italy, Argentina and Turkey) at locations with significant corn borer pressure. Fumonisin levels in Bt hybrids were compared to their near isogenic controls. When averaged across all 180 sites for 2 consecutive years, fumonisin levels for several different Bt hybrids were approximately 50% lower in the United States [12]. In Argentina, results during one season with one Bt hybrid tested at over 50 locations found fumonisin levels on average 60% lower. In Turkey, one Bt hybrid tested for 2 consecutive years had fumonisin levels 85% lower than controls. Field trials in France (several years) and Italy (one year) at multiple locations also found consistently lower fumonisin levels in Bt hybrids [13,14]. At some locations, the reduction in fumonisin levels

was large enough to make the difference between an unacceptable crop and one that was safe for consumption.

Building on this initial success, the next offering of Bt maize will produce Cry proteins that will provide better protection against a wider variety of insect pests such as fall armyworm, corn earworm in addition to corn borers. The overall strategy is to produce maize varieties that are much less susceptible to environmental stress factors in the field and therefore yield more than conventional varieties. A secondary benefit of these improvements should be reduced susceptibility to fungal infection and mycotoxin contamination. For example, the recent introduction of another Bt maize variety protected against corn rootworm feeding (YieldGard Rootworm®, registered trademark of Monsanto Technology, LLC) demonstrated superior performance this year in parts of the midwest United States afflicted with prolonged drought. The maintenance of a healthy root system presumably afforded better absorption of water and nutrients. The introduction of herbicide resistant maize varieties can also improve nutrient availability by reducing weed competition that competes for the same nutrients. The combination of root, stalk and ear protection against insect feeding as well as herbicide tolerance in the same maize plant can reduce plant stress significantly. Ongoing research is identifying genes that reduce drought stress and others that improve the efficiency of nitrogen uptake from the roots. Combining these important agronomic traits with those already available will further enhance the ability of improved maize varieties to withstand a broad variety of plant stress factors. We anticipate that this will lead to further reduced fungal infection and mycotoxin contamination. Thus, biotechnology has opened up many opportunities to significantly reduce mycotoxin contamination of food crops.

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Applications of forecasting deoxynivalenol in wheat using DONcast for grain producers, the grain industry and regulators

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Forecasting *Fusarium* toxins is useful to prevent their entry into the food chain. Wheat fields under an array of agronomic practices were sampled for deoxynivalenol (DON) content at harvest across Ontario from 1996 to 2004. The variation in toxin levels associated with year and agronomic effects was estimated from simple linear models. In wheat, environment effects accounted for 48% of the variation in DON across all fields, followed by variety (27%), and previous crop (14 to 28%). A robust site-specific, DON forecast (DONcast) which accounts for 80% of the variation in DON, was commercialised for wheat and has been used to make fungicide spray decisions in Ontario Canada for more than 5 years and this model is delivered online, sponsored by the crop protection industry and the Ontario Wheat Producers Marketing Board (http://www.ownweb.ca/lib/fusarium.cfm).

There is growing interest amongst producers to use this tool pre-harvest to make marketing decisions and for grain handlers to use the tool for grain sourcing. In Uruguay, DONcast is used to alert growers, regulators and grain handlers of pending problems with DON in locally grown wheat (http://www.inia.org.uy/online/site/157852I1.php). In France a pilot study is underway to investigate the utility of DONcast under European conditions. In Iran, where there is a desire to be self sufficient in wheat, DONcast will be used retrospectively to determine the risk (frequency and intensity) of exposure to DON and to identify wheat growing regions at highest risk.

This model is robust when used to make decisions above or below decision thresholds in the range from the detection limit to 5 ppm. For example from field data collected in France in 2004, 72% of samples were predicted correctly to contain either above or below a threshold of 1.0 ppm DON, and 83% of samples were predicted correctly to be above or below a 2.0 ppm threshold. Most of the inaccurate predictions were false positives. Similarly, in Uruguay in 2004, 68.3 and 74.8% of samples were predicted correctly to be above or below a threshold of 1.0 and 2.0 ppm, respectively, with the number of false positives at 8 and 12%, for these two thresholds.

DONcast is very sensitive to heading date, varietal susceptibility to *Fusarium* and the management of previous crop residue, and it has successfully taken these factors into account. It is unrealistic to put DONcast in the same class as a semiquantitive analytical test, but it can be used in a similar vein as a qualitative test, with the advantage of pre-harvest, remotely sensed, forecasting for producers, grain marketers and regulators alike.

Technologies for the reduction of mycotoxin contents in wheat

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Mycotoxins are metabolic products of toxigenic fungi, which cause human and animal health problems. The food and feed industry is obliged to reduce the content of mycotoxins to the limit values given by law. In the grain processing industry other possibilities than thermal treatment must be used for mycotoxin reduction, since functional flour characteristics already change at a temperature of 60°C.

Wheat can be contaminated with mycotoxins. The mycotoxin content of wheat depends on numerous different factors, such as grain variety, seeds, soil preparation, crop rotation, fungicides, weather conditions, and storage conditions. A high quality and purity of finished grain-based products is required by law, consumers, bakeries, and other flour and grain processing industries. The milling industry is in the centre of the production chain and, therefore, has to ensure that the mycotoxin content of its products remains within the legal limit values. The damage in grain caused by mycotoxins, can differ considerably. Some parts can only be superficially contaminated, while other parts can be partially or completely spoiled. Therefore, different cleaning methods are necessary.

The unique peeling process, developed by Bühler, was presented in 2003 and since that time this process is used worldwide with great success. This process results in a substantial decrease of the mycotoxin content concentrated at the grain surface.

Further novelties have been introduced in the field of grain processing, e.g., a new high capacity gravity separator (Gravomat, Bühler AG). This apparatus was developed particularly for the selection of shrivelled kernels and DON contaminated grains, which differ by density from healthy grains. The accurate sorting technology enables a precise classifying of wheat into four fractions with a different DON contamination. The separation of heavily contaminated wheat fractions reduces the overall mycotoxin contamination of the main product. In combination with the peeling process and an efficient aspiration a substantial DON reduction can be obtained.

Optical sorters are more and more used within the grain cleaning process by the milling industry. By this modern technology, an accurate separation of discoloured kernels can take place. Furthermore, machines of the newest generation are even able to separate kernels with just small spot defects. In the grain milling industry, colour sorters are mainly used to eliminate ergot to avoid a possible contamination of grain with ergot alkaloids.

Last but not least, the grain milling industry has to take into consideration additional consumer demands. The production process has to be completely transparent, e.g., a total traceability of the production chain is expected. Therefore, modern wheat processing plants are fitted with quite complex systems, which assist in keeping control all the time. In case of complaints or doubts the production process can be tracked and traced enabling any actions, if necessary.

Mycotoxin analysis: an overview of classical, rapid and emerging technologies

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This presentation covers classical as well as latest activities in analytical methods for mycotoxin determination and the advances of coherent quality assurance. The majority of mycotoxin analyses carried out in the laboratories are still based on physicochemical methods, which are continually improved. For example, immunoaffinity columns and multifunctional clean-up columns have become of increasing importance and in some areas of mycotoxin analysis they have more or less displaced conventional liquid-liquid partitioning or column chromatography during clean-up. The need for rapid yes/no decisions on the other hand has lead to a number of new screening methods. In particular, rapid and easy-to-use test kits based on immunoanalytical principles or the generation of artificial macromolecular receptors employed in molecularly imprinted polymers (MIPs) have made good progress.

Further research in mycotoxin analysis is pursued in the field of biosensors and also the potential of infrared spectroscopic techniques as screening method has been demonstrated. In the area of multi-mycotoxin analysis the most promising development was observed in mass spectrometry. The great potential of LC-MS/MS for screening and identification of a number of mycotoxins as well as their metabolites has recently been demonstrated and will also be discussed.

At the same time, several interlaboratory studies in the field of mycotoxin analysis revealed problems proven by high between laboratory standard deviation and non-traceable results. This not only shows the necessity of reliable methods and well defined performance characteristics but also the need for appropriate calibrants of defined concentration and stated purity. Several (certified) reference materials are already available or under production, incl. ochratoxin A in wheat, deoxynivalenol (DON) in maize and wheat, and zearalenone in maize. With these measures important steps towards traceability of results in mycotoxin analysis have been achieved.

Multimycotoxin analysis by LC-MS/MS in a single sample extract

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Until now mycotoxins have been determined mainly by single compound analytical methods, based on immunoassay clean-up. These methods were collaboratively studied for aflatoxins (Stroka et al., 2000), for ochratoxin A (Entwisle et al., 2000) and fumonisins B_1 and B_2 (Visconti et al., 2001). The immunoassay was considered to be so specific that confirmation was hardly necessary. Increasing quality demands changed these views in the nineties. Methods were developed in which mass spectrometry was applied for confirmation purposes, still based on individual mycotoxins. Hurst et al. (1991) applied thermospray MS for the confirmation of aflatoxins in peanuts, Xiao et al. (1995) used electron impact MS to elucidate ochratoxin structures and Holcomb et al. (1993) choose fast atom bombardment MS to confirm the presence of fumonisin B_1 in food. In our laboratory the need for confirmation emerged when application of deoxynivalenol (DON) assays revealed peaks in the chromatogram that could even be interfering with the DON peak. We started to use the LCQ for confirmation of DON as described by Berger et al. (1999). While doing so the Scientific Committee of Food (2000) published opinions on zearalenone, fumonisins and trichothecenes on the EU website. Such opinions are preceding Commission Regulations, i.e. the opinion on ochratoxin A was published in 1998 and the corresponding regulation was published in 2000. To maintain legal limits it would therefore be preferable to determine mycotoxins in different types of matrices in one single extract by a routine analysis. This is also helpful for HACCP control purposes.

Facing the expected legislation in the future we set out to find a way to analyse as many mycotoxins as possible in one LC-MS run. This principle is originating from the mycological field, where measurements preferably are made in crude fungal extracts to identify mould species (Smedsgaard and Frisvad, 1996). They applied single quadrupole equipment to *Penicillium* isolates, in which they identified 13 mycotoxins, of which ochratoxin A, citrinin, penicillic acid and roquefortine C are the most important. To perform quantification and identification at the same time a triple quadrupole is needed. In that case quantification can be carried out on the parent ion and identification is obtained from characteristic daughter ions. Kussak et al. (1995) applied this technique for the determination of aflatoxins in dust, Lukacs et al. (1996) identified fumonisins in corn with it and Razazzi et al. (1999) determined nivalenol and DON in wheat. But in all these cases the authors still applied clean-up. Janssens and Franken (2000, Technical Laboratory Rotterdam, the Netherlands, personal communication) compiled a method for not only trichothecenes, but also aflatoxins and ochratoxin in a single run in feed. We set out to optimise, extend and validate it for food, as lower limits exist for human consumption. Especially attention was paid to the matrix effect. The result on the first 13 mycotoxins was presented at the Second World Mycotoxin Forum (Spanjer et al., 2003). Since then the method is extended with some 10 mycotoxins more at the XI IUPAC meeting in 2004, until 26 up to now, October 2005.

Materials and methods

Chemicals
All solvents were analytical-reagent or HPLC grade. Water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). Mycotoxins were purchased from Sigma (St. Louis, MO, USA). Stock and standard solutions are prepared by routine procedures.

Analytical procedure
25g grounded sample material is mixed with 100ml 80/20 v/v acetonitril-water during 2 hours. The filtered extracts are diluted 4 times with water. 20 µl of the diluted extract is injected in

the LC-MS system. This consisted of a Waters Alliance 2695 HPLC system (Waters, Milford, MA, USA), coupled to a Quattro Ultima triple quadrupole mass spectrometer (Micromass, Manchester, UK), equipped with a pneumatically-assisted electrospray interface, operating in positive mode, at a capillary voltage of 2.5 kV, a desolvation temperature of 450°C, variable cone voltage (see table), cone gas flow of 100 l/hr and a desolvation gas flow of 600 l/hr, both nitrogen gas. Collision gas was argon at 0.8 bar on pressure regulator. For each mycotoxin the parent and two daughter ions were recorded. Dwell time was 0.02 s.

Calculations were performed by the Masslynx & Quanlynx software. Separation was achieved on a 150 x 3.2 mm ID Alltima C_{18} (5 µm) column (Alltech, Breda, the Netherlands) at a column temperature of 30°C. The gradient was composed from solvents A (0.1% formic acid in water) and B (0.1 % formic acid in acetonitril) at a flow rate of 0.3 ml/min. The solvent program started with 80% A. In 12 minutes the gradient developed to 30% A, which was hold for another 5.5 minutes.

Results

Thus far the method is developed to include aflatoxins B_1, B_2, G_1 and G_2, ochratoxin A, DON, nivalenol, 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol, fumonisins B_1, B_2, and B_3, diacetoxyscirpenol, zearalenone and derivatives, fusarenon-X, T2-toxin, HT-2 toxin, citrinin, cyclopiazonic acid, ergotamin, penicillic acid, roquefortin and sterigmatocystin. Applying the method to food samples revealed the existence of a matrix effect, which was expected due to the lack of clean-up. Logically it depends on the sample type. Validation data are presented for aflatoxins in peanut and fig and ochratoxin and DON in wheat. The matrix effect is shown for aflatoxin B_1, ochratoxin A and DON in peanut and cornflakes.

In several routine samples more then one mycotoxin was actually measured. Results on maize and buckwheat samples, rye samples in which ergot alkaloids were identified and the comparison between the CEN method and this multimycotoxin method for aflatoxin B_1 in peanut are presented. For babyfood samples in which lower aflatoxin and ochratoxin A levels are set in the legislation we had to apply immunoassay again. But then these very low limits were easily achieved by the LC-MS.

Discussion

To separate all compounds a gradient is needed that starts with a high percentage of water, which is unfavourable for the ionisation process in the ion source. In the ESI^+ mode the most prominent ion is the protonated molecular $[M+H]^+$ ion, the parent (m/z). To validate the identity of the parent, these ions are fragmented into daughter ions with argon gas in the collision cell of the triple quadrupole. Quantification was carried out on the parent ion. Identification was based on the ratio of daughter ions and retention time. We did not investigate differences between positive or negative electrospray. Razzazi et al. (1999 and 2002) considered positive ion mode to be preferred for type A and negative mode for type B trichothecenes. Tuomi et al. (1998) experienced positive ion mode to be clearly more effective for trichothecenes. Lagana et al. (2003) proved this by data for the water/acetonitril/ formic acid eluent. From the presented data it is clear that the matrix effect, as indicated by Rundberget et al. (2002) for penitrem A, has to be determined for every separate matrix-mycotoxin combination. Biselli et al. (2004) and Krska et al. (2005) published similar attempts, but focused mainly on trichothecenes, which they worked out in detail.

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(a complete list will be published in the conference book)

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Immunoaffinity clean-up/fluorescence detection methods for mycotoxins

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Purification of extracts in the analysis of mycotoxins in agricultural and food commodities, feeds or biological fluids is a crucial phase, especially when low detection limits are required. The use of antibody-based immunoaffinity columns (IACs) for the purification of the extracts offers a number of advantages when compared to other commonly used clean-up procedures. They include specificity of the antibody that eliminates extraneous interferences in the extract, applicability to complex matrices (i.e. feed coffee, cheese, blood), optimum performances in terms of precision and accuracy within a broad range of concentrations covering the area of practical interest, rapidity of performances, reduction in use of hazardous solvents and possibility of automation. The main disadvantage of using IAC is the high cost of the columns due to the larger quantity of antibody per analysis. Regeneration of IACs for their reuse has been investigated by several authors.

IACs are commercially available for aflatoxins B_1, B_2, G_1 and G_2 (AFB_1, AFB_2, AFG_1, AFG_2), aflatoxin M_1 (AFM_1), ochratoxin A (OTA), fumonisins B_1, B_2 and B_3 (FB_1, FB_2, FB_3), zearalenone (ZEN), deoxynivalenol (DON) and T-2 and HT-2 toxins. Extracts after IAC clean-up are analysed either by high-performance liquid chromatography (HPLC) equipped with a UV or fluorescence detector (FD) or by a dedicated fluorometer allowing more rapid determination. HPLC with fluorescence detection generally gives high sensitivity, selectivity and repeatability of measurements and specific labelling reagents are currently available for the derivatisation of non-fluorescent mycotoxins to form fluorescent derivatives. IACs combined with HPLC/FD are routinely used for the determination of AFB_1, AFB_2, AFG_1 and AFG_2 (after derivatisation with bromine), AFM_1, OTA, ZEN, FB_1, FB_2 and FB_3 (after derivatisation with OPA reagent), T-2 and HT-2 toxin (after derivatisation with 1-anthroylnitril) in several matrices.

Recently, commercial multi-mycotoxin immunoaffinity columns have been used for simultaneous determination of OTA and ZEN or AFB1, AFB2, AFG1, AFG2, OTA and ZEN by HPLC/FD with good accuracy and precision. Automated systems for the HPLC determination of aflatoxins and OTA using IAC clean-up have been also described.

Several methods using HPLC/FD and IAC clean-up have been recently validated by collaborative studies for various mycotoxins in a number of food commodities, and have been adopted as official or standard methods by the AOAC International or the European Standardization Committee (CEN). In particular, methods for the determination of aflatoxins in corn, raw peanuts and peanut butter (AOAC Official Method 991.31), AFB_1 and total aflatoxins in peanut butter, pistachios, figs and paprika (999.07), OTA in barley (2000.03), AFM_1 in liquid milk (2000.08), OTA in roasted coffee (2000.09), OTA in wine and beer (2001.01), FB1 and FB2 in maize flour and cornflakes (2001.04), aflatoxins in animal feed (2003.02) and OTA in green coffee (2004.10) by immunoaffinity column clean-up and HPLC with fluorescence detection have been approved as official methods, first or final action, by AOAC International.

Standardisation activities in mycotoxin methodology

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European standardisation in the CEN working group Biotoxins

Both ISO (International Standardisation Organisation) and CEN (European Committee for Standardisation) are active in the field of standardising methods of analysis for mycotoxins. Currently, CEN is the most active organisation of the both. The working group Biotoxins (WG 5) of the European Technical Committee CEN/TC 275 – ''Food analysis – Horizontal methods'' selects and elaborates methods of analysis for mycotoxins that are to become European standards. The methods need to be established and validated. The standards can be used for mycotoxins, which are subject to legal limits. When used for this purpose, the main functions of the standards are to enable food manufacturers to determine with reasonable certainty whether a production batch may be put on the market. On the other hand, the standards enable regulatory authorities to determine equitably whether foodstuffs on the market comply with legal limits.

The "ISO and CEN way": consensus and ''all parties concerned''

CEN standardisation uses the consensus principle and is based on the ''all parties concerned'' approach. This sometimes has the disadvantage that it can take several years to complete a standard. Especially when parties have different views on the content. On the other hand, the end result is usually broadly accepted by all the parties that were involved in the process.

Currently, about fifty experts from fifteen different CEN countries are active in preparing the European standards in the CEN working group. They come from, amongst others, companies, food inspectorates, government departments, commercial and research laboratories. The group is chaired by the Food and Consumer Product Safety Authority (VWA). VWA plays a pro-active role in the development of the European standards. The Dutch standardisation organisation (NEN) runs the secretariat of the group.

Criteria for selection of methods of analysis

The CEN working group Biotoxins decided to establish some criteria to guide in the selection between several methods of analysis. These performance criteria have been laid down in a CEN Report (CR 13505:1999). They are based on published data, collected from official reports on European interlaboratory studies on the respective mycotoxins (certification studies, method validation studies). Where these performance characteristics are absent or limited in availability, the criteria are estimated based on the experiences and opinions of the experts of the CEN working group Biotoxins. CEN Report CR 13505 gives information concerning method performance, which can be expected from experienced analytical laboratories. It may contain useful information for example for CEN members, the European Commission, the EFTA secretariat or other governmental agencies or outside bodies.

CEN standards and European legislation

The link between CEN standards and European legislation is growing stronger. This can for example be seen from the recent draft mandate from the European Commission to CEN. The mandate concerns the establishment of standardised methods of analysis and sampling in the field of mycotoxins. The standards are intended to enable the control in a harmonised way of the compliance with the provisions of Community legislation in the field of mycotoxins

in food. The draft mandate is related to Regulation (EC) No. 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.

This Regulation establishes in Europe a general framework for official feed and food safety controls to be performed by national authorities along line the food chain. Analytical methods accepted by CEN will be used for performing these controls, and future development of European standardisation is foreseen.

Already published European standards

The CEN working group Biotoxins of CEN/TC 275 has published fourteen European standards so far on the analysis of mycotoxins. Amongst them are standards for e.g., aflatoxin B and G, ochratoxin A, fumonisins B_1 and B_2, and patulin. Commodities for which they have been validated are: cereals and cereal products, shell-fruits and maize, peanuts, pistachios, figs, paprika powder, barley, roasted coffee, wine, beer and apple juice.

Sampling and sample preparation also belong to the scope of the working group. A recent publication for example deals with the issue of sample comminution for mycotoxin analysis. This so-called CEN Technical Report describes an extensive comparison study between dry milling and slurry mixing.

Further information

If you are interested and want to know more about the European standardisation activities in the field of mycotoxins, you can contact the secretariat of the CEN working group:

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How to deal with measurement uncertainty?

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Analytical results form the basis for any decision taking process in official food control (OFC) concerning mycotoxins (acceptance or rejection of goods). Therefore the correct interpretation of any analytical result is of key importance. Especially the fact that analytical results are associated with an uncertainty (probability dispersion around the result, within which the "true" value most likely will fall) is of utmost significance, as the basis for any decision (rejection) must be "beyond reasonable doubt". This recently led to European legislation on mycotoxins in which uncertainty statements have to be taken into account when comparing analytical results with legislative limits [1].

Uncertainty is a function of several factors that can depend on e.g., the analyte concentration, the matrix to be analysed, the method used, etc. Therefore there is no "universal" or "general" figure, but uncertainty has to be estimated for each different analytical scenario.
Several ways exist to estimate (or measure) uncertainty and extensive monographs have been published on this matter [2,3]. These documents supply a scientific sound, detailed and logic basis for the principles of measurement uncertainty (MU).

One of the main aims in analytical laboratories implementing MU is, that the estimation/establishment of MU should be logical and scientific but in addition to this also realistic and practical (easy) to establish, where possible. With a focus on this aim, several position papers, guidelines and procedures have been drafted to facilitate an estimate of uncertainty by practical means. The basis of these documents is identical: "Providing means to establish a reasonable/realistic estimation of the uncertainty". Nevertheless the concepts of approaching uncertainty might differ. For example, some approaches involve the use of precision data derived from collaborative studies; others use mainly data from in-house experiments.

The issue on how to deal with MU has been discussed at EU expert level, which led to a report that aims to give guidelines on this matter with respect to OFC [4]. This report lists recommended procedures – as mentioned above – for the estimation of MU that are considered as equally valid for the use in OFC.

This presentation will give an overview on the recommended procedures and discusses the main impact that MU has on daily laboratory procedures and data interpretation.

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The potential carry-over of various mycotoxins into animal products

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Although it is generally accepted that the carry-over of the *Fusarium* toxins deoxynivalenol (DON) and zearalenone (ZON) into foods of animal origin is rather low, and does not significantly contribute to human exposure [1,2], there is still a need to monitor the transfer of these toxins for several reasons. Both DON and ZON, among other mycotoxins, play a central role in animal feeding as they are frequently detected in feedstuffs at concentrations, which might cause adverse effects in livestock.

Earlier carry-over data, often obtained from kinetic studies employing radioactively labelled toxins, revealed variable results and practically relevant toxin concentrations from naturally contaminated feedstuffs were often not considered. Therefore, we combined feeding experiments with dairy cows, pigs and poultry with carry-over studies when examining the adverse effects of these *Fusarium* toxins from contaminated feedstuffs. Moreover, a number of varying feeding conditions which could potentially influence the transfer of DON and ZON or of their metabolites were considered in this way.

For example, the carry over of DON into the milk of dairy cows was examined in dependence on feeding level of the cows [3] as we hypothesised that a faster feed transit through the digestive tract occurring with higher feed intakes would concomitantly decrease the time available to degrade DON to de-epoxy-DON by rumen microorganisms. If this were to be the case, then it could explain the occurrence of the not metabolised parent toxin DON in the blood and milk of cows as it became evident from some unpublished reports. However, within the range between 5.6 to 20.5 kg dry matter (dm) intake per cow and day, and a dietary DON concentration of 3.4 mg/kg dm, approximately 94-99% of the flow of DON plus de-epoxy-DON at the duodenum occurred as de-epoxy-DON. A comparable metabolite profile was detected in blood serum and milk, suggesting the small intestine as the main site of absorption of both compounds and a minor role of hepatic de-epoxidation. That the small intestine can be viewed as the main site of DON-absorption is supported by the *in vitro* findings of Dänicke et al. [4] who demonstrated the intact rumen epithelium to be an efficient barrier to DON. The carry-over rates expressed as the ratio between the excretion of DON or de-epoxy-DON with milk and DON intake ranged between 0.0001 and 0.0002, and 0.0004 and 0.0024, respectively. It was concluded therefore that in healthy cows DON is detected in physiological samples mainly as de-epoxy-DON, and that the level of feed intake does not influence the metabolite profile. The carry over rates of DON and de-epoxy-DON increased significantly with the milk yield although this was observed at a very low level. At the tested dietary ZON concentration of 0.062 mg/kg dm, we were not able to detect any traces of ZON or its metabolites in the milk [5].

In growing bulls fed a ration containing 0.76 mg ZON/kg dm, ZON residues were detected only in bile, where β-ZOL amounted to approximately 68 % of total metabolites, whereas the respective percentages of α-ZOL and ZON were 8 % and 24 % [6]. Supplementing the contaminated ration with a detoxifying agent even increased the concentration of ZON-residues in bile. Residues in serum and blood cake, muscle, kidney, liver, fat from the kidney cavity and back fat were all below the detection limits of the used HPLC-method.

In fattening pigs, we examined the effects of practically relevant DON-concentrations of up to 1 mg per kg diet on DON residues in muscle and liver and calculated approximate carry-over factors as the ratios between the concentration of DON in tissue and diet of 0.002 to 0.003 [7]. Pelleting of the diets, which was found to improve nutrient digestibility, did not increase

the tissue residues. In a further experiment with fattening pigs, the effect of the feeding regimen on DON residues was examined [8]. No differences in tissue residues could be detected when the DON-containing diet was fed either *ad libitum* or restrictively. The carry-over factors were approximately 0.002, 0.004 and 0.01 in muscles, liver and kidney, respectively, at a dietary DON concentration of approximately 7 mg/kg. De-epoxy-DON was also detected in livers at this dietary concentration and its proportion of the sum of DON plus de-epoxy-DON reached approximately 8 %. This proportion was found to vary even at a higher level between 16 and 45 % in livers of gilts fed diets up to approximately 9.7 mg DON/kg [9]. In the same experiment, the transfer of ZON was examined. Its concentration in the experimental diets was increased up to approximately 0.36 mg/kg. No residues of ZON or its metabolites were detected in livers of the gilts, but total ZON residues were present in bile up to 596 ng/g. The mean proportion of α-zearalenol (ZOL), β-ZOL and ZON of the sum of all three compounds were 52, 3 and 45 %, respectively. This metabolite profile is different from that found in bile of immature female pigs fed diets up to 0.42 mg ZON/kg diet, where Döll et al. [10] reported mean proportions of α-ZOL, β-ZOL and ZON of 26, 2, and 72 % at comparable total residues of up to approximately 575 ng/g. Not only does the metabolite profile seem to be different, but also the carry-over of these toxins. A mean carry-over factor of 0.02 was found for the liver of these piglets. Therefore, the age of the pigs at slaughter seems to play a role when evaluating the transfer of ZON and its metabolites to the liver.

This carry-over factor is markedly higher than for laying hens. Dänicke et al. [11] reported a mean carry-over factor, calculated for total ZON residues, of approximately 0.005 at a dietary ZON-concentration of 1.1 mg/kg fed over a period of 16 weeks. No ZON-residues were detected in breast meat, abdominal fat, yolk or albumen. Supplementing of the contaminated diet with a detoxifying agent did not prevent the transfer of ZON-residues to the liver or even to the bile. Moreover, neither DON nor de-epoxy-DON could be detected in yolk or albumen of eggs from the same study at a dietary DON-concentration of 11.9 mg/kg dm [12]. In Pekin ducks, neither DON nor de-epoxy-DON could be detected in plasma or bile at dietary DON concentrations up to 6-7 mg/kg [13], suggesting only a minor role of carry-over of DON in this species. Dietary ZON concentrations of 0.05 to 0.06 mg/kg resulted in a dose-dependent increase in the concentration of ZON-residues in bile, but were lower than the detection limits in the liver.

Taken together, the carry-over of DON and ZON into edible tissues of farm animals seems to be rather low, and tested nutritional manipulations (level of feed intake in dairy cows, feeding regimen for pigs and pelleting of pig diets, supplementing laying hen and growing bull diets with a detoxifying agent) had no effects on the already low DON- and ZON-residue concentrations.

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Current concepts in the etiology and prevention of mycotoxicoses in livestock and poultry

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The frequency and severity of mycotoxicoses appears to be increasing globally. Analytical surveys indicate that while aflatoxin has long been recognised as a significant problem, *Fusarium* mycotoxins are now understood to cause major economic losses in both temperate and tropical climates. It is also now clear that combinations of mycotoxins arising from blends of contaminated ingredients in finished feeds can cause toxicological synergism that increases the severity of mycotoxicoses. This is particularly true for *Fusarium* mycotoxins that result in conditions such as immunosuppression and increased production losses arising from disease challenge.

When mycotoxins cannot be eliminated from raw materials due to adverse climatic conditions preharvest or due to poor storage facilities, therapeutic and preventative strategies can be employed to minimise production losses and to ensure safety and wholesomeness of foods of animal origin. Therapeutic strategies include nutrient fortification, which minimise mycotoxin-induced damage to target tissues such as liver and promote mycotoxin metabolism and excretion. Preventative strategies include the use of supplements containing enzymes of bacterial origin, which degrade mycotoxins in the intestinal lumen thereby minimising toxicity. Another preventative approach is the use of organic and inorganic mycotoxin adsorbents, which minimise intestinal absorption of mycotoxins and promote their faecal excretion. Such adsorbents are non-digestible large molecular weight polymers that adsorb small mycotoxin molecules through various mechanisms. The efficacy of preventative strategies can be determined *in vitro* and *in vivo. In vitro* assays have been most often applied to mycotoxin adsorbents. The efficiency of adsorbence can be determined in test tubes under controlled conditions or using more sophisticated incubation models that mimic the conditions of the gastrointestinal tract. Only *in vivo* studies, however, can include environmental factors such as housing density and disease challenge in tests of efficacy.

Studies of the feeding of combinations of *Fusarium* mycotoxins to starter pigs indicate that appetite suppression is the major cause of reduced growth. Such diets also result in reduced appetite when fed to sedentary horses. When metabolic energy requirements are increased, however, such appetite suppression is largely overcome. This has been observed in gestating sows and exercised horses. Laying hens also more readily consume *Fusarium* mycotoxin-contaminated grains compared to broiler chicks, turkeys and ducks. The behaviour of reduced feed intake serves as a natural mechanism to protect animals from the adverse effects of mycotoxins on metabolism. When such behavioural mechanisms are overridden, greater metabolic toxicity is seen.

When mycotoxins cannot be eliminated for animal and poultry feeds, preventative strategies can be employed to maximise production efficiencies and ensure food safety.

The potential of dietary mycotoxin sequestering agents

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Mycotoxins are toxic chemical by-products of fungi that frequently occur as contaminants of food. In historical context, the mycotoxin problem from mouldy foods is longstanding, unavoidable and seemingly inextricable. Hazardous mycotoxins that have been implicated in disease and death in man and animals include the aflatoxins, trichothecenes, fumonisins, zearalenone, ochratoxins, and ergots. Of these primary toxins, aflatoxin B_1 is a direct acting mutagen and a very potent carcinogen. Moreover, outbreaks of aflatoxicoses in humans have been well-documented in countries where warm, humid climates are conducive to the growth of *Aspergillus* fungi and the production of toxic metabolic products.

Research in our laboratory at Texas A&M University has focused on the development of innovative sorption strategies for the detoxification of the aflatoxins. In particular, the laboratory has employed isothermal analyses and molecular modelling techniques to characterise and design clay-based materials for the enterosorption (and inactivation) of aflatoxins. The findings are of direct relevance to human health in rural communities and developing countries, where occurrence of these agents and human exposure is often elevated. One aim of the laboratory is to understand the surface chemistry and mechanisms involved in the interactions of processed clay minerals. A calcium montmorillonite clay (i.e., NovaSil or NS) has been shown to prevent the adverse effects of aflatoxins in various animals when included in the diet. Studies have also confirmed that NS does not protect animals against other mycotoxins, and it does not interfere with the utilisation of important vitamins and micronutrients in the diet. Results have shown that NS clay binds aflatoxins with high affinity and capacity in the gastrointestinal tract, resulting in a notable reduction in exposure from these poisons.

This same technology, which should be culturally acceptable in developing countries, may one day be applicable and sustainable for use in human foods. A recent Phase I clinical trial in Texas has confirmed the safety of NS for further study in humans. Future objectives are to assess the safety and efficacy of NS in Phase II intervention studies in Ghana, where NS clay will be delivered by mouth using capsules before meals. In these studies, biomarkers of aflatoxin exposure in urine and blood will be measured to determine the consequences of NS treatment on aflatoxin bioavailability from the diet. Eventually, the preferred delivery of NS clay may be through its inclusion in common foods such as peanut butter and cereals or as an additive to vitamins and condiments.

In summary, enterosorption strategies based on NS clay hold great promise for the management of aflatoxins. The NS clay remedy is novel, inexpensive and easily disseminated. Since clay minerals are structurally and chemically diverse, many are ineffective and/or nonselective for the aflatoxins. Based on our research, all aflatoxin sequestering clays should be rigorously evaluated *in vitro* and *in vivo*, and should meet the following criteria: (i) favourable thermodynamic characteristics of ligand sorption; (ii) tolerable levels of priority metals and dioxins/furans based on JECFA/WHO recommendations; (iii) efficacy in multiple animal species; (iv) safety in long-term studies; and (v) negligible interactions with vitamins, micronutrients and other mycotoxins.

Adsorptive chemical properties of yeast cell wall based product toward several mycotoxins

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No technology is actually available to totally eliminate mycotoxins from the food and the feed chain. Many studies carried out during the last decade indicate that organic adsorbents such as yeast cell walls can be added to contaminated food or feeds to selectively bind mycotoxins, allowing the toxins to pass through the digestive tract without any negative effect on animals or carry over to edible animal products such as milk, eggs or meat and thus be detrimental to consumers. Our work was devoted to better understand the potentialities and the chemical mechanisms involved in the adsorption process of organic binders toward zearalenone, which has been taken as model mycotoxin.

In this respect, using an *in vitro* methodology to test the binding capacity of individual components of *Saccharomyces cerevisiae* differing in their cell wall glucan/mannan/chitin content, we observed that ß-D-glucans, which are composed of linear chains of ß-(1,3)-D-glucans branched with ß-(1,6)-D-glucan side chains, are mainly involved in adsorption of zearalenone (affinity rates up to 30%) in aqueous solution. An affinity rate approaching 50 % was reached with the alkali-insoluble fraction of yeast cell wall ß-D-glucans. The interaction was governed by a co-operative relationship between the toxin and ß-D-glucans for the low ZEN concentrations, and the Hill's model was selected to fit the adsorption curve (R^2 = 0.969; RSD = 0.296 µg/ml). The affinity is greatly depending on the quantities of ß-D-glucans present in the cell wall and their structure related to the network organisation of ß-D-glucans involving single and/or triple helix structure. Furthermore, the decrease of chitin content after extraction of ß-D-glucans demonstrated its negative implication in adsorptive process due to the rigidifying properties of chitin toward the ß-D-glucan network, restricting ZEN accessibility to the interactive binding sites in the ß-D-glucan helix.

NMR investigation and equilibrium studies using bound and free toxins balanced according to various environmental conditions indicated that weak chemical linkages such as hydrogen and Van der Waals bonds occurred between ß-D-glucans and both the hydroxyl groups of the phenol moiety of ZEN and the ketone group of the non-phenolic ring. These results were used together with X-ray spectra of mycotoxins to produce several *in silico* models showing the ZEN molecule caged inside the helix-shaped ß-(1,3)-D-glucans, which were firmly stabilised by ß-(1,6)-D-glucan branched side chains. The stability of [ß-D-glucans + ZEN] complexes is explained by the extraordinary geometric similarities between ZEN spatial organisation and the single-helix conformation of six ß-D-glucopyranose residues per turn of the ß-(1,3)-D-glucan chain previously determined by X-ray diffraction. Interaction stability was assessed calculating the potential energy of the complex.

This scientific approach improves our knowledge of the chemical complexation mechanisms of organic adsorbents towards ZEN, and of powerful methods for investigating these mechanisms. Furthermore, our work confirmed the adsorptive properties of ß-D-glucans for five other mycotoxins: aflatoxin B1, deoxynivalenol, fumonisin B1, ochratoxin A and patulin. Affinity rates varied widely between toxins due to their structural and physico-chemical disparities. Nevertheless, we concluded that ß-D-glucans may have strong affinities for mycotoxins exhibiting "aflatoxin-like", "deoxynivalenol-like" or "zearalenone-like" structures.

Detoxification of mycotoxins by biotransformation

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The main problem of dealing with mycotoxins in animal nutrition is the big number of different molecules in that group of secondary fungal metabolites. This leads to differences in chemical and physical properties resulting in a great variety in their mode of actions. Secondly we know that it is very difficult to control mycotoxin contaminations. Although we are using preventive strategies to reduce the production of mycotoxins on the field, during harvest and storage, contaminations still occur. For this reason, detoxification strategies have been developed to protect livestock animals receiving “low quality grains” that are often contaminated with mycotoxins.

The most often used approach to deactivate mycotoxins in animal feeds is the application of clay minerals capable of binding fungal metabolites and hence reducing the resorption in the gastrointestinal tract. However, there is a great drawback of this method, because it has been reported several times that in feeding experiments this method just works against aflatoxins (AF). These data could also be confirmed by our own studies using different clay minerals for the detoxification of ochratoxin A (OTA). For these reasons further strategies for combating mycotoxins in food- and feedstuffs need to be developed.

A very promising method for detoxifying mycotoxins is biotransformation. Certain enzymes and microorganisms have the capability to transform mycotoxins to metabolites, which are non-toxic. This process is very specific, irreversible and not only limited to a certain group of mycotoxins as it is the case with aflatoxin-binders. The only prerequisite for biotransformation (enzymatic or microbial degradation) is the availability of respective microorganisms and/or enzymes capable of detoxifying fungal toxins. This can also be the drawback of this method, because the great variety of mycotoxins with different molecular structures requires different microorganisms or enzymes to degrade the whole spectrum of mycotoxins occasionally occurring in feed and feedstuffs. Furthermore it is very crucial to use microorganisms which are not able to produce metabolites being more toxic than the parent compound. Dealing with biotransformation also requires good analytical skills, microbiological experience as well as knowledge in toxicity assays. However, looking up in the literature, it can be seen that biotransformation is not really a new issue. The first microbes capable of degrading certain toxins were already published in the sixties. Sargeant et al. [1] described a mixture of complex lactones (aflatoxins) in groundnuts which were responsible for a serious outbreak of the “turkey X disease” in England 1960. Only a few years later there were the first reports on the bacterium *Flavobacterium aurantiacum* NRRL B-184, which is able to detoxify AF [2]. Since then some other papers on microorganisms like *Mycobacterium fluoranthenivorans* sp. nov or *Aspergillus flavus* both capable of degrading AF have been published [3,4]. Although first attempts of biotransformation and degradation of aflatoxins were made in the sixties, no practical application has been published so far. One reason for this might be the fact that the application of certain clay-based adsorbents leads to very good results in practice.

Concerning mycotoxins belonging to the group of trichothecenes a lot of detoxification studies using rumen fluid and intestinal contents were performed in the eighties and nineties. Rumen fluid was chosen because ruminants are known to be very resistant against toxic effects of trichothecenes, like deoxynivalenol (DON). King et al. [5], Swanson et al. [6], He et al. [7] and Kollarczik et al. [8] conducted successful *in-vitro* trichothecene transformation experiments with ruminal or gut microflora. Mixed cultures of anaerobic microorganisms are capable of detoxifying DON by enzymatic reduction of the 12,13-epoxy-group to a diene, resulting in the known metabolite DOM-1, which was first described by Yoshizawa et al. [9].

DOM-1 appeared non-toxic in toxicity studies of Kollarczik et al. [8]. However, no pure culture of the DON-biotransforming strain could be isolated. Binder et al. [10] were the first who described a novel strain of *Eubacterium* sp. having the ability to biotransform DON to DOM-1. Up to now this is the only pure culture of a microorganism, which is able to detoxify DON by biotransformation. Based on the *Eubacterium* strain (BBSH 797) a mycotoxin deactivating feed additive was developed and tested in several feeding trials [11].

El-Sharkawy and Abul-Hajj [12] published that the fungus *Gliocladium roseum* is able to open the lacton ring of zearalenone resulting in a detoxification because the metabolite of this reaction does not any longer fit to the binding site of the estrogen receptor. Duvick et al. {13,14] patented *Rhodococcus erythropolis* and *Norcardia globulera* as ZON degrading strains. There are no reports available stating that those microbes were used for a practical application in feed. We recently found a yeast strain belonging to the genus of *Trichosporon*, which is able to degrade zearalenone to a not yet identified metabolite. However, several tests using oestrogen-receptor-based *in vitro* assays revealed, that the resulting metabolite is non-estrogenic.

Only 4 years after the discovery of ochratoxin A (OTA) [15] it was described that the enzyme carboxypetidase A is able to cleave OTA to phenylalanine and the non-toxic metabolite OTalpha [16]. Further microorganisms capable of detoxifying OTA are *Phenylobacterium immobile* [17] and *Acinetobacter calcoaceticus* [18]. Recently some reports on the OTA-detoxification capabilities of *Aspergillus* and *Rhizopus* fungi were published. None of these strains were useful for the development of an OTA deactivating feed additive and therefore we started a screening for detoxifying microorganisms [19,20]. More than 20 OTA-cleaving microorganisms could be isolated. In a selection process a novel yeast strain turned out to have the best requirements to get an ingredient in an animal feed additive for OTA deactivation. This yeast strain was characterised [21] and tested in a feeding trial with chickens [22].

Exophiala spinifera, *Rinocladiella atrovirens* and an aerobic bacterium (ATCC 55552) were the first isolates described for fumonisin (FUM) detoxification. Due to some limitations (e.g. FUM has to be the only carbon source present) the above mentioned microorganisms cannot be used as animal feed additives. Therefore we did an extensive screening of microbial strains derived from strain collections and various different natural habitats for the presence of FUM degrading microbial activity. While a screening under anaerobic culture conditions (pig intestines), as well as the testing of nearly 150 organisms from strain collections did not show positive results, FUM transforming activity could be detected in one soil sample and a number of maize samples. Trials in order to isolate the respective fumonisin degrading microorganisms out of the enriched cultures resulted in a number of strains, whose FUM degrading activity could be proven. The most promising bacterial and yeast strains were further characterised with regard to a general taxonomic description, and to different aspects of their toxin degradation behaviour (effective FUM concentration, time course of fumonisin transformation, degradation behaviour under different physiological conditions). Some of the isolates are currently evaluated for their use as a fumonisin detoxifying feed additive.

The limitation of clay minerals, which just can deactivate aflatoxins, can be overcome by the use of the biotransformation concept. We have selected certain microorganisms, which have proven their efficacy in detoxifying trichothecenes, zearalenone, ochratoxins and fumonisins. However, besides of the above mentioned groups of mycotoxins, there are reports on several other fungal toxins occurring in feed commodities and some fungal metabolites still need to be discovered. Such “unknown” mycotoxins can directly affect the animal or can have some synergistic effect when occurring together with already known mycotoxins. Therefore we need hepatoprotective and immune stimulating substances in addition to binding agents and microorganism or enzymes to alleviate mycotoxicosis caused by the “less important” mycotoxins.

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Mycotoxin feed issues in Latin America

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Latin America has had several mycotoxin feed issues, almost all the mycotoxins are present, but only the most important mycotoxin outbreaks will be described.

- Argentina. Aflatoxicosis and ochratoxin A (OTA) in the poultry industry from several feeds based on maize, sorghum and sunflower, with high mortality and economical losses. OTA has been reported in grapes and wines; altertoxin I and altenuene in sunflower; Fusariotoxins deoxynivalenol (DON) in wheat and fumonisins in maize.
- Bolivia. Several *Fusarium* toxins; Brazil nut culture with aflatoxins; studies of OTA in human milk.
- Brazil. Peanuts from this country were the cause of the death of 100,000 turkey in the UK, called "turkey X disease", which was the first issue about aflatoxins known worldwide. Also the presence of fumonisins in maize in the Southern region is noted. The poultry industry suffers from aflatoxicosis, OTA and T-2 toxin and a monitoring system for cereals (maize, barley sorghum) and feed has been applied. Feed for bovine cattle has also been contaminated with OTA and aflatoxins. There was a fumonisin poisoning issue of catfish (*Ictalurus punctatus*) used in cat feed. Furthermore patulin in apple juice export; aflatoxins in cattle, pigs and poultry industry are frequently occurring; aflatoxin M_1 (AFM_1) in milk, egg and rice; citrinin in cheese and pig kidney; moniliformin in maize; OTA in coffee; zearalenone in sorghum and rice; citrinin, OTA and aflatoxins in black beans.
- Costa Rica. The big problem is aflatoxicosis in maize due to the tropical conditions of the country.
- Cuba. Aflatoxins in cereals, cocoa, coffee and spices, as well as patulin in mango.
- Guatemala. Aflatoxins in maize.
- Mexico. In 1986 an outbreak of equine leukoencephalomalacia happened in the States of Jalisco and Mexico – fumonisins were not known then – and the mortality rate was high. In 1995, the death of 200,000 chickens in Tapachula, State of Chiapas, was due to aflatoxicol. In Iguala, State of Guerrero, the death of 40,000 chickens by T-2 happened; the poultry feed came from Argentina by ship. Aflatoxins, fumonisins, zearalenone and DON in maize from Chiapas and Michoacan. The presence of aflatoxins in milk and cereals has been frequent, and with maize as the staple food for animal and human consumption, the problem is increasing. Another problem is zearalenone and T-2 in Yucatan from imported maize used as feed for pigs. Silage feed has been contaminated with aflatoxins, nivalenol, OTA, T-2 toxin, DON and zearalenone. Also OTA in green coffee; aflatoxins in dry chilli and peanuts.
- Peru. Some reports about killing strains of *Fusarium oxysporum* mycotoxins in coca and non-coca plants.
- Uruguay. Ergot is a problem in cattle; aflatoxins and Fusariotoxins in cereals.
- Venezuela. Mainly aflatoxins, fumonisins, DON, OTA, zearalenone and citrinin in feed based on maize, wheat, oil seeds, cotton seed, peanut, coffee, sorghum, sunflower, rice (also paddy rice), milk and yucca. In 2003 there was an outbreak of aflatoxins that caused high mortality rate in thousands of dogs.

SPOTLIGHT PRESENTATIONS

Mycotoxin analysis – from screening to confirmation: the ochratoxin A case

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Globally, there is an increased awareness in mycotoxins. This is reflected in an increasing number of commodity regulations and also by growing industry and consumer demand for knowledge regarding the presence or absence of mycotoxins. Legislation and demand are one thing, but there must be means to obtain the required information. These methods differ depending on the level of accuracy required and the technical expertise available.

On the example of ochratoxin A, a mycotoxin produced by specific *Aspergillus* and *Penicillium* species and found frequently in cereals, dried fruits and coffee, but also in products from animal origin, multiple approaches and possibilities for testing will be shown. On one end you will find easy-to-use methods such as test cards (Ochracard), which require only minimal laboratory equipment, no measurement equipment and limited technical expertise. Using this test kit one receives a cut-off result showing if the sample is below or above a certain value.

For more precise information about the contamination level of a sample, the method of choice for screening is an enzyme immunoassay (ELISA). This type of assay requires only a basic laboratory infrastructure. The RIDASCREEN® ochratoxin A or RIDASCREEN®FAST ochratoxin A. ELISAs provide semi-quantitative results with a visual evaluation or quantitative results using a spectrophotometer. To obtain more precise information, an increased level of technical knowledge, better infrastructure and slightly more elaborate sample preparation are required. Nevertheless, these methods can easily be implemented in laboratories, which are not specialised in mycotoxin analysis yet.

As a confirmation method high performance liquid chromatography (HPLC) is most prevalent. Performing HPLC, there is a higher demand on the quality and purity of sample preparation. To achieve this, sample extracts have to be cleaned by immunoaffinity columns using highly specific antibodies to clean and concentrate a specific mycotoxin (Ochraprep®).

The presentation will show the principles behind all these technologies, give their advantages and disadvantages to determine “fit for purpose” application, and will also give information about the correlation of results.

New developments and applications for mycotoxin detection

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L.M. Streppel and P.J.M. van Wichen
Euro-Diagnostica B.V., the Netherlands

Euro-Diagnostica is committed to achieving the highest level in the supply of a qualitatively superior package of diagnostic products tailored to Food Safety Control. We service customers all over the world with a team of experienced product managers with an in-depth scientific background. We make use of a worldwide network of distribution partners who are dedicated to the goals of our organisation, providing the best possible solutions for our customers.

Euro-Diagnostica has two platforms for mycotoxin screening in various matrices. These comprise the Flow-Through Rapid Test and the Enzyme-Immunoassay (EIA). The Flow-Through Rapid Test is innovated and optimised to suit end-user applications under non-laboratory conditions. Signal differentiation between positive and negative samples has been enhanced for visual interpretation (New Device). The test is simple to the extent it can be carried out without any laboratory training. It is also rapid with results obtained within 10 minutes. It is far less costly as it does not require equipment. The Flow-Through Rapid Test is currently available for aflatoxin B_1 (AFB_1), ochratoxin A (OTA), and zearalenone (ZEA). These have been validated for matrices ranging from simple cereals to the more difficult spice matrices with at least 95% accuracy. Since the market introduction, about six months ago, we have especially aimed at OTA detection in green coffee. We dare to say that our customers are enthusiastic about this unique product.

Euro-Diagnostica B.V. has developed and validated a sensitive ELISA method for screening for the presence of AFB_1. According to Commission Regulation (EC) no 683/2004 of 13 April 2004, the maximum level of AFB_1 in food intended for babies, infants and young children is 0.1 µg/kg (100 ppt). The EIA for AFB_1 is sensitive (standard curve ranging from 2.5 to 40 ppt) and shows minimum effects for the different matrices. The method is used to control food intended for babies, infants and young children for AFB_1 at levels down to 30 ppt.

Both the Flow-Through Rapid test for the qualitative screening of mycotoxins and the EIA for quantitative analysis of AFB_1 are cost effective and reliable aids for your screening laboratory.

Quantitative determination of aflatoxin in grain from 0 to 25 ppb using a lateral flow assay and reader

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A quantitative lateral flow assay method, termed the Charm Rosa Aflatoxin MRL (Quantitative), for aflatoxin B_1 detection between 0 and 25 ppb is presented for grains. The kit has a limit of detection of under 2 ppb. This kit is similar to the Charm Rosa Aflatoxin (Quantitative) kit that recently passed GIPSA defined guidelines for 5-100 ppb total aflatoxin detection for corn using a 70% methanol extraction. In Europe, ethanol may be the preferred solvent for extraction and the Charm Rosa Aflatoxin MRL (Quantitative) kit has been calibrated using either a 70% methanol extraction or a 50% ethanol extraction.

The general procedure is as follows: (i) a milled/ground sample is extracted with solvent; (ii) a 100 µl portion of extract is added to 1 ml dilution buffer; (iii) a 300 µl portion of the diluted extract is added to a lateral flow test strip; (iv) the test strip is incubated for 10 minutes at 45°C; and (v) the test strip is removed and read in the calibrated reflectance reader. The reader provides a numerical result from 0 to 25 ppb by comparing the binding intensity of 2 test lines and a control line. Corn, wheat, cracked corn, popcorn, soybeans and rice have been successfully tested with this kit using both solvent extraction methods.

A new fluorescence based test kit for rapid and quantitative determination of deoxynivalenol in grains

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The contamination of food and feed by mycotoxins has a big impact on the food industry. Various toxins cause different symptoms, ranging from reduced feed intake in farm animals up to causing cancer in humans.

According to a new Regulation of the Commission of the European Communities [1], Member States have to adapt from July 1st, 2006 maximum levels for *Fusarium* toxins, such as deoxynivalenol (DON), zearalenone and fumonisins in certain foodstuffs. Such an approach ensures that food business operators apply all possible measures to prevent/reduce the contamination as far as possible in order to protect public health. The maximum level for deoxynivalenol in unprocessed cereals other than durum wheat, oats and maize is set to 1250 µg/kg, for unprocessed durum wheat and oats 1750 µg/kg. No specific methods for the determination of *Fusarium* toxins levels in foodstuffs are required by legislation, laboratories may choose any technique provided that the selected method meets the proposed criteria [2].

For deoxynivalenol contaminations below 500 ppb performance characteristics require a recovery in the range from 70% to 120% (RSDr % ≤ 40, RSD_R % ≤ 50). The analytical procedure of the new fluorescence-based test takes up to 12 minutes for extract purification, derivatisation and measurement. The advantage of this system is its speed and easy procedure providing quantitative DON determination. It can be learned quickly and performed by a person with little or no chemical training and experience. It is designed for single sample testing, and supplies extremely fast detection. To ensure international standard, the internal validation procedure for wheat, maize and barley is performed regarding to EU Directives 2005/38/EC and USDA/GIPSA requirements.

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2. Commission Directive 2005/38/EC of 6 June 2005.

Immunoaffinity column technology – multiple analyte columns with HPLC and LC/MS: a view toward the future of mycotoxin testing

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The question of how to manage mycotoxins in the world's food supply continues to be a concern for scientists and lawmakers. Regulations are in place for many mycotoxins, and more are proposed while awaiting approvals by their respective countries and trade regions. Demand is increasing for analytical methods that detect mycotoxins at very low levels while maintaining high standards for accuracy and precision. VICAM immunoaffinity columns, coupled with HPLC, LC/MS and fluorometry, offer versatile and efficient analytical methods which are trusted by scientists and technicians in more than 100 countries.

VICAM is pleased to introduce the next generation of immunoaffinity column technology: multi-analyte immunoaffinity columns (also known as Combi-columns). Combi-columns offer the advantage of a single sample preparation, which isolates several mycotoxins from one sample. Combi-columns are used with HPLC or LC/MS for detection and quantitation with a wide range of sample matrices.

Improved methods and the ability to combine several major mycotoxins into a single assay will provide more efficient ways of monitoring contamination levels while ensuring the safety and quality of the world's food, grains and animal feeds.

Ultra performance LC-MS/MS for improved analysis of mycotoxins

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This study describes a method for the determination of multiple mycotoxins in food using Liquid Chromatography-Mass Spectrometry. Aflatoxins and ochratoxin A are subject to EU legislation for some years. Deoxynivalenol (DON) legislation is drafted and EU member states are requested to maintain the proposed level in advance. EC is considering legislation for *Fusarium* toxins like fumonisins, T-2, HT-2, zearalenone and nivalenol. All these legal limits are set for a variety of matrices. Analysis is mainly carried out by HPLC after immunoassay clean-up or in a kind of ELISA procedure. This implies application of single mycotoxin analyses. Trichothecenes can be analysed as a group, which has been done firstly by GC-MS.

The last years LC-MS is introduced in mycotoxins analysis, but until now applications still are published as single mycotoxin analysis or as analysis of a group of similar compounds. To maintain legal limits it would be preferable to determine mycotoxins in different types of matrices in one single extract by a routine analysis. Such as to avoid any additional steps or further purification of a food matrix extract.

After obtaining a triple quad with Acquity Ultra performance liquid chromatography (UPLC) we therefore set out to combine published and unpublished LC-MS methods to obtain a method in which mycotoxins subject to actual and upcoming interest could be analysed in one extract by a single (UP)LC-MS run by means of multiple reaction monitoring. With the described method it is possible to determine aflatoxins, ochratoxin A, DON, 3-acetyl-deoxynivalenol (3-Ac-DON), fumonisin B_1 and B_2, diacetoxyscirpenol (DAS), zearalenone (ZON), T-2 toxin and HT-2 toxin. The use of UPLC contributes to the shortening of analysis time and improved sensitivity. This study report presents the results of a multi-mycotoxin method with which EC regulations can be obtained in a less laborious way.

The success and failures of mycotoxin adsorbents in basic research and field applications

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The last few years have brought an incredible amount of information about the nature of absorbents that can be used to decrease the bioavailability of mycotoxins. As a result, new natural strategies for controlling the disease processes associated with mycotoxins have been elucidated. Since these fungal toxins are often effective at low concentrations, it has been necessary to develop new mechanisms for understanding the basic adsorption processes involved in toxin clearance. It is clear that simple “binding assays” do not reflect the efficacy of many of the adsorbent materials that interact with mycotoxins. Absorption kinetics based on overall capacity, molecular folding shifts and affinity are now becoming available. All of these will be useful in defining the most efficient applications and strategies for producing new and more useful sequestering agents. Studies with the yeast cell wall based product, Mycosorb are setting new standards for evaluating adsorbents and their effects.

The future holds many challenges. Despite years of effort looking at the basic adsorption mechanisms and the chemistry involved, *in vivo* studies demonstrating the toxin sequestering ability of specific agents currently used for the control of mycotoxicosis have yet to be completed in a meaningful way. Assays based on specific pathological indicators are insensitive and fail to provide useful description of control strategies. They also fail to clearly define the hidden effects resulting from interactions between mycotoxins. It is important that future work in this area defines specific biomarkers for tracking mycotoxin-associated intoxication so that we can clearly establish the value of prevention strategies. In addition, the fate of many toxins in animal systems is still unknown and the true pharmacokinetics of these toxins remains unclear. Studies which track labelled mycotoxins within the animal’s body are also needed to clearly define the role of mycotoxin sequestering agents in the gastrointestinal tract.

Mycofix® Plus – three combined strategies guarantee success in mycotoxin control

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Mycotoxins are toxic chemical products formed by fungal species that colonise crops in the field or after harvest and thus pose a potential threat to human and animal health. In animal husbandry five classes of mycotoxins are of major concern, namely aflatoxins, trichothecenes, zearalenone, ochratoxin A and fumonisins. Due to their diverse structure these fungal toxins are able to cause a great variety of acute symptoms. Moreover they are able to suppress the immune system of animals at quite low concentrations.

In spite of all efforts to prevent the formation of mycotoxins in the field and during storage significant contaminations still occur. To alleviate negative effects on animals, successful detoxification strategies are needed. Specifically treated clay minerals can be used to successfully "bind" aflatoxins, but their adsorption capacity against other mycotoxins is very limited or even zero. A novel strategy to control the problem of mycotoxicoses in animals is the application of microorganisms capable of biotransforming mycotoxins into non-toxic metabolites. The microbes act in the intestinal tract of animals prior to the resorption of the mycotoxins.

BBSH 797, a bacterial strain belonging to the genus of *Eubacterium* able to deactivate trichothecenes by reduction of the epoxide ring, has been described [1,2]. Its mode of action was proven *in vitro* and *in vivo.* Further a novel yeast strain, capable of degrading ochratoxin A and zearalenone, was isolated and characterised. Ochratoxin A was detoxified by cleavage of the phenylalanine moiety resulting in the amino acid and the isocumarin derivate ochratoxin α. The ZON-metabolite was not identified but tests in cell cultures revealed that the metabolite was no longer estrogenic. Due to the yeast's affiliation to the genus *Trichosporon* and to its main property to degrade mycotoxins the strain was named *Trichosporon mycotoxinivorans*.

Mycofix® Plus combines biotransformation and adsorption, which ensures an effective control against all agriculturally relevant mycotoxins taken in with contaminated feeds. Selected plant and algae extracts that counteract effects of non-degradable and non-adsorbable toxins complete the product and ensure the product's superiority to commercially available mycotoxin binders.

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POSTERS

Progress on major mycotoxins
P1 – P8

P1 *Mycotoxins in foodstuffs of plant origin in the Czech Republic – results from the official monitoring programme and import control 2003 - 2005*
S. Baršová, Z. Svec and P. Cuhra
Czech Agriculture and Food Inspection Authority, Inspectorate in Prague, Czech Republic

P2 *Comparison of Fusarium toxins contents in cereal based food and feed products*
S. Biselli and C. Hummert
Eurofins / Wiertz-Eggert-Joerissen, Germany

P3 *Aflatoxin M_1 and M_2 in Dutch infant formula*
G. Boonzaaijer and W.A. van Osenbruggen
TNO Quality of Life, the Netherlands

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[1]University of Arizona, Division of Plant Pathology and Microbiology, USA, [2]CFSAN, Food and Drug Administration, USA and [3]Agricultural Research Service, USDA, University of Arizona, USA

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P1

Mycotoxins in foodstuffs of plant origin in the Czech Republic – results from the official monitoring programme and import control 2003 - 2005

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The object of the mycotoxins monitoring programme in the Czech Republic is to check compliance with maximum levels of mycotoxins laid down both in Commission Regulation (EC) No. 466/2001 and in directive No. 305/2004 Coll. published by Ministry of Health and to monitor the mycotoxins levels in foods to yield data for the evaluation of exposure of population to mycotoxins. Mycotoxins which are involved in monitoring programme are following: aflatoxin B_1, sum of aflatoxins (B_1, B_2, G_1, G_2), ochratoxin A, patulin, deoxynivalenol and sterigmatocystine. The mycotoxin monitoring as well as the control of mycotoxins content in imported products are carried out by the Czech Agriculture and Food Inspection Authority. The quality management system of the inspections and the connecting activities of the Czech Agriculture and Food Inspection Authority has been implemented and certified according to the ISO 9001:2000. The import control, including sampling at point of entry, is in compliance with the community legislation and focused especially on import of groundnuts, nuts and dried fruit from the third countries. Samples intended for monitoring are collected by trained and educated inspectors in supermarkets, markets, retails, warehouses and at point of entry. All analyses have been carried out in one specialised laboratory of Czech Agriculture and Food Inspection Authority in Prague and methods used for analyses are fully validated and accredited. As a part of quality assurance system laboratory has been participating in number of proficiency testing, i.e. FAPAS, CHEK. Since the year 2003 about 1,300 samples of cereals, cereal products, groundnuts, nuts, baby and infant food, dried fruits, fruit products, candies, cacao products, spices, juices and coffees have been analysed. Results are presented separately for each year according to the commodities and to the purpose of sampling (import control, monitoring and compliance).

P2

Comparison of *Fusarium* toxins contents in cereal based food and feed products

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Around 1,000 cereal-based samples have been analysed by a recently developed multi-toxin method using HPLC-MS/MS technique. Herewith, the simultaneously detection of several mycotoxins (e.g., type A- and B-trichothecenes, and zearalenone) is possible. In this presentation the presence of deoxynivalenol and zearalenone in comparison to the relatively rarely investigated type A-trichothecenes (HT-2, T-2 toxin) in different cereal-products is discussed. With regard to upcoming regulations in the EU, samples were additionally screened for HT-2 and T-2 toxin to gain an insight view of the most hazardous food groups. Despite, up to now no concrete limit for T-2 toxin is in discussion, the degree of contamination in food products is of special concern for food products, which should place on the market to avoid possible risk for consumers. The used method proved to be extremely sensitive for T-2 toxin with LOD below 1 ppb, therefore a comprehensive data set was achieved.

P3

Aflatoxin M_1 and M_2 in Dutch infant formula

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Aflatoxin B_1 and B_2 contaminated feedstuffs can result in milk (products) contaminated with aflatoxin M_1 and M_2. Conversion ratios vary from 0.3 to 1%. Aflatoxin M_1 is less toxic than aflatoxin B_1 (LD50 0.46 mg/kg bw; 1 day old duckling), however, this mycotoxin is a source of concern for the infant food industry. In most countries legislation exist regarding aflatoxin M_1. Only a few countries have legislation regarding aflatoxin M_2 in milk and milk products. EU regulation 683/2004 allows a maximum level of 0.025 µg aflatoxin M_1/kg in milk ready to drink. Equivalent to this is approximately 0.15 µg aflatoxin M_1/kg in dry milk powder. Recently TNO was faced via international trade with demands concerning maximum levels of aflatoxin M_2 in infant formula. As a result, a fast method was developed by TNO, involving immunoaffinity clean-up. After extraction, the extract is cleaned up by Aflaprep M columns (R-Biopharm Rhone), which have also a strong cross-reaction with aflatoxin M_2. After the cleaning step, the extract is eluted and diluted. Separation and detection take place at an HPLC system with fluorescence detection. A baseline separation was obtained between aflatoxin M_1 and M_2. A successful validation was performed in infant formula (powder). Hereafter an investigation was performed, 22 infant formulae both powder and ready to drink products were analysed. One powder sample appeared to be positive for both aflatoxin M_1 and M_2. The amount was for M_1 0.67 µg/kg and for M_2 0.10 µg/kg. Converted to ready to drink amounts we calculated a contamination of 0.11 µg/kg (M_1) and 0.02 µg/kg (M_2). This exceeds the maximum allowed concentration in the EU, which underlined the necessity of monitoring.

P4

Survey for aflatoxin and ochratoxin A contamination in EU dry-fruit imports from Turkey with special regard on figs

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In February 2004 the German government established special limits for ochratoxin A in dry fruits and figs, where the maximum values were set to 2 ppb in dry fruits and 8 ppb in figs, respectively. With respect to this regulation and within their due diligence efforts German importers analysed in 2004 almost 50% of the entire fig imports from Turkey into Germany for aflatoxin and ochratoxin. All sample were obtained by application of EU sampling procedures laid down in EU 98/53, analyses were carried out by LC with fluorescence detection after slurry homogenisation and immunoaffinity clean-up. The presentation gives an overview about the ratio of contamination of figs and dry fruits with aflatoxin and ochratoxin, two of the most predominant mycotoxins. An estimation of the percentage of samples exceeding legal limits of EU or German regulations is available. The study allows a comparison of aflatoxin and ochratoxin levels in products harvested in 2002, 2003 and 2004, which allows conclusions regarding annually occurring differences based a broad set of results. First information regarding possible differences between conventionally and organically grown products are available.

P5

The S strain of *Aspergillus flavus* is associated with the highly contaminated maize that resulted in deadly aflatoxicoses in Kenya during 2004

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Maize (*Zea mays* L.) has been cultivated in Africa for over 400 years and is the dominant cereal crop of the continent providing over 40% of consumed calories for some countries, including Kenya. However, in addition to acquisition of needed calories, maize consumption in Africa frequently results in exposure to aflatoxins, potent hepatotoxic human carcinogens. Several *Aspergillus* species are known to contaminate maize with aflatoxins and the species may be divided into both strains and vegetative compatibility groups. The aflatoxin-producing fungi vary widely in many characteristics. This variation may indicate differential adaptation to locations, crops, or natural ecological niches. Both ability to infect and decay crops and aflatoxin-producing capacity differ among aflatoxin-producing fungi. Thus, the potential of these fungi to contaminate crops with aflatoxins also varies. Determining the most important causes of a contamination event requires considering both the aflatoxin-producing potential of the fungi present and frequency with which they occur in the contaminated crop. Because aflatoxin producers vary in adaptation, growth and reproduction strategy, differentiating the most important causal agents from other aflatoxin producers might be an important step in the development of long-term methods to reduce contamination. During January to June 2004, 317 cases of acute aflatoxicosis in Eastern and Central provinces of Kenya were identified, with a case-fatality rate of 39%. The epidemic was caused by ingestion of maize with aflatoxin concentrations up to 4,400 ppb. Although aflatoxins have been associated with a lethal food poisoning in Kenya three times since 1981, investigations into the fungi causing the underlying contamination events have not been reported. The current investigation sought to identify the most important aflatoxin-producers associated with the poison Kenyan maize from 2004. A total of 1,223 isolates of *Aspergillus* section *Flavi* from 103 maize samples taken during the 2004 epidemic from 3 severely affected districts (Matchakos, Makueni, Kitui) were characterised. Over 97% of the section *Flavi* isolates were *Aspergillus flavus* and the reminder (2.3%) were *A. parasiticus*. No other aflatoxin-producing fungi were detected. *A. flavus* can be delineated into two major morphotypes, called the S and L strains, which vary in both fruiting habit and aflatoxin-producing ability. The majority (73%) of the *A. flavus* isolated from the Kenyan maize belonged to the S strain. This is the highest S strain incidence so far reported from any crop from any location, worldwide. S strain isolates produced much greater quantities of aflatoxins than the L strain isolates (665 µg aflatoxin B_1/g mycelium, n=117; versus 40 µg aflatoxin B_1/g mycelium, n=30). Incidence of the S strain increased with average aflatoxin content from 69% in samples with <20 ppb total aflatoxins to 94% in samples with >1,000 ppb total aflatoxin. Only S strain isolates were recovered from 5 of 6 samples with >1,000 ppb. These results indicate that the severe aflatoxin contamination experienced in rural Kenya in 2004 was caused primarily by the S strain of *A. flavus*. Because of the ecological and physiological divergence of the S strain from other *A. flavus* strains, development of methods to prevent contamination should take into account the characteristics of this distinct etiological agent.

P6

Analysis of wines, grape juices and cranberry juices for *Alternaria* toxins

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Samples of red and white wine made in Ontario (VQA), British Columbia (VQA), Québec ("vins artisanals"), imported wines (from Italy, South America and the USA) and Canadian and US grape juices and cranberry juices were analysed for the *Alternaria* mycotoxins alternariol (AOH) and alternariol monomethyl ether (AME). After cleanup on aminopropyl SPE columns, AOH and AME were initially determined by reversed phase LC with UV detection. Positive sample extracts were re-analysed by LC-tandem negative ion electrospray mass spectrometry (MS/MS) in multiple reaction mode. Overall mean method recoveries measured by LC-UV were 93% for AOH and 81% for AME. Limits of detection in wine (and juice) by LC-UV for AOH were 0.8 (0.4) ng/ml and for AME were 0.5 (0.4) ng/ml; they were below 0.01 ng/ml by LC-MS/MS. As determined by LC-MS/MS, AOH was found in 13/17 Canadian red wines at levels of 0.03 to 5.02 ng/ml and in 7/7 imported red wines at 0.27-19.4 ng/ml usually accompanied by lower concentrations of AME. Red grape juice (5 positive/10 samples) contained only sub ng/ml levels of AOH or AME except for one sample (39 ng AME/ml). White wines (24 samples), white grape juices (3 samples) and cranberry juices (5 samples) contained little or no AOH/AME.

P7

Incidence and levels of aflatoxins B_1, B_2, G_1 and G_2 in breakfast and infant cereals sold in Canada

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349 Retail breakfast cereals (corn-, wheat-, rice-based and mixed-grain) and infant cereals (based on rice, soy, barley, and mixed-grain) were collected across Canada from 2002 to 2005. They were analysed for aflatoxins B_1, B_2, G_1 and G_2 using a modified AOAC International official method with immunoaffinity column clean-up. Determination of aflatoxins was by HPLC using post-column derivatisation with pyridinium hydrobromide perbromide and fluorescence detection. More than 50% of the breakfast cereals and 50% of the infant cereals had detectable levels of aflatoxin B_1 (AFB_1)(LOD 0.002 ng/g); 4% of the breakfast cereals and 1% of the infant cereals had AFB_1 levels exceeding 0.10 ng/g (the EU maximum limit for AFB_1 in baby foods and processed cereal-based foods for infants and young children). Levels were 0.002 to 1.00 ng/g for AFB_1, 0.002 to 0.14 ng/g for AFB_2, 0.008 to 0.27 ng/g for AFG_1 and 0.008 to 0.048 ng/g for AFG_2.Thus incidence of aflatoxins in breakfast and infant cereals was high, but the concentrations found in most samples were within international guidelines. This information will be necessary for risk assessment of aflatoxins in cereals consumed by Canadians.

P8

Determination of citrinin and ochratoxin A by *Penicillium verrucosum* in olives

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Mycotoxins in olives have been investigated by several researchers and penicillic acid (Oral and Heperkan, 1999) and citrinin (Sısmanoglu and Heperkan, 2004) were determined. Different studies exist that detect aflatoxin both in olives and olive oils (Yassa et al., 1994; Toussaint, 1997; Daradimos et al., 2000), however other researchers indicate that olives are weak substrates (Mahjoub and Bullerman, 1987; Eltem, 1996; Leontopoulos et al., 2003) for aflatoxin production. Citrinin is produced by different fungi such as *Penicillium citrinum*, *P. verrucosum*, *P. expansum, Aspergillus terreus*, *Monascus ruber*, and *M. purpureus* in different foodstuffs (Vinas et al., 1993; Blanc et al., 1995; Pitt and Hocking, 1997; Hajjaj et al., 1999; Xu et al., 1999; Frisvad and Thrane, 2002). Ochratoxin A mainly produced by *A. carbonarius*, *A. ochraceus* and *P. verrucosum* (Mantle and Chow, 2000; CAST, 2003). In this study two different type of olives namely black and green olives were inoculated with *P. verrucosum* spores for their ability to produce citrinin and ochratoxin A. Olives were stored at 25°C for 30 days. Citrinin production was detected by thin layer chromatography under long wave UV light, ochratoxin A was studied by HPLC. *P. verrucosum* which is a citrinin and ochratoxin A producer, produced both ochratoxin A and citrinin in olives. As a result both black and green olives are suitable substrates for citrinin and ochratoxin A production by *P. verrucosum*. Citrinin is nephrotoxic (Krogh et al., 1973; Frank, 1992) and genotoxic (Föllmann, et al., 1998) in humans kidney damage appears to be a likely result of prolonged ingestion. Ochratoxin A and citrinin have been suggested as the cause of Balkan endemic nephropathy (Pfohl-Leszkowicz et al., 2002). The presence of citrinin and ochratoxin in food cause health problems. Therefore sustainable strategies should be established.

P9

Effects of roasting with lemon juice and/or citric acid on degradation of aflatoxins in contaminated pistachio nuts

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Aflatoxins (AF) are highly toxic and carcinogenic secondary metabolites and have been detected in various food commodities including pistachio nuts. A great deal of effort has been made to completely eliminate the toxin or reduce its content in foods. We have recently reported that heat inactivates AF in contaminated pistachio nuts. In Iran, roasting with lemon juice is traditionally used. In this study, the efficacy of roasting with lemon juice and/or citric acid on reduction of AF in highly contaminated pistachio nuts (AFB_1 levels of 268 and 383 ng/g) was tested. All applied treatment protocols showed some degree of AF degradation (up to 93% for AFB_1 and 83.5% for AFB_2). Although roasting of pistachio nuts with 30 ml water, 30 ml lemon juice and 6 g citric acid at 120°C for 1 hour and roasting samples with 30 ml water, 15 ml lemon juice and 4.5 g citric acid at 120°C for 1 hour produced significant degradation of AFB_1, they changed physical appearance of pistachio nuts. However, roasting of pistachio nuts with 30 ml water, 15 ml lemon juice and 2.25 g citric acid at 120°C for 1 hour, reduced level of AFB_1 to 49% of initial level without a noticeable change in appearance of samples. In addition, we observed a synergistic effect between heating and lemon juice/citric acid in AFB_1 degradation. It has been shown that acidification with citric acid is an efficacious and safe procedure in reducing AF levels in maize. It could, therefore, be concluded that roasting with lemon juice and citric acid is a useful and safe method in degradation of AF in naturally contaminated pistachio nuts.

P10

Control of European corn borer (*Ostrinia nubilasis*) in maize and mycotoxin contamination

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European corn borer (ECB), *Ostrinia nubilalis* Hübner, causes severe damage to corn crops by reducing yields with its feeding activity. *O. nubilalis* ear feeding has been also shown to be highly associated with fungal contamination and subsequent mycotoxin accumulation. These metabolites are a large and diverse group of naturally occurring fungal toxins, some of which are strongly implicated as agent of toxic disease in humans and animals. More precisely, *Fusarium verticillioides*, able to produce a group of toxins called fumonisins, has been associated with ECB injury and can be spread by insect larvae. In the last years the ECB infestation is became more severe in North Italy, where more than 80% of the ears evidences cavities at harvest. The Department of Agronomy of the University of Turin since 1996 coordinates a multidisciplinary research project to valuate the most important techniques in order to prevent mycotoxins contamination in corn kernel before harvesting. Among several crop practices studied, the ECB control showed an important effect in order to control moulds development and toxin production. To analyse the effect of this phytophagous insect more than 300 grain samples were analysed, since 2000, with high performance liquid chromatography methods (HPLC) researching the relationship between the main mycotoxins of cereals and the ECB activity and control. Data collected confirm that *F. verticillioides* and fumonisins are highly associated with ECB activity. The comparison of healthy and injury ears show that ECB larvae infestation increased *Fusarium* ear rot incidence (+86%) and severity (+96%). Contamination of fumonisin B_1 was generally one ten time higher in ears damaged by *O. nubilalis* respect healthy ears, and was positively related to the number of cavities per ears. Particularly, apical tunnels proved to increase fumonisin contents (R^2=0.80; n=12), while correlation was weaker for middle and basal ones. On the other hand, also in wet and cold years, ear infection from other *Fusarium* species, like *F. graminearum*, able to produce zearalenone and trichothecenes, still showed no significant effect of ECB activity. Use of pyrethroids or other insecticides against the second generation's larvae is an important tool for the prevention of insect activity (reduction of 50% of insect severity). A complete schedule of applications, concerning the stages of the plant and of the insect was studied in order to improve the effectiveness of the control. The distribution after 6-7 days from ECB's flight peak, is able to reduce *Fusarium* ear rot incidence (55%) and severity (65%) and fumonisin B_1 contamination (79%); a delay of the distribution generally was less effective. Insecticides decrease fumonisins at a lower rate for early seeding time respect late seeding or late maturing hybrids. The comparison between different molecules and products, use of single and double treatment were also studied.

P11

Field strategy for mycotoxin control in maize

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Fumonisins, zearalenone and deoxynivalenol can significantly compromise quality and commercial value of maize. Their occurrence is related to diffusion of *Fusarium* ear rot, caused mainly by *Fusarium verticillioides*, and *Gibberella* ear rot caused by *F. graminearum*, which are favoured by distinctly different conditions. The Po Valley includes a wide range of environments that differ in temperature, relative humidity and water availability and therefore are suitable for growing different maize hybrid maturity classes. The project aims at defining a site-specific field strategy for maize crop that can reduce mycotoxins contamination. Sampling and data collection in the different environments was carried out by the Technical Service of Pioneer Hi-Bred Italia. A particular attention was put on *Fusarium* ear rot development, which is strongly correlated with kernel injuries due to insect and particularly to the high pressure by European corn borer (*Ostrinia nubilalis*). Several crop management choices can contribute to control fumonisins contamination. Among those variables planting time, harvesting time and insect control showed to be the most effective. Significant differences in the fumonisins occurrence have been frequently observed; nevertheless early planting time (early March in most of Po Valley environments) always reduced grain fumonisins content, compared to later planting time. At the same time, final fumonisins concentration increases with the delay of harvesting time. Early planting leads to earlier tasseling and harvest time and therefore aims at mismatching the main activity of ECB and the period of higher susceptibility of plants to insect damages. Insecticides application was a successful tool to control ECB incidence and significantly reduced fumonisins content. The insecticide efficiency was correlated to the development stage of the insects and, therefore, to the application time. Even if *Gibberella* ear rot was less common in the experimental period, some field strategies, dealing with fertilisation and harvest time, seem to be more effective in order to control zearalenone and deoxynivalenol rather than fumonisins. In conclusion, this research has clearly demonstrated with evidence the possibility to improve significantly corn grain quality, increasing at the same time yield and farmer income. To implement good crop management techniques for maize production quality and its further improvement, there is a need to communicate technical information at farm level. The widespread Pioneer Hi-Bred technical network and agronomic support activities may play a key role in this perspective.

P12

Targeting stress-response genes for control of aspergilli: target-gene based bioassays using antifungal natural compounds

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Signal transduction and stress-response genes of fungal pathogens play important roles for exerting virulence and pathogenesis. For example, mutants of fungal pathogens lacking a two-component histidine kinase or a Mitogen Activated Protein Kinase (MAPK) gene have been shown to have significantly reduced virulence compared to respective wild-type strains. To discover more stress-response genes critical for virulence and mycotoxin biosynthesis, and as potential targets for control, we constructed an *in silico* database of transduction/ stress-response pathway genes of *Aspergillus flavus*. The database was based on orthologs of the yeast, *Saccharomyces cerevisiae*, because its entire genome had been sequenced and well annotated, whereas that of *A. flavus* had not. Moreover, many genes in *S. cerevisiae* are interrelated to genes of many fungal pathogens. For example we demonstrated functional complementation of an antioxidative stress gene from *A. flavus*, mitochondrial superoxide dismutase (sodA), in a sod2 yeast mutant. This complementation verified that *S. cerevisiae* deletion mutants could serve as a model system for indirectly studying *A. flavus* functional genomics and discovery of target genes for fungal control. We next developed a bioassay system, using yeast, to screen phenolic natural compounds for anti-aflatoxigenic or antifungal activity. Many fungitoxic phenolics are produced during fungal infection in plants, and detoxification of these compounds/fungicides by fungi is necessary for their successful pathogenesis. Deletion mutants of yeasts were used to ascertain genes (or their products) being affected by any active compounds. After identifying active compounds using the yeast screening system, we tested the active compounds on *A. flavus*. The results were parallel between the fungi, demonstrating the usefulness of yeast for rapid screening. We were able to identify signal transduction and antioxidative stress response genes important to fungal tolerance. Targeting the antioxidative stress response system with certain compounds (e.g., vanillyl acetone) in combination with strobilurin-fungicides had a synergistic antifungal effect against both fungi. This provides evidence that antifungal activity of known fungicides can be enhanced with natural compounds. Application of yeast bioassays and functional genomics for controlling other fungal pathogens such as *A. fumigatus* are discussed.

P13

Re-evaluating the pH effect on mycotoxin synthesis and differentiation in *Aspergillus nidulans* (*Emericella nidulans* (Eidam Winter)) and *A. parasiticus* (Speare)

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Aflatoxins (AF) are the most extensively studied mycotoxins since their discovery, mainly due to their carcinogenic, genotoxic, teratogenic and immunosuppressive properties. Their biosynthetic pathway, the genetic elements that control their synthesis and the physiological factors that influence it have been broadly described. However, due to the complexity of the regulation of all those elements, prevention of the production of these mycotoxins has not yet been achieved. *Aspergillus parasiticus* and *A. nidulans* have been used as a tool to investigate aflatoxin (AFB_1) and sterigmatocystin (ST) synthesis. Many ambiental factors influence their production, mainly the pH of the substrate. The objectives of this study are: (i) to elucidate the role of external pH on differentiation and mycotoxin synthesis at the onset of fungal development and (ii) to determine the involvement of the Pal/Pac signalling pathway in the regulation of ST synthesis in *A. nidulans* following Arst and Peñalva´s criteria. Several strains were used to achieve this purpose: A. *parasiticus* ATCC 16992, *A. nidulans* FGSC26, the acidity mimicking mutant MAD134 (biA1, palA1, wA3), the alkalinity mimicking mutant MAD135 [biA1, $pacC^c14$ (5-492)], and the parental strain MAD002 (biA1) (Pal/Pac mutants kindly donated by Dr. Miguel A. Peñalva. CSIC, Madrid, Spain). Fungal growth, conidiation and AF production by *A. parasiticus* were quantified after 72 h of incubation at 28 °C in PDB at four different pH values using adequate buffers. The same parameters and ST production were evaluated for *A. nidulans* after 72 h of incubation at 37°C in minimal Käfer medium (without being pregrown). Two nitrogen sources were used: sodium nitrate ($NaNO_3$) or ammonium tartrate. Sucrose (5%) was used as the carbon source, with or without phosphate buffer (pH 6.5, 100 mM) or citrate buffer (pH 3 or pH 6.5 100 mM). Microscopic morphology was also evaluated. All of the evaluated parameters in *A*. *parasiticus* reached their highest values at acid pH (4.5). However, *A. nidulans* behaviour was different. In medium with sodium nitrate as the nitrogen source and without citrate buffer (final medium pH 8.35), the *A. nidulans* FGSC26 strain produced 1250 ng ST/mg mycelium. On the contrary, when pH was maintained with citrate buffer 100 mM at pH 3.0 or at pH 6.5, the fungus scarcely produced ST. On the other hand, the Pal/Pac mutants showed that in this medium (alkalinisation conditions), the alkalinity mimicking mutant MAD135 produced 4 times less ST when compared to the parental strain MAD002 (which produced 12 ng ST/mg mycelium), but the acidity mimicking mutant MAD134 produced even less. When the parental strain MAD002 was grown in medium with ammonium tartrate without phosphate buffer (acidification conditions) it did not produce ST, but in the presence of this buffer (pH 6.5) it produced 60-fold less ST than when grown in alkalinisation conditions. In this same medium (acidification conditions), the alkalinity mimicking strain MAD135 produced 2 times less ST than in alkalinisation conditions regardless of the presence of phosphate buffer and the acidity mimicking mutant MAD134 did not produce ST in this medium regardless of the presence of buffer. These results show that *A. parasiticus* produces more AFB_1 in acidic conditions (pH) and *A. nidulans* in alkaline conditions. Furthermore, the acidity mimicking mutant MAD 134 and the alkalinity mimicking MAD135 were able to produce ST in alkalinity. The results show that *A. parasiticus* behaviour is different to that of *A. nidulans* when referred to pH response. Transcription levels of the genes flbA and aflR, in the wild type and Pal/Pac mutant strains of *A. nidulans* at different pH values are being analysed.

P14

Fusarium mycotoxins in UK cereals

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Fusarium head blight of small grain cereals in temperate regions of Europe, America and Asia can be caused by a number of *Fusarium* species. *Fusarium* species can produce a wide range of mycotoxins including several type A (e.g., HT-2 and T-2 toxins) and type B (e.g., deoxynivalenol and nivalenol) trichothecenes and others, such as zearalenone, which can be harmful to humans and animals. Trichothecenes inhibit protein synthesis and may result in anorexia and reduced weight gain at low concentrations and diarrhoea, emesis, haemorrhage, circulatory shock and death at high concentrations. Zearalenone is oestrogenic and can cause various fertility problems in a number of animal species due to hyperoestrogenism. The European Commission has set maximum permitted limits for deoxynivalenol and zearalenone in cereals and cereal products to be introduced in July 2006. Limits for HT-2 and T-2 combined and fumonisins are to follow. For unprocessed wheat and barley the forthcoming maximum limits are 1250 ppb for deoxynivalenol and 100 ppb for zearalenone. For unprocessed durum wheat and oats the forthcoming maximum limits are 1750 ppb for deoxynivalenol and 100 ppb for zearalenone. *Fusarium* head blight pathogens are known to infect cereals primarily at anthesis and the weather conditions during this period are important parameters, which affect the incidence and severity of infection. Consequently, the abundance of *Fusarium* mycotoxins produced can also vary between seasons and across regions. Two investigations, funded by the UK Food Standards Agency and Home-Grown Cereals Authority, were started in 2001 and 2002 to assess the level of *Fusarium* mycotoxin contamination in UK cereal production and the extent to which agronomic factors such as variety, crop rotation, land cultivation and fungicide application affect this contamination. Each year ca. 300 samples of wheat and 100 samples each of barley and oats from fields of known agronomy were collected and analysed for ten trichothecenes and zearalenone. Results from the first four years have shown that the incidence of mycotoxins were generally low with few samples exceeding the forthcoming legislative limits. For wheat, only five mycotoxins (deoxynivalenol, nivalenol, HT-2, T-2 and zearalenone) were detected in more than 5% of the samples tested. Of these deoxynivalenol was the predominant mycotoxin found although zearalenone exceeded the forthcoming limits in more samples than deoxynivalenol (3.6% compared to 2.6%). For barley, the concentrations of all mycotoxins were lower than found in wheat and no samples exceeded forthcoming legislative limits. For oats, the concentrations of deoxynivalenol and zearalenone were lower than found in wheat and no samples exceeded forthcoming legislative limits. The type A trichothecene concentrations were notably higher compared to wheat samples. However, most of the contamination appears to be on the husk and not on the edible portion and this should be considered when limits are set for HT-2 and T-2 in the future. Results will be modelled using the field data to identify agronomic factors important in the determination of mycotoxin content of harvested cereal grains. This analysis will be used to determine “Good Agricultural Practice” to minimise mycotoxin contamination of UK cereals.

P15

Investigations into the effect of flowering on the production of mycotoxins in wheat by *Fusarium graminearum*

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Flowering time is regarded to be the most susceptible period for primary infections of wheat spikes. During this period lemma and palea of the florets spread apart and *Fusarium* spores might fall into the opened florets. Later they can germinate in the closed florets and cause an infection. Although many investigations have been done, the infection process is still under dispute. With our experiments we addressed the question whether spores reaching the inner side of the florets cause higher infection levels than those which are on the outer side. Therefore, we tried to imitate open (chasmogamous) and closed (cleistogamous) flowering under constant infection conditions by placing defined amounts of macroconidia of *Fusarium graminearum* (Schwabe) (telemorph *Gibberella zeae* (Schw.) Petch) into a floret and between two florets. Three days after the injection of 240 conidia into the florets, spikelets exhibited strong bleaching and visible mycelium or in few cases only mycelium without bleaching. On the contrary, no bleaching at all was observed after the injection of 240 conidia between the florets. The results from the rating of the symptoms are in accordance with the measured mycotoxin concentrations in the spikelets. The average amount of Deoxynivalenol (DON) after injection between the florets was 2.3± 2.3 µg per spikelet. After injection into the florets a significantly increased average of 9.0±5.5 µg DON per spikelet was measured. To improve the comparability, spikelets located at the same spike were treated with the different injection procedures. In addition we carefully chose florets at similar growth stages and shortened the incubation time from three to two days. DON amount after injection into the florets strongly exceeded the respective amount found after injection between the florets. While amounts of DON with a mean value of 0.02±0.04 µg per spikelet was found after injection between the florets after injection inside the florets DON increased significantly to 0.8±0.8 µg DON per spikelet. Deviations of DON values among spikelets treated with the same injection procedure were still fairly high. The experiments further showed that the amount of spores reaching the inside of the florets is of crucial importance for the level of the infection. After doubling the amount of spores injected into the florets from 120 to 240 conidia of *F. graminearum*, we found an increase of DON concentration in the spikelets by a factor of three. In conclusion, our experiments quantitatively verified that the *Fusarium* infection level of wheat spikelets is approximately proportional to the number of spores reaching the inner part of the florets that is especially determined by both the area of the exposed inner surface of the floret and the time this surface is exposed. In addition, the infection probability of spores placed at the outside of the florets is significantly lower compared to spores inside the floret, which is in accordance with previous experiments. Hence, influencing the flowering process by breeding might provide a natural cure against infections with *Fusarium* head blight

P16

Application of HACCP to control mycotoxins in dry grind ethanol byproduct production

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Ethanol has been used as a fuel source in the United States since the early 1900's and is seeing a renewed and increased interest as an alternative to petroleum. Today most fuel ethanol is produced by the dry grind mill process creating a valuable co-product, distillers dried grain with solubles (DDGS). Approximately 3.2 to 3.5 million metric tons of DDGS are produced annually in North America, which is generally used as animal feeds. Mycotoxin contamination of corn processed in dry grind ethanol production may both contaminate the DDGS and stress the yeast during fermentation thereby lowering ethanol yields. During the last three decades, the Hazard Analysis Critical Control Point (HACCP) system has been gradually introduced and applied successfully by the food industry to introduce risk assessment based evaluations for potential contamination of food products with pathogenic micro-organisms and physical and chemical safety hazards, including mycotoxins. HACCP is a pro-active, highly structured, systematic quality management system that includes the identification, evaluation and control of hazards in the entire agricultural system. As a result of the increased importance of mycotoxins in feed and food safety, this paper recommends that a HACCP based approach be incorporated in the Maize-based ethanol production process.

P17

Two-dimensional profiles of germination, growth and fumonisin B_1 production by *Fusarium* section *Liseola* in relation to environmental factors

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In recent years, *Fusarium* species belonging to section *Liseola* have attracted much attention because of their ability to produce fumonisins. Among them, *Fusarium verticillioides* and *F. proliferatum* are the major producers. A number of surveys have shown that a high percentage of samples of corn-based feed are contaminated by fumonisins (Wilson et al., 1990; Ross et al., 1991). They have also been found in samples intended for human consumption (Sydenham et al., 1991; Pittet et al., 1992). In the present year, the EU Commission has adopted regulation 856/2005 regarding *Fusarium* mycotoxins, in which maximum levels of FB_1+FB_2 between 0.2 and 2 µg g^{-1} are proposed for maize and maize products. The two-dimensional profiles of the environmental factors, water activity and temperature, on colonisation by *F. verticillioides* and *F. proliferatum* and FB_1 production *in vitro* and in maize grain have been determined. Germination was possible between 5-37°C at 0.88 a_W, the growth between 5-35°C and 0.90 a_W, while FB1 production was restricted to 10-37°C and 0.93a_W. Overall, the *Fusarium* species have a wide temperature range favourable for growth. Consequently, provided a a_W level of <0.93 is maintained, post-harvest spoilage and FB_1 production will be prevented at any temperature regime. However, higher moisture levels (>0.93 a_W) pre-harvest in the field, particularly in high humidity conditions would almost certainly result in fumonisin accumulation in the ripening maize grain. An increase in fumonisin concentration during storage would be an indicator of poor management of the grain post-harvest.

P18

Ribosomal resistance to trichothecene toxins

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Trichothecenes are a diverse class of sesquiterpenoid secondary fungal metabolites which act as inhibitors of eukaryotic protein synthesis. Trichothecene production is a suspected virulence factor of many plant pathogenic fungi (e.g., *Fusarium* spp.). The most prevalent trichothecene, deoxynivalenol (DON), can accumulate in infected grain, and poses a threat to human and animal health. We have identified mutations in the yeast ribosomal protein L3 which lead to DON resistance and have attempted to engineer trichothecene resistance in transgenic plants by introduction of a plant RPL3 cDNA containing such an alteration conferring resistance in yeast. Such transgenic tobacco lines show improved ability to adapt to DON, but not high level constitutive resistance. Toxin-dependent utilisation of the engineered ribosomal protein L3 limits trichothecene resistance [1]. The RPL3 gene family of hexaploid wheat (6 genes) was characterised by sequencing and heterologous expression in yeast. Three SNP-based markers could be developed which allowed the mapping of these TaRPL3 genes by using two double haploid mapping populations. One of the genes maps to a previously identified region containing a *Fusarium* resistance QTL (Qfhs.ifa-5A, resistance to initial infection). Yet, no amino-acid alterations were found in the *Fusarium* resistant wheat cultivars. cDNAs of all homeologs were cloned and expressed in toxin sensitive *Saccharomyces cerevisiae*. The wheat cDNAs can complement the yeast RPL3 gene disruption mutant, and small differences in the susceptibility against DON between the homeologs were observed. Despite the lack of mutations conferring high level resistance, we hypothesise that differential utilisation of TaRPL3 isoforms with different properties may lead to differences in basal DON resistance. In a screen for yeast genes conferring DON resistance when overexpressed in yeast we identified a gene coding for another ribosomal protein. As many other small subunit ribosomal proteins it is encoded by two genes in yeast. We have constructed a deletion mutant containing an epitope tagged copy under control of a regulatable promoter (MET3) as sole source of this protein. In this yeast strain DON resistance is dependent on the expression level of the small subunit ribosomal protein, which seems to play an important role in translational fidelity.

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P19

Ecophysiological approaches to improving biocontrol of *Penicillium verrucosum* and ochratoxin in moist grain using the yeast *Pichia anomala*

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Pichia anomala has been found to reduce mould growth and ochratoxin A (OTA) production. The major hurdle in production of commercial biocontrol agents (BCAs) has been the lack of production of appropriate formulations. Of particular importance is the conservation of viability and ecological competence after application. With this in mind, studies were conducted to develop formulations of *P. anomala*, which would have these attributes. Studies with fluidised bed-drying examined several additives for conservation of viability and showed that cottonseed flour + skimmed milk was the best treatment. Yeast cell osmoprotection was also employed. The biocontrol efficacy of formulated *P. anomala* cells was tested at the laboratory scale and results showed that they inhibited *Penicillium verrucosum* and OTA production. Formulation additives were found to have no adverse effect on mould growth and OTA production. Furthermore, modified yeast cells with increased levels of trehalose and arabitol gave similar efficacy as fresh cells. A subsequent pilot scale study, using malfunctioning airtight silos containing moist grain, showed that addition of fresh cells or formulated *P. anomala* cells both effectively also control *Penicillium roqueforti*.

P20

Inactivation of the *Fusarium* toxin deoxynivalenol by glucosylation

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During the infection of small grain cereals and maize *Fusarium graminearum* produces the mycotoxin deoxynivalenol (DON). It has been demonstrated that the production of the trichothecene DON, which acts as an inhibitor of eukaryotic protein synthesis, contributes to the virulence of *Fusarium* (presumably by interfering with the expression of plant defense genes). We have cloned an *Arabidopsis* gene, which can inactivate the *Fusarium* toxin DON. A yeast strain highly sensitive to DON was used as a host for an expression library and a UDP-glucosyltransferase (UGT) gene conferring resistance to DON (DOGT1) was identified (Poppenberger et al., 2003). The metabolite DON-3O-glucoside is inactive in inhibiting protein synthesis (tested with a wheat germ extract *in vitro* translation system). Overexpression of the DOGT1 gene in *Arabidopsis* led to increased DON resistance of seedlings. Using a doubled haploid population of wheat segregating for *Fusarium* resistance we could show, that the ability to convert DON into DON-3O-glucoside co-localises with the main *Fusarium* resistance QTL (Qfhs.ndsu-3BS), which was previously identified as governing resistance to fungal spread in the wheat ear (Lemmens et al., in press). The *Arabidopsis* DOGT1 gene is located in a cluster of 6 highly similar genes, but surprisingly the protein with the most closely related sequence is not protecting against DON. By making hybrid proteins and functional testing in yeast we characterised structural features essential for substrate specificity of these UGTs. Interestingly nivalenol, which has just one additional hydroxyl group, escapes detoxification by DOGT1. The formation of toxin glucosides which leads to inactivation of virulence factors seems to be an important mechanism of plants conferring resistance to necrotrophic fungal pathogens. Yet, glucosylation can also lead to the formation of “masked mycotoxin”, as the DON-glucoside escapes routine detection methods but may be reactivated in the digestive tract of animals.

Acknowledgements

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P21

Deoxynivalenol exposure in UK adults: reduction by dietary intervention

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Mycotoxins are fungal metabolites found in a wide range of foodstuffs including cereals. In animals the *Fusarium* mycotoxin deoxynivalenol (DON) causes feed refusal, decreased weight gain, cardiotoxicity, teratogenicity and immunomodulation. Despite concerns in the European Union over chronic DON exposure in humans there are no biomarker data to indicate prevalence and level of exposure or to help evaluate health effects. Consequently we have developed methodology to assess human exposure by measuring DON in urine samples. In the current study we assessed the level of urinary DON in relation to diet in 21 healthy UK volunteers (8 males, 13 females), aged 21-59, from an initial recruitment of 29 people. A detailed semi-weighed food diary was used to assess the amounts and types of foods consumed over 48 hours prior to a morning urine collection. Following a 4-day intervention period, in which major sources of wheat and corn were avoided (specifically bread, pasta and breakfast cereals), a second urine sample was collected. A food diary was also kept on day 3 and 4 of the intervention to assess compliance. Following overnight digestion with beta-glucuronidase, urinary DON was extracted and quantified by immuno-affinity enrichment and LC-MS. DON was detected in 21/21 (100%) urine samples prior to intervention. The geometric mean level in individuals on a free diet was 8.4ng DON/ml urine (95%CI 6.3-11.2). Following the intervention the level of DON was non-detectable in 9/21 (42.9%) individuals and geometric mean levels were about 10-fold lower (0.7ng/ml urine, 95%CI: 0.4-1.2) ($p<0.0001$). In summary, all participants in this UK study were consuming DON contaminated food and when major sources of wheat and corn were avoided urinary DON levels were markedly reduced. Furthermore the work demonstrates that this biomarker approach will permit the exploration of the health consequences of DON exposure.

P20

Mycotoxin control in the maize food chain

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Maize production and processing, from field to final products for food and feed, determined mycotoxins content. Studies carried on from 1996 in Italy, underline the opportunity to prevent and control toxins contamination along all the chain. Data from more than 3000 analyses on samples collected from field, post harvest and processing, underline the importance of each single step of maize chain. The main aspects that have been analysed could be summarised as follows.

- Pre-harvest. Studies of techniques able to reduce toxigenic fungi development were conducted from 1996 in several environment of North Italy. The most important techniques are hybrid selection, sowing time and density, fertilisation, European corn borer control and harvesting time. Late maturing hybrids had zearalenone and deoxynivalenol contamination levels from 3 to 4 times higher compared to early hybrids. Late sowing (May) has always a *Fusarium* toxins occurrence significantly higher than early sowing times (March and April). High nitrogen fertilisations increased significantly zearalenone content, while fumonisins are increased by N deficiency (+ 80%). Balanced fertilisation seems to assure generally lower contamination.
- Post-harvest and first processing. Accurate cleaning and fast drying of maize kernel are able not only to stop fungi development but also to reduce mycotoxins concentration. Grain cleaning immediately after drying reduces all the mycotoxins: the reduction rate ranged between 30 to 40%, with regard to different cleaning equipments and kernel characteristics.
- Dry milling processing. As a consequence of grain processing toxins are re-distributed among the different components. A study was carried on in an Italian maize mill from 2002, analysing 14 lots of 2002-2004 maize productions. The level of grain decontamination obtained with processing was defined. Meals and grits had a medium content more than 5 times lower than mycotoxin contamination of unprocessed grain. Flour contamination was 2-3 times lower than unprocessed grain. On the contrary, germ and animal meal (bran) had a higher content than unprocessed grain. Therefore, mycotoxins tend to concentrate in bran and germ. Endosperm, fraction from which derived meals and grits, is only partially contaminated.

In conclusion, dry milling is a selective process of decontamination able to improve significantly maize meals. A careful analysis of the possible decontamination method pointed out the importance to start the chain from controlled and healthy maize kernels. It is possible to control mycotoxins content with the use of good agricultural practices, good management practices and trough traceability from field to the post harvest processing.

P23

Early warning of *Fusarium* mycotoxin risks by modelling and quantitative detection of fungi and mycotoxins in animal feed production chains

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Regulation of maximum permitted mycotoxin levels in cereal products forces all stakeholders in the production chain to have clear-cut procedures to control the quality of batches and to fine tune logistics in such a way that their risk is minimised. Apart from preventing problems at the farm level as far as possible, conditions during and after harvest are of major influence on mycotoxin levels. This study focuses on three aspects, which may improve forecasting the occurrence and severity of mycotoxin producing fungi as well as levels of different mycotoxins. *Fusarium* head blight in small grain cereals is caused by a complex of species with specific population dynamics. Factors at the farm level are studied to improve our understanding of the dynamics of these species using quantitative PCR and relate the dynamics to management practices. Existing and newly collected field data are analysed and modelled to increase the prediction accuracy for *Fusarium* spp. as well as the mycotoxin levels before and after harvest. Different trading and processing routes are assessed to determine critical control points for the most risky food and feed chains of cereal products. The knowledge acquired will be used to direct further research and anticipate on future policies with regard to mycotoxin regulation and food safety.

P24

Fast, sensitive polyclonal antibody-based ELISA test for the detection and quantitation of the mycotoxin zearalenone in food commodities

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A fast, very sensitive polyclonal antibody-based Enzyme Linked Immunosorbent Assay (ELISA) test to detect and quantitate the mycotoxin zearalenone (ZEA) in food and feed commodities was developed. An immunogen of ZEA-oxime-BSA conjugate was used to develop the antibodies in rabbits as host animals. A ZEA-horseradish peroxidase conjugate was prepared and used in the assay. The assay is a competitive direct ELISA (CD-ELISA) in a microwell format. Free ZEA in the sample or ZEA standard controls competes with enzyme-labelled ZEA for the antibody binding sites in a 5-minute incubation step. The absorbance signal generated by substrate reaction with the bound enzyme in the second 5-minute incubation step is inversely proportional to ZEA concentration. Zearalenone is extracted from samples with 70% methanol (1:5 w/v) by shaking for 5 minutes, then the sample extract is diluted 1:5 (v/v) with water and used for analysis. The limit of detection of the assay is 0.40 ng/ml of ZEA (10 ppb). The concentration of ZEA that is required for 50% binding inhibition is 2.7 ng/ml (67.5 ppb). Cross-reactivity of zearalenone related compounds were assessed. The inter- and intra-assay variability of the test is 3.9 and 3.6%, respectively. The mean recovery of zearalenone from spiked wheat, corn, barley and feed were 90, 85, 93, and 87%, respectively. The assay can be used to detect and quantitate zearalenone in corn, wheat, barley, and feed samples within 10 minutes.

P25

Enzyme-linked immunosorbent assay for determination of zearalenone in cereals and biological fluids

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Zearalenone (F-2 toxin) is an oestrogen-like compound produced by some *Fusarium* species, particularly occurring in different cereals (maize, wheat, barley) and causing hyperestrogenism in swine, cattle and poultry. Zearalenone is converted to trans-α- and cis-β-zearalenol in the liver and these metabolites can be detected in milk, serum and urine. Among these metabolites the trans-α-zearalenol is three to four times more estrogenic than F-2, while β-zearalenol has almost the same activity as F-2. Both F-2 and zearalenol can diffuse from blood into peripheral tissues (uterus, mammary gland and hypothalamus) where they exert estrogenic effects. Our objective was to develop and validate a monoclonal antibody based direct ELISA test for rapid and quantitative determination of zearalenone in cereals and its metabolites in different biological fluids (milk, sera) and animal tissues. Cereal samples have to be extracted with acetonitril containing mixture (89%) and dilute the extract 1:25 before the test, sample volume is 50 μl. Milk and serum samples can be measured by this method without dilution, tissue extracts require 1:10 dilution, in either case the volume of sample is 20 μl. The measuring range of F-2 in cereals is 25-400 ng/g, in milk and sera is 0.5-10 ng/ml with a detection limit of 25 ng/g for cereals, 0.2 ng/ml for milk and 0.4 ng/ml for porcine serum. The measuring range of F-2 is 10-200 ng/g in tissue samples, with detection limit of 10 ng/g. This zearalenone ELISA test enables quick and quantitative detection of zearalenone and its major metabolites as “zearalenone equivalents”.

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P26

Sampling variability – investigation of variability associated with testing lots of wheat kernels for deoxynivalenol and ochratoxin A (case study truck)

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In June 2005 the new Commission Regulation 856/2005 for *Fusarium* toxins (deoxynivalenol, zearalenone, fumonisins, HT-2/T-2 toxin) has been published. In parallel a sampling procedure similar to the existing one for the investigation of ochratoxin A (Commission Directive 2002/26/EC) came out. *Fusarium* toxins such as deoxynivalenol (DON) and zearalenone are produced by several species of *Fusarium*. Wet and cold weather conditions during flowering promote infection, resulting in head blight in wheat, barley, oats and rye. Since *Fusarium* infections occur during growth period the *Fusarium* toxin distribution throughout a given lot of grain kernels should be much more even compared to ochratoxin, which is a prominent example for intoxication as result of fungi attack during storage. Toxins from storage fungi are often concentrated in spots, while for the variability of *Fusarium* toxins stratification effects depending on handling and manipulation through the harvest process have probably the highest impact [1,2]. Beside the toxicological aspect, which has to be considered for the food chain, grain producers are facing significant economic consequences in those years with excessive *Fusarium* infections. Altogether, this leads to the need of a reliable sampling standard with the lowest possible variability. However, the sampling protocol mentioned above was primary developed to investigate storage toxins and recommends 100 incremental samples (100 g each) for a lot between 20 t and 50 t. The incremental samples have to be homogenised in total, from the resulting homogenate the laboratory sample is taken. The entire sampling and homogenation procedure is costly an especially time consuming. During harvest “time” is the most limiting factor. Within the present study the distribution of deoxynivalenol and ochratoxin A infected kernels in a lot of 26 t of wheat was investigated to develop a practical sampling plan which can be used effectively to avoid storage of highly contaminated lots and to save time within the trade of grains. The study delivered clear results for the sampling variability for DON in comparison to ochratoxin A. On the same occasion the sampling plan of the directive 2005/38/EC was compared to the procedure recommended by the ICC standards as well as GIPSA standards of the grain inspecting handbook. In north European countries the risk of *Fusarium* toxins in freshly harvested cereals is much higher compared to ochratoxin A, therefore it is more desirable to have a fast screening assay to evaluate the deoxynivalenol concentration directly on site. Different commercially available membrane immunological methods were compared and validated against a reference method based on LC-MS/MS.

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P27

Mycotoxins and certified reference materials – a short overview

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Certified reference materials (CRM) play an important role in quality control and method development in mycotoxin analysis. The Reference Materials Unit of the Institute for Reference Materials and Measurements (IRMM) develops, produces, certifies, monitors and distributes highest quality reference materials. In the mycotoxin field the IRMM produces two different types of CRMs: calibrants (mostly as solutions) and matrix materials. Certified calibrants are *inter alia* used for calibration and spiking experiments. Calibrants are not only certified for their identity but also for their mass concentration. The mass concentration of certified calibrants is through their gravimetrical preparation directly traceable to the SI. The substances used for the preparation of calibrants undergo a thorough purity and identity assessment. The mass concentration is certified with its associated uncertainty. The uncertainty includes contributions from gravimetrical preparation, possible inhomogeneity and long term stability assessments of the calibrant. Certified matrix materials are mainly used for method validation and quality control measurements. The key benefit gained from using a certified matrix reference material is an assessment of the trueness of the used method. Mycotoxin matrix materials from the IRMM resemble as closely as possible a real sample. Therefore only naturally contaminated raw materials are used for the production of these CRMs. Matrix materials are certified for the mass fraction of the toxin in the matrix accompanied by an uncertainty budget. This uncertainty budget includes, as the uncertainty for the calibrants, contributions from possible inhomogeneity, possible instability and the characterisation study. Mycotoxin CRMs are not only an important tool in method development and quality control but they are also a tool to guarantee the comparability of measurements between different laboratories. The range of available mycotoxin CRMs nearly covers all mycotoxins regulated in European Regulation EC/466/2001 and European Directive 2002/32/EC in their latest amendments.

P28

Using fluorescence polarisation for mycotoxin detection – a new generation of rapid and quantitative immunoassays

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Mycotoxins pose a threat to human and animal health through the ingestion of contaminated food. The Food and Agriculture Organization (FAO) of the United Nations has estimated that 25% of the world's food crops are contaminated by mycotoxins each year. Food commodities most susceptible to mycotoxin contamination are cereals, nuts, coffee, cocoa, spices, oil seeds, peas, beans and fruit, particularly apples. Since most mycotoxins are chemically stable, they tend to survive storage and processing, even at elevated temperatures. Being toxic in very low concentrations requires sensitive and very reliable methods for detection. Failure to achieve a satisfactory detection performance with resulting false positives and negatives can lead to significant economic losses. Rejected cargoes and disputes arising from unreliable analytical results can be extremely costly. Rapid and reliable analytical results are therefore critically important for the food and feed industry enabling testing on-site during delivery. The fluorescence polarisation immunoassay technique is a practical and appropriate solution to rapid and reliable mycotoxin detection, allowing quantitative determination in the ppb range within 5 minutes. Fluorescence polarisation immunoassay is a technique to measure molecular orientation and mobility using polarised light and fluorescent tracer. When excited with polarised light, tracers attached to molecules with high molecular weight emit a high level of polarised fluorescence, while small tracers emit a low level of polarised fluorescence. In an immunoassay application, the changes in fluorescence polarisation between free and bound tracers can be rapidly and accurately measured and related to the concentration of unlabeled mycotoxins (analyte) present in a sample. Different fluorometer configurations have been compared and evaluated:

- Excitation. Two light sources have been evaluated: xenon arc lamp and light emitting diodes (LED). Neutral density filters and interference filters were utilised in order to achieve stable excitation intensities at the desired wavelength (for fluorescein: 485 nm, DF12). A polarise was used in the vertical direction.
- Emission. T-format design with polarises, interference filters and photo multiplier tubes (PMTs). One PMT measured the horizontally polarised component of fluorescence intensity; the other PMT measured the vertically polarised component. Emission filters (for fluorescein: 520 nm, DF 35) were used to reduce background light intensities.

Fluorescence polarisation immunoassays are simple mix-and-read homogenous assays, which do not require immobilisation, incubation or washing steps. Using Aokin's mix-and-read protocol, a sample with unknown amount of mycotoxin, fluorescently labelled mycotoxin tracer and anti-mycotoxin antibody are mixed together and placed in a cuvette. Mycotoxin and fluorescently labelled mycotoxin tracer bind competitively with the anti-mycotoxin antibody. The amount of mycotoxin in the sample is determined from the comparison of the time resolved polarisation values of sample against a calibration curve. Results will be presented for the detection of zearalenone and deoxynivalenol in wheat. A market launch is planned for 2006.

P29

Development of a LC-MS/MS method for the simultaneous determination of mycotoxins on cellulose filters and in fungal cultures

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The development of a rapid LC-MS/MS method for the detection of mycotoxins possibly related to the Sick Building Syndrome on filters and in fungal cultures is presented. The method describes the simultaneous determination of 16 mycotoxins. Because fungi-surface sampling as regards the Sick Building Syndrome, happens by scraping off fungal material and vacuuming onto cellulose filters, these two media were used as samples. They were spiked with nivalenol, deoxynivalenol, zearalenone, diacetoxyscirpenol, T-2 toxin, verrucarol, verrucarin A, neosolaniol, sterigmatocystin, roridin A, ochratoxin A, aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2, which can be produced by isolates from fungi-damaged buildings. De-epoxy deoxynivalenol was used as internal standard. *Penicillium nalgiovense* was cultivated on agar substrates for several days, scraped off and spiked with 16 mycotoxins. The samples were extracted twice with ethyl acetate and dichloromethane, respectively. After shaking and centrifugation, the supernatants were combined and cleaned-up using solid-phase columns. The eluate was dried and redissolved in 200 µL mobile phase consisting of methanol:water (30:70 v/v). Cellulose filters were spiked with 16 mycotoxins and extracted with methanol:water (30:70 v/v). The supernatant was dried under nitrogen and redissolved in 200 µl mobile phase. Sample extracts were injected on a C_{18} reversed-phase SunFire analytical column (Waters, Milford, MA, USA) and separated using a Waters Alliance 2695 XE HPLC system. A mobile phase of variable mixtures of ammonium acetate (10 mM) and sodium acetate (20 µM) in methanol (solvent A) and in water (solvent B) at a flow rate of 0.3 ml/min was used. The analytes were identified and quantified on a Micromass Quattro Micro triple quadrupole mass spectrometer (Waters, Milford, MA, USA). The mass spectrometer was operated in the positive electrospray ionisation (ESI+) mode using multiple reaction monitoring (MRM). For all the mycotoxins the precursor ion and the cone voltage resulting in the most abundant precursor ion were determined. Afterwards product ions and collision energy were determined. The limit of detection (LOD) was determined by spiking 11 blank samples with all mycotoxins concerned at 5 different levels. The LOD of the compounds varied from 50 pg/µl for deoxynivalenol to 0.009 pg/µl for roridin A.

P30

Detection and quantification of mycotoxins using multiple-use, in-line immunoaffinity chromatography combined with HPLC

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A well-established method for monitoring of mycotoxin contamination is the use of an immunoaffinity column (IAC) for sample preparation prior to analysis with HPLC. In general the supports used for these applications are based on natural polysaccharide matrices. However, due to their limited mechanical strength, in-line sample preparation is not easily performed and only a small fraction of the eluate is injected onto the analytical column leading to an unnecessary loss in sensitivity of the method. Silica gel is undoubtedly the most widely used chromatographic support because of its mechanical strength. In this application, an adequately wide-pore silica gel treated with Grace's proprietary surface passivation technology is used as a support for the immobilisation of antibodies, which selectively recognises aflatoxins B_1, B_2, G_1 and G_2 or ochratoxin A. The resulting immunoaffinity column can be coupled in-line with the analytical column. As a result, fully automated sample preparation and analysis is feasible leading to an increase in reproducibility, sensitivity and sample-throughput. Besides general performance characteristics, these immunoaffinity columns have been validated for their stability and were found to be reusable for as many as 200 analyses, resulting in relatively low cost per analysis ($30/analysis) compared to othersample preparation techniques ($40-$260/analysis).

P31

Isotope labelled internal standard for better quantification of deoxynivalenol in cereals

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Deoxynivalenol (DON), known as vomitoxin, is a trichothecene mycotoxin produced by several *Fusarium* species causing many harmful effects for animal and people. It has been found to be a common toxic contaminant in grain and agricultural commodities. Since DON is one of the most frequently analysed mycotoxins in Europe, fast analytical methods with high accuracy and low detection limits gain more and more importance. During the past ten years no other technique in the area of chemical analysis developed so rapidly than liquid chromatography-mass spectrometry (LC-MS). It can be used for analytes within a wide range of different polarities. Further advantages are a straightforward sample preparation, which is not requiring chemical derivatisation and little limitations by molecular mass. Differences in ionisation efficiencies and hence in signal intensities between standard substances and substances influenced by interferences, e.g., from the matrix are setting a limit to LC/MS methods. This problem can be overcome by adding an internal standard to the sample, which behaves similar to the analyte and therefore can correct for recovery losses during the sample preparation process and ion suppress effects in the MS ion source. The molecular structure and the physicochemical properties of an IS should be as close to the target analyte as possible. For HPLC/MS, the best internal standards are (non-radioactive) isotope marked molecules of the respective analyte. We have developed a LC-MS method for quantification of deoxynivalenol using fully isotope labelled $^{13}C_{15}$-DON. Different strategies with and without internal standard were performed to show suitability of $^{13}C_{15}$-DON as internal standard compound. This study shows that an IS can successfully correct fluctuations during extraction, clean-up, separation and ionisation of the sample. Good agreements with the certified values of the reference materials (maize, wheat) were reached. It could be shown that either an efficient clean-up or use of a suitable IS, are crucial for obtaining accurate results by LC-MS/MS.

P32

The detection of aflatoxins by optical biosensor analysis

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Aflatoxins are a group of closely related toxic secondary metabolites produced by certain strains of the fungi *Aspergillus flavus* or *Aspergillus parasiticus*. They have been shown to be highly carcinogenic to laboratory animals, primarily targeting the liver, and have acute toxicological effects on humans. There has also been great interest on the effects of long-term exposure to low levels of aflatoxins due to the positive association between dietary aflatoxins and human hepatocellular carcinoma. Many countries, therefore, have attempted to limit exposure by imposing regulatory limits on aflatoxins in a wide range of foodstuffs and animal feeds. Traditional techniques for the detection and/or quantification of aflatoxins include TLC or HPLC, or immunoanalytical methods such as ELISAs. These methods, however, are either laborious, inconsistent and require skilled laboratory workers, or are not sufficiently sensitive to detect levels of aflatoxins to the regulatory limits. An assay has been developed to detect and quantify aflatoxin B_1 using a Biacore biosensor, utilising the phenomenon of Surface Plasmon Resonance (SPR). It is anticipated that this assay will overcome the problems associated with other techniques and provide a high throughput, automated system with detection levels consistent with aflatoxin regulatory requirements.

P33

Direct *in situ* measurement of mycotoxin concentrations

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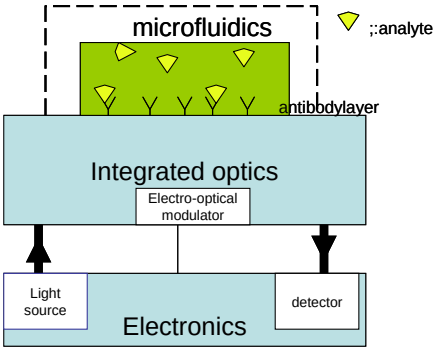
Up to now determination of mycotoxin concentrations in food and feed is complicated, time consuming and relatively expensive. A serious obstacle is that in the food and feed batches, if present, mycotoxin bursts arise on isolated and in advance unknown locations and many samples out of one batch have to be analysed. Also the mycotoxins are often incorporated in a matrix and in a pre-treatment step the mycotoxins have to be extracted by a solvent in order to enable a subsequent determination of the concentration. A further complicating factor arises from the low values of the maximum residue levels as given by legislation, being in the order of magnitude of 1-0.1 µg/kg and as a consequence at present sophisticated measurement methods are applied which have to be performed by skilled people in the laboratory. OptiSense has started the development of a small hand-held optical sensing system, which will enable direct, *in situ*, short time measurement of small concentrations of several mycotoxins in the extract solutions. The sensing system consists of an optical chip, of which the sensitive region is provided of a thin chemo-optical interface layer and which is assembled with a small electronic system for processing the output signal and presenting the required data. The chemo-optical interface layer encompasses receptor molecules (e.g., antibodies) which can associate selectively with a specific mycotoxin. As a result the optical properties of that layer are modified and an integrated optical circuit, as a part of the optical chip, converts these small changes into a change of the optical output signal. The major strength of this system [1] is its large resolution and hence low detection limit enabling direct detection of low concentrations of relatively small molecules. This is obtained by a well designed interplay of optics and electronics; the system enables to monitor very small, association induced, changes of the interface layer, a change of its effective thickness of 10^{-5} nm which is approximately equivalent to a weight change of 0.01 pg/mm^{2}. For realistic values of the association constant of the antibodies (10^{9}-10^{10} /mol), it is envisaged that mycotoxin concentrations down to about 1 pg/ml can be measured. Of course attention is paid to aspects such as cross selectivity, non-specific adsorption, regeneration, re-use, response time and reliability. Also a simple microfluidic system will be incorporated for enabling a proper transport of the sample (and if desired other) solutions to the sensitive interface. For measuring simultaneously concentrations of various mycotoxins an array of sensors will be implemented in one single optical chip each of them being provided of an interface with receptors with high association potential for a specific mycotoxin. If provided of other types of receptors the sensing system enables measurement of low concentrations of other analytes, which are complementary to the specific receptor.



Acknowlegdement
Supported by the Dutch Ministry of Agriculture, Nature and Food Quality, OptiSense is at first focusing on the determination of aflatoxins in cacao. It is planned that first sensing systems of this type will enter the market in 2007.

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Validation of screening test kit Ochracard for the determination of ochratoxins in wine

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Ochratoxin A is produced by various moulds of the genera *Aspergillus* and *Penicillium*, and is a known carcinogen and nephrotoxin of animals and humans. Ochratoxin A can be found in a variety of foodstuffs including cereals, dried vine fruits, coffee and wine. In January 2005, the European Commission fixed a limit of 2.0 µg/l (ppb) for OTA in wine, therefore it is important to validate qualitative screening methods, at detection levels equal to and around the European legislative limits. In response to the new legislation R-Biopharm Rhône introduced Ochracard, a qualitative screening test for the determination of ochratoxin A in cereal, dried fruit, coffee and wine. The following study aims to assess the performance of Ochracard on 30 naturally contaminated Italian red wine samples. Ochratoxin A is extracted from the sample, filtered and passed through an immunoaffinity clean-up column before being diluted and applied to Ochracard. Ochratoxin conjugate is then applied to the membrane and any unbound conjugate is removed by washing. A colourless substrate is added and the card is left to develop for 5 minutes. Finally a stop solution is applied to the membrane. A purple spot must appear at the control site to indicate that the test is valid (i.e. that all of the reagents have been added to the card in the correct order as advised). A purple spot at the sample site shows that the contamination level is less than 2.0 µg/l (ppb). No colour at the sample site indicates contamination at a higher level than the legal limit of 2.0 µg/l (ppb). The 30 red wines analysed with Ochracard, were also purified using Ochraprep immunoaffinity columns and the results confirmed using HPLC with fluorescence detection. The following table summarises the results obtained.

Sample	Ochracard result (ppb)	HPLC result (ppb)
1	less than 2	0,52
2	more than 2	3,79
3	less than 2	0,17
4	less than 2	0,98
5	less than 2	0,09
6	more than 2	4,18
7	less than 2	0,18
8	less than 2	0,89
9	more than 2	2,89
10	less than 2	1,13
11	more than 2	3,80
12	more than 2	4,50
13	more than 2	2,96
14	more than 2	1,26
15	less than 2	0,20
16	more than 2	2,28

Sample	Ochracard result (ppb)	HPLC result (ppb)
17	more than 2	2,20
18	more than 2	2,50
19	more than 2	2,59
20	more than 2	2,04
21	more than 2	6,20
22	more than 2	8,80
23	less than 2	1,98
24	more than 2	2,09
25	less than 2	1,65
26	less than 2	0,04
27	less than 2	1,22
28	less than 2	1,35
29	more than 2	2,02
30	less than 2	1,42
31	less than 2	1,37 (1,5 certified value)
32	more than 2	2,89 (3,0 certified value)

With the exception of one wine sample (no. 14) the results obtained using Ochracard were in agreement with HPLC. As part of the validation study, two wine reference materials (no. 31-32) were also analysed using Ochracard and using Ochraprep with HPLC and both methods confirmed the certified values. In conclusion Ochracard was found to be a suitable screening test for analysis of ochratoxin A in wine at the legal limit of 2.0 µg/l (ppb).

P35

The efficiency of different extraction solvent mixtures used in analyses of aflatoxins from a certified peanut meal reference material

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Four analytical methods with different extraction solvent mixtures were used in a study to compare the efficiency of the extraction step in analysis of aflatoxins from a reference material with a certified value of 206 ±13 µg aflatoxin B_1/kg. The extraction mixtures used were chloroform/water (100+10), acetonitril/water (60+40), acetone/water (85+15) and methanol/water (80+20). The found analytical values from the extraction mixtures were after correction for recovery 211, 204, 163 and 120 µg/kg, respectively. The small amounts of aflatoxin B_2 in the reference material followed the same pattern.

Table 1. Recovery experiments; recovery and standard deviation of added aflatoxin B_1 and B_2 at 36 µg/kg level.

Extraction solvent	No. of analyses	AFB_1 (%)	AFB_2 (%)
Acetone/water (85+15)	5	78.9 ± 5.6	79.5 ± 5.9
Methanol/water (80+20)	5	76.1 ± 2.8	85.7 ± 1.1
Chloroform/water (100+10)	3	74.6 ± 3.6	80.0 ± 3.6
Acetonitril/water (60+40)	3	109.6 ± 1.8	111.3 ± 0.5

Table 2. Analytical results.

Extraction solvent	No. of analyses and sample type	AFB_1	Corr. AFB_1	AFB_2	Corr. AFB_2	No. of analyses and sample type	AFB_1	AFB_2
Acetone/ water (85+15)	3 High level	124.7±14.1		18.2± 1.7		3 Blank material	0.73	0.15
	5 Mix high + blank*	131.3±7.7		19.0±1.0				
	8 Summary of all	128.8±10.1	163	18.7±1.2	23.1			
Methanol/ water (80+20)	3 High level	92.4±8.5		12.7±1.3		3 Blank material	0.60	0.12
	5 Mix high + blank*	90.1±2.3		12.8±1.0				
	8 Summary of all	91.0±5.0	120	12.8±1.0	14.6			
Chloroform/ water (100+10)	3 High level	157.3 ±22.1	211	21.5±2.2	26.9	-		
Acetonitril/ water (60+40)	3 High level	223.4± 10.3	204	30.0±0.7	27.0	-		

* 5 g high level + 20 g blank material

P36

In-house validation of a method for patulin determination in apple fruit derivatives at 10 µg/kg level

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A rapid and simple HPLC method for quantitation of patulin in apple derivative products at 10 µg/kg level has been adopted by our laboratory. This method is based in a recent published method for patulin determination in J.AOAC (Vol. 88, no. 2, 2005) which has been tested through an interlaboratory study. In-house validation parameters obtained in four apple or fruit- based matrix (apple juice, infant fruit puree, infant apple compote and cider) are presented. The RSD_r in all cases, at 10 µg/kg and 30µ g/kg spiking level, is less than 16%. And recovery means are between 63.8 and 73.0%. In cider, recovery is lower (58.6-60.7%) due to patulin unstability in alcoholic media. We fulfil the performance characteristics set for patulin analysis at 10 µg/kg level in the Directive 2003/78/CE.

P37

Development of a HPLC-MS/MS based method for screening the most relevant types of mycotoxins in a single chromatographic run

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Toxic metabolites produced by miscellaneous species of fungi growing on plants include a variety of structurally different species. This has lead to the development of many analytical methods dealing with single classes of compounds including a limited number of target analytes. Lately, additive and synergistic effects have been observed concerning the health hazard posed by mycotoxins [1,2], resulting in the search for multiresidue methods for the simultaneous screening of different classes of mycotoxins. The development of such a method is, however, a difficult task, as both ionic (moniliformin is an acid with a pKa value of 1.7) as well as very apolar compounds (such as the enniatins) are included in the list of analytes. As a consequence, the conditions during sample preparation and chromatographic separation are a compromise and may be far from optimal concerning certain target substances. Furthermore, LC/MS, which is the only method capable of a selective and simultaneous detection of a large number of analytes in complex matrices, is prone to suffer from unpredictable signal suppression due to matrix effects. In this work, a HPLC-MS/MS method has been developed for the separation and the detection of over 40 mycotoxins including trichothecenes, zearalenone derivatives, fumonisins, aflatoxins, ochratoxins, enniatins and ergot alkaloids at the ppb-level (and below) in a single chromatographic run. The optimisation of the method is described and its potential as well as its limitations concerning a simultaneous screening of the analytes in food and feed samples are discussed.

Acknowledgements
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P38

Quantitative determination of aflatoxin in grain from 0 to 25 ppb using a lateral flow assay and reader

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A quantitative lateral flow assay method, termed the Charm Rosa Aflatoxin MRL (Quantitative), for aflatoxin B1 detection between 0 and 25 ppb is presented for grains. The MRL for grain is 5 ppb aflatoxin B_1 and this kit has a limit of detection of approximately 2 ppb. This kit is similar to the Charm Rosa Aflatoxin (Quantitative) kit that recently passed GIPSA defined guidelines for 5-100 ppb total aflatoxin detection for corn using a 70% methanol extraction. In Europe, ethanol may be the preferred solvent for extraction and the Charm Rosa Aflatoxin MRL (Quantitative) kit has been calibrated using either a 70% methanol extraction or a 50% ethanol extraction. The general procedure is as follows: (i) a milled/ground sample is extracted with solvent; (ii) a 100 µl portion of extract is added to 1 ml dilution buffer; (iii) a 300 µl portion of the diluted extract is added to a lateral flow test strip; (iv) the test strip is incubated for 10 minutes at 45°C; and (v) the test strip is removed and read in the calibrated reflectance reader. The reader provides a numerical result from 0 to 25 ppb by comparing the binding intensity of 2 test lines and a control line. Corn, wheat, cracked corn, popcorn, soybeans and rice have been successfully tested with this kit using both solvent extraction methods.

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Feasibility study for the production of certified calibrants for the determination of deoxynivalenol and other B-trichothecenes

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The objective of the project was to develop the ability to produce and certify calibrants of deoxynivalenol (DON), 3-acetyl-deoxynivalenol (3-Ac-DON), 15-acetyl-deoxynivalenol (15-Ac-DON) and nivalenol (NIV) for their concentrations interrelated to the monitoring of these mycotoxins in cereals, food and feed. Certified reference materials (CRMs) of this kind are required to ensure the comparability and traceability of measurements of analytical test laboratories. Besides, good analytical performance is needed for internal and export control by cereal handling companies and food industries to reduce *Fusarium* infestation and toxin contamination of European cereals. The use of certified calibrants is an important element in the quality assurance system of laboratories analysing B-trichothecenes. Systematic errors resulting from calibration with inconvenient calibrants could in future be avoided through a regular check by means of certified calibrants of these toxins. The research work started with studies on the isolation, purification and characterisation of solid DON, 3-Ac-DON, 15-Ac-DON and NIV. The purity assessment was carried out by 10 complimentary analytical methods and showed purity levels >97%. As a result, toxins of highest purity with well characterised impurities were available for the gravimetrical preparation of calibrants. Their mass concentration was checked within an intercomparison study among the main partners. The determination of common molar absorption coefficients for the selected B-trichothecenes in acetonitril was also carried out by the main partners. Molar absorption coefficients of 6805±122, 6983±104, 6935±101, and 6955±145 l mol^{-1} cm^{-1} were determined (with a confidence interval at 95%) for DON, 3-Ac-DON, 15-Ac-DON and NIV, respectively. The stability studies (short and long time) were performed to obtain information about the storage and transport conditions: Stability of all the toxins in acetonitril up to 40°C for a duration of 12 months was confirmed during this studies. In an intercomparison study concluding the project, ampoules containing the gravimetrically prepared calibrants of the single toxins as well as ampoules containing a mixture of DON, 3-Ac-DON, 15-Ac-DON and NIV in acetonitril were sent to the 13 European participants. Although the intercomparison suffered somewhat from a high number of outliers, the expected decrease of the coefficient of variation (CV) was clearly demonstrated. Results furthermore indicated that the use of toxin mixtures as calibrants is feasible. The certified calibrants for DON (IRMM-315) and NIV (IRMM-316) will eventually be available from the Institute for Reference Materials and Measurements, IRMM, of the European Commission presumably by the beginning of 2006.

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P40

Rapid determination of ochratoxin A in rice and bread by immunoaffinity column clean-up and liquid chromatography using a monolithic column with fluorescence detection

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A fast and sensitive HPLC method using a monolithic column was developed for the determination of ochratoxin A (OTA) in rice and bread. The samples were extracted with an acetonitril/water mixture and diluted with phosphate buffer saline. The aqueous extracts were then purified using immuoaffinity columns (IAC). OTA was directly quantified by HPLC with fluorescence detection using a Chromolith Performance (RP-18e, 100 mm×4.6 mm) column. The method was validated by analysis of rice and bread spiked samples at 3 different levels of 2, 5, and 10 ng/g, for 3 days. The retention time of OTA at the flow rate of 2 ml/min was 2.5 min. Limit of detection (LOD) was estimated as 0.06 ng/g. Validation of this method gave average recoveries of 88.3% and 109.7% for rice and bread samples, respectively. The result of analysis of OTA in a certified reference corn sample also showed that the method is accurate. The between-day coefficient of variation on rice and bread samples spiked at 5 ng/g was found to be 9.6% and 5.9%, respectively. Compared to other existing methods, the present method provides a remarkably low LOD of 60 pg/g and is simpler (no enrichment step of the IAC eluate) and much faster. This method was applied to the screening of 18 rice samples (imported and locally produced) and 18 bread samples collected from retail market in Tehran, Iran in 2005. Seven rice samples were positive for OTA at levels varying from 0.06 to 1.66 ng/g with a mean (± SD) of 0.38 (± 0.61) ng/g. None of the samples was above the Iranian maximum tolerated level of 5 ng/g. Regarding bread, none of the samples was found to be contaminated with OTA. This is the first report of OTA contamination in rice in Iran.

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A new AgraStrip™ total aflatoxin lateral flow test

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Lateral flow tests called AgraStrip™ were validated to test total aflatoxins at cut-off levels of 4ppb, 10ppb and 20ppb, respectively. The test is a one-step lateral flow immuno-chromatographic assay based on an inhibition immunoassay format. Antibody-particle complex is dissolved in assay diluent and mixed with sample extract in microwells. The mixed content is then wicked onto a membrane, which contains a test zone and a control zone. The test zone captures free antibody-particle complex, allowing colour particles to concentrate and form a visible line. A positive sample with total aflatoxins greater than the cut-off level will result in no visual line in the test zone. Alternatively, a negative sample with total aflatoxins less than the cut-off level will form a visible line in the test zone. The line will always be visible in the control zone regardless of the presence of aflatoxins. The test is a rapid semi-quantitive method with assay result to be obtained within 5 minutes. Validation studies assessed accelerated stability; accuracy in corn, peanuts, soybean and wheat; and ruggedness of the test kits. Results indicated the tests are accurate, rugged and effective for semi-quantitive measuring total aflatoxins greater or less than the cut-off levels.

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Effect of deoxynivalenol (DON) content of the concentrate on milk yield and milk quality

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Thirty-two early to mid lactation multiparous Holstein cows were used in a randomised block design for a 10 week trial at INIA La Estanzuela, Uruguay to determine the effects of deoxynivalenol (DON) and Mycosorb (modified glucomannan, Alltech, Inc., Nicholasville, KY) on milk yield, milk composition, somatic cell count (SCC), body weight gain (BWG), body condition scores (BCS) and hepatic activities. Four concentrates were prepared to contain 0 (T1); 2.5 (T2); 5.0 (T3) or 5.0 ppm of DON plus 1% of Mycosorb (T4). Cows grazed on a grass/clover pasture (estimated 10 kg dry matter intake daily) and offered 25 kg of corn silage and 6 kg of experimental concentrates per head per day. This feeding allowed us to offer 0 ppm DON, 15 ppm DON, 30 ppm DON or 30 ppm DON + 6 g Mycosorb per cow daily. Milk yield was recorded and milk samples were taken from Monday through Friday each week throughout the trial. Prior to feeding the experimental diets, milk yield was recorded and milk samples were collected and analysed to determine milk composition. Cows were also bled to determine aspartate aminotransferase (AST) and gamma glutamyl transpeptidase (GGT) base levels. Weekly averages of milk and component yields, milk contents, BWG and BCS were used as repeated measures from week 2 through 10. Cows were individually bled to determine concentration of AST and GGT on weeks 4 and 8. Yield of milk, protein, lactose and non-fat solids, and contents of milk fat, protein, lactose, non-fat solids and total solids did not differ ($P>0.10$) among treatments. Fat corrected milk yield was lower ($P<0.05$) for T3 (19.6 kg/d) compared to T1 (21.8 kg/d), T2 (22.2 kg/d) and T4 (21.6 kg/d). Milk fat content and fat yield were different ($P<0.05$) between T3 (3.30%, 0.746 kg/d) and T2 (3.72%, 0.841 kg/d). Total solid yield was lower ($P<0.05$) for T3 (2.605 kg/d) compared to T1 (2.851 kg/d), T2 (2.858 kg/d) and T4 (2.815 kg/d). Somatic cell count was higher ($P<0.05$) for T3 (333.1 x 1000 scc/ml) compared to T1 (108.3 x 1000 scc/ml), T2 (116.3 x 1000 scc/ml) and T4 (79.2 x 1000 scc/ml). No differences ($P>0.10$) were recorded among treatments for BWG as well as for BCS; neither difference ($P>0.10$) was recorded among treatments for AST or GGT blood levels. It was concluded that daily intakes of 30 mg of DON depressed milk fat content and fat corrected milk yield, and increased the SCC. Utilisation of Mycosorb corrected the effects of daily intakes of 30 mg of DON on lactating dairy cows.

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Effect of dietary Mycosorb™ on aflatoxicosis in broilers. I. Performance, serum biochemistry and haematology parameters

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The amelioration of aflatoxicosis in broiler chickens was examined by feeding two concentrations of yeast cell wall derived mycotoxin binder (Mycosorb™, Alltech, KY, USA). Mycosorb, incorporated into the diet at 0.5 and 1 g/kg, was evaluated for its ability to reduce the detrimental effects of 2 mg total aflatoxin (AF; 82% AFB_1, 5.50% AFB_2, 10.20% AFG_1 and 1.58% AFG_2) in diet on growing broiler chicks from 1 to 21 d of age. A total of 240 male broiler chicks *(Ross-308)* were divided into 6 treatment groups [control, AF, Mycosorb (0.5 g/kg), AF plus Mycosorb (0.5 g/kg), Mycosorb (1 g/kg), and AF plus Mycosorb (1 g/kg)]. Compared to control, AF treatment significantly decreased body weight gain from week 2 onwards. AF treatment also caused significant decreases in serum total protein, albumin, total cholesterol, triglyceride, glucose, inorganic phosphorus, creatinine levels and alanine aminotransferase (ALAT) activity but increased the aspartate aminotransferase (ASAT) activity. Red blood cell, haematocrit, haemoglobin, thrombocyte, and lymphocyte counts and tibial crude ash levels were significantly reduced by AF treatment, while significant increases were seen in heterophil counts. The addition of Mycosorb (1 g/kg) to an AF-containing diet significantly improved the adverse effects of AF on haematological parameters, total protein, albumin values and ASAT activity. Mycosorb (1 g/kg) also partially improved body weight gains (59%) and the other biochemical parameters influenced by AF treatment. The addition of Mycosorb (both 0.5 and 1 g/kg) to the AF-free diet did not cause any considerable changes in the investigated values. These results clearly indicated that Mycosorb (1 g/kg) addition effectively diminished the adverse effects of AF on the investigated values. Also, the higher dietary concentration of Mycosorb (1 g/kg) was found more effective than the lower concentration (0.5 g/kg) against the adverse effects of AF on the variables investigated in this study.

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Effect of Mycosorb™ on aflatoxicosis in broilers. II. Gross examination and histopathology

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The present study was conducted to evaluate the efficacy of a yeast cell wall derived mycotoxin binder (Mycosorb™, Alltech, KY, USA) to counteract the toxic effects of aflatoxin (AF) in broilers. Mycosorb, incorporated into the diet at 0.5 and 1 g/kg, was evaluated for its ability to reduce the detrimental effects of 2 mg AF/kg diet on growing broiler chicks from 1 to 21 d of age. A total of 240 male broiler chicks *(Ross-308)* were divided into 6 treatment groups (Control, AF, Mycosorb (0.5 g/kg), AF plus Mycosorb (0.5 g/kg), Mycosorb (1 g/kg), and AF plus Mycosorb (1 g/kg)). A detailed necropsy was conducted and the pathological examinations were performed on the liver, bursa of Fabricius, thymus, spleen and kidney.
The AF treatment caused moderate to severe hydropic/fatty degeneration in hepatocytes of liver and tubular epithelium of kidneys, follicular depletion in bursa of Fabricius, thymus and spleen. Mycosorb additions to the AF-containing diet at the levels of 0.5 g/kg and 1 g/kg diminished the severity of pathological changes, slightly and moderately, respectively. The number of affected organs was also significantly ($P<0.05$) reduced in the group given 1 g/kg Mycosorb added to AF-containing diet, compared to the AF-group. These results clearly show that Mycosorb addition effectively diminished the adverse effects of AF on the pathological changes investigated in this study and that the higher concentration of Mycosorb (1 g/kg) was more effective than the lower concentration (0.5 g/kg).

P45

Transmission of ochratoxin A into ewe's milk following a single or chronic ingestion of contaminated feed

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Ochratoxins, a group of highly toxic metabolites produced by some species of *Aspergillus* and *Penicillium*, are commonly found in foods and animal feeds. Ochratoxin A (OTA), the most important toxin of this family, is nephrotoxic, hepatotoxic, teratogenic and carcinogenic in animals and was recently classified by the International Agency of Research on Cancer (IARC) as a class 2^B, possible human carcinogen. Ruminants, thanks to the action of rumen microorganisms, have the capacity to hydrolyse OTA into the less toxic compound OTα. However, not all the ingested toxin is degraded in the rumen. The undegraded OTA is absorbed in the gastrointestinal tract and can contaminate animal products posing a potential risk for consumers. Although the presence of OTA has been reported in bovine milk, the transmission from contaminated feed into ruminants' milk has never been investigated. In this study the effect of a single or chronic OTA ingestion on the transmission of the toxin and its metabolite into milk was examined in dairy ewes. Six dairy ewes in late lactation were divided in two lots that received 5 (dose 1) and 30 µg (dose 2) OTA/kg body weight/day. Contaminated wheat that was experimentally inoculated with a toxigenic A*. ochraceus* strain was the toxin source. Daily doses of ground wheat were orally administered as a bolus once a day to ensure that animals received consistently the desired quantity. The exposition lasted for 28 days. OTA and OTα in plasma and milk were determined on days: -7, 0, 1, 9, 15, 22, and 28 of the experiment. OTA was detected in plasma and milk in a dose dependent manner. Chronic ingestion increased the toxin carry-over into milk. After a single oral administration, the mean concentration of OTA was 26.9$\pm$2.6 and 108.4$\pm$9.0 ng/l for dose1 and 2, respectively. After 28 days of administration, the toxin concentration in milk was 3 times higher than the concentration at day 1. The OTA milk:plasma ratio did not change throughout the experimental period but it was also dose dependent. It was 0.058$\pm$0.02 and 0.082$\pm$0.04 for dose 1 and 2, respectively. Concentrations of OTA that can be found in naturally contaminated feeds escape ruminal degradation and can be transmitted, although in low quantities, into ewe's milk.

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Occurrence of mycotoxins in animal feedstuffs of Thailand

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Mycotoxin contamination of human food and animal feed is a worldwide problem, because mycotoxins are naturally occurring toxins. They may enter the human and animal dietary system by indirect or direct contamination. Indirect contamination of foods or feeds can take place when an ingredient of a process has previously been contaminated with toxin-producing fungi and while the fungi may be killed or removed during processing, the mycotoxins will mostly remain in the final product. Contamination of cereals constitutes the main point of entry of many mycotoxins into food and feed chains. Therefore, human ingestion of mycotoxins will mainly occur from the consumption of mycotoxins in residues and metabolites in animal-derived foods such as milk or meat products. Thailand is in the tropical area, where it is hard to avoid mould-contaminated food and feed. So, the main purpose of this study was to investigate the possible incident of mycotoxins in animal feeds. In this study a survey has been carried on the natural occurrence of mycotoxins in commercially available animal feed samples. The study was divided into two parts, the first part was to identify the genus of mould and the second part was to determine the mycotoxins in these animal feeds. The results showed that all the samples were contaminated with moulds. These moulds are *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., and non-septate fungi. For determination of mycotoxins, the results showed that aflatoxin B_1 was detected in 23/25 samples (92%), and the average was 7.56 ppb. Ochratoxin A was detected in 3/10 samples (30%) in levels of 10.48, 11.14 and 12.35 ppb. Deoxynivalenol was detected in 13/15 samples (86%), and the average was 33.77 ppb. T-2 toxin was detected in all samples (10 samples), and the average was 6.91 ppb. Multi-mycotoxin contamination was determined from 10 samples. The results revealed that 3/10 samples were contaminated with 4 mycotoxins (aflatoxin B_1, ochratoxin A, deoxynivalenol and T-2 toxin), and 7/10 samples were contaminated with 3 mycotoxins (aflatoxin B_1, deoxynivalenol and T-2 toxin). From the results obtained in this study a high risk for human health is suggested, because of the possibility of indirect exposure through meat and other animal products. So, further studies are required to determine mycotoxin residues in meat and animal products.

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Toxicity of different trichothecene mycotoxins on growth performance, immune responses and efficacy of a mycotoxin degradation enzyme in pigs

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The purpose of this study was to investigate a defined concentration of trichothence mycotoxins, deoxynivalenol (DON) and zearalenone (ZON), incorporated into feeds on growth performance, blood biochemistry, and immune response of pigs and the toxic alleviating effects of a mycotoxin degradation enzyme (MDE). A total of 48 weaning pigs was randomised, allotted to four treatments including control, toxin 1 (DON and ZON), toxin 2 (DON, ZON, and MDE), and MDE alone for a 6 weeks challenge trial; two replicates per treatment. The results of different criteria, including growth performance, serum biochemistry parameters, alveolar macrophages activity, antibody titers for PR vaccine, and cytokines gene expression profile, showed that DON and ZON caused a consistent impairment in pigs after 6 weeks exposure. Based on the histopathology and blood biochemistry findings it is suggested that the combination of DON and ZON caused a chemical multi-organ toxicity in pigs and that MDE provides a partial and complete toxic sparing effect from different trichothecene combinations.

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A review of the interactions between mycotoxins and immunity in poultry

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Mycotoxins are secondary metabolic products of fungi (moulds) and they are toxic to animals or humans. Major mycotoxins are aflatoxins, trichothecenes, zearalenone, ochratoxins and fumonisins. Their ingestion by farm animals can result in specific symptoms such as liver damage or egg production being affected. Besides, mycotoxins can also affect the immune system of birds. Interactions between mycotoxins and pathogenic bacteria. Increased mortality has for example been reported in chickens given a feed containing aflatoxin and infected with different strains of *Salmonella*. The presence of ochratoxin A in feed of chicks increased mortality and the severity of the infection with *E. coli*. Similarly, birds given a feed containing fumonisin B_1 had more difficulties to handle *E. coli* infections. Interactions between mycotoxins and coccidiosis. Combination of aflatoxin and coccidia in Japanese quail-chicks resulted in higher incidence of the disease. Feeding birds with diets contaminated with T-2 toxin may alter the efficacy of monensin and other related antibiotics used in the treatment of coccidiosis. Likewise, an interaction between *Eimeria tenella* and ochratoxin A has been reported in chicks. Interactions between mycotoxins and viruses. Different publications describe the interaction between mycotoxins and Newcastle disease. Fumonisins, moniliformin, cyclopiazonic acid and T-2 toxin, as well as aflatoxins, reduced the production of antibodies following the vaccination against this viral disease. The impact of aflatoxin on severity of infectious bursal disease (IBD) was also studied.

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A review of the interactions between mycotoxins and immunity in pigs

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Mycotoxins are secondary metabolic products of fungi (moulds) and they are toxic to animals or humans. Major mycotoxins are aflatoxins, trichothecenes, zearalenone, ochratoxins and fumonisins. Their ingestion by farm animals can result in specific symptoms such as liver damage, reproduction problems, kidney failure, or lung damage. Beside specific symptoms, mycotoxins can also affect the immune system. For example, the negative effect of mycotoxins on resistance to pathogenic bacteria has been described as early as in the late seventies. Outbreaks of salmonellosis were reported in the presence of aflatoxins in feed. Aflatoxin also increased susceptibility to swine dysentery. Ingestion of ochratoxin A contaminated feed can increase susceptibility to salmonella infection in piglets. Different authors have reported other effects of ochratoxin A on immunity, such as decreased lymphocytes, increase of apoptotic phagocytes, eosinophils, leukocytes and neutrophils and reduced phagocytosis. Another observed interaction is the one between Fumonisin B1 and *Escherichia coli* in piglets. Significantly higher populations of the bacteria were present in the intestines of animals given a contaminated feed compared to piglets that had received no fumonisin. Mycotoxins can also reduce the efficacy of vaccination. Aflatoxin has for instance a negative effect on the development of acquired immunity to swine erysipelas. More recently it was observed that fumonisin B1 can reduce the production of antibodies against *Mycoplasma agalactiae*.

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New methods for efficacy testing of mycotoxin inactivators

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Mycotoxins are secondary metabolic products of fungi (moulds) and they are toxic to animals or humans. Major mycotoxins are aflatoxins, trichothecenes, zearalenone, ochratoxins and fumonisins. Their ingestion by farm animals can result in specific symptoms such as liver damage, reproduction problems, kidney failure, or lung damage. About twenty years ago, use of so-called “mycotoxin binders” has given a new perspective to the control of mycotoxins. For instance, hydrated sodium calcium aluminosilicates (HSCAS) have high affinity for aflatoxin B1 and can be successfully used in the feed to prevent aflatoxicosis. Other compounds have been tested, but controversial results have been observed. One of the main limitations of the “mycotoxin binders” is that their efficacy is restricted to a few mycotoxins. They are effective against so-called polar mycotoxins, such as aflatoxins. In the case of other mycotoxins, such as trichothecenes, binding efficacy is generally very poor, if not zero. Recent research indicates that the biotransformation of mycotoxins, using live microorganisms or enzymatic preparations, gives promising results. It has been demonstrated that some enzymes can transform the structure of mycotoxins such as deoxynivalenol or zearalenone, which are very difficult to bind efficiently. The products obtained from the reaction are less toxic compounds. The application of such enzymatic transformations to the feed sector gives new opportunities because enzymes can have a specific action and their reaction, compared to binding, is not reversible. The search for efficient combinations of mycotoxin binders and enzymes can be facilitated by using a system designed to simulate the digestive tract. A small intestinal model that can be applied to mycotoxin tests has been developed and is called “gut simulator”. The gut simulator mimics the digestive tract and allows studying interactions between feed, mycotoxins and mycotoxin-deactivating substances in “real” conditions. This offers a clear advantage when compared to the classical in-vitro tests where only pH is controlled and other parameters are not taken into account. Indeed large differences were found between the classical in vitro tests and the gut simulation model. Using this equipment, a screening of different binders, enzymes, and other substances, has been possible.

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Efficacy of a glucomannan-containing yeast product (Mycosorb®) and hydrated sodium calcium aluminosilicate to reduce the individual and combined toxicity of aflatoxin and T-2 toxin in commercial broilers

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A feeding trial was conducted on commercial broilers for a period of 35 days to determine the efficacy of a glucomannan-containing yeast product (Mycosorb®, Alltech, Inc.) and hydrated sodium calcium aluminosilicate (HSCAS) to reduce the individual and combined effects of aflatoxin (AF) and T-2 toxin (T-2) on the performance, organ weights and immune status of broiler chickens. Twelve dietary treatments (4x3 factorial) consisting two dietary levels each of AF (0 and 2 mg/kg), T-2 toxin (0 and 1 mg/kg), Mycosorb (0 and 1 kg/ton) and HSCAS (0 and 10 kg/ton) were tested on 720 commercial broiler chickens divided at random into 36 replicates of 20 chicks of equal sex ratio. Weight gain and feed intake were recorded weekly. Organ morphology and antibody titers for Newcastle disease (ND) and infectious bursal disease (IBD) were measured on 35th day. AF and T-2 toxin individually decreased weight gain and increased feed conversion ratio (FCR) ($p<0.05$). AF alone ($p<0.05$) increased weights of liver, kidney, gizzard and spleen and reduced the thymus and bursal weights. T-2 toxin ($p<0.05$) increased the liver and gizzard weights and decreased the thymus weight. Both AF and T-2 toxin when fed individually affected ND and IBD titers in a significant manner. Significant interaction between AF and T-2 toxin was observed for their additive effects on weight gain, FCR, organ weights and antibody titers. Addition of Mycosorb ($p<0.05$) improved weight gain, feed conversion efficiency and restored organ weights. Supplementation with Mycosorb significantly improved antibody titers against ND and IBD. Supplementation with HSCAS ($p<0.05$) resulted in improvement in weight gain and restored organ weights in the groups fed AF alone, but not in T-2 toxin fed groups. HSCAS inclusion did not influence FCR in toxin fed groups. Addition of HSCAS ($p<0.05$) improved the antibody titers against ND and IBD only in AF fed groups. Addition of a glucomannan-containing yeast product (Mycosorb®, Alltech, Inc.) was found to be effective in averting the individual and combined toxicity of aflatoxin and T-2 toxin in commercial broilers, while HSCAS was effective only against aflatoxin.

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Use of a glucomannan-containing yeast product (Mycosorb®) to reduce the effects of ochratoxin A in broiler chickens

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A biological experiment was conducted to evaluate the ability of a glucomannan-containing yeast product (Mycosorb®, Alltech, Inc.) to reduce the toxic effects of ochratoxin A in broiler chickens. Two levels of ochratoxin A (0 and 500 ppb) and two levels of Mycosorb (0 and 0.05%) were tested in a 2x2 factorial arrangement. Experimental details are: (i) two hundred and eighty broiler chicks were reared in deep litter system for five weeks; (ii) production performance, gross pathological changes, blood parameters and serum biochemical changes were evaluated and (iii) data pertained were analysed by one-way ANOVA and means were compared by Duncan's multiple range test. Feeding of ochratoxin contaminated diet resulted in a significant reduction in body weight gain (17.6%), increased gamma glutamyl transferase activity (38.5%), decreased total proteins (16.4%) and hematocrit values (11.3%). Supplementation of the diet with Mycosorb significantly ($P \leq 0.05$) improved the body weight gain (3.6%), total proteins (11%) and hematocrit values (4.7%). Furthermore, Mycosorb showed beneficial effects by decreasing gamma glutamyl transferase activity (14.7%) and reducing mortality. The results clearly indicate that the inclusion of the glucomannan-containing yeast product (Mycosorb) at 0.5 kg/ton of feed is beneficial in counteracting the adverse effects of ochratoxin A in broiler chickens.

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Mycotoxins in grass and maize silage for dairy cattle

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The feed ration of dairy cows consists of three types of feed: concentrate, by-products and forages. Forages generally represent 50-80% of the feed ration of dairy cows. The main forage crops in Europe are grass, fed fresh or as silage or hay, and maize, generally fed as whole crop silage. In contrast to ingredients for concentrate feeds there is little information available on mycotoxin levels in grass and maize. Maize and, to a lesser extent, grass may be infected in the field by deoxynivalenol (DON) or zearalenone (ZEA) *Fusarium* species. These moulds do not survive the silage fermentation process but DON and ZEA remain unchanged. DON and ZEA in feed are of concern because of their possible impact on animal health and productivity. Maximum tolerance levels in the Netherlands for DON and ZEA in feed for dairy cows are 3.0 and 0.50 mg/kg, receptively (on total feed ration basis). Due to their extremely low carry over to milk DON and ZEA in feed are not of concern with respect to the safety for human consumption of milk. This study was carried out to provide information on the levels of mycotoxins in grass and maize silage produced in the Netherlands in 2002, 2003 and 2004. Samples from 120 grass silage and 140 maize silage clamps at Dutch dairy farms were collected between June 2002 and November 2004. Samples were taken by core sampling four to eight weeks after ensiling. Samples were air-dried 20 h at 65°C and milled. A multi-analyte LC-MS method was used for the detection of 14 mycotoxins. DON and ZEA were found frequently in maize silage. DON was detected above the limit of quantification (LOQ) of 0.25 mg/kg in 70% of maize silages, varying from 40% in 2002 to 98% in 2004. The average concentration in positive samples was 0.85 mg/kg, varying from 0.45 mg/kg in 2003 to 1.0 mg/kg in 2004. The maximum concentration varied from 1.0 mg/kg in 2003 to 3.1 mg/kg in 2004. One sample exceeded 3.0 mg/kg. ZEA was detected above the LOQ of 0.025 mg/kg in 49% of maize silages, varying from 10% in 2003 to 85% in 2004. The average concentration in positive samples was 0.17 mg/kg, varying from 0.12 mg/kg in 2003 to 0.18 mg/kg in 2004. The maximum concentration varied from 0.15 mg/kg in 2003 to 0.94 mg/kg in 2004. Five samples exceeded 0.50 mg/kg. Grass silage proved a much less important source of DON and ZEA than maize silage. None of the 120 samples tested contained DON and seven samples (6%) contained a low level of ZEA (average concentration 0.09 mg/kg; maximum concentration 0.31 mg/kg). Aflatoxins (B_1, B_2, G_1 and G_2), ochratoxin A, T-2 and HT-2 toxin, di-acetoxyscirpenol and sterigmatocystin were detected in none of the samples. Fumonisins (B_1 and B_2) were detected in two maize silages samples (up to 34 mg/kg). Roquefortin C was detected in one grass silage sample (0.08 mg/kg). It is concluded that maize silage is an important source of DON and ZEA. Since it usually accounts for 25-40% of the diet of dairy cows, maize silage forms a significant contribution to the total exposure of cows to these mycotoxins.

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Aflatoxin B_1 intoxication in piglets: consequences on liver drug metabolising enzymes and on immune response

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The aim of the study was to investigate the consequences of *in vivo* intoxications with increasing doses of aflatoxin B_1 (AFB_1) on liver drug metabolising enzymes and immune response in a monogastric animal model sensitive to this mycotoxin. Twenty six-weeks-old piglets were randomly allotted in four experimental groups. For 28 days, they had free access to food contaminated or not with AFB_1 0 µg/kg (control group), AFB_1 385 µg/kg, AFB_1 867 µg/kg or AFB_1 1807 µg/kg. All piglets received two ovalbumin subcutaneous injections by days 4 and 15. Blood samples were weekly collected for measurement of immuno-globulins, lymphocytes proliferation and cytokines production. After euthanasia, organ samples were collected. Liver glutathion *S*-transferase (GST) and cytochrome-P450 dependent activities were quantified. AFB_1 decreased weight gain and induced dose-dependent lesions in liver. AFB_1 intakes did not alter GST, P450 3A N-demethylase and P450 2E hydroxylase activities. Significant decreases in total microsomal P450 content and in P450 1A O-dealkylase activities were observed in the liver of piglets exposed to the highest dose of AFB_1. Conversely P450 2B and P450 2C N-demethylase activities were weakly increased. AFB_1 ingestion increased IgA plasmatic levels, but did not affect the anti-ovalbumin IgG production. By contrast, AFB_1 inhibits the anti-ovalbumin cellular-mediated immune response, it was delayed and weaker in the animals exposed to AFB_1. In summary, high AFB_1 contaminated feed consumption decreased liver P450 1A but not P450 3A activities, two P450 isoforms mainly implicated in AFB_1 oxidation. P450 2B and P450 2C are minor P450 isoforms in pig; their weak increased activities might be better explained by an imbalance in the expression of the various P450 than by a specific effect of AFB_1. Concerning the immune system, this study showed a significant toxic effect of AFB_1 on the cellular immune response post-immunisation and support the hypothesis that AFB_1 intake may alters vaccine efficacy.

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P55

In vivo protective effects of dietary esterified glucomannan on aflatoxin B_1 intoxication in piglets

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The aim of the study was to investigate the potent protective properties of esterified glucomannan against aflatoxin B_1 (AFB_1) intoxication *in vivo* in piglets, a monogastric animal model sensitive to this mycotoxin. Two main targets of AFB_1 were explored: liver drug metabolising activities and the immune system. Twenty-two six-weeks-old piglets were randomly allotted in four experimental groups. For 28 days, they had free access to food contaminated with AFB_1 2 µg/kg (control group), AFB_1 482 µg/kg, AFB_1 968 µg/kg or AFB_1 1912 µg/kg and complemented with esterified glucomannan (2 kg/t). All piglets received two ovalbumin subcutaneous injections by days 4 and 15. Blood samples were weekly collected for measurement of immunoglobulins, lymphocytes proliferation and cytokines production. After euthanasia, organ samples were collected. Liver glutathion *S*-transferase (GST) and cytochrome P450-dependent activities were quantified. AFB_1 intake decreased weight gain even when esterified glucomannan are included in feed, but lesions in liver are mainly observed in animals exposed to AFB_1 1912 µg/kg. AFB_1 intakes did not alter GST, P450 3A and P450 2B N-demethylase, and P450 2E hydroxylase activities, but decreased total liver microsomal P450 content and P450 1A O-dealkylase activities in piglets exposed to the highest dose of AFB_1. A weak increase in P450 2C N-demethylase activity was observed in these animals. In our four experimental groups AFB_1 did not affect the IgA plasmatic level neither the anti-ovalbumin IgG production. No alteration of the anti-ovalbumin cellular-mediated immune response was observed in groups consuming AFB_1 contaminated feed complemented with glucomannan when compared to control group. In summary, the addition of esterified glucomannan in AFB_1 contaminated feeds ameliorated liver functions in animals exposed to AFB_1 968 µg/kg. AFB_1 still decreased hepatic P450 1A but not P450 3A activities. The investigations on the immune system indicated that when associated with esterified glucomannan, AFB_1 did not inhibit the specific anti-ovalbumin immune response. These observations support the hypothesis that esterified glucomannan present some protective effects against AFB_1 intoxication and significant immuno-stimulatory properties.

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Education in the diagnosis of poultry mycotoxicosis

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Mycotoxins are quite abundant in raw materials as at least 1/3 of the raw materials produced worldwide are known to contain mycotoxins. The knowledge we have up to now on mycotoxins for poultry have revealed that their negative effect on performance is related to reduced immune function, reduced reproductive performance, reduced growth rates and/or poor vaccination response to viral diseases. The question what levels of mycotoxins can be considered to be safe is dependent on factors as (i) chemical class of mycotoxin, (ii) presence of other mycotoxins (combined effect?), (iii) exposure time to toxins, and (iv) health status of animals. So there is no real guideline to say what is the safe level besides just saying that the only safe level is the level of total absence. The real issue is that diagnosis of (poultry) mycotoxicosis under field conditions is not yet well known by the field and feed mill veterinarians. The European branch of Alltech Biotechnology Centre, the research centre of Alltech Inc. (Lexington, Kentucky, USA) has therefore started up the initiative to set up training sessions in diagnosis of poultry mycotoxicosis. In Europe, this led to the First European Mycotoxin Posting Session, organised in Hungary in May 2005 and hosted by Prof. Elisabeth Santin (Universidade Federal do Paraná, Curitiba, Brazil). Prof. Santin has large experience in education of field diagnosis of mycotoxins in Brazil. At two locations, 4 times 15 animals were fed uncontaminated or contaminated feed as indicated in Table 1. Pictures and a DVD of the posting session were made to be used as an aid to both poultry veterinarians and company technicians who are faced with suboptimal performance in their flock, and which want to assess if mycotoxins are to blame. This DVD pinpoints on the different morphological signs, which have to be investigated to make a correct diagnosis.

Table 1. Feed mycotoxin levels of the different treatments.

Groups	**Treatments**	**Aflatoxin** (ppm)	**T-2 toxin** (ppm)	**Mycosorb** (kg/t)
1	Control	0	0	-
2	Aflatoxin andT-2 toxin	0,2	0,4	-
3	T-2 toxin	0	0,4	-
4	Aflatoxin andT-2 toxin and Mycosorb	0,2	0,4	2

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Impact of fumonisin B_1 on the pig immune response

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Mycotoxins are secondary metabolites of fungi that may contaminate animal and human feeds at all stages of the food chain. Their global occurrence constitutes a major risk factor for human and animal health. Mycotoxins can also alter normal immune function when present in food at levels below observable overt toxicity. Fumonisins are a family of cytotoxic and carcinogenic mycotoxins produced by *Fusarium verticilloides* and *F. proliferatum*, natural contaminant of maize. Fumonisin B_1 (FB_1) causes species-specific toxicological effects in laboratory and domestic animals including pigs. It has been reported as being carcinogenic in rodents and has been implicated as a contributing factor in human oesophagal cancers. A series of experiment were performed to analyse the effect of this mycotoxin on the immune response of piglets and the consequences in term of susceptibility to infection and efficacy of the vaccinal immune response. Gastrointestinal epithelium is the first tissue exposed to food contaminants, toxins and pathogens. In a first step, ingestion of low doses of FB_1, was shown to increases the intestinal colonisation in piglets orally infected by an opportunistic pathogenic strain of *E. coli*. In these animals an increase bacterial translocation from the intestinal tract to extra-intestinal organs such as lung and lymph node was also observed. Using the porcine epithelial intestinal cell line, FB_1 was demonstrated to alter the cell morphology and inhibit in a dose dependent manner their proliferation by blocking the cells in G0/G1 phase of the cell cycle. FB_1 also decreases the TEER of intestinal cell monolayer, however this effect could only be seen after a prolonged exposure to the toxin. This decrease TEER was associated with an increased translocation of the bacteria across the cell monolayer. FB_1 also decrease in a dose dependent manner the production of IL-8, a cytokine involved in the recruitment of neutrophils. This inhibition was observed both *in vitro* on epithelial cell treated with the toxin and *in vivo* on ileal fragments from piglets that had ingested the toxin for 7 days. We hypothesised that by decreasing the intestinal inflammatory response and by altering the barrier function of the epithelium, ingestion of FB_1 increases the intestinal colonisation and the bacterial translocation by pathogenic bacteria. Consumption of feed contaminated with high doses of FB_1 induces pulmonary oedema in swine. Thus the effect of this toxin on lung inflammation caused by *P. multocida*, a secondary pathogen, which can generate a respiratory disorder in predisposed pigs was investigated. Ingestion of toxin or bacterial infection did not affect weight gain, induced no clinical sign or lung lesion, and only had minimal effect on bronchoalveolar lavage cell composition. By contrast, the combined treatment with fumonisin culture material and *P. multocida* delayed growth, induced cough, and increased BALF total cells, macrophages and lymphocytes. Lung lesions were significantly enhanced in these animals and consisted of subacute interstitial pneumonia. TNF-α, IFN-γ and IL-18 mRNA expression was also increased. This indicates that ingestion of low doses of FB_1 predisposes pigs to lung inflammation. Although, the bioavailability of FB_1 is low, analysis of sphingosine/sphinganine ratio in the serum indicate that this toxin have a systemic effect. The effect of FB_1 on the systemic immune response was thus investigated. We demonstrated that ingestion of FB_1 does not interfere with the global production of immunoglobulin but decreases the specific antibody response mounted during a vaccination protocol. This effect was more pronounced in male than in females. Antibody synthesis being controlled by the balance between Th1 and Th2 cytokines, the effect of FB_1 was analysed on cytokine synthesis. *In vitro* exposure of peripheral blood mononuclear cells to FB_1 decreases the synthesis of IL-4 (Th2 cytokine) synthesis whereas it increases IFN-γ production (Th1 cytokine). FB_1 also decrease lymphocyte proliferation and block them in the G_1 phase of the cell cycle. We hypothesised that the anti-proliferative effect of this mycotoxin combined with the effect of the toxin on the cytokine balance may explain its effect on vaccine efficacy. Taken together our data indicate that FB_1 alters both the innate and the specific immune response. This alteration may have some consequences for animal and human when eating FB_1 contaminated feed or food.

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Effects of feeding grains naturally-contaminated with *Fusarium* mycotoxins on hepatic fractional protein synthesis rates of laying hens and the efficacy of a polymeric glucomannan mycotoxin adsorbent in prevention of these effects

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Cytotoxicity of trichothecene mycotoxins has been attributed to the inhibition of protein synthesis and has been experimentally linked to the 12,13-epoxytrichothecene nucleus. Trichothecenes bind to the 60S ribosomal subunit and interact with peptidyl transferase. It has been shown *in vitro* that T-2 toxin inhibited hepatic protein synthesis in rats. The effect of trichothecenes on *in vivo* protein synthesis rates has not yet been determined. Experiments were conducted, therefore, to evaluate the effects of feeding grains naturally-contaminated with a combination of *Fusarium* mycotoxins on hepatic fractional protein synthesis rates (FSR) of laying hens. Thirty-six, 32-week-old laying hens were fed diets formulated with (i) uncontaminated grains, (ii) contaminated grains or (iii) contaminated grains +0.2% polymeric glucomannan mycotoxin adsorbent (GMA, Mycosorb^{TM}, Alltech Inc., Nicholasville, KY, USA) for a period of four weeks. Hepatic FSR were measured *in vivo* by the flooding-dose method. Birds were weighed and then injected intravenously with a flooding dose of L-phenylalanine (L-Phe; 1.50 mmol/kg body weight) containing L-[ring-$^{2}H_5$] Phe at 40 mol% (0.60 mmol/kg body weight) and L-Phe at 60 mol% (0.90 mmol/kg body weight) dissolved in sterile saline. Fifteen minutes after injection, birds were killed. Liver was excised and removed immediately and frozen. The isotopic enrichment of L-[ring- $^{2}H_5$] Phe in tissue free and bound pools was determined by gas chromatography-mass spectrometry using n-propylheptafluorobutyrate derivatisation. The feeding of contaminated grains decreased hepatic FSR in laying hens compared to controls after four weeks. The hepatic FSR of birds fed the control diet and contaminated grains+GMA were not different. It was concluded that the *in vivo* hepatic FSR of laying hens was inhibited by the feeding of grains naturally contaminated with *Fusarium* mycotoxins and this may explain some of the adverse effects seen when contaminated grains are fed to laying hens.

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Effects of feeding grains naturally-contaminated with *Fusarium* mycotoxins on performance and metabolism of first parturition sows during lactation, and the efficacy of a polymeric glucomannan mycotoxin adsorbent in preventing these effects

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Piglets fed diets containing grains naturally contaminated with *Fusarium* mycotoxins such as deoxynivalenol (DON) and fusaric acid exhibit reduced average daily feed intake (ADFI) and average daily body weight change (ADBWC) with no effect on gain to feed ratio. There is little information relating to the effects of feeding sows with grains naturally contaminated with *Fusarium* mycotoxins. Therefore, 36 first parturition sows were used in a completely randomised block design to study the effects of feeding grains naturally contaminated with *Fusarium* mycotoxins on performance and metabolism and the prevention of these effects by supplementation with a polymeric glucomannan mycotoxin adsorbent (GMA, Mycosorb^{TM}, Alltech, Inc., Nicholasville, KY, USA). Lactation diets consisted of corn, wheat and soybean meal and were fed over three weeks prepartum and three weeks after farrowing. The diets were: (i) control; (ii) contaminated grains (5.5 mg/kg DON, 0.5 mg/kg 15-acetyl DON and 0.3 mg/kg zearalenone); and (iii) contaminated grains (5.7mg/kg DON, 0.5 mg/kg 15-acetyl DON and 0.3 mg/kg zearalenone)+0.2% GMA. Twelve sows were allotted per treatment. Allowance of feed was *ad libitum*. Refused feed was collected and weighted daily. Means were compared with Tukey's test and significance was declared at $P<0.05$. Feeding of contaminated diets after farrowing reduced ADFI ($P<0.05$) compared to control. Feeding of contaminated diets after farrowing resulted in body weight losses ($P<0.05$) compared to controls. Feeding of contaminated grains+GMA, however, did not result in a significant loss in body weight compared to controls. There was no effect of diets on serum concentrations of beta-OH butyrate, haptoglobin, blood protein, albumin, globulin, albumin: globulin ratio, urea, creatinine, glucose, cholesterol, nor on activities of alkaline phosphatase, gamma glutamyltransferase, aspartate aminotransferase, creatine kinase, glutamate dehydrogenase, total bilirubin, conjugate bilirubin and free bilirubin the day of farrowing. On day 7 post farrowing blood urea concentrations were lower for sows fed contaminated grain compared to controls ($P<0.05$). Body weight gain of piglets to weaning was not affected by diet. Protein, fat and lactose concentrations of milk were not affected by diet on the day of farrowing nor during lactation. It was concluded that feeding first parturition sows with a diet containing grains naturally contaminated with *Fusarium* mycotoxins reduces ADFI, and results in body weight losses during the lactation period. The result of a reduction of feed intake during lactation is the mobilisation of body tissues to support milk production, but milk composition was not changed by the effect of feeding a diet contaminated with *Fusarium* mycotoxins. Supplementing diets containing grains naturally contaminated with *Fusarium* mycotoxins with a polymeric glucomannan mycotoxin adsorbent prevents body weight losses of sows.

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Prevention of the effects of feeding grains naturally contaminated with *Fusarium* mycotoxins to gestating gilts with a polymeric glucomannan mycotoxin adsorbent

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Average daily feed intake (ADFI) and average daily gain (ADG) of piglets are depressed by *Fusarium* mycotoxins such as deoxynivalenol (DON) and fusaric acid, although gain to feed ratio of piglets is not affected. There is a lack of information concerning the effects of feeding sows grains naturally-contaminated with *Fusarium* mycotoxins. Therefore, 36 first parturition sows were used in a completely randomised block design to study the effects of feeding grains naturally contaminated with *Fusarium* mycotoxins on performance and metabolism and efficacy of a polymeric glucomannan mycotoxin adsorbent (GMA, Mycosorb^{TM}, Alltech, Inc., Nicholasville, KY, USA) in prevention of these effects. Gestation diets consisted of corn, wheat and soybean meal and fed over three weeks prepartum. The diets were: (i) control; (ii) contaminated grains (5.5 mg/kg DON, 0.5 mg/kg 15-acetyl DON and 0.3 mg/kg zearalenone); and (iii) contaminated grains (5.7 mg/kg DON, 0.5 mg/kg 15-acetyl DON and 0.3 mg/kg zearalenone) grains+0.2% GMA. Twelve gilts were allocated per treatment. The daily feed allowance was 2.5 kg/gilt and refused feed was collected and weighted daily. Means were compared with Tukey's test and significance was declared at $P<0.05$. Diets had no ($P>0.05$) effects on ADFI. Feeding contaminated grain reduced ADG ($P<0.05$) compared to controls diets, however, ADG was similar ($P>0.05$) between control diet and the diet that containing contaminated grains + GMA. Gain to feed ratio was not affected ($P>0.05$) by diets. There was no effect of diet ($P>0.05$) on serum concentrations of beta-OH butyrate, haptoglobin, total protein, albumin, globulin, albumin: globulin ratio, urea, creatinine, glucose, cholesterol, or on activities of alkaline phosphatase, gamma glutamyltransferase, aspartate aminotransferase, creatine kinase or glutamate dehydrogenase or on concentrations of total bilirubin, conjugated bilirubin or free bilirubin. There was no effect of diet on body weight of piglets at birth. The percentage of stillbirths was higher ($P<0.05$) for litters from those gilts that were fed the contaminated diet compared to those litters from gilts that were fed with the diet containing the contaminated grains+GMA. The number of piglets born alive was higher ($P<0.05$) for litters of gilts fed the diet containing the contaminated grain+GMA compared to the other two treatments; there were no differences between treatments in total piglets born. It was concluded that the feeding of a diet containing grains naturally contaminated with *Fusarium* mycotoxins to first parturition sows during gestation affects ADG, and increases the percentage of stillbirths. Supplementing contaminated diets with GMA prevents these effects.

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Isolation and characterisation of microorganisms for the biological inactivation of fumonisins

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Fumonisins are a group of mycotoxins produced by *Fusarium verticillioides* and *F. proliferatum*. They are naturally occurring contaminants of cereal grains and especially maize worldwide. The biological effects of fumonisins include pulmonary oedema in pigs, leukoencephalomalacia in horses, and liver cancer and nephrotoxicity in rats. Fumonisins are also considered potentially carcinogenic to humans. Since chemical and physical decontamination strategies are unsatisfactory, biological detoxification by microbial degradation of the toxic substances is a viable alternative. We isolated and characterised microorganisms with the specific ability to metabolise fumonisin B_1 (FB_1), the most abundant and most important representative of this group of mycotoxins. Environmental samples including intestinal contents of pigs, maize with natural fumonisin contamination, and soil samples were enriched by incubation in fumonisin-containing growth media, and screened for fumonisin transforming activity. Degradation of the mycotoxin was detected in soil and maize samples, and several pure cultures were isolated from the enriched cultures. The fumonisin degrading activity of such new strains was studied and found to follow a previously reported two-step pathway. New bacterial and yeast strains were taxonomically characterised and found to belong mostly to alpha-Proteobacteria and to the previously described as fumonisin-metabolising yeast strain *Exophiala spinifera*. Fumonisin detoxification was studied with various FB_1 concentrations, under various physiological conditions and in heterogeneous food and feed related matrices. The fumonisin degradation products were non-toxic in a duckweed phytotoxicity assay. Presently, the suitability of newly isolated strains as feed additive and their potential to degrade fumonisins in the intestinal tract of animals are being investigated.

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In vitro investigations of various adsorbents for their ability to deactivate aflatoxin B_1

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The contamination of animal feed with mycotoxins represents a worldwide problem for the animal industry. Amongst the major mycotoxins affecting animal health is aflatoxin B_1 (AFB_1), a very potent mycotoxin commonly produced by *Aspergillus flavus* and *A. parasiticus.* The most applied method for protecting animals against aflatoxicosis is the utilisation of mineral clays mixed into the feed in order to bind the mycotoxins efficiently in the gastro-intestinal tract. This strategy has been given considerably attention in the last years and HSCAS (hydrated sodium calcium aluminosilicates) are amongst the adsorbents reported to adsorb AFB_1 with high affinity and high capacity and to prevent animals from aflatoxicosis. All mycotoxin binding agents should be tested for efficiency, safety and performance in sensitive animals before their inclusion in animal diets. Since feeding trials are time consuming and very expensive a strategy to pre-screen materials with the potential to bind AFB_1 *in vitro* was established. Based on the obtained results highly promising sorbent materials should be ranked for further *in vivo* studies. In the course of this research project various minerals, mainly Ca-, Na- or acid treated- bentonites, were examined to obtain information concerning adsorption efficacy, specificity and the mechanism of the adsorption process. Because it was claimed that yeast cell walls can also be used as mycotoxin-adsorbents a series of products containing mannanoligosaccharides (MOS) und ß-D-glucans were also tested for their ability to bind AFB_1. All experiments during this study of binding materials were done in parallel to a reference product. Our aim was to investigate and find other adsorbents with improved performance compared to the reference. Adsorption experiments were performed in buffer solutions in order to evaluate the ability to bind AFB_1 at diverse pH-values and different times of incubation. To determine the strength of binding, already bound AFB_1 was subjected to extraction by methanol and the chemisorption index was calculated. Moreover isothermal analysis to determine values for the maximum adsorption capacity and the type of adsorption process (chemisorption/physisorption) were performed. Determination of adsorption in simulated gastrointestinal (GI) fluid to examine binding under physiological conditions and interactions with GI-matrix was carried out as well. In addition, unspecific binding capability of the materials regarding selected vitamins was examined, because through the choice of a specific adsorbent the adsorption of essential nutrients, particularly if their concentrations in feed are much higher compared to those of the mycotoxin, should be avoided. Characterisation of the materials is currently conducted using X-ray diffraction (XRD) as well as simultaneous thermoanalysis (differential scanning calorimetry (DSC) and thermogravimetry (TG)) in order to localise a relationship between the capacity to bind mycotoxins and mineral composition. Further investigations also include adsorption tests with other common mycotoxins like zearalenone, ocharatoxin A and fumonisin B_1.

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The chemical mechanisms involved in the complex-forming properties of organic adsorbent extracted from *Saccharomyces cerevisiae* toward mycotoxins

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Mycotoxins are present throughout the food chain. They are found primarily in plants (cereals, fruits and vegetables) and their derived products eaten by humans or animals, and thus they can be harmful to them. Many studies carried out during the last decade indicate that organic adsorbents such as yeast cell walls can be added to contaminated food or feeds to selectively bind mycotoxins, allowing the toxins to pass through the digestive tract without any negative effect on animals or carry over to edible animal products. Recent researches have set the basis of the interaction between zearalenone (ZEN), a non-steroid estrogenic mycotoxin produced by *Fusarium* spp., and yeast cell walls from *Saccharomyces cerevisiae*. Our research programme has been conducted to discriminate among the components of the yeast cell wall, the molecule(s) involved in the interaction with mycotoxins and to study the stability of the complex under several pH conditions. Cell wall of *S. cerevisiae* is composed of beta-D-glucans, mannans and chitin. Our results indicated that beta-D-glucans, which consist in an assembly of linear chains of beta-(1,3)- D-glucans branched with beta-(1,6)-D-glucan side chains, are mainly involved in the adsorption of zearalenone (ZEN, affinity rates A up to 50%) but also of several other major mycotoxins such as aflatoxin B_1 (AFB_1, A>90%), deoxynivalenol (DON, A>40%) or patulin (PAT, A>46%) in aqueous solution. Chitin has a negative impact on the adsorptive process due to its rigidifying properties toward the beta-D-glucan network, restricting toxin accessibility to the interactive binding sites whereas mannans are not involved in the interaction with mycotoxins. Experiments conducted using NMR indicated that weak chemical linkages such as hydrogen and Van der Waals bonding occurred between the hydroxyl groups of beta-D-glucans and the hydroxyl, ketone and lactone groups of mycotoxins. *In vitro* binding capacity tests (39°C, 90 min, shaking) were carried out to study the incidence of different pH in the environment surrounding the complex, by analogy to the digestive conditions found in animals (pH 3., 6.0, 8.0). After centrifugation, the supernatant was collected for free ZEN content analysis using HPLC and thus, the amount of ZEN bound by beta-D- glucans was calculated. The experimental data were plotted according to the Hill mathematical model. The mean affinity of beta-D-glucans for AFB_1, DON, OA, PAT and ZEN was respectively 90.0%, 41.5, 10.7, 46.0 and 48.1%. However, these affinity rates differ from acid to basic pH conditions. In this respect, basic pH conditions (pH 8.0) were not appropriate to establish the interaction between beta-D-glucans and AFB_1, DON or ZEN (<10%), whereas PAT was highly bound at pH 8.0 (46.4%) and slightly bound in acidic conditions (<10%). AFB_1 was poorly bound at pH 3.0 (<10%) and was highly complexed at pH 6.0 (63.7%). No significant difference was observed between pH 3.0 and 6.0 in the adsorption of DON by MTB-100™, respectively equal to 38.1 and 41.5%. In the case of OA, only small differences were observed between the three pH values (between 10.5 and 24.2%). Finally, acidic pH conditions were more appropriate for the adsorption of ZEN (up to 62.3%) than near to neutral (45%) or basic conditions (<10%). Thus, we can conclude that the adsorption affinity is driven by the huge structural and physico-chemical disparities among mycotoxins and by their geometrical complementaries with the beta-D-glucan sites of interaction. Between mycotoxins, beta-D-glucans had high affinity for "aflatoxin-", "deoxynivalenol-" and "zearalenone-like" structures.