THE
INTERNATIONAL NETWORKING
CONFERENCE FOR THE FOOD
AND FEED INDUSTRY

FINAL PROGRAMME

ABSTRACTS OF LECTURES & POSTERS

the second World Mycotoxin Forum

GRAND HOTEL
HUIS TER DUIN
NOORDWIJK
THE NETHERLANDS

17-18 February 2003

Final Programme
Abstracts of Lectures & Posters

the second

World Mycotoxin Forum

The international
networking conference for
the food and feed industry

17 and 18 February 2003
Noordwijk aan Zee, the Netherlands

Welcome at the second World Mycotoxin Forum!

Dear participant,

We are pleased to present you the lecture and poster abstracts of the second World Mycotoxin Forum, the international networking conference for the food and feed industry.

The World Mycotoxin Forum's main objectives 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.

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 Organising Committee,

Daniel Barug

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; and
- the posters are grouped according to theme and then in an alphabetical order according to the first author.

PROGRAMME

Monday 17 February 2003

08.45 *Opening of the second World Mycotoxin Forum*

Plenary meeting

Mycotoxins - worldwide

Chair: Dr. Alain Pittet
Nestlé Research Center, Switzerland

09.00 *Chair's introduction*

09.10 *Current issues in Europe*
Dr. Angelo Visconti
Institute of Sciences of Food Production, Italy

09.35 *Current issues in USA*
Dr. Deepak Bhatnagar
U.S. Department of Agriculture, USA

10.00 *Current issues in South America*
Dr. Maya Pineiro
Food and Agriculture Organization (FAO), Italy

10.30 Coffee/tea break

11.00 *Current issues in Southeast Asia and Australia*
Dr. John Pitt
Food Science Australia, Australia

11.25 *Current issues in Africa*
Dr. Gordon Shephard
Medical Research Council, South Africa

11.50 *Mycotoxins and regulations: an update*
Hans van Egmond, M.Sc.
National Institute of Public Health and the Environment, the Netherlands

12.15 *Discussion*

12.30 Lunch break

Monday 17 February 2003

Parallel session 1

Prevention and control

Chair: Dr. Rebeca López-García
Logre International, Mexico

13.30 *Chair's introduction*

13.45 *Plant breeding as a tool to prevent mycotoxins*
Dr. Thomas Miedaner
University of Hohenheim, Germany

14.15 *Fighting mycotoxin producing fungi in the field*
Dr. Paul Nicholson
John Innes Centre, UK

14.45 *Post-harvest formation of mycotoxins: a lack of knowledge or action?*
Dr. Monica Olsen
National Food Administration, Sweden

15.15 Coffee/tea break

Case studies

15.45 *Reduction of fumonisin levels in Bt corn*
Dr. Robert Grogna
Monsanto Europe, France

16.05 *Making wine safer: the case of ochratoxin A*
Dr. Zofia Lawrence
CABI Bioscience, UK

16.25 *Biocontrol of aflatoxins in peanuts*
Dr. John Pitt
Food Science Australia, Australia

16.45 *Discussion*

17.00 End of parallel session 1

Monday 17 February 2003

Parallel session 2

Specific food issues

Chair: Jan Willem van der Kamp, M.Sc.
International Association for Cereal Science and Technology, Vienna

13.30 *Chair's introduction*

13.45 *Risk assessment for mycotoxins*
Dr. Samuel Page
World Health Organization (WHO), Switzerland

14.15 *The implementation of EU controls on imported food*
Hans Jeuring, M.Sc.
Inspectorate for Health Protection and Veterinary Public Health, the Netherlands

14.45 *The fate of mycotoxins during cereal processing*
Keith Scudamore
KAS Mycotoxins, UK

15.15 Coffee/tea break

Case studies

15.45 *Peanuts, aflatoxin and the Origin Certification Program*
Dr. Thomas Whitaker
U.S. Department of Agriculture, USA

16.05 *Mycotoxins in spices: the red pepper story*
Dr. Dilek Heperkan
Istanbul Technical University, Turkey

16.25 *Safe organic vegetables: the carrot-Alternaria model*
Dr. Ruud van den Bulk
Plant Research International, the Netherlands

16.45 *Discussion*

17.00 End of parallel session 2

Monday 17 February 2003

Spotlight presentations

A special presentation facility for sponsors and exhibitors to promote their products in more detail

Spotlight presentations 1

Chair: Dr. Angelo Visconti
Institute of Sciences of Food Production, Italy

17.00 *In vivo demonstrations of the efficacy of a polymeric glucomannan mycotoxin adsorbent in prevention of Fusarium mycotoxicoses*
Prof.dr. Trevor K. Smith
University of Guelph, Canada

17.15 *Neogen Corporation: developing and marketing products dedicated to food and animal safety*
Dr. Mohamed M. Abouzied
Neogen Corporation, USA

17.30 *Charm Sciences aflatoxin M1 and B1 assays: rapid, quantitative, low-cost results*
Annie Baumgartner
Charm Sciences

17.45 *A new quantitative detection kit to monitor Fusarium in cereals*
Dr. Gert H.J. Kema
Plant Research International, the Netherlands

18.00 End of spotlight presentations 1

Spotlight presentations 2

Chair: Ton van Osenbruggen
TNO Nutrition and Food Research, the Netherlands

17.00 *Analysis of deoxynivalenol by DONprep immunoaffinity column*
Simon Bevis
R-Biopharm Rhône, UK

17.15 *Romer Labs® New AgraQuant® ELISA line for the detection of mycotoxins*
Johann Binder
Romer Labs, Germany

17.30 *Recent applications of immunoaffinity columns in the analysis of mycotoxins*
Nancy Zabe
VICAM, USA

17.45 End of spotlight presentations 2

Tuesday 18 February 2003

Parallel session 3

Sampling and analysis

Chair: Tom Nowicki
Canadian Grain Commission, Canada

08.45 *Chair's introduction*

09.00 *Sampling and sample preparation*
Dr. Thomas Schatzki
U.S. Department of Agriculture, USA

09.30 *Development of certified reference materials for mycotoxins*
Dr. Ralf Josephs
Institute for Reference Materials and Measurements, Belgium

10.00 *Quality assurance with regard to mycotoxin analysis*
Dr. Sebastian Kastrup
Wiertz Eggert Jörissen, Germany

10.30 Coffee/tea break

Case studies

11.00 *Mycotoxin analysis: current challenges*
Dr. Aldo Rizzo
National Veterinary and Food Research Institute, Finland

11.20 *Multi-residue method for mycotoxin analysis*
Dr. Jean-Claude Motte
Veterinary and Agrochemical Research Centre, Belgium

11.40 *Application of in vitro bioassay for the screening of mixtures of trichothecenes*
Dr. Osamu Tajima
Kirin Brewery, Japan

12.00 *Discussion*

12.15 Lunch break

Tuesday 18 February 2003

Parallel session 4

Specific feed issues

Chair: Dr. Reinder Sijtsma
Nutreco International, the Netherlands

08.45 *Chair's introduction*

09.00 *Moulds and mycotoxins in silage*
Prof.dr. Johanna Fink-Gremmels
Utrecht University, the Netherlands

09.30 *Effects of dietary mycotoxins on the quality of animal products and on human health*
Dr. Bert Veldman
Institute for Animal Nutrition 'De Schothorst', the Netherlands

10.00 *Mycotoxins - concerns for their occurrence in pet foods*
Dr. John Richard
Romer Labs, USA

10.30 Coffee/tea break

Case studies:

11.00 *The use of a dynamic in vitro model of the gastrointestinal tract in studying mycotoxin adsorbents*
Dr. Evelijn Zeijdner
TNO Nutrition and Food Research, the Netherlands

11.20 *The efficacy of mycotoxin binders proven with a dynamic in vitro model of the gastrointestinal tract*
Dr. Giuseppina Avantaggiato
Institute of Sciences of Food Production, Italy

11.40 *Mycotoxin formation on inundated area*
Dr. Monique de Nijs
TNO Nutrition and Food Research, the Netherlands

12.00 *Discussion*

12.15 Lunch break

Tuesday 18 February 2003

Final plenary meeting

Further down the line

Chair: Hans van Egmond, M.Sc.
National Institute of Public Health and the Environment, the Netherlands

13.30 *Conclusions of parallel session 1*
Dr. Rebeca López-García
Logre International, Mexico

13.50 *Conclusions of parallel session 2*
Jan Willem van der Kamp, M.Sc.
International Association for Cereal Science and Technology, Vienna

14.10 *Conclusions of parallel session 3*
Tom Nowicki
Canadian Grain Commission, Canada

14.30 *Conclusions of parallel session 4*
Dr. Reinder Sijtsma
Nutreco International, the Netherlands

14.50 Coffee/tea break

15.15 *Communicating the risk of mycotoxins to consumers*
Dr. Teresa Gruber
Council for Agricultural Science and Technology, USA

15.45 *Mycotoxin research: progress and future prospects*
Prof. Naresh Magan
Cranfield University, UK

16.15 *Closing the second World Mycotoxin Forum*
Jan Willem van der Kamp, M.Sc.
International Association for Cereal Science and Technology, Vienna

16.30 End of conference

LECTURES

Mycotoxins - current issues in Europe

Angelo Visconti

Institute of Sciences of Food Production (ISPA), Italy

A considerable amount of research funds have been invested by the European Union in order to provide a better understanding of consumer requirements and to ensure a healthy, safe and high quality food supply leading to reinforced consumer confidence in the safety of the food supply. Different tools have been effectively used at this regard throughout several EU programmes, including shared cost projects, thematic networks, concerted actions, conferences and seminars, training and mobility fellowships, etc.

Mycotoxins and toxigenic fungi are chemical and biological food contaminants occurring naturally in the field and/or afterwards during storage and transformation and have received particular attention by the European Commission. To these contaminants a “cluster” of projects has been devoted by the V Framework Programme within the Key Action 1 “Food, Nutrition and Health”, with the co-ordination of some nine projects aimed to prevent the risk of mycotoxins exposure throughout the food chain. These projects involve different studies on several mycotoxins including *Fusarium* toxins (hazard analysis, pre- and post-harvest control and decontamination strategies, molecular tools for early detection of mycotoxigenic species, etc.), ochratoxin A (HACCP, mapping of species, ecology, prevention strategies, diagnostic systems and early detection of ochratoxigenic fungi, mechanism of carcinogenicity, risk assessment and integrated management in grape and wine), *Alternaria* toxins (in relation to safety of organic vegetables-carrot as a model).

Food Safety policy in the European Union is based on a comprehensive, integrated approach of risk analysis (risk assessment, management and communication) throughout the food chain, from farm to table. Risk assessment provides the scientific foundation upon which the risk analysis process is built, and comprises of hazard identification, hazard characterisation, exposure assessment and risk characterisation steps. The European Union aims to harmonise legislation between the countries of the Union, and has established regulatory limits for controlling aflatoxins B1, B2, G1 and G2 in cereals, nuts, nuts products and dried fruit, aflatoxin M1 in milk, and ochratoxin A in cereals and dried vine fruits. Maximum levels for ochratoxin A in coffee beans, cocoa beans, dried fruit, wine, beer, spices and grape juice are likely to be set by the end of 2003. Maximum levels for patulin in apple juice, other apple products and other fruit juices are currently under discussion.

Over the past two decades the Commission has supported collaborative studies for improving and validating methods of analysis of mycotoxins or other food contaminants and the production of relevant reference materials. Progressive harmonisation of the analytical methods for most food contaminants has been pursued through the implementation of the results of specific Commission programmes (M&T, SMT, etc.) in close collaboration with the European Committee for Standardisation (CEN) and other international standardisation bodies. The actual trend in the control of food contaminants is the use of biotechnology and nanotechnology for developing rapid, simple and automated test kits aimed to generate on-site and real-time data on the incidence of target contaminants in food and feedstuffs.

Mycotoxins - current issues in USA

Deepak Bhatnagar[1], Gary A. Payne[2], Thomas E. Cleveland[1] and Jane F. Robens[3]

[1]USDA/ARS/SRRC, USA, [2]North Carolina State University Raleigh, USA
and [3]USDA/ARS/NPS, USA

Mycotoxins are secondary metabolites produced by filamentous fungi that cause a toxic response (mycotoxicosis) when ingested by higher animals. *Aspergillus, Fusarium* and *Penicillium* are the primary genera that produce these toxins in the human food chain. Mycotoxins can contaminate human foods and animal feeds through fungal growth prior to and during harvest, or from improper storage following harvest. Post-harvest contamination can be minimised by careful handling during harvest and proper storage conditions. Pre-harvest contamination, on the other hand is beyond man's direct control because toxin production is governed by environmental conditions which may be conducive to fungal growth and mycotoxin formation.

In the U.S., the Food and Drug Administration (FDA) has set regulatory limits on the permissible levels of certain mycotoxins in food and feed based on health concerns. Regulatory limits have been established for aflatoxins, and guidelines have been issued in the U.S. for other mycotoxins of public health concern including deoxynivalenol, fumonisins and patulin. FDA has issued no regulations or guidelines for ochratoxin A content at this time.

These regulatory guidelines (within U.S. as well as those enforced internationally) and crop losses due to disease caused by mycotoxingenic fungi have a put a tremendous economic burden on U.S. agriculture. It is estimated that the mean direct economic annual costs of crop losses from just three mycotoxins, namely aflatoxins, fumonisins, and deoxynivalenol, are estimated to be $932 million. Additional losses can be from a number of unseen problems such as reduction in yield (both crops and animal products), enhanced health care costs, and losses incurred in associated industries following crop losses due to mycotoxin contamination. Therefore, there is significant emphasis being placed on devising (i) sensitive, specific, non-destructive and rapid procedures for detecting fungal and toxin contamination of crops and (ii) effective strategies for controlling pre-harvest contamination of commodities such as corn, peanuts, tree nuts and cotton.

Novel methods for detection of mycotoxins

In addition to more sensitive TLC, HPLC, ELISA and GC techniques now available, sensitive and versatile high-resolution MS and GC tandem methods are coming to the market. New biosensors are being developed using fibre optics, hyperspectral and near infrared imaging.

Understanding the basic biology of toxigenic fungi

The genetics and biology of aflatoxin, trichothecene and fumonisin biosynthesis have been investigated in significant detail, and many of the genes and/or enzymes involved in toxin formation have been identified. Genomic efforts, such as Expressed Sequence Tag (EST) microarrays, cosmid clone sequencing, chromosome sequencing, and large-scale whole genome sequencing, on toxigenic and non-toxigenic *Aspergillus* and *Fusarium* species have been made in recent years. The technological breakthroughs in genomics research will almost certainly promote a revolution in our understanding of the biology and genetics of these filamentous fungi for the control of mycotoxin contamination in food and feed. This information will assist us in establishing a better understanding of the factors affecting mycotoxin formation, ecology and epidemiology of toxigenic fungi, and a genetic analysis of the host plant-fungal interactions.

Preharvest control of mycotoxigenic fungi

Significant in-roads have been made in establishing various control strategies such as development of atoxigenic biocontrol fungi that can out compete their closely related, toxigenic cousins in field environments, thus reducing levels of mycotoxins in the crops. Potential biochemical and genetic resistance markers have been identified in crops, particularly in corn, which are being utilised as selectable markers in breeding for resistance to aflatoxin contamination. Prototypes of genetically engineered crops have been developed which: (i) contain genes for resistance to the phytotoxic effects of certain trichothecenes, thereby helping reduce fungal virulence, or (ii) contain genes encoding fungal growth inhibitors for reducing fungal colonisation. Gene clusters housing the genes governing formation of trichothecenes, fumonisins and aflatoxins that have been elucidated are being targeted in strategies to interrupt the biosynthesis of these mycotoxins. Ultimately, a combination of strategies using biocompetitive fungi and enhancement of host plant resistance may be needed to adequately prevent mycotoxin contamination in the filed. To achieve this, plants may be developed that resist fungal infection, interrupt mycotoxin biosynthesis and/or reduce the toxic effects of the mycotoxins themselves.

Other issues

In addition to preharvest control of mycotoxin contamination in crops, the specific role and mode of action of mycotoxins in human health needs to be examined in detail. For example, the immunosuppressive nature of mycotoxins, the exposure to multiple mycotoxins, the interaction of mycotoxins with dietary components, role of fumonisins in cancer, and the risks of ochratoxin and cyclopiazonic acid exposure must be elucidated. Certainly, the development of biomarkers is of prime interest to allow early detection of mycotoxin exposure. The role of mycotoxins, if any, on indoor air quality needs to be examined including determining the primary fungi and mycotoxins that may be involved. And, with the events of bioterrorism in the last decade, particularly with respect to the potential for use of mycotoxins as bioterrorism agents, rapid detection and detoxification procedures for these compounds need to be developed. These latter processes, wherever technically feasible, could help in establishing effective post-harvest methods for decreasing or eliminating mycotoxins in human food and animal feed.

Mycotoxins - current issues in South America

Maya Pineiro

Food and Agriculture Organization of the United Nations (FAO), Italy

Mycotoxins affect a wide range of agricultural products, including most grain crops, which are the backbone of developing economies, and in particular in South America. Contamination of these susceptible commodities occurs as a result of environmental conditions in the fields, as well as improper harvesting, storage and processing operations. Many other factors, including poor infrastructure, managerial and economical constraints, provide additional negative aspects and increase mycotoxin levels in the region. The recently held meetings provide an insight into the main challenges facing the region and some trends for addressing the mycotoxin problem. These key problems may be grouped into three major areas: inadequate food control systems (FCS); lack of prevention and control policies and strategies; and insufficient awareness. Main obstacles encountered are insufficient government and private resources; outdated FCS; scarce or no action plans for prevention and control; and lack of communication. The approach to addressing the mycotoxin problem in South America can be considered two-fold: technical and organisational/managerial, and would depend on the specific situation of the countries involved. The future of this region awaits careful assessment of each country's mycotoxin situation with subsequent formulation of action plans for priorisation and implementation of defined technical and organisational strategies. FAO activities in support of mycotoxin prevention and control (policy advice, technical assistance projects, training workshops, publications, etc), including the work of Codex and JECFA, are instrumental in providing the up to date information and technologies, and disseminating effective strategies to developing countries.

Mycotoxins - current issues in Southeast Asia and Australia

John I. Pitt and Ailsa D. Hocking

Food Science Australia, Australia

Australasia and Asia cover a very large part of the globe, so this paper will be confined to the situation in Australia and Southeast Asia, areas where the authors have firsthand knowledge. It is likely that problems in most other areas in the region will be similar, except for New Zealand, which has a cooler climate and different issues.

In Australia, the dominant mycotoxin problem has been, and remains, that of aflatoxins in peanuts. Peanuts are mostly grown under dry culture in Australia and, as occurs elsewhere in peanut growing areas of the world, drought stress before harvest is a major factor causing aflatoxin production. In bad seasons, i.e. seasons when inadequate rainfall causes temperature and moisture stress before harvest, more than half the crop may be affected to some degree. Aflatoxins in Australian peanuts are reduced by good farm management practice, then at intake to shelling plants, lot (truck loads) are segregated into grades depending on aflatoxin content. After the nuts are shelled, colour sorters are used to remove discoloured kernels, and this process also removes most of those that contain aflatoxins. Chemical testing for aflatoxin content then provides quality assurance. Rejected lots, i.e. nuts showing more than 5 µg/kg aflatoxin, are further processed. Blanching removes skins and roasting increases the discoloration of infected nuts, so that subsequent colour sorting removes essentially all aflatoxin, and that is confirmed by further assays. By this means, aflatoxin can be reduced consistently to acceptable levels, but at a high monetary cost to farmers and shellers. A program is underway to reduce aflatoxins by competitive exclusion, using nontoxigenic strains of *A. flavus* added to fields in which peanuts are grown. The approach has given promising results.

A second mycotoxin problem in Australia is the potential presence of ochratoxin A (OA) in grapes and grape products. This results from infection by *Aspergillus carbonarius*, a crop - mycotoxin association discovered only in the past few years. Grape juice and wines can be affected by growth of *A. carbonarius* before grapes are harvested, as the result of damage by plant pathogenic fungi or skin splitting due to unseasonal rain. OA occurs in wines from throughout the world, but levels are usually low. In Australia, good quality control before and during wine making ensures OA is kept to very low levels. In dried vine fruits, the possibility of mechanical damage during harvesting and the prolonged time available for fungal growth during drying increase the probability of OA being formed, and being present at higher levels. OA occurrence in cereals, significant in Europe and Northern North America, is not a problem in Australia, because the cause, *Penicillium verrucosum*, is a low temperature fungus of rare occurrence here.

Fusarium toxins sometimes occur in cereals in Australia, but probably to a lesser extent than in grains from any other region. Fumonisins may be found as the result of *F. verticillioides* (= *F. moniliforme*) and related species, but fumonisins are not important in the context of human health because maize forms only a small part of the Australian diet. Trichothecene toxins are sometimes present, especially deoxynivalenol (DON) in wheat, due to the growth of *F. graminearum*. However, high grade wheats from the main Australian wheat belts are usually free of this toxin, as maturation commonly occurs under hot and dry conditions unsuitable for the growth of *F. graminearum*.

In Southeast Asia, the problems of mycotoxins, although caused by the same fungi, are quite different in magnitude. Lower standards in farm management, drying practice and storage combined with all too frequent adverse weather conditions of rain and high humidity are conducive to mycotoxin formation in a wide range of crops.

A major study we undertook several years ago examined all major food commodities on sale in Indonesia, Thailand and the Philippines. More than 1700 samples were examined and 35,000 fungal isolates identified. From these identifications, the potential for mycotoxin production could be deduced and commodities analysed for major mycotoxins. Some commodities, especially beans of all types and rice, both paddy and polished, had only low levels of infection with potentially toxigenic fungi, and negligible levels of major mycotoxins. Some other crops, however, were heavily infected by toxigenic fungi.

Nearly 1000 mycotoxin assays were carried out during this study. As in Australia, the major mycotoxin of concern in Southeast Asia and other tropical Asian areas is that of aflatoxins. The major crops affected are peanuts and maize, with the effect on human health varying, depending more on food consumption patterns than climatic factors. In Thailand, aflatoxin in the diet mainly comes from peanuts, but the problem is to a large extent controlled because peanuts are often screened for aflatoxin or colour sorted by hand before retail sale. In the Philippines, maize is the staple diet and here aflatoxins in maize are a considered the major problem. In Indonesia, both maize and peanuts are consumed: peanuts are a major dietary staple for most Indonesians and levels of aflatoxin are of great concern. Especially in Indonesia, levels of aflatoxins in peanuts and maize are unacceptably high. Indeed calculations based on our data on levels of aflatoxins in peanuts and maize in Indonesia indicate that up to 20,000 people per annum die of liver cancer induced by aflatoxins. The problem of aflatoxin in foods is compounded by high rates of infection with hepatitis B and with parasitic liver diseases. Little has been done thus far to reduce this problem.

The other major cause for concern was the high level of *Fusarium* species in some commodities, especially of *F. verticillioides* (= *F. moniliforme*) in maize. Some isolates of this species were found to be capable of producing fumonisins, but fumonisins were not assayed in this study because of the technical difficulties involved in this assay until recently. The most common *Fusarium* species in Southeast Asian commodities was *F. semitectum*, but this species has only a low potential to produce mycotoxins. The risk from trichothecene toxins was apparently low because *F. graminearum* was uncommon.

Current issues in Africa

Gordon S. Shephard

Medical Research Council, South Africa

The current mycotoxin issues in Africa reflect the concerns of the developing world. They impinge on aspects of food safety, health and agriculture and need to be considered in an African context. The issue of food safety in Africa is one which interacts with and is frequently subjugate to issues of food security, especially in geographic areas where food shortages are caused by recurrent natural weather phenomena such as drought and political issues such as past or current conflict. Agricultural land is commonly worked by small scale or subsistence farmers. These communities are reliant on the consumption of homegrown crops, irrespective of the quality considerations normally applied in the developed world. FAO data for 1998-2000 indicated that of the 22 countries listed with equal or greater than 35% undernourishment, 17 are in Africa.

The major health challenges facing Africa include injuries and violence, the evolving microbial threats of TB, malaria and HIV/AIDS, and problems in malnutrition and children's' health. Nevertheless, mycotoxins are an important problem as evidenced by occasional outbreaks of human mycotoxicoses and the role of aflatoxins in liver cancer in west Africa and fumonisins in oesophageal cancer in South Africa. In addition, the extent to which the immunomodulatory effects of aflatoxins with depressed cell-mediated immunity contribute to the infectious disease burden is difficult to quantify, but undoubtedly significant. Aflatoxin exposure in populations in certain African countries occurs throughout life, with aflatoxin having being detected in cord blood and human breast milk.

Of the agriculturally important mycotoxins, aflatoxin and fumonisin contamination have been reported from a number of African countries. Little information is available on deoxynivalenol and zearalenone levels apart from some South African data, while human exposure to ochratoxin A has been reported in the north African countries of Tunisia and Egypt.

Although regulatory authorities attempt to reduce mycotoxin exposure to levels below the tolerable daily intake (TDI) values set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the reality of subsistence diets, in which levels of cereal (maize) consumption of 400 g/person/day or more are possible, implies that only the best quality cereal, generally not available to these communities, will suffice to prevent these TDI values being exceeded. For these subsistence farmers, the factors that influence the quality of harvest include both lack of information on optimal cultivars, lack of finance or cash crops to afford these commercial varieties, fertilisers and insecticides, and lack of information on optimal crop storage conditions. In addition, more information is needed on the effects of traditional rural food processing in reducing mycotoxin levels.

In order to address the mycotoxin problems in Africa, food sufficiency must be achieved. Although national governments may institute regulatory policies, the needs of small farmers for resources and information must be addressed. At national level, there is a need for adequate budgets and increased food testing capacity. Although trade globalisation may be seen as a means of achieving the foreign income required, the institution of more stringent regulations by the importing developed nations, may lead to reduced trade flows and higher mycotoxin intakes in the producing countries in Africa.

Mycotoxins and regulations: an update*

Hans P. van Egmond and Marco A. Jonker

National Institute of Public Health and the Environment (RIVM), the Netherlands

Regulations for mycotoxins have been established in food and animal feed in many countries since the late 1960's to protect the consumer from the harmful effects that mycotoxins may cause. Various factors influence the decision-making process of setting limits for mycotoxins. These include scientific factors such as the level of human exposure, data about effects on man and animals, as well as the availability of methods of sampling and analysis. Economical factors such as commercial interests and sufficiency of food supply have their impact as well. Over the last 2 decades there have been various international inquiries on worldwide limits and regulations for mycotoxins. Several of these were carried out by RIVM for the Food and Agriculture Organization (FAO) of the United Nations. The latest completed inquiry resulted in the publication "Worldwide regulations for mycotoxins 1995. A compendium" (FAO Food and Nutrition Paper 64, 1997). Since this publication appeared many new limits and regulations for mycotoxins have come into force or are in development; therefore the information contained herein is rapidly changing, and FAO decided to produce an update of this document, in co-operation with RIVM.

International inquiry

In 2002, we held an international inquiry among the Agricultural Services of the Dutch Embassies around the world, with the request to gather up-to-date information from the local authorities on the situation regarding mycotoxin regulations, in as many countries of the world as possible. Where this procedure did not lead to the desired information, personal contacts were used. The questions in the inquiry concerned:

- the existence of mycotoxin regulations;
- the types of mycotoxins and products for which regulations are in force or proposed, together with maximum permissible levels;
- the authorities responsible for control of mycotoxins;
- the use of official and published methods of sampling and analysis;
- the disposal of consignments containing inadmissible amounts of mycotoxins.

By the end of 2002 data were received from 86 countries. Together with information gathered in previous inquiries, detailed information is now available about the existence or absence of specific mycotoxin limits and regulations in human food and animal feed, in 117 countries. All data were inspected, interpreted to the best of our knowledge and tabulated. The processing and correction stages are not completed yet, and it will probably take until the end of 2003 before the whole exercise can be finalised. Yet, the collection of updated information made it possible to give some first impressions and to come to some provisional conclusions.

Tour-du-monde

On a worldwide basis, at least 98 countries had mycotoxin regulations for food and/or feed in 2002, an increase of approx. 30% compared to 1995. The total population in these countries represents approx. 90% of the world's inhabitants. Mycotoxins for which currently (proposed) limits and regulations exist include the naturally occurring aflatoxins, aflatoxin M1, agaric acid, deoxynivalenol, diacetoxyscirpenol, the fumonisins B1, B2 and B3, HT-2 toxin,

* Please note that at the time of publication the figures mentioned were not yet final and may therefore be subject to further adjustments.

ochratoxin A, patulin, phomopsins, sterigmatocystin, T-2 toxin and zearalenone. On a regional basis the following provisional observations can be made:

- Africa
  - 14 countries with known regulations (54% of inhabitants of the region)
  - majority of countries: regulations unknown or non-existent
  - several countries indicate: regulations should be developed
  - most regulations exist for aflatoxins
  - most detailed regulations: Morocco
- Asia/Oceania
  - 25 countries with known regulations (89% of inhabitants of the region)
  - regulations for total aflatoxins dominate in food; regulations for aflatoxin B1 dominate in feed
  - harmonised regulations exist for Australia and New Zealand
  - "exotic" regulations exist in Australia/New Zealand: agaric acid and phomopsins
  - most detailed regulations: China and Iran
- Europe
  - 38 countries with known regulations (99% of inhabitants of the region)
  - limits for many different matrix-toxin combinations
  - EU harmonised regulations exist for aflatoxins and ochratoxin A
  - EU harmonised recommendations exist for deoxynivalenol
  - EU harmonised regulations are in development for patulin, several *Fusarium* toxins in foods, baby foods and feedstuffs
  - most detailed regulations: several candidate-EU countries
- Latin America
  - 19 countries with known regulations (92% of inhabitants of the region)
  - harmonised aflatoxin regulations exist in MERCOSUR member states
  - aflatoxin regulations are mostly set for total aflatoxins
  - most detailed regulations: Uruguay
- North America
  - 2 countries with known regulations (100 % of inhabitants of the region)
  - aflatoxin regulations are set for total aflatoxins
  - Canada: detailed tolerances for *Fusarium* damaged kernels (% FDK) and for ergot (% by weight); HT-2 toxin regulated in feed.
  - USA: detailed tolerances for total fumonisins (B1, B2, B3) in a wide variety of foods and feedstuffs.

Provisional conclusions

Comparing the situations in 1995 and 2002, it appears that in 2002 more mycotoxins are regulated in more commodities and products, whereas tolerance limits generally tend to decrease. Regulations have become more diverse and detailed with newer requirements regarding official procedures for sampling and analytical methodology. At the same time, several regulations have been harmonised between countries belonging to economic communities, or they are in some stage of harmonisation. Nevertheless the regulatory requirements remain substantially different across many countries.

Plant breeding as a tool to prevent mycotoxins

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Mycotoxin contamination is a continuous threat for the production of small-grain cereals and maize that are among the world's most valuable crops for food and feed. The most important mycotoxins are deoxynivalenol, nivalenol, and zearalenone caused by *Fusarium* head blight of small-grain cereals and *Gibberella* ear rot of maize in the humid-temperate regions, fumonisins and moniliformin caused by *Fusarium* ear rot of maize in the warmer climates, and aflatoxins caused by *Aspergillus* ear rot of maize in the sub-tropical and tropical areas. Resistance to mycotoxins and disease symptoms in cereals is quantitatively inherited with no genotype being free of disease. Genotypes, however, highly differ in the amount of mycotoxins and disease severity and these differences can be successfully exploited by plant breeding. Generally, resistant germplasm has a much lower mycotoxin concentration than susceptible material, even under favourable epidemic conditions for the fungus. For establishing this correlation in all diseases and large sets of genotypes across locations and years, rapid and efficient mycotoxin analyses have to be developed. In plant breeding, ELISA techniques that are established for most of the mycotoxins mentioned, play an important role because other cheap and reliable high-throughput analytical methods are missing.

Resistance selection is hampered by the high importance of the environment (locations/years) on the genotypic differentiation, the different resistance components, and the growth stage-dependent resistances in all diseases. These factors require an extremely high experimental input (field experiments, large number of environments, different inoculation treatments). It is an outstanding challenge for the breeder to combine grain yield and other agronomic traits with reduced mycotoxin concentrations in superior varieties. Molecular markers will help in transferring important quantitative trait loci from inferior germplasm to adapted breeding materials (marker-assisted backcrossing), or in selecting within progenies of crosses between susceptible and resistant genotypes (marker-assisted selection). Additionally, improved mycotoxin assays, optimised selection procedures, and gene technology approaches should support and further accelerate the breeding progress in future to result in a more healthy crop for animals and humans.

Fighting mycotoxin producing fungi in the field

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A number of *Fusarium* and *Aspergillus* species are important pathogens of wheat and maize in Europe and worldwide infecting floral organs and contaminating grain with a range of mycotoxins that are harmful to human and animal consumers. Fusarium head blight of wheat and gibberella ear rot of maize caused by *F. graminearum* or *F. culmorum* leads to contamination by trichothecenes. Infection of maize by *F. vertillioides* and *F. proliferatum* may lead to accumulation of fumonisins. In addition, maize may become contaminated with aflatoxins through infection by *A. flavus* or *A. parasiticus*. It is highly likely that integrated approaches will be required to overcome these diseases and reduce the risk of mycotoxins entering the human and animal food chains. Herein we review some of the current and potential methods to control the causal agents and reduce the associated risk of mycotoxin contamination. Work in our laboratory centres on Fusarium head blight of wheat (FHB) and this will be reflected in this presentation. FHB is a disease complex that contains both toxin-producing and non toxin-producing species a fact that is of considerable significance. *Fusarium graminearum* is the major pathogen worldwide, while *F. culmorum* tends to predominate in maritime regions such as the UK where non toxin-producing species such as *Microdochium nivale* also occur.

The use of fungicides to control FHB is currently widespread but control can be erratic and mycotoxin levels have been found to increase in some instances following fungicide application. Molecular diagnostic assays are providing insight into the interactions between FHB species that contribute to this effect. While fungicides such as tebuconazole and metconazole have good activity against *Fusarium* species others, such as azoxystrobin, have greater efficacy against *M. nivale*. It has been shown that *M. nivale* and *F. culmorum* are antagonistic and it is hypothesised that fungicides such as azoxystrobin preferentially inhibit *M. nivale* allowing the *Fusarium* species to colonise the host tissues to a greater extent, leading to higher levels of mycotoxin. Even where the most effective fungicides are applied, adequate control of disease and mycotoxin accumulation may not be achieved. Thus fungicides, alone, are unlikely to provide sufficient protection against this disease and its associated mycotoxins.

The breeding of varieties that are highly resistant to infection and accumulation of mycotoxins is problematic due to the polygenic nature of the resistance and the influence of environmental factors on disease and mycotoxin accumulation. However, significant progress is being made for both wheat and maize, aided by analysis of quantitative trait loci (QTL), molecular mapping and marker-assisted selection. A single locus is unlikely to provide sufficient resistance to FHB to protect the crop under high disease pressure. For this reason it is highly desirable to identify loci on different chromosomes to facilitate combining them to enhance FHB resistance. Combining different resistance mechanisms within a single variety should minimise the risk that the resistance will be overcome by changes in the pathogen(s), in addition to increasing the level of FHB resistance.

Whatever the source or genetic basis for FHB resistance it is essential that reduced symptoms are accompanied by reduced levels of trichothecene mycotoxins. Highly resistant varieties tend to restrict accumulation of DON but few studies have demonstrated that disease and DON reduction is due to the action of the same gene(s). Recent work has shown that QTL for reduced disease are coincident with those for reduced DON accumulation indicating that for some sources, at least, a reduced level of mycotoxin should accompany reduction in disease.

Biocontrol agents suitable for use in agriculture have also been identified and some offer an alternative to fungicides as a means of controlling disease and mycotoxin contamination. Several genetic modification approaches are also being investigated that may complement the resistance available to conventional breeding programmes. The role of agronomic practices in reducing disease may be somewhat limited but should not be overlooked as part of an integrated approach to limit the diseases and reduce the risk of mycotoxins contaminating grain. In the long term the development of highly resistant varieties offers the greatest promise of preventing contamination of grain with mycotoxins without the need for intervention by growers when conditions are conducive to infection.

Postharvest formation of mycotoxins: a lack of knowledge or action?

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This presentation will mainly deal with the formation of ochratoxin A and aflatoxins as examples of postharvest formation of mycotoxins. These mycotoxins are being regulated in many countries and continue to be of special concern, especially the aflatoxins. The postharvest formation of these mycotoxins is not strictly and in some commodities the formation occurs mainly preharvest. It all depends on the toxin producing species and where the ecological circumstances are favourable for toxin production. New and old knowledge concerning the postharvest toxin formation will be presented and put in perspective.

Toxicological evaluation of aflatoxins and ochratoxin

JECFA (Joint FAO/WHO expert committee on Food Additives) evaluated aflatoxins (B and G forms) the last time at its 49th meeting in 1998. Aflatoxins are considered to be human liver carcinogens and aflatoxin B1 is the most portent of the aflatoxins. The potency of aflatoxins in hepatitis B carriers is substantially higher (~ 30 times) than the potency in non-carriers. Since aflatoxins are genotoxic carcinogens, no tolerable daily intake (TDI) can be allocated. However, JECFA estimated the carcinogenic potency of aflatoxin B1 to be about 0.01 cancers per year per 100 000 persons per ng/kb body weight per day in non-carriers and about 30 times higher in hepatitis B carriers.

JECFA evaluated ochratoxin A at its 56th meeting in 2001. Ochratoxin A is nephrotoxic and causes renal carcinogenicity. The mechanism of action is still debated, both genotoxic and non-genotoxic mechanisms have been proposed. The committee retained the previous established provisional tolerable weekly intake (PTWI) at 100 ng/kg b.w. (corresponding to approximately 14 ng/kg b.w. per day).

Estimates of tolerable daily intake for ochratoxin A, based on non-threshold mathematical modelling approaches or a safety factor/threshold approach, have ranged from 1.2 to 14 ng/kg b.w. per day. The Scientific Committee for Food of the European Commission (SCF) considered it would be prudent to reduce exposure to ochratoxin A as much as possible, ensuring that exposures are towards the lower end of the range of tolerable daily intakes of 1.2-14 ng/kg b.w. per day which have been estimated by other bodies, e.g. below 5 ng/kg b.w. per day.

Ochratoxin A in cereals

Cereals normally account for 50-80% of average consumer intake of OTA. Consequently, prevention of OTA formation by specific moulds in cereals would have a significant impact on levels of human exposure. The aim of OTA PREV project, a project within the Fifth Framework Program, is to identify the key elements in an effective HACCP programme for OTA in cereals, and provide tools for preventative and control procedures. New knowledge resulting form this project will be presented and compared with common knowledge and practice. *P. verrucosum* is the only ochratoxin producer found in cereals in Europe even though black and ochre aspergilli have been isolated, but they have not produced ochratoxin A. Investigations of farms in Denmark, Sweden and the UK indicates that *P. verrucosum* contaminate the grain during or after harvest and that the source is the contaminated environments of combines, dryers, silos etc.

Aflatoxins in tree nuts

The formation and control of aflatoxins in pistachios has been well investigated and there is a lot of knowledge found in the literature. The pistachio (*Pistacia vera*, Anacardiaceae) is a semidry stone fruit consisting of a single kernel enclosed in a thin, bony shell, which is surrounded by the hull. The shell partially splits to varying extents at least a month before maturity and harvest. Normally, the hull does not rupture when the shell splits in the immature pistachio fruit. However, in a small percentage of the pistachios, the shell and the still adhering hull splits together. This hull rupture, often referred to as "early splitting", is a very important event for infection with the aflatoxin producing fungi *Aspergillus flavus/parasiticus*. The rupture exposes the kernel to airborne fungal spores or insects, like the navel orange worm (*Amyelios transitella* (Walker)), which might carry fungal spores on their bodies. An "early split" nut is characterised by a distinct, dark and smooth-edged split on the hull. The oldest "early splits" have rough and shrivelled hulls and contain the highest levels of aflatoxin and may contain 99% of all aflatoxin detected.

Infection with *A. flavus/parasiticus* before harvest may lead to further build up of aflatoxin after harvest if drying is slow or if storage and transportation is under high humidity. Prevention of aflatoxin in pistachios can be achieved in one or two ways: by preventing the infection in the first place or by modifying the environment to inhibit mould growth and mycotoxin production. Since the primary infection of the pistachio with *A. flavus/parasiticus* takes place already in the orchard, the most important preventive measure after harvest is to prevent further growth of the fungi and accumulation of toxin. To improve stability and avoid further contamination, the pistachios should be dehulled and dried to a moisture content of 5-7%, corresponding to a water activity (a_w) of less than 0.70 at 25ºC, as soon as possible after harvest, usually within 48 hours. Pistachios, with aflatoxin formed already during the preharvest period, can be sorted out and discarded during the processing.

Knowledge about the formation of aflatoxin in Brazil nuts is much scarcer. The Brazil nut tree (*Bertholletia excelsa*) is native in the Amazon basin. It grows to great height, up to 50 m (averaging 40 m). The seeds of *B. excelsa* are known in English as Brazil nuts. The edible products are the seeds produced within a capsular woody fruit (pod), which is extremely hard. It takes 15 month for the fruit to develop after they have been set and may contain some 20 seeds per fruit. The ripe fruits fall mostly in January and February during the rainy period. Very little is known about how the Brazil nut is infected by *Aspergillus flavus/parasiticus*. It is possible that conidial germination and penetration of *A. flavus/parasiticus* through the floral parts of the young kernels is a source of infection. Indeed, rotted kernels have been observed inside the hard shell of the Brazil nut fruit, suggesting premature invasion and colonisation of young nuts. It has also been observed that kernels looking fresh on the outside could sometimes be mould-affected on the inside. The fruits are mostly collected in the morning because the weather is generally better, and because there is less danger of accidents from fruit fall, a particularly dangerous part of Brazil nut collection. The fruits, which weigh from 0.5 to 2.5 kg, fall from heights of 40 to 50 m. They acquire such velocities when they fall that upon impact they often become embedded in the soil.

Fruits should be gathered as soon after they fall as possible in order to minimise insect and fungal attack to the seed, and to limit the number of seeds carried away by animals (agoutis). After the fruits are collected, they are split open with a machete or an axe, and the seed are removed. The seeds are placed in water to clean them of mud and to determine which are bad. Seeds that sink are good while those that float to the surface are culled out. During storage and transport the seeds are aerated by moving their position.

Despite the fact that Brazil nuts have an outer shell covering which may protect them during collection, handling and storage, cracks can develop permitting an entrance for fungal

contamination. In addition, the poor management of temperature and humidity parameters during transportation further facilitates fungal growth and associated aflatoxin production. Studies have shown that provided moisture content are kept below 10-11% /equivalent to 0.70 water activity), then Brazil nuts can be stored safely.

Ayerst & Budd (in 1960, before aflatoxin was described) performed very interesting studies on the decay Brazil nuts, due to moulds, during transport and storage of Brazil nuts. The first one was performed on groups of samples, with different moisture content (MC), transported between Manaus and England and stored in England. The degree of deterioration during storage was estimated by the increase in the proportion (%) of decayed nuts. There was no deterioration at 29% MC and very little at 26 and 23%, but considerable deterioration occurred at 20 and 15%! In the second experiment samples of nuts, with different initial MC, were stored in England at 15ºC and 70% RH. Moisture contents were checked at 2, 6 and 12 month. There was a slight initial deterioration of the wettest samples (about 27% MC) which did not increase after 2 month. In samples of 23 and 17% MC progressive deterioration occurred throughout the storage period, but in the samples at 10-11% MC there was no deterioration even after a year. The differences were statistically significant (χ^2 test). The authors explain the interesting findings in these two experiments by the fact that the nuts with highest MC have health living cells. The robust shells are effective barriers to insects and mites and prevent bruising or surface damage to the kernel, which would encourage fungal attack. Even if this outer layer of cells is broken, in nuts with high MC, healing occurs and this is associated with suberisation of cell walls and the presence of dark brown inclusions in other cells. This supports the idea that there is a strong connection between the metabolism of the nut and its resistance to decay. The authors concluded that deterioration could be reduced either by maintaining the nuts at above 25% MC or by drying them from this level to about 12% MC over a short period.

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Links

http://www.slv.se/OTAPREV
http://www/who.int/pcs/jecfa/jecfa.htm
http://www.europa.eu.int/comm/food/fs/sc/scf/index_en.html

Reduction of fumonisin levels in grain from Bt Corn

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All over the world, corn can be infected with fungi that produce mycotoxins in various quantities according to climatic conditions. The most ubiquitous fungi infecting corn are *Fusarium verticilloides* and to some extent *Fusarium proliferatum* which cause ear rot and produce fumonisin mycotoxins. Other species, such as *Fusarium graminearum*, produce deoxynivalenol and zearalenone mycotoxins in small grain cereals as well as in corn grain. Environmental factors such as damage from insects, heat, water stress and genetic susceptibility are known to predispose corn plants to infection with fungi [1,2].

Biotechnology has made it possible to develop corn hybrids that are protected against lepidopteran corn boring pests such as *Ostrinia nubilalis* (European corn borer), *Sesamia* sp. and *Diatraea saccharalis*. The coding sequence for Cry1Ab protein derived from the soil bacteria *Bacillus thuringiensis* (Bt) has been introduced into corn plants. Bt microbial spray insecticides that contain Cry1 Ab and other Cry insect control proteins have been safely used in agriculture for over 40 years [3]. Bt insect control Cry proteins are non-toxic to mammals and other non-target organisms. Using a CaMV 35S gene promoter, the Cry1Ab protein is produced throughout corn plant tissues providing season-long protection against several generations of corn borer insects of the Lepidopteran family. While season-long protection has benefits for the farmer and the environment following reduced application of chemical insecticides, there is growing evidence of another potentially important health benefit of Bt protected corn; reduction of fumonisin mycotoxin contamination of corn grain.

Scientists at Iowa State University were the first to report that Bt protected corn had reduced ear rot and fumonisin contamination when compared to near isogenic, non-transgenic controls [4]. This reduction was attributed to reduced insect damage to the corn kernels caused by corn borers, decreasing *Fusarium* infection. *Fusarium* species can enter wounded corn tissues damaged by insects. This protection was most evident in varieties (such as Monsanto's event MON 810 - YieldGard CornBorer®)* where the Cry protein was expressed in all plant issues throughout the growing season. Additional field trials With YieldGard®(*) CornBorer hybrids have been conducted in Turkey, the United States, Argentina and France.

In the Cukurova (Adana) region of southern Turkey, second crop corn, sown after wheat harvest, is a common practice. Hot and dry weather conditions prevail in that region. With four to five generations per year, *Ostrinia nubilalis* and *Sesamia nonagrioides* cause significant reduction in grain quality and yield (up to 100% in second crop corn). In that region, one replicated trial was implemented end of June, 2001. Hybrids included for comparisons were: the near isogenic control hybrid DK626, the Bt version of DK626 and a non-Bt commercial hybrid for reference. The 4 blocks were divided in 2 sub-blocks. One sub-block of each replicate received 3 applications of the insecticide lambda-cyhalotrine at 2-3 week intervals, starting the 3rd week of July. Before harvest, samples of 25 plants per plot were collected and dissected to count the number of larvae per plant. Disease index was calculated from 50 plants per plot using the following scale: 1, no ear rot; 2, ear rot on 1-10%

* YieldGard® is commercialised under the trademark YieldGard CornBorer® in the United States.

of kernels; 3, 11-20%; 4, 21-30% and 5, ear rot visible on over 31% of kernels. Among the fungi identified at harvest, *Fusarium verticilloides* represented 70% of the fungal biomass.The 4 central rows of each plot were harvested in October to measure the grain yield. Several sub-samples of grains were also collected in each plot, mixed and used for fumonisin B1 + B2 + B3 quantification by ELISA method. Results are presented in Table 1.

Table 1. Comparison of the number of larvae per plant, disease index and fumonisins concentration in grains, between Bt and non-Bt version of the DK626 hybrid.

	Ostrinia larvae/plant	Sesamia larvae/plant	Disease index	Yield MT/ha 15%	Fumonisins [PPM]
UNTREATED					
DK626Bt	0.02	0.03	1.54 b	10.53	2.5 c
DK626	1.42	5.28	2.74 a	6.91	17.5 ab
Ref. hybrid	1.35	8.64	3.05 a	7.19	18.1 a
TREATED (4)					
DK626Bt	0.01	0.04	1.77 b	11.63	2.6 c
DK626	1.37	4.06	2.41 a	8.31	15.6 b
Ref. hybrid	1.58	7.01	2.81 a	9.14	16.6 ab

Without insecticide application, there was a 7-fold reduction of fumonisins in the grain of DK626Bt by comparison with its near isogenic version, DK626. The disease index was also significantly reduced by 56%. Both results are clearly linked to the excellent control of *Ostrinia nubilalis* (98.5%) and *Sesamia nonagrioides* (99.4%). Grain yield was increased by 52%. Application of insecticide over non-Bt hybrid resulted in limited control of *Sesamia* (23%), but had no significant impact on fumonisins reduction. No significant advantage was seen with the applications of insecticide to DK626Bt.

In the United States, a large number of strip trials (48 rows by 91.5 m) were established in 2000 (92 locations, 11 states) and 2001 (61 locations, 12 states). Several pairs of hybrids were compared at each location. Grain samples of 0.9-2.2 kg were collected in each strip. Fumonisins B1, B2 and B3 were measured by HPLC method. For the year 2000, levels of total fumonisins (FB1 + FB2 + FB3) were above quantification (0.5 ppm) in 53 out of 200 YieldGard CornBorer / control pairs that were analysed. Thirty-eight comparisons out of 53 showed lower fumonisin level in the Bt hybrid, higher fumonisin levels were found in the grains of Bt hybrids in 9 comparisons. On average the level of fumonisins was reduced from 3.29 to 1.48 ppm (55%). Considering the trials with the highest infection rate, at 15 sites, fumonisin levels were above 4 ppm for control hybrids, but only 4 sites in the case of Bt hybrids. In 2001, 161 Bt / control pairs were analysed. Average fumonisins level was reduced from 4.88 (controls) to 2.62 ppm (YieldGard CornBorer). Ninety-six comparisons out of 131 showed lower levels of fumonisins in Bt hybrids compared to controls, a reverse situation was observed for 28 other comparisons. In terms of maximum level, the concentration of fumonisins was above 10 ppm at 14 sites in the case of the near isogenic controls and above 10 ppm at 6 sites with the YieldGard CornBorer hybrids.

In the 2000/2001 season, 57 strip trials were implemented across 3 provinces of Argentina. In that country, the major lepidoptera pests that bore tunnels in corn plants are *Diatraea saccharalis* and *Helicoverpa zea.* An average yield increase of 12% was gained by achieving control of those insects with YieldGard. Aflatoxins and deoxynivalenol were found at some sites, but only fumonisins B1 + B2 (analysed by HPLC) were present at all locations. On average, fumonisins concentration was reduced 66% from 5.63 (near isogenic control) to 1.93 ppm (Bt hybrid). In terms of frequency of high concentrations, more than 10 ppm was measured at 10 locations for conventional hybrid, but only one site with the YieldGard hybrid.

In France, YieldGard hybrids and their near isogenic controls were also evaluated in strip trials at 39 locations over 3 years, mainly in the southwest and central regions. *Ostrinia nubilalis* was observed in both regions whereas *Sesamia nonagrioides* was only present in the southwest area. The number of larvae per plant (sample of 25 plants per strip) was

counted as well as the percentage of ears showing visible fungi infection were compared for 66 comparisons of Bt / conventional non-Bt hybrids. The number of larvae per plant ranged from 0 to 5.3, with an average of 1.23 for controls. In 61 out of 66 comparisons, Bt corn reduced the number or larvae per plant by over 95%. Regarding the frequency of ear rot, in 59 comparisons ear rot was less frequent with Bt hybrids. For mycotoxin quantification, 1-2 kg grain samples were collected in each strip. Fumonisin B1 was quantified by a HPLC / fluorescence detector method. With conventional near isogenic control hybrids, 16 out of 68 comparisons showed fumonisin concentrations above 1 ppm, 6 concentrations were above the recommended threshold of 3 ppm and 3 (all in 2001) were above 5 ppm. All Bt hybrids had fumonisin levels in grain well below 1ppm.

All results reported above show clearly that there is a consistent trend of average reduction of fumonisin concentrations with YieldGard hybrids. But most important are the frequent reduction of high fumonisin levels that may pose health risks to humans and farm animals. These results show also that there is a relationship between control of corn borer insects and reduction in fumonisin levels.

Reduction in fumonisin levels could have important health implications for corn grown in countries where mycotoxin contamination is high and corn represents a major portion of the diet. In China, parts of Africa and in parts of Latin America, countries with high corn consumption, significant fumonisin contamination occurs leading to fumonisin exposures considerably in excess of the adult PMTDI (2 µg/kg body weight/day). In Europe, due to the recent classification of fumonisin B1 in the 2B group (possibly carcinogenic) there is more and more attention given to the control of this mycotoxin.

Protection of corn crops against boring insect pests can reduce ports of entry to fungi that produce fumonisins leading to decreased contamination of corn grain with this mycotoxin. The application of biotechnology has the potential to reduce mycotoxin contamination of corn, improving its safety for consumption. To more fully evaluate the potential reduction of fumonisin levels in Bt corn grown in other world areas, additional trials are being initiated with Bt corn grown in South Africa, the Philippines, South America and other countries.

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Making wine safer: the case of ochratoxin A

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Grape can be a product for immediate consumption as table grapes, or for longer-term storage in the form of dried grape (raisins, sultanas) or as a processed product (grape juice or wine). In 1996 a mycotoxin, ochratoxin A (OA) was found in grape processed products. Surveys conducted in several countries confirmed the presence of OA in wine, with higher concentrations being found in southern regions, and increased amounts in the order white<rose<red.

An EU project commenced in 2001 aimed at assessing the risk of OA in table grapes and wine in Europe with the aim of reducing the amount of toxin through an integrated management of production and processing. All European countries of the Mediterranean basin are involved, together with the United Kingdom and Israel.

Preliminary results indicate that OA producing fungi are already present on the grapes in the vineyard prior to harvest. Grape variety does not appear to have as much an influence as does location of the vineyard. Weather patterns also influence levels of OA. Furthermore, the OA producing fungi isolated from grapes are different to those found on cereals.

Results obtained from the application of various adjuvants aimed at reducing and/or eliminating OA levels in wine will be discussed. In addition, preliminary results on the effects of temperature and water activity on growth and toxin production by ochratoxigenic fungi isolated during these studies will be reported.

Biocontrol of aflatoxins in peanuts

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Aflatoxins remain the most important mycotoxin problem in the world, and peanuts are a major source of these toxins. Control of aflatoxin levels in peanuts on a commercial scale is possible by colour sorting and aflatoxin assays on shelled peanuts, and this is widely practised in developed countries. However, this procedure is expensive. In consequence, a great deal of effort has been devoted to developing methods for reducing aflatoxin formation in peanuts, but traditional approaches using plant breeding have almost always been unsuccessful. In Australia, the cost penalties imposed by shelling companies on farmers for excessive levels of aflatoxin in peanuts are very high, so methods for reducing or preventing aflatoxin formation remain an imperative. Current approaches in the USA and Australia centre on biocontrol or, more correctly, competitive inhibition, using non-toxigenic strains of *A. flavus* and/or *A. parasiticus*. The rationale behind this approach is that spores of non-toxigenic strains of these species added to peanut soils will compete with the naturally occurring toxin forming strains for sites for growth on developing peanuts. In sufficient numbers, so that non-toxigenic spores outnumber toxigenic spores by 2:1 or preferably 20: 1, aflatoxin levels in shelled peanuts may be reduced by 90% or more. In practice, the non-toxigenic strains to be used must be carefully chosen. They must not only be totally incapable of producing toxins, but they must also be competitive under field conditions. Ideally, the strains should be taken from soils or nuts in the same area as they are distributed. Because *A. flavus* is a known (though weak) human pathogen, the introduction of non-toxigenic spores on the large scale requires care. It is likely that distribution over plants or soil by spraying or dusting will never be permitted. In Australia, we have chosen to spread the spores directly onto soil in a carrier, by means of standard farm machinery such as fertiliser spreaders. After looking at alternatives, the carrier system we chose is cracked barley, to which the spores of the selected non-toxigenic isolates are added in molasses. The cracked barley (or wheat or other small grain) and molasses system has several advantages: cracking the barley increases the number of particles, assisting uniform distribution in soil, and reduces germination by the barley (a major problem in using live seed); the molasses acts a s a vehicle for good distribution of the non-toxigenic spores on the grain, reduces dust from the cracked grain, aids adherence of the spores to the grain, and increases the nutrient status of the grain. Nutrient status is important: a major advantage of the use of grain as a substrate is that it assists growth of the spores on the grain, so that the added fungi sporulate in the field situation, producing a multiplier effect on spore numbers in the soil. This multiplier effect may be up to 1000 fold in laboratory experiments. Field trials of this technology are under way in Australia, with some promising results. On some occasions aflatoxin levels in soil treated with non-toxigenic fungi have shown a 95% reduction in aflatoxin compared with controls. It is expected that a commercial process will be developed soon.

Risk assessment for mycotoxins

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Risk assessments for mycotoxins and other food contaminants that are to a significant extent unavoidable present considerable challenges, particularly from international perspectives. The Joint Expert Committee on Food Additives and Contaminants (JECFA) of the Food and Food and Agriculture Organization (FAO) and the World Health Organization (WHO) of the United Nations is tasked with carrying out mycotoxin risk assessments that are used by the Codex Alimentarius Commission and some Member States in their risk management decisions. The following mycotoxins have been evaluated by JECFA: aflatoxins, dexoynivalenol, fumonisins, ochratoxin A, patulin, T-2/HT-2 toxins, and zearalenone.

In 1987, the aflatoxins were first evaluated by JECFA, and Environmental Health Criteria 70 "Principles for the Safety Assessment of Food Additives and Contaminants in Food" was published. Since that time considerable changes have taken place in the procedures and complexity of assessments of chemicals in food. There have been significant advances in chemical analysis, toxicological assessment, and risk assessment procedures. The paradigm of risk analysis with definitions of the terms and tasks of risk assessment, risk management, and risk communication has been shaped. As a result of these changes, FAO and WHO have initiated a project to update and harmonise the common principles of the risk assessment chemicals in food, including natural constituents, additives, contaminants, and residues of pesticides and veterinary drugs (Update Project). The outcome, "Principles and Methods for the Risk Assessment of Chemicals in Food", will be a dynamic web-based guide and incorporate by consensus new considerations, principles and methods as they evolve. The risk assessments for mycotoxins that JEFCA has carried out will be discussed in view of some of the problems encountered and the potential for improvements in risk assessment approaches.

Uncertainties associated with the lack of adequate data and the fact that mycotoxin contamination can have serious impacts on trade and food sufficiency require good communication between the risk assessors and risk managers in formulating the questions to be addressed by the risk assessment. The risk assessment paradigm of hazard identification, hazard characterisation, exposure/intake assessment, and risk characterisation must be an iterative process.

Hazards associated with most mycotoxins were first recognised from toxicity in livestock resulting from mycotoxin-contaminated feed. As studies in experimental animals and *in vitro* were carried out, other adverse effects were identified. In animal studies, mycotoxins have a wide variety of mechanisms of toxicity, including genotoxic and non-genotoxic carcinogenesis. However, there are limited epidemiological studies clearly associating mycotoxin contamination with adverse effects in human populations. This may be a result of the inadequate statistical power of most epidemiological studies to detect effects at the levels of contamination that are usually present. As advances in molecular epidemiology to identify biomarkers are applied, suspected adverse effects may be confirmed and unrecognised effects determined.

Hazard characterisation approaches for the mycotoxins usually have been divided on the basis of genotoxicity or absence thereof. If the chemical is non-genotoxic, then a threshold for toxicity is generally accepted. A no-observed-adverse- effect-level (NOAEL) or lowest-observed-adverse-effect-level (LOAEL) can be determined. Based on the type of critical

effect, the dose-response characteristics, and the overall quality of the data, a safety factor that takes into account species differences and variations in humans is selected to establish the threshold for the adverse effect in humans. The derivation of safety factors is an issue that will be addressed in the Update Project. In carrying out hazard characterisation for food contaminants, JECFA established in 1972 the concept of provisional tolerable weekly intake (PTWI) to contrast with the acceptable daily intake (ADI) concept for food additives. Tolerable intake represents the maximum acceptable level of a contaminant in the diet. JECFA also established the endpoint of provisional maximum tolerable daily intake (PMTDI) for food contaminants that are not known to accumulate in the body. In 1978 JECFA introduced the concept of an "irreducible level" for potent carcinogens, such as the aflatoxins. This is the ALARA (as low as reasonably achievable) principle. More recently for aflatoxins B_1 and M_1, JECFA has carried out quantitative risk assessments to address risk management questions based on the comparisons of proposed standards. he difficulties with the risk assessments for food contaminants such as acrylamide, ethyl carbamate, and chloropropanols demonstrate the need for the development of guidelines for the use of quantitative risk assessment procedures for chemicals in food. The International Programme on Chemical Safety is carrying out an in-depth review of these approaches and will develop general principles for the use of quantitative risk assessment techniques.

The quantitative evaluation of the likely intake of mycotoxins on an international basis is extremely problematic. Food consumption patterns vary tremendously. Production and processing practices vary, and these data are often not available from developing countries. The selection of the food or food groups that contribute significantly to dietary exposure is a critical consideration. The WHO Global Environmental Monitoring System/Food Contamination Monitoring and Assessment Programme (GEMS/Food) is expanding its number of regional diets from five to thirteen in order to more accurately cluster the various food consumption patterns around the world. However, the GEMS/Food database does not currently support exposure assessment for subpopulations such children, pregnant women or the elderly, and these are issues of increasing concern. In most of the JECFA reviews of mycotoxins, the analytical data on the levels of contamination were often inadequate from developed countries and non-existent from developing countries. Since most mycotoxin contamination is heterogeneous, sampling is an important consideration in the development of information on the levels of contamination. Data from national food balance sheets or from national food consumption surveys, where available, can be used to obtain more accurate assessments at the national levels. Total diet studies, which can provide individual data on contaminant levels in foods as consumed, may provide the best estimate of exposure for national populations and subgroups at risk. Historically, JECFA has had available mainly pooled data for mycotoxin contamination, so intake estimation was based on a combination of mean food consumption levels with weighted mean contamination levels. The complexity of assessing individual exposures and in order to analyse particular situations, probabilistic approaches need to be applied. This is occurring at the national level, and is being considered by JECFA and the Update Project. While most mycotoxin risk assessments by JECFA have been for chronic exposure, acute exposure can be an issue for some, such as deoxynivalenol. The Update Project is also developing guidance for the establishment of acute reference doses.

For the fumonisins, ochratoxin A, deoxynivalenol, patulin, and T-2/HT-2 toxins, JECFA concluded that intakes below the PMTDI/PTWI present negligible health risks. For aflatoxins B1 and M1 in non-hepatitis B/C positive populations, the carcinogenic effects between the two maximum levels proposed would be impossible to demonstrate. These risk characterisations included discussions of the considerable uncertainties involved, particularly those resulting from inadequacies in the intake data and lack of information on toxicokinetics and mechanisms of actions. In addition, JECFA has emphasised the importance of prevention and control measures.

Improved data on toxicokinetics, the application of modelling approaches for dose-response characterisation and exposure assessment, and new technologies to measure biomarkers of exposure, effect, and susceptibly will result in significant improvements in risk assessments. Optimal data requirements for dose-response modelling may require some changes in animal testing protocols and in the collection of analytical data. However, for international considerations, appropriate intake data may remain a limiting factor in the risk assessment.

The implementation of EU controls on imported food

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Inspections of consignments of food at ports of entry play an increasingly important part in food controls. Until 1993, the situation in Europe was that the bulk of imported foods were allowed to enter the European Union unchecked at the point of entry. These foods were liable to point of sale checks and sampled in importers premises by the Member State health authorities. Since detailed legislation came into force, consignments of animal products from third countries (non-Member States) are controlled at the outside border of the community since 1993. For non-animal foodstuffs there is no detailed harmonised legislation on imports and as a consequence there may exist in EU-countries different policies on food imports and control measures.

A working party of the Food Law Enforcement Practitioners in Europe developed in 1993 an import control system for non-animal products. In some countries of the European Union (EU), import controls of some foodstuffs were introduced.

From the recent activities of a new FLEP Working Party 'Mycotoxins', it can be concluded that there is still a need for harmonised EU-legislation on imports of non-veterinarian products from third countries.

Introduction

Foods are now more widely distributed than years ago. Consumers are further removed from the source of the foods bought, either by time or distance. Each year about 10 million tonnes of foodstuffs enter into the European Union (EU) through the port of Rotterdam alone. Therefore, it is important to obtain assurance that the products, produced and/or processed far away from the final consumers, are safe and of good quality. Food inspection at ports of entry plays an increasingly important part in food controls.

There are obvious advantages in checking imported foods at the point of entry where effective action can be taken, as opposed to point of sale where authorities will typically only respond to complaints. In the latter, action taken is isolated and can result in non-complying foods elsewhere in the marketplace from the offending supplier, going undetected.

Import controls ensure there is greater consistency in the treatment of 'domestic' and imported foods with all foods inspected for compliance against a single standard. Point of entry checks will have a deterrent effect and will encourage importers to take more care in buying products outside the Community (and in turn will ensure the EU is not seen as a convenient market for sub-standard or low quality foods rejected elsewhere around the world). Efforts to negotiate government to government agreements for the recognition of certification will provide additional safeguards about the environment, processing and handling controls in food factories in third countries.

Increased surveillance of food imports at the port of entry will relieve much of the pressure currently on Member State health authorities, which have been seeking to deal with non-complying food imports. These arrangements will allow the Member State authorities to review their priorities and may enable them to redirect some of their resources to priority areas in the domestic sector.

Food law enforcement practitioners

Food Law Enforcement Practitioners (FLEP) is an informal grouping (forum) of European food law enforcement practitioners representing the management of food control interests in the European Union and European Free Trade Area (EFTA). The terms of reference include acquaintanceship, the exchange of information and co-operation between European colleagues in order to develop mutual confidence and trust in the resolution of enforcement problems. The forum aims to promote good practice, support the principle of the European 'Home Authority' and offer an enforcement perspective of directives, regulations, food standards and guidelines.

In 1993 a FLEP Working Party on 'Import controls' proposed a system for an imported foods inspection program for non-animal products. Under these proposed food inspection arrangements, all imported foods from third countries would be subject to a substantially more extensive inspection regime to ensure they complied with EU food standards. The imported foods would be liable to point of entry physical checks (food: all foodstuffs not excluding raw materials, ingredients, and additives, except the animal products for which there are separate provisions).

The program sought to provide a uniform requirement for point of entry monitoring of imported foods to check their compliance with the EC food standards. All imported foods from third countries would be liable to testing, with the frequency of inspection and the checks to be performed determined by the assessed health risks (acute and long term) associated with the food, past reports of non compliance or the past unsatisfactory record of the supplier. The range of checks to be carried out at the time of importation would include product safety, residue concentration and biological impurities.

The current situation

The situation in 2001 has changed little from the position in 1993. For example: consignments of nuts and pistachios are transported under customs control (T1-document) through ports to internal destinations within the EU. They are released by customs in great quantities and the content is then transported back to other countries. This gives rise to ‘backdoor-problems’ – for example, in the Netherlands high risk consignments which are released are controlled by the Dutch authorities. Goods may be cleared through other ports with less stringent controls; as a result, now and then consumer packages with products that do not fulfil the EU-legislation can be found in Dutch supermarkets.

An example: import controls and mycotoxin legislation

A FLEP Working Party ‘Mycotoxins’ studied in 2001 the enforcement activities for mycotoxins by enforcement bodies in Europe. The measures that are taken by enforcement bodies were evaluated and several recommendations were formulated. The conclusions and recommendations from the report will be presented.

Main conclusions were:

- For aflatoxins a strict enforcement policy is necessary. Lots with excessive levels should not enter the food chain. An exception is when physical treatments are possible to reduce the contamination. Physical treatment must be carried out under supervision of the enforcement bodies.
- There is still a need for harmonised legislation on imports of non-veterinarian products from third countries.

The fate of mycotoxins during cereal processing

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It is important to protect the consumer from the effects of a mycotoxin in the food supply when risk assessment suggests that level of exposure is likely to be undesirable. This can be tackled by developing protocols that minimise the formation of the mycotoxin in the field or store or by introducing legislation backed up by effective monitoring systems. The European Community has introduced maximum permissible limits for aflatoxins, ochratoxin A and patulin in specific products and are actively involved in considering which further mycotoxins need regulation and the limits that are appropriate. For those commodities such as cereals that undergo significant processing, the concentration of a mycotoxin in a food item that reaches the consumer may be considerably lower than in the raw harvested crop. If this relationship can be established, it may be possible to set limits at a higher level for the raw product than for the processed food without compromising human safety, especially when mycotoxins appear closely associated with a particular crop, e.g. deoxynivalenol in wheat and fumonisins in maize. This will protect the consumer without unnecessarily penalising the cereal industry. This paper provides a brief review of the fate of some important mycotoxins during cereal processing and refers to recent studies of ochratoxin A in cereals to illustrate some key points.

Cereals are invaded by fungi both in the field and after harvest so have the potential for multitoxin contamination. The specific mycotoxin(s) formed will depend on a number of factors but particularly on the climate and the type of cereal. Thus cereals may be contaminated by any of the main mycotoxin groups: aflatoxins, ochratoxin A, deoxynivalenol and other trichothecenes, zearalenone and fumonisins (maize). Most of these mycotoxins are quite stable although factors to be considered during processing such as temperature, pH, the presence of other constituents or enzymic action may cause reaction and breakdown to occur.

While the general trend associated with most processing is to reduce the mycotoxin concentration present, there are 2 specific circumstances that must also be considered. Firstly, in milling it is usual for a mycotoxin to be unequally distributed among the milled fractions, such that in some parts e.g. commonly bran, a concentration of mycotoxin may occur. A similar principle applies to wet milling processes. Secondly, chemical reaction may occur so that while concentrations of the mycotoxin may be reduced, a toxic reaction product might be formed. In general this possibility has been much less studied although a hydrolysed toxic product has been reported from fumonisin and toxic products from citrinin.

Ochratoxin A has been shown to be a frequent contaminant of cereals in parts of Europe and elsewhere and also occurs in coffee, dried fruit and other stored produce. Evaluation of its risk to the consumer has resulted in the introduction of statutory maximum permissible limits within the EC for ochratoxin A in cereals (5 µg/kg) and derived products and foods (3µg/kg) and limits are expected soon for other food commodities. Recent research in Europe has addressed some of the problems posed by ochratoxin A and the EC has funded a study under the 5th Framework Programme aimed at prevention and managing ochratoxin A in cereals. One aspect within this study has examined the fate of ochratoxin A in wheat through the food chain including milling, baking and extrusion processing. Preliminary results are presented to show how concentrations of ochratoxin A change during these processes including the extent to which they can be reduced by procedures such as surface scouring of the whole wheat grains. Ochratoxin A is redistributed during milling so that it is reduced in

white flour fractions but increased in offal and bran. The scouring stage can assist in removing some of the mycotoxin and by combining this process with the removal of the bran and offal fractions achieves a reduction of approximately 65% in the concentration of ochratoxin A in bread the baked from white flour under pilot scale studies. In contrast the concentrations of ochratoxin A in wholemeal flour were hardly affected by extrusion.

Results from this ochratoxin A study will be discussed with regard to current regulations, and used to illustrate many of the economic considerations facing the food and animal feed industries in handling and disposal of contaminated fractions and in consumer protection, A detailed study of the fate of mycotoxins in cereals needs to consider many processes such as cleaning, milling, baking, cooking, extrusion, malting and brewing. Most studies reported have been carried out in the laboratory, or at the best at pilot scale, so it remains difficult to assess how well this work relates to the full industrial scale. It is area with many gaps in current knowledge but with considerable potential to minimise the risk to human and animal health by careful and informed management of the raw materials.

Peanuts, aflatoxin and the Origin Certification Program

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The European Union (EU) and U.S. peanut industry have agreed to an Origin Certification Program (OCP) to test U.S. export peanuts for aflatoxin at the port of origin and certify that the peanuts meet EU aflatoxin regulation. The OCP does not preclude random testing of lots for aflatoxin at the port of entry. For domestic use, the U.S. Department of Agriculture (USDA) requires three 22 kg laboratory samples to average less than 15 total ng/g for acceptance. The EU requires one 30 kg laboratory sample to test less than 15 total ng/g (8B1) for raw peanuts destined for further processing and three 10 kg laboratory samples to all test less than 4 total ng/g (2B1) for finished peanuts sold for direct human consumption. The U.S. proposals to the EU was to use the official USDA 22 kg sample for raw peanuts or divide the USDA 22 kg sample into three 7.3 kg samples for finished peanuts. The EU agreed, but in addition requires grade certificates for traceability and all aflatoxin test results for verification. The U.S. exporter uses the first USDA 22 kg sample test results for screening lots. The second USDA 22 kg sample is tested according to EU protocol for lot certification. The origin certification program will reduce lots rejected at the port of entry, reduce the disruption in supply for the importer, reduce economic losses for the exporter and the importer, and maintain EU standards for consumer safety. The origin certification program is an example of an agreement between two countries that is mutually beneficial to both while maintaining high standards for consumer safety.

Mycotoxins in spices: the red pepper story

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Many spices are exposed to fungal contamination as well as other microorganisms at various stages of production. Yeasts are rarely found but high levels of mould including *Aspergillus* and *Penicillium* are commonly encountered. *Fusarium* and *Alternaria* were also isolated in some areas of the world and the isolated moulds produced different types of mycotoxins. Although type and frequency of the moulds may vary widely from spice to spice, toxic mould contamination will cause mycotoxin production. Many strains of moulds, while growing under favourable conditions, produce metabolites that are toxic to humans, animals and birds. Aflatoxins are a group of mycotoxins produced by genus *Aspergillus*, particularly *A. flavus, A. parasiticus* and *A. nomius*. Two other species found recently are also aflatoxin producers, namely *A. ochraceoroseus* and *Emerciella venezuelensis*. Aflatoxins were the most frequently isolated mycotoxin from different spices.

Red pepper, obtained from fresh red pepper by drying and grinding is widely used as a spice all over the world as well as Turkey. Aflatoxin contamination of spiced roasted nuts and processed meats were also observed to be correlated with the addition of spices. During harvesting and sun drying, red pepper can be contaminated with moulds. Red pepper is grown and harvested mainly in the southeast and eastern parts of Turkey and is known by the name of its region of origination. In this study, a total of 36 samples of red pepper grown in 4 different regions were examined to determine aflatoxigenic moulds and aflatoxins. Dilution plate and direct inoculation techniques were used together in order to determine mould contamination. For the enumeration and isolation of moulds DRBC was used as a standard medium. Aflatoxin analysis was performed by afla-test column followed by high-pressure liquid chromatography (HPLC). 13 of 36 Samples were contaminated with aflatoxigenic moulds. Aflatoxins were determined only in 5 samples with a value between 10.5 - 31.1 ppb. This was higher than the limits of both the Turkish Codex and EC Standards. In the previous years exported red pepper has been rejected due to the high mycotoxin content above standard limits. Such incidences have been reported in Europe in the past. Since Turkey is one of the red pepper exporting countries efforts should be made to prevent or reduce contamination and subsequent growth of moulds during processing and storage of red pepper. This is also important to avoid economical loses.

Safe organic vegetables: the carrot-*Alternaria* model[*]

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European agriculture is confronted with a growing demand of consumers for organic vegetables. However, undesirable components such as mycotoxins pose a challenge to the safety of organic vegetable products. An EU-project was started in 2001 with the overall objective to develop strategies to ensure a safe organic food supply by developing detection methods, anticipating mycotoxin risks, tracing the sources of contaminants in the food production chain, and eliminating the risk factors. The research is done with the model carrot-*Alternaria*. This group of fungi contains known producers of harmful mycotoxins, which may enter the food chain via infected seeds.

Establishing methods for detection of *Alternaria* and analysis of *Alternaria* mycotoxins

Suitable methods for detection of the mycotoxin-producing *A. alternata* and *A. radicina* on various types of carrot plant material, in particular seeds and roots, have been established. The methods are based on plating on selective agar media or non-selective blotters, after which evaluation for fungal growth takes place.

HPLC-based analytical methods for simultaneous determination of the principal *Alternaria* mycotoxins in rice cultures of *A. radicina* and *A. alternata* were developed. To monitor mycotoxin accumulation in carrots it was necessary to clean up the extracts on a polymeric based cartridge or a C_{18} cartridge before HPLC analysis. The protocol for determination of radicinin, tenuazonic acid, altertoxin I, and alternerariol methyl ether in carrots by high performance liquid chromatography and UV detection is available in CEN format.

Establishing the basic understanding of *Alternaria* mycotoxin production

Various strains of *A. radicina* and *A. alternata* from diverse sources were isolated and tested for their virulence and mycotoxin production. In particular for *A. radicina* isolates differences in virulence were detected. With regard to mycotoxin production, *A. radicina* isolates could produce high amounts (> 1000 µg/g) of radicinin when cultured on rice, and *A. alternata* isolates produced high amounts of tenuazonic acid and lesser amounts of alternariol, alternariol methyl ether, and altertoxin I. The incubation temperature affected the mycotoxin production. Studies on production of mycotoxins by the fungi on carrot slices are ongoing.

Monitoring myxotoxin accumulation in the production chain

On 3 locations in Europe (DK, NL, and F) organic carrots were produced from the same seed batches contaminated with *Alternaria*. The site of production had a large influence on the contamination of the carrot roots produced. With regard to the analysis for mycotoxins, preliminary experiments have shown that stored carrots with clear symptoms of fungal damage did not contain detectable levels of mycotoxins. However, strains of *A. radicina* isolated from carrot roots showed a different metabolite profile when grown on rice and carrot slices. In particular, *A. radicina* produced radicinin when grown on rice, whereas the same fungus produced other compounds, but not radicinin, when grown on carrot slices. Epi-

[*] Acknowledgement**:** This research is financially supported by the European Commission, Quality of Life and Management of Living Resources Programme (QoL), Key Action 1 on Food, Nutrition and Health.

radicinol was identified and a second, so far unknown, compound is being characterised. The toxicity of both compounds will be determined.

Control measures

Control measures to prevent the introduction of *Alternaria* and mycotoxin accumulation in the carrot production chain and that are acceptable to organic farmers were studied. Bioassays to test carrot genotypes for differences in resistance towards *A. radicina* were developed, and will be used to select carrot accessions with improved levels of resistance for use in breeding programmes. Furthermore, antagonistic microorganisms selected for their control efficacy and low cytotoxic activity are currently being tested for optimisation of production, formulation and storage, and will be used for seed and plant treatment studies. Plant based compounds which inhibit the growth of *Alternaria in vitro* will be tested as seed treatments as well. Seed production experiments are ongoing and should lead to measures enabling an *Alternaria*-free organic carrot seed production.

Benefits

A total strategy to reduce the risk of mycotoxins in organic carrots and products derived from them will be developed. This will advance the production of safe organic carrots and processed products, and will contribute to the health of the European consumer and the promotion of organic farming. Where possible, the results will be extrapolated to other organic crops.

Sampling and sample preparation

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The present manuscript investigates the effect of experimental parameters on the mean and variance of measurements of contaminants in subsampled granular commodities. Subsampling involves the homogenisation of a generally large (10 kg) granular sample, followed by taking an aliquot thereof for actual analysis. Currently two methods of homogenisation are in use, dry grinding and wet slurry grinding. This makes studies of homogenisation particularly pertinent, since dry grinding is in common use in the U.S. (a producing region), while labs in the E.U. (a consuming region) are using wet grinding. The problem is approached here both theoretically and experimentally, using aflatoxin determination in pistachios as an example.

The theoretical approach to variance follows that used to elucidate the large sampling variances commonly observed in tree nuts and similar commodities. These variances arise from the broad distribution of aflatoxin among individual granules (kernels). Homogenisation reduces the particle size, thus greatly increasing the number of particles/unit weight, allowing one in turn to obtain aliquots with variances no larger than (or even smaller than) those obtained in the sampling step. The resulting variance is, accordingly, related to the particle size distribution as well as to the size of the aliquot. Experimental tests are run by first dry grinding a large sample, testing a portion thereof, followed by wet grinding and retesting. A number of grinding parameters were investigated, among which were time of grinding, solvent used for wet grinding (water/methanol), grinding head used in the slurry grinder, amount of solvent and extraction fluid composition. The theory of subsampling was tested by grinding under assorted conditions and by use of the measured particle size distribution, to good effect. It was noted that the variance obtained by wet grinding was substantially totally accounted for by the variance of the final chemical analysis, while dry grinding introduced an additional variance which could not be reduced to below roughly twice the analytic variance. The variance due to sampling (the initial step in the analysis) was, as expected, much larger than either of the other two contributions,

Experiments further showed that the mean value obtained by wet grinding exceeded by 30% that obtained by dry grinding when pistachios were ground in water. Results in other commodities may differ, in some cases even reversing the trend (see poster of Spanjer et al.). A theoretical basis for this effect could not be found, but may be associated with either of two effects which could not be controlled. One involved the difficulty of maintaining a homogeneous slurry while withdrawing a subsample, due to rapid settling of the slurry. Such settling was routinely observed, but probably was not enough to account for the 30% difference in means observed. The other might be an increased extraction of aflatoxin from a ground pistachio matrix by the presence of water in the slurry (prior to the actual aflatoxin extraction step).

In light of the much larger variance introduced by sampling, the subsampling variance is virtually negligible. The change in mean can be removed by calibration, or better by inter-laboratory agreement to use the same subsampling protocol, with adjustment of acceptance level, if needed. The simpler and faster equipment cleanup between samples needed in wet grinding argues for standardisation on the latter method.

Development of certified reference materials for mycotoxins

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In the recent years has been an increased public awareness for mycotoxins, secondary fungal metabolites that may contaminate food and animal feed (e.g. cereals, maize, peanuts and products thereof). These toxic secondary metabolites are produced both pre- and postharvest by a diverse variety of fungi imperfecti, especially by the genera *Aspergillus* and *Fusarium*, which are known to occur in zones of warm and moderate climate respectively.
Aflatoxins B1, B2, G1 and G2 are the main naturally occurring mycotoxins formed by moulds of the genus *Aspergillus*. Besides its high level of acute toxicity aflatoxin B1 (AfB1) is widely regarded as the most potent liver carcinogen. Aflatoxin M1 (AfM1) is a toxic hydroxylated derivative of AfB1, which is formed and excreted in the milk of lactating animals after the ingestion of AfB1 contaminated feed. Deoxynivalenol (DON) belongs to the large group of trichothecenes and is one of the most prevalent *Fusarium* toxins in Europe described by various surveys on the natural occurrence. Ingested trichothecenes initiate a wide range of effects, e.g. skin irritation, reduced consumption of feed, feed refusal, emesis, diarrhoea and multiple haemorrhages. They are also known to act as immunosuppressants and potent inhibitors of protein and DNA synthesis [1].

The frequent occurrence of mycotoxins in food and animal feed, the high consumption of these products and the high risk associated herewith led to the establishment of measures to control the mycotoxin contamination. The importance of mycotoxins as a food safety hazard is reflected in the existence of regulations controlling the maximum limits for mycotoxins at national [2] and European Commission level [3].

The analytical difficulty and the economic importance of controlling mycotoxin levels in food and animal feed led the BCR (Community Bureau of Reference) to fund several projects on the preparation of certified reference materials (CRMs) for various mycotoxins [4-6]. Because of the good acceptance of these CRMs and the ability to ensure comparability and traceability further on it was required to prepare and certify new batches of AfM1 in milk powder, AfB1, B2, G1 and G2 in peanut meal and peanut butter and DON in wheat and maize. The common IRMM approach for the production, certification and evaluation of the uncertainty for food CRMs will be exemplarily described for three materials of AfM1 in milk powder. Hence, two milk powder materials with different mass fractions of AfM1 (BCR-283R and -284R) as well as an uncontaminated milk powder material BCR-282R were prepared in-house by IRMM.

In compliance with the "Guide to the Expression of the Uncertainty in Measurement" (GUM) [7] the uncertainty of a CRM should consider all sources relevant to the user. This includes the uncertainty of the batch characterisation (u_{char}), but also uncertainties related to possible between-bottle variation (u_{bb}), instability upon long-term storage (u_{lts}) and instability during transport to the customer (u_{sts}). The combined standard uncertainty for a CRM can thus be expressed as:

$$U_{CRM} = \sqrt{u_{char}^2 + u_{bb}^2 + u_{sts}^2 + u_{lts}^2}$$

The ISO guides 31 and 34 [8,9] require producers of CRMs not only to include contributions connected to homogeneity and stability in the combined standard uncertainty but also certifying bodies to state shelf lives for their CRMs to inform the user about the expected lifetime of the CRMs.

The uncertainty contribution of the homogeneity was calculated by use of an ANOVA

approach. Therefore, 31 samples of both BCR-283R and -284R taken at regular intervals from the filling sequence were analysed in triplicate in random order for the AfM1 content. The homogeneity of the uncontaminated milk (BCR-282R) was demonstrated by analysing 31 samples taken at regular intervals from the filling sequence for the protein content.
The approach to calculate the uncertainty of stability relies on regression analysis of stability data with subsequent testing the slope of the regression line for significance. When the slope is found statistically insignificant, a shelf life is chosen and the uncertainty connected to this time is estimated. For the short- and long-term stability (STS and LTS) both BCR-283R and -284R were tested for the AfM1 content employing isochronous designs at times of 0, 1, 2, and 4 weeks for 40 °C, 18 °C, and 4 °C as well as 0, 6, 12, and 18 months for 4 °C and -18 °C respectively. In addition, a stability monitoring scheme over a long period of 9 years has been started [10].

In general, the estimation of the uncertainty of the batch characterisation will be performed by a model based on analytical results submitted or an approach based on the full uncertainty budgets submitted by the participants. First the batch characterisation was performed by means of an interlaboratory study of 12 expert laboratories. For the determination of AfM1 in milk powder the participants mainly employed a method based on ISO 14501 [11] with minor modifications. This method is carried out by reversed-phase high performance liquid chromatography (RP-HPLC) followed by fluorescence detection (FLD) subsequent to an immunoaffinity column (IAC) clean-up.
In order to compare both models for the estimation of the uncertainty of the characterisation IRMM was interested in the measurement uncertainties of the analytical methods employed by the participants. The measurement uncertainties were assessed by IRMM in compliance with the GUM for each analytical method and participant based on additional data collected during the interlaboratory study.

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Quality assurance with regard to mycotoxin analysis

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During the last 40 years the interest for mycotoxin analysis has enormously developed. Numerous methods for the determination of mycotoxins have been established and been used in monitoring programmes, import/export control measures, etc. These analyses are an important link in the whole chain of quality control, and some requirements are essential for producing reliable analytical data.

The knowledge of the material which has to be analysed has always to be considered very detailed which also shows the importance of information about the sample. There are differences between retail products and bulk ware as well as goods produced under similar conditions or such which are blended from many different origins. After definition of certain batches or lots, the sampling procedure follows. Sampling in the past has been an empirical task due to the fact that mycotoxins are extremely uneven distributed within a lot. Using statistical and mathematical models, this can be changed and compensated by a mathematical basis.

Since January 1st 1999, EC directives for aflatoxins were put in practice, which consisted of sampling plans resulting in sample weights of up to 30 kilogram. These 30 kg must be grinded and mixed completely without any dividing. The use of water slurries ant the advantages for sample preparation are shortly discussed.

For the following analysis the use of reference or official methods is recommended. Standardised or published validated methods are possible alternatives. In general, every analytical method should have proven its performance, both by in-house validation and inter-laboratory trial according to relevant norms or protocols. For other purposes the use of commercial available test kits might be appropriate but false-positive results have to be confirmed by reference or validated methods.

Reference materials are invaluable tools for the analyst. Reference materials should be used as a matter of course for the analyst as well as proficiency tests. These tests are essential for quality control. Other recommendable tools are control charts, either for standards or for mycotoxins in a special matrix.

Before reporting the measurement uncertainty has to be taken into account. Although the specification of uncertainty is demanded by ISO 17025, it is still not clear how to apply it or how to obtain this factor. There are different proposals for the assessment of uncertainty, but most of them are not practicable for an analytical laboratory. It is a suggestion to take the performance data of a method which has been validated in a collaborative trial. The Reproducibility relative standard deviation RSD_R is a useful variable to characterise the uncertainty of a method.

Mycotoxin analysis: current challenges

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The need for more precise and sensitive mycotoxin analytical methods is growing mainly because of the changes in the legislation of food contaminants. Maximum levels for aflatoxins and ochratoxin A have already been assessed and in the near future these levels will be set for some other toxins, too. Monitoring the levels of different mycotoxins in food requires also more sensitive analytical methods for data acceptable for risk assessment purposes.

Systematic collaborative studies are needed to admit new standardised methods for individual mycotoxins. These methods will utilise novel improved laboratory techniques and equipment. Traditional laborious techniques will be replaced with more convenient ones like high-performance liquid chromatography (HPLC) and gas chromatography (GC). In sample preparation, immunoaffinity-columns with specific antibodies will replace the general sorbents to gain more sensitivity to the methods.

Recent approaches in combining liquid chromatography with mass spectrometry (LC-MS) will, however, be the most powerful technique in mycotoxin analysis in the future. With this technique structural elucidation of single compounds can be done more effectively and new toxins can be discovered more easily. Also, development of so-called multitoxin-analysis will probably be increasing and more data about the samples will be obtained with this technique.

Accreditation of the laboratories officially engaged in food analysis will guarantee the general working practises and quality control level. For instance, regular participation in method proficiency testing is nowadays a common demand in most of the quality handbooks.

Trichothecene analysis with GC-MS is a routine method in our laboratory. Although very simple in principle, the method includes many critical points that need to be taken into account for the most precise results. The ways of solving these problems will be discussed in more detail. Also, examples of utilising LC-MS in mycotoxin analysis will be presented.

Multi-residue method for mycotoxin analysis

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Many analytical methods have been developed for the determination of mycotoxins including TLC, HPLC, GC and ELISA. Due to the quite different properties of the mycotoxin families, most of the procedures of extraction and clean up were developed for the determination of only one specific mycotoxin. This result is very unsatisfactory because even if the result of the analysis is negative, other mycotoxins could be present in the tested sample. Cereal grains may be infested by mycotoxins that where produced by mycelia living on the fields but also by mycotoxins produced only during the storage by specific mycelia.

The goal of our research is to develop a method that could be used for the quantitative and semi-quantitative determination of the most current mycotoxins such as trichothecenes (NIV, DON, T-2, HT-2, 15 and 3AcetylDON) and macrocycles (ZEN, ZEL). Along with these mycotoxins, other acidic mycotoxins would also added such OTA, citrinine, moniliformine and fumonisins.

The main steps are the extraction, the purification, the analysis and the detection, respectively. The extraction step has to be non-specific and the yields have to be sufficient. Indeed, we have to chose an extraction solvent that can extract the maximum of each of the present mycotoxins whatever can be their physico-chemical properties: hydrophilic or hydrophobic, neutral or acidic. The second step is the separation between the acidic and the neutral mycotoxins by the way of anionic exchange columns. The trapped mycotoxins and the non-retained neutral mycotoxins were then purified following their own specific way. Only one analytical method could be developed in order to get all the mycotoxins baseline separated by the same gradient. A second gradient can be used in order to help for the cross determination of the mycotoxin identity.

This paper will present the general strategy developed in our laboratories for the yet semi-quantitative determination of the trichothecenes in the presence of OTA, ZEN and ZEL. The analytical flow chart will be discussed and samples would be presented illustrating the critical steps.

Application of *in vitro* bioassay for the screening of mixtures of trichothecenes

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Trichothecene mycotoxins are a group of over 150 structurally related secondary metabolites produced by members of *Fusarium* mould. Several trichothecene mycotoxins are often found in combination in infested cereal grains, but in general it is difficult to simultaneously detect all possible trichothecenes by chemical analysis. Also, the vast majority of toxicological studies have focused on the toxic effect of single trichothecene and there is clear lack on information on combined toxicity. Because of their natural co-occurrence and our lack of knowledge on combined effects, there is an increasing concern about the hazard of exposure to mixtures.

Generally the compounds which have similar mode of action and only differ in their potencies follow the basis of dose-addition. Therefore our first question is whether the dose-addition is applicable for estimation of toxicity after simultaneous exposure of multiple trichothecenes.

In our first study, whole mixture experiments with five trichothecenes were conducted in an *in vitro* bioassay using DNA synthesis inhibition in L929 mouse fibloblast cell line as an end point. The effect of the mixture was much higher than the effect of individual trichothecene. The dose-addition rule rather than response-addition rule appeared to be applicable to describe the toxicity of the mixtures. This result suggests the mechanism of a similar joint action among the selected trichothecenes.

Based on the assumption that combined action of multiple trichothecenes may enhance the sensitivity of bioassay to detect contamination of trichothecenes, we applied the *in vitro* bioassay for the screening of barley grains contaminated with trichothecenes mixtures. Series of studies were performed to test the extract of barley spiked with a mixture of trichothecenes and inoculated with *Fusarium* fungi producing several trichothecenes. Finally, naturally contaminated barley samples were applied to the assay. Trichothecenes were extracted from barley samples with acetonitrile /water and partially purified by a charcoal-alumina clean up column. One portion of the clean up samples was applied to the bioassay. The other portion was subjected to GC/ECD analysis after HFBI derivatisation for chemical determination. The sensitivity of the bioassay varied among the trichothecenes but as far as HT-2 toxin, T-2 toxin, DAS are concerned, 50 ppb of each could be detected by the bioassay. Further the toxicity was more pronounced when cells were exposed to the mixture of these three or five including DON and NIV. Observed IC50 of the mixture of three and five trichothecene fitted in well with predicted IC50 based on the dose-addition rule, suggesting that detection limit of the bioassay could be lowered by increasing the number of toxins present. In the experiments of *Fusarium* inoculated and naturally contaminated barley samples, one or more kinds of trichothecenes were detected by GC analysis and the toxicity was observed by the bioassay. The order of the toxicity of the samples corresponded to the contamination level but the observed toxicities of heavily contaminated samples were more than the predicted ones on the basis of dose-additivity that was calculated by the summation of T-2 equi-effective dose for each toxin detected in the sample. These results may suggest that some toxins other than detected by GC analysis might be produced in the barley samples.

In conclusion, the bioassay may alert for the presence of the toxins not covered by chemical analysis and therefore it seems to be a complementary tool for the detection of mycotoxin contamination in raw materials.

Moulds and mycotoxins in silage

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Fungal invasion of feed commodities and subsequent formation of mycotoxins has been identified as a prominent risk factor to feed quality, regarding feed palatability, nutritional value ultimately affecting animal health and productivity [1]. Fungal invasion and mycotoxin formation can occur at the pre-harvest stage, as well as post harvest during storage. Whilst mould prevention is possible during the entire feed producing process, mycotoxins can not readily be inactivated or destroyed once formed. Ensiling is one of the oldest processes applied in feed preservation to prevent the growth of microorganisms including fungi [2]. The preservative effect results from acidification of the feed commodity, by means of the natural flora of lactic acid-producing bacteria, often supported by intentionally addition of starter cultures and organic acids, and by creating an anaerobic microclimate by compression of feed material. Under practical conditions, however, poor quality of the raw material and managerial defaults in the technical process may be responsible for insufficient preservation allowing undesirable growth of anaerobic and micro-aerobic acid-tolerant fungi and other microorganisms including yeasts [3]. Fungal species, among others *Penicillium roqueforti*, *Byssochlamus* spp., *Aspergillus fumigatus* and *Trichoderma* are adapted to these conditions and are thus the most frequently determined contaminants of ensilaged feeds [4,5]. These mould species are known to be toxinogenic. Mycotoxin formation including *P. roqueforti* toxins as well as *A. fumigatus* toxins, has been found not only under experimental conditions but also by analysis of field samples [6,7].

P. roqueforti and its toxins

Penicillium roqueforti is the most frequently found mould species in silage and is known to produce as major toxins roquefortin C, patulin, mycophenolic acid, PR-toxin, and penicillic acid [8-10]. Mycotoxin production in naturally contaminated material has found to be time dependent following a sequence in which penicillic acid is the first measurable metabolite appearing in measurable concentrations as early as 13 days after inoculation of silage, followed by patuline (22-27 days), mycophenolic acid (approx. 36 days) and PR-toxin (49 days) [11]. This production pattern hinders not only a successful toxin monitoring in ensilaged commodities but affects also the evaluation of actual adverse health effects in animals. *P. roqueforti* metabolites such as roquefortine C and patulin are known neurotoxins, and clinical signs in ruminants may comprise muscle weakness and lack of co-ordination [12]. However, these symptoms may be confined to high dose exposure, thus having a low incidence in farm animals. This observation can be explained by a rather poor oral bioavailability of the toxins, particularly in ruminant species. In contrast, cattle may be the most sensitive animal species regarding the antimicrobial effects of the other *P. roqueforti* toxins, such as penicillic acid, mycophenolic acid and PR toxin [13]. Their antimicrobial activity has been recognised immediately after identification of these toxins, and we recently confirmed these early findings [14]. Exposure of cattle to these toxins, which also exert local inflammatory responses, will result in ruminal dysbacterioses and ruminitis, as observed regularly as a consequence of exposure to mouldy silage.

Aspergillus fumigatus and its toxins

A. fumigatus is the second most prominent toxinogenic mould species in silage [15]. Its known mycotoxin spectrum comprises a variety of tremorgenic mycotoxins [16]. These groups of indole-derivatives including for example verrucologen, fumitremorgens, and penitrem A has been reported to induce tremor and other signs of neurotoxicity in laboratory

animal species and livestock, including sheep [17]. Again, the low oral bioavailability of these compounds might provide the explanation as to why these neurological symptoms are rarely seen under practical conditions (in contrast to intoxications with Lolitrems and ergovalins originated form endophytic *Neotyphodium* species).

A. fumigatus is a very common mould species, which can be found on decaying soils and plant materials. Exposure to *A. fumigatus* spores may result in fungal infections, particularly of organs exposed readily by external invasion such as the upper airways, the mammary gland and the uterus during partus. Antigens against *A. fumigatus* have been found in serum of cattle with experimentally and spontaneous systemic mycotic infections [18]. Exposure to *A. fumigatus* spores correlates with airway hypersentivity of horses, and systemic infections in cattle with bronchitis, mastitis and incidental abortion [19]. However, *A. fumigatus* can also produce toxins inside the tissues, as demonstrated in cattle, suffering from therapy-resistant mastitis and in humans with long aspergillosis caused by *A. fumigatus* [20].

Fusarium species and their toxins

Fusarium species have also been found on ensilaged products, especially in corn silage [21]. Investigating the possible consequences of *Fusarium* species, having a high prevalence in corn (maize) plants, it become evident that silage may not only contain fusariotoxins, already formed prior to harvest and being stable under the conditions created by ensiling, but the possibility of toxin production by moulds growing on the surface or in oxygen containing cavities cannot be excluded [22,23]. In particular, the toxins deoxynivalenol and zearalenone have been detected as fungal metabolites originating from pre- and post-harvest fungal growth [24]. Both toxins will exert detrimental effects, particularly in pigs. Deoxynivalenol affects the gastrointestinal tract, resulting in reduced nutrient absorption and growth retardation and also exerting a pro-inflammatory and immunosuppressive effect [25], whereas zearalenone is known to bind to estrogen receptors, thereby causing hyperestrogenism and impaired fertility [26]. The fore-stomach flora of ruminants will degrade the toxins, but this process is saturable depending on the competence of the ruminal flora.

In conclusion, low quality silage is susceptible to invasion by toxinogenic fungi, and toxins formed at the pre-harvest stage seem to remain largely intact during the ensiling process. Mould invasion will reduce the palatability and acceptance of ensiled feeds and induce in allergic reactions. Moreover, evidence is increasing that mycotoxins are also formed regularly under the conditions of ensiling, comprising an additional health risk. Among the biological properties of the toxins, their neurotoxic effects, but also the antimicrobial properties must be considered, particularly in ruminants, which traditionally consume large amounts of silage in the winter season.

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Effects of dietary mycotoxins on the quality of animal products and on human health

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A variety of crops are susceptible to fungal invasion and in turn might be contaminated with mycotoxins, originating from the secondary metabolism of moulds. It is estimated that 25% of the world's crop production is contaminated to some extend with these mycotoxins. Contamination of food and feed commodities with mycotoxins affect human and animal health. Exposure of humans may result directly from consumption of plant derived foods or may be a result of carry over of mycotoxins and their metabolites into edible animal products such as milk, meat and eggs, comprising the indirect route of human exposure. The following overview is based on the resultant of a desk study, initiated by the Dutch Product Board of Animal Feed with the aim to implement control measures for mycotoxins in the entire production chain.

Prevalence of mycotoxins in feed commodities

The most frequently found toxinogenic mould species in feed commodities belong to the genera *Aspergillus, Fusarium, Penicillium* and *Claviceps.* This may result in the presence of the following mycotoxins:

- Aflatoxins (Aflatoxin B1, B2, G1, G2), which have to be expected in tropical and subtropical products, such as oil seeds, nuts, corn and rice. At the same time, these products might be contaminated with cyclopiazonic acid.
- Ochratoxins (particularly ochratoxin A), which at least in Northern Europe has to be expected in grains, nuts, peas and sometimes in grass pellets. Ochratoxin A is known to occur also in grapes, wines and coffee beans. Contaminated commodities may also contain citrinin.
- Zearalenone is predominantly formed in corn (maize - entire plant) and has also been found in soybean, cereals and grains. It is worthwhile to mention, that zearalenone might be present in the plant material in its conjugated form, resulting in an underestimation of the degree of contamination.
- Deoxynivalenol, representing type B trichothecenes, is particularly found in wheat, but may also contaminate barley and corn, corn silage and corn cob mix and even hay. Next to DON, other type B trichothecenes such as nivalenol might be present in the same commodity. DON is also found in millers' by-products at various levels.
- T-2 toxin, representing Type A trichothecenes has been found in cereals and grains as well as straw and hay.
- Fumonisins (of which the major representative is Fumonisin B1) are predominantly found in corn (maize) and products thereof. At present there is no evidence that fumonisins occur in (North) European feed commodities, but they may be present in imported commodities, originating from regions with a subtropical climate.
- *Penicillium roqueforti* toxins can be found in ensilaged feeds due to the preference of this mould for an acidic environment. The high prevalence of *P. roqueforti* toxins in ensilaged feeds requires further investigations to determine the kinetics of toxin production.

Taking into consideration that all above-mentioned toxins are able to induce clinical signs of intoxication at high levels and are known to impair health and productivity of animals at lower levels, a monitoring programme for these most relevant mycotoxins (in Europe) should be considered. The design of monitoring programmes should take into account the biology of mould invasion and mycotoxin formation in specific feed commodities, as total monitoring of all feed commodities is virtually impossible.

Adverse effects impairing animal health and productivity

The detrimental effects of mycotoxins for production animals can be summarised as follows:

- Poultry. Poultry is very sensitive to aflatoxins (> 150 µg/kg). Problems caused by aflatoxins are scarce in the Netherlands, due to the implementation of EU regulation and thus the inspection of imported raw material.
  Poultry is sensitive to OTA (<500µg/kg), the major effect being a decrease in resistance towards infectious agents. The nephrotoxic effects of OTA result in dirty eggshells, caused by urine spots.
  Poultry is also very sensitive to group A thrichothecenes (T-2-toxin, < 400 µg/kg: DAS, < 500 µg/kg), but less sensitive to group B trichothecenes (DON, < 2,500 µg/kg; NIV, > 2,500 µg/kg). The first recognisable symptom of T-2-toxin exposure is the appearance of cheese-like necrotic-spots at the mucosal side of the bill. Furthermore, T-2 exposure results in reduced egg-production of layers and decreased hatchability of eggs.
  Poultry is not very sensitive to ZEN (> 25,000 µg/kg), FB (> 100,000 µg/kg) and other mycotoxins. However, the sub-clinical and zootechnical effects of a prolonged low-level exposure with mycotoxins to poultry remain to be investigated.
- Pigs. One of the most important mycotoxins causing serious problems in European pig production in recent years is the mycotoxin deoxynivalenol (DON). DON inhibits feed ingestion or even induces feed refusal thus affecting performance. Moreover, as member of the trichothecenene family, DON has a pro-inflammatory effect in the gastrointestinal-tract and increases sensitiveness to infections. Pure crystalline DON is clearly less toxic (> 4,000 µg/kg) than DON originating from natural contaminated raw material (> 1,000 µg/kg), in which most likely other trichothecenes are present at the same time. T-2 toxin, as mentioned above, may induce direct dermal toxicity following exposure to toxin-containing dust.

  Breeding sows and particularly gilts are extremely sensitive to the estrogenic effect of ZEN, which will cause a decrease of reproduction-results (< 180 µg/kg). In young piglets, discoloration and enlargement of nipples and vulva are early clinical signs of mycotoxin exposure.

  Pigs are sensitive to OTA, which primary causes kidney-damage. Clinical signs include polyuria and polydipsia followed by a decrease in feed intake. Furthermore, the immunosuppressive effects of OTA have been reported to increase in pigs the sensitivity towards infections.

  Exposure to ergot alkaloids (< 0.3 %) decreases the number of vital piglets and decrease litter size.

  Pigs are sensitive to fumonisins which may induce cardiomyopathia and pleural edema (PPE). However, due to the poor oral bioavailability of fumonisins, high toxin levels (> 25,000 µg/kg) are necessary to induce these clinical symptoms.

Animal health risks and exposure assessment

Based on the average and the maximum concentration of mycotoxins in raw feed materials, the actual exposure to mycotoxins of the different categories of farm animals can be calculated. The outcome of these calculations allow the following conclusions:

- The observed ZEN-concentrations in feeds are high enough to adversely affect health and reproduction of gilts and sows.
- The measured DON concentrations are sufficient to reduce performance in pigs.
- The other above mentioned mycotoxins, AFB, OTA, FB and T-2 + HT-2 occur less frequently in feed commodities or at low concentrations. Thus, adverse effects induced by theses toxins seem to be less significant in Western Europe.

Carry over

To estimate carry-over rates, the rate of absorption of mycotoxins from the gastrointestinal tract, its tissue distribution, metabolism and rate of excretion, need to be evaluated in an assessment of possible risks to public health resulting from the ingestion of animal-derived food commodities. In summary it can be stated that:

- Aflatoxins are generally well absorbed, rapidly metabolised (hepatic first pass effect) resulting in reactive epoxides, which are considered to be responsible for the DNA damage and cellular necrosis in the target animal. However, the carry-over into edible tissues is lower than the excretion of one of the aflatoxin B1 metabolites, aflatoxin M1, into milk. Aflatoxin M1 is of public relevance as it retains the mutagenic and genotoxic potential of aflatoxins. Due to low exposure rate towards aflatoxins in Europe (as the consequence of EU regulations), the risk of undesirable residues in edible tissues is negligible.
- Fumonisins are poorly absorbed from the gastrointestinal tract (ca. 1%). Following metabolism they are rapidly excreted. Thus, residues in edible tissues seem to be of no public health significance.
- Trichothecenes are well absorbed, but rapidly metabolised and excreted. No accumulation seems to take place in animal tissues and thus the public health risk related to residual amounts of trichothecenes in animal derived foods seems to be of no significance.
- Ochratoxin A, cyclopiazonic acid and citrinin bind to plasma-proteins once absorbed, resulting in accumulation and a prolonged residence time in the animal body. Total body clearance varies considerably between species. Moreover, accumulation of ochratoxin in kidneys and to a lesser extend in liver, has been reported, which resulted in regulations for pig organs and meat in some EU member states. However, it is generally accepted that the exposure of consumers towards OTA, CIT and CPA originates predominantly from plant-derived foods and only to a small percentage from animal derived foods.
- Zearalenone is well absorbed and rapidly metabolised in farm animals into two major metabolites, alfa-zearalenol and beta-zearalenol. Alfa-zearalenol formed by pigs has a higher, beta-zearalenol (formed as major metabolite in ruminants) a lower affinity towards oestrogen receptors. Little is known on the elimination constants of the metabolites in farm animals.

As to current knowledge, no clear indications were found that carry-over of mycotoxins to animal products poses a serious risk to human health. The contribution of animal products to human exposure by mycotoxins is much lower than that of plant products.
In conclusion, mycotoxins in animal feedingstuffs may pose a risk for animal health and performance but carry-over of mycotoxins to animal products should under modern agricultural practice not pose a human health hazard if proper control measures are in place, preferably based on HACCP principles.

Mycotoxins - concerns for their occurrence in pet foods

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The pet food industry is a rapidly growing aspect of the economy on a worldwide basis and it is projected that sales of dog and cat food by the year 2010 will reach $41.3 billion. Incorporated ingredients in pet food often include milled products of corn and wheat. Both can be major sources of mycotoxin contamination. Of primary concern, relative to these toxins, are the aflatoxins and deoxynivalenol while other mycotoxins such as zearalenone, ochratoxin, fumonisins, ergot alkaloids, and penitrem are either potentially problematic or suspected causes of minor outbreaks of disease in pet animals. Although good tests are available for mycotoxins in the major commodities processed for pet food, some outbreaks still have occurred in the United States and South Africa resulting in recalls of the product.

Additional concerns are based on the following:

- dogs will seek out certain mould-contaminated products and ingest them;
- certain mycotoxins that are immunosuppressive and cause reproductive problems can occur in pet food;
- mycotoxins are suspected as causative agents in undiagnosed health problems in pet animals;
- commodities known to be sources of mycotoxins (developed in the field as unavoidable contaminants) are used as major ingredients in pet food;
- pet food manufacturing is a growing, major industry worldwide;
- pet food manufacturers have spent billions of dollars on brand recognition and want to protect their brand name;
- litigation relative to mycotoxins is costly and time consuming;
- a need to comply with regulations within a country;
- regulations are not uniform worldwide and some pet food manufacturers may not have sufficient in-house testing and regulations; and
- there is growth in consumer awareness regarding mycotoxins.

Even though there are occasional outbreaks of mycotoxicoses from pet food, because of our concerns, pet food is less likely now to be contaminated with mycotoxins than in previous times. Improvements can still be made by use of adequate testing involved in a total quality assurance program for mycotoxins integrated into the pet food manufacturer’s overall quality assurance program. This is the key to avoiding future outbreaks of mycotoxicoses in pet animals and product recalls.

The use of a dynamic *in vitro* model of the gastrointestinal tract in studying mycotoxin adsorbents

Evelijn E. Zeijdner and Robert Havenaar

TNO Nutrition and Food Research, the Netherlands

Improvement of mycotoxin binding adsorbents is an important issue for the feed industry. For assessment of the efficacy and specificity of mycotoxin adsorbents and subsequently to inhibit the bio-accessibility of mycotoxins in the gut, there is a great need for laboratory models with a high predictive value. The reason is simple: it is not possible to perform studies in animals with all varieties of adsorbents in different types of feed matrices. However, the question is: what is the relevance of the results from these models in comparison to the situation in animals? This paper describes a dynamic, computer-controlled system that mimics the successive kinetic events in the gastrointestinal tract (GI tract) and has been validated in comparison to *in vivo* studies. How TIM is applied successfuly in testing the efficacy of mycotoxin binders, will be presented by Dr. Guiseppina Avantaggiato.

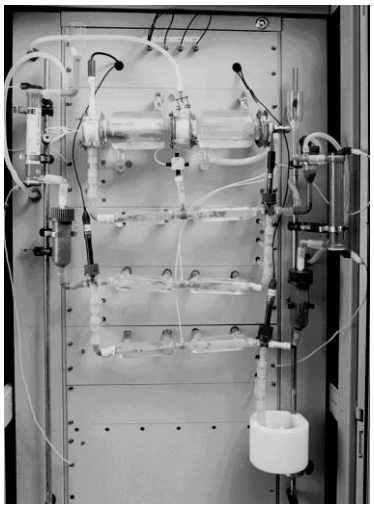

Figure 1. Picture of the TNO dynamic model of the stomach and small intestine (TIM-1).

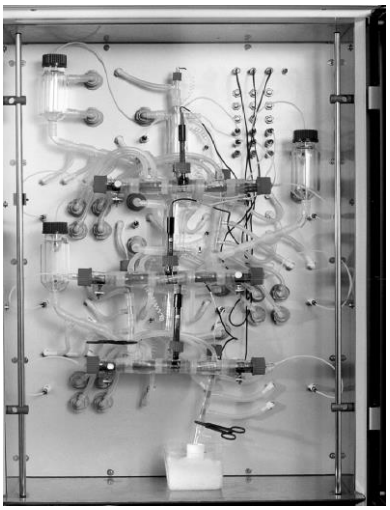

Figure 2. Picture of the TNO dynamic model of the large intestine (TIM-2).

TNO gastrointestinal models (TIM)

At our institute, a multicompartmental, dynamic, computer-controlled model (TIM) has been developed, which closely simulates the *in vivo* conditions of the stomach and small intestine (TIM-1; Figure 1) and of the large intestine (TIM-2; Figure 2) of humans (babies, young adults or elderly) and monogastric animals (dogs, pigs and calves) in relation to their feeding states and type of ingested food/feed. The models simulate the peristaltic movements in the GI tract, mixing and moving the contents gradually through the stomach and the intestines. This allows mimicking the exact gastric emptying and intestinal transit times that are observed in humans and monogastric animals for various types of food and feed products. Simulated salivary, gastric, biliary, and pancreatic secretions are introduced into the corresponding compartments using computer-controlled pumps. In this way physiological pH values, concentrations of electrolytes, activities of digestive enzymes, essential for the digestion of food, as well as the concentration of bile, for emulsifying the fat particles and the production of micelles, are simulated throughout the GI tract. The large intestinal model (Figure 2) includes a complex, metabolic active micro biota of human or animal origin. During the digestion process the absorption of water, digested food compounds, solubilised (active) compounds and microbial metabolites, takes place from the consecutive intestinal

compartments of the model. For this purpose hollow-fibre membrane systems are hooked up at the jejunum and ileum compartment, respectively, or placed inside the large intestinal compartment.

Physiological relevance

Compared to other *in vitro* models of the GI tract, TIM has specific advantages. Unique for these models is that it is a dynamic system. This means that the successive physiological conditions in the stomach and segments of the intestines of humans and animals are closely simulated. Examples are: the gastric emptying patterns, gastrointestinal transit times in combination with the changing pH values, and concentrations of electrolytes, enzymes and bile salts during passage of the food through the GI tract. Nevertheless, it is good to realise the limitations. There are no real feed back mechanisms, no mucosal cells inside the model, and there is no immune system. To overcome the absence mucosal cells, we use cultured monolayers of intestinal mucosal cells on membranes and intestinal segments in combination with TIM.

The strength of these laboratory models is that the experiments can be performed under strictly controlled conditions, resulting in a high repeatability for a series of products, in contrast to human and animal studies, which show large biological variations. Besides average physiological situations, more extreme conditions can be mimicked as well. For example individuals producing too much gastric acid, or too little gastric or pancreatic enzymes, or a fast passage time. To perform *in vivo* studies under these conditions, it is necessary to turn to specific patient groups, which is often very complicated and leading to ethical constrains.

Validation and application in agro-feed area

To ensure that the results obtained in TIM are relevant for the human or animal situation, extensive validation studies, related to several areas of food research, have been performed. For these validation studies *in vivo* data are used from previous and ongoing experiments with humans and animals carried out in our Institute and in other institutes. We describe below some of these validation and application studies which are of special interest for novel agricultural products and post-harvest processing methods.

Digestion of foods and availability for absorption of nutrients
Validation experiments related to the digestibility of various food products for humans, dogs, pigs, and calves were carried out simulating the average physiological conditions found for humans and for the animal species involved. The results of protein digestion experiments showed that the digestibility coefficients for these products had a strong correlation with the digestibility data obtained from ileal fistulated pigs and calves (Figure 3) and for dogs. The digestion of carbohydrates, such as starches, can be followed in the model by the intestinal absorption of glucose units via the membrane systems. The glucose concentration curves inthe absorbed fluids showed a good correlation with plasma glucose levels of diabetic humans after the intake of starches in similar products (Figure 4).

The model has also been validated for the availability for absorption of phosphorus, calcium, magnesium, and iron from different types of foods and food supplements, in comparison with literature data from humans and fistulated pigs. The experiments were also aimed on the release of minerals from the phytate (inositol phosphate) complex by the enzyme phytase from natural origin in cereals and from fungal origin as pig feed additive. Phytase significantly increased the intestinal absorption of iron and phosphorus, which was in good agreement with reported *in vivo* results.

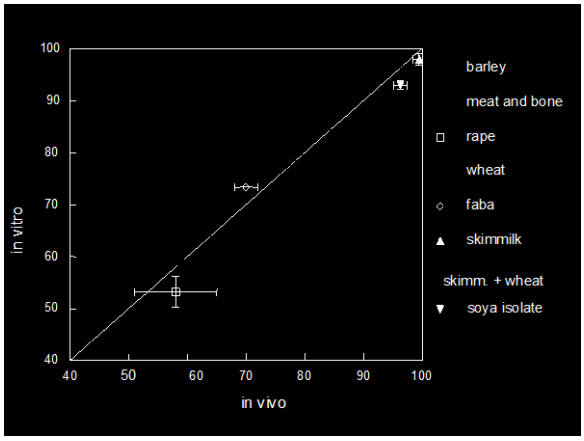

Figure 3. Correlation between *in vitro* and *in vivo* digestibility of different types of proteins.

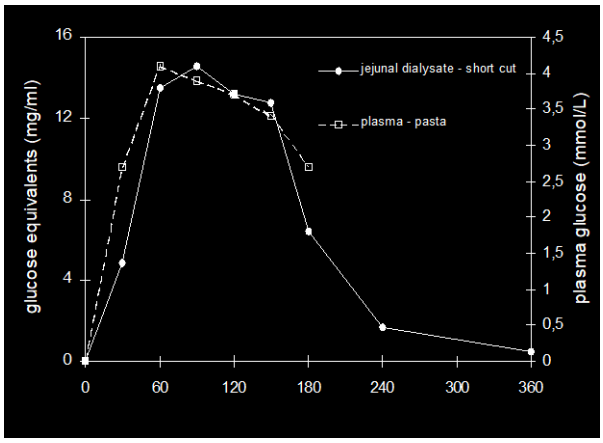

Figure 4. Glucose concentrations in dialysate of TIM-1 (solid lines) and in plasma of humans (dotted lines) after the intake of pasta.

Functional foods and bio-active compounds

With respect to functional foods, such as probiotics, the systems are used to study the stability and functionality of beneficial bacteria versus pathogens. The survival of probiotic lactic acid bacteria in the TIM systems showed a good similarity with human trials. Application of these models in pharmaceutical research is validated in comparison to human studies with several active compounds (such as acetaminophen, cephalosporines) and drug delivery systems (such as controlled release, immediate release). In a project on alternatives for laboratory animals we studied the availability for absorption of mutagenic compounds and the interaction with anti-mutagenic food ingredients. These studies demonstrate the predictive value of these GI tract models in relation to functional foods and pharmaceuticals, whereas they can be used as tool for selection and therewith speed up the development of the product.

Fermentation of dietary fibres

Dietary fibres from various cereals and crops used as functional ingredient for improved bowel functions. These dietary fibres are partly- or non-digestible in the stomach and small intestine and thus reach the large intestine where they are available as substrate for the intestinal micro biota. The GI tract model is used to investigate these fibres in novel foods in relation to their stability or digestibility as well as to their rate of fermentation by the human (or animal) intestinal micro biota. During short term fermentation studies in the large intestinal models we analyse the production of short chain fatty acids, carbon dioxide, hydrogen, and methane. In long-term experiments, during prolonged intake of a substrate, we can study the adaptation of the microorganisms, which can result in a faster fermentation by enzyme induction, and/or in a shift in the composition of the micro biota. This can induce, for example, increased numbers of beneficial bacteria and inhibition of potential pathogens, or shifts in the production of microbial enzymes, such as glucuronidase, glucosidase and azoreductase, ammonia, or off-flavour compounds.

Safety evaluation of novel and genetically modified foods

The GI tract models are used for the safety evaluation of (novel) foods and food ingredients. An example is a TNO project on food allergy, focussed on existing food proteins with known allergenic potential and on novel proteins, whether or not derived by gene-technology in plants. In TIM, simulating the GI conditions of babies, children and adults, we study the resistance of these proteins against the proteolytic enzymes in the stomach and first part of the small intestine. In general, potential food allergens are more resistant against digestion

than non-allergenic proteins, possibly also depending on the maturation of the digestive tract. By isolating and studying the polypeptide fragments by protein- and immuno-blots, we try to predict the allergenic potential of novel proteins and peptides.

In relation to genetically modified foods (GM food, e.g. cereals, crops) or ingredients (e.g. GM enzymes, proteins) we investigate gene stability or the risk of gene transfer. For example the stability of naked DNA during passage through the stomach and intestine, and the transfer of genes from the GM food (e.g. antibiotic resistance markers) into the microorganisms present in the large intestine.

Conclusions

There is a growing market for foods with claims of high nutritional quality and specific health benefits. The availability of a validated laboratory model without ethical constraints makes it possible to apply tests on the nutritional quality of (novel) foods and the efficacy of functional foods and functional additives, such as mycotoxin binders, from an early stage of the development of the product and process. In the presented dynamic, computer-controlled model (TIM) the main physiological aspects of the real-life GI tract are simulated. Validation studies for a broad range of applications showed the value of this model in relation to extrapolation of the results to the human or animal situation. TIM can be used successfully for various scientific research topics as well as to assess the nutritional quality and safety of (novel, GM) foods and ingredients, and to test the efficacy new food processing methods on the quality and safety of foods, before a final product is put onto the market.

References

A list of references in relation to the presented items can be provided on request.

The efficacy of mycotoxin binders proven with a dynamic *in vitro* model of the gastrointestinal tract*

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Removing mycotoxins from contaminated feedstuffs is of great interest in animal nutrition and there is an increasing demand for effective decontamination methods. The addition to the diet of some indigestible adsorbent materials that tightly bind mycotoxins in the gastrointestinal (GI) tract is one of the practical methods to detoxify mycotoxins. A wide range of inorganic materials has been tested *in vitro* for mycotoxin inactivation and several commercially available products are used in the livestock industry as mycotoxin binders. However most of these materials show different affinities for different groups of mycotoxins and/or nutritional factors, and their arbitrary inclusion in animal feeds could negatively affect animal health. Moreover, a large number of adsorbent materials have been tested only *in vitro*. Several *in vitro* methods have been used to assess toxin binding to clay minerals and do not consider the complexity of the gastrointestinal (GI) tract with respect to the bioavailability of the toxin. *In vivo* adsorption experiments are complicated by physiological variables and the composition of feed, requiring further investigations to assess properly the efficacy of mycotoxin binders. The development of rapid and reliable pre-screen methods for testing adsorbent/mycotoxin combinations alternatively to animal experiments is highly desirable. Such an approach, consisting in the use of a laboratory model which mimics metabolic processes of the GI-tract, is presented herein.

A GI-model simulating the GI conditions of healthy pigs was used to evaluate, in both jejunal and ileal compartments, the intestinal absorption of zearalenone (ZEA) and the efficacy of activated carbon and cholestyramine in reducing toxin absorption. Zearalenone contaminated wheat (4.1 mg/kg) was fed to the GI-model, as such or with the inclusion of these adsorbent materials at different levels. During 6 h digestion, almost all the toxin released from the food matrix, corresponding to 32% of the ZEA intake (247 µg), was rapidly absorbed. Zearalenone absorption occurred mainly in the jejunal compartment (22%), while 10% absorption occurred in the ileal compartment. A significant reduction of ZEA absorption was found after inclusion of activated carbon or cholestyramine, with a more pronounced effect exhibited by activated carbon. In particular, ZEA absorption was reduced by 84% and 50%, respectively, when 2% activated carbon or cholestyramine was added to the meal. In the jejunal and ileal compartments, these materials behaved similarly in reducing ZEA absorption. No delay was observed in the transit of both adsorbents with respect to the transit of the meal through the GI-tract of the model, as the strong sequestering effect of 2% of activated carbon or cholestyramine occurred already during the first two hours and was persistent for the rest of the experiment. Among the four inclusion levels tested in the study (0.25%, 0.5%, 1% and 2%, w/w), the maximum effect in avoiding the intestinal absorption of ZEA was reached with 1% of activated carbon and 2% of cholestyramine. It can be concluded that, activated carbon and cholestyramine are good candidates for ZEA detoxification by inducing the formation of ZEA-adsorbent complexes that avoid the gastrointestinal absorption of this toxin. This is the first time that a laboratory GI-model has been used to study the effectiveness of adsorbent materials in reducing the intestinal absorption of mycotoxins. The GI-model represents a reliable tool that can be used in alternative to animal experiments to select the most promising mycotoxin binder.

* Acknowledgments: This work was supported by the European Commission, contract QLK1-CT-1999-996: Hazard analysis control of food contamination: prevention of Fusarium toxins entering the human and animal food chain.

Mycotoxin formation on inundated area*

Monique de Nijs[1], K.A. van Hoeij-de Boer[1], W.A. van Osenbruggen[1] and J.E.J. Kamps[2]

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In the near future, global warming will, most probably, result in increased precipitation in the upstream areas of the rivers in the delta of the Netherlands. As a consequence, an increase in water flow and increased number and longer peak periods of rainwater drainage can be expected. The current system of water drainage in the Netherlands is not up to this challenge. Global warming will also result in rise of the sea water level, making drainage even more difficult. There is, therefore, an increased demand for retention areas to prevent flooding of residential areas. This means that designated areas will intentionally be flooded in case of peakflows. In addition to the traditional areas of washlands among the rivers, these areas involve the new designated 'emergency-polders' (grassland areas). The effects of inundation on the quality of grasslands in the emergency-polders and thus on animal health are currently unknown. One of the concerns is that inundation of the grasslands may result in explosive growth of *Fusarium* moulds and subsequently in mycotoxin production in the grass. This might harm the health of animals in case the grass is used for fodder or silage. A study was carried out to estimate the risks of inundation of grasslands in regard to mycotoxin formation. Published literature data were used and experts in the field of mycotoxins were interviewed.

Grasses can be infected by endophytic moulds or by moulds occurring in the soil. Endophytic moulds grow in symbiosis with the plant and play a role in the defence of the plant against insects, parasites and other infections. Most endophytic moulds are found in grasses in tropical arid areas such as *Lolium perenne* L. (Perennial Ryegrass) and *Festuca arundinacea* Shreb. (Tall Fescue grass). The symbiotic moulds living on these grasses can form large amounts of mycotoxins when stress occurs, usually at periods of cold stress. Animals feeding on these grasses can develop 'Fescue foot' or 'Ryegrass staggering'. Although these grasses gain much from symbiosis with these moulds when grown in tropical arid areas, the same grasses can grow well in the temperate climate zone without the symbiotic moulds. Soil moulds are always present. The amount depends on the soil type, previous plants and humidity. Moulds infect the plants in periods of stress, such as i) mechanic damage, ii) draught or high humidity, or iii) high nitrogen content, iv) use of herbicides and v) short mowing or grazing. Mycotoxins produced by *Fusarium* that can be expected in grasses in the Netherlands are zearalenone (ZEA) and the trichothecenes T-2 toxin and deoxynivalenol (DON). The mycotoxins tend to accumulate in the capitula (flower heads) more than in the leafs.

Washlands have been inundated for centuries in the Netherlands. No reports linking animal health problems to mycotoxins in the grasses are available. There are several reasons: i) grasses occurring in the washlands are adapted to the inundation, ii) inundation only took place in winter, iii) there is no use of fertiliser in the washlands and iv) the soiltype allows good drainage after inundation. However, emergency-polders can differ from the traditional washlands at various points posing a serious risk for mycotoxin formation. It was concluded that excess formation of mycotoxins can take place in grassland after inundation. However, the use of relevant culture management will minimise the risks. This will be discussed in the presentation.

* The work is carried out by order of RIZA in the project 'Onderzoek naar risico voor het ontstaan van mycotoxinen in geïnundeerd grasland', RI-3723. The authors are indebted to Mr. Ing. J.E.J. Kamps for his advice.

Mycotoxin research progress and future prospects

Naresh Magan

Cranfield University, UK

At the present time we are examining the critical control points in the food production chain as part of a prevention strategy to minimise the contamination of food raw materials with mycotoxins. For some mycotoxins more a significant amount of knowledge is already available. For others, e.g. grapes and wine, less is known. In the next 5-10 years we will have to consolidate and move forward from the solid and not insignificant advances already made or being made in a range of complimentary areas.

Regulation and EU directives have been the main driver in finding methods for the detection and control of entry of mycotoxins into the food chain. Looking to the future there are areas where significant advances need to be made for enabling prevention strategies to be implemented. These include:

- use of genomics and proteomics approaches to develop resistance to the fungi or to
- switch off production of mycotoxins;
- rapid and at/on line diagnostics relevant to EU limits for mycotoxins need to be produced and be cost-effective;
- the toxic effects of low concentrations of mixtures of mycotoxins is urgently needed for more accurate TDI values for specific mycotoxins to be obtained;
- are there emerging and new secondary metabolites produced by spoilage fungi which may be mycotoxins;
- decision support systems pre- and post-harvest are required including online expert systems for predicting risk from contamination on a regional and area specific level;
- effective production of toxin-free animal feeds are also necessary especially where mixed tropical and temperate raw materials are used for production.

The question arises as to how these questions can be effectively answered. We need close collaboration between research scientists in academia and institutes and industry to enable these challenges to be met in the medium term.

SPOTLIGHT PRESENTATIONS

*In vivo d*emonstrations of the efficacy of a polymeric glucomannan mycotoxin adsorbent in prevention of *Fusarium* mycotoxicoses

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A series of experiments have been conducted to determine the efficacy of a polymeric glucomannan mycotoxin adsorbent (GMA) in preventing *Fusarium* mycotoxicoses in broiler chickens, laying hens, starter pigs and mature horses. The source of mycotoxins in each experiment was a blend of naturally contaminated corn and wheat which was obtained from farms in southern Ontario, Canada. The finished feeds were analysed and found to contain measurable amounts of deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), zearalenone (Z) and fusaric acid (FA). The following compounds were determined to be below the limits of detection: 1) 3-acetyldeoxynivalenol, 2) nivalenol, 3) T-2 toxin, 4) iso T-2 toxin, 5) acetyl T-2 toxin, 6) HT-2 toxin, 7) T-2 triol, 8) T-2 tetraol, 9) fusarenon-X, 10) diacetoxyscirpenol, 11) scirpentriol, 12) 15-acetoxyscirpentriol, 13) neosolaniol, 14) zearalenol, 15) fumonisin, 16) aflatoxin B1, 17) aflatoxin B2, 18) aflatoxin G1, and 19) aflatoxin G2.

In several experiments, broiler chicks were fed varying levels of contaminated grains with and without 0.2% GMA for 56 days. Weight gains, feed intake, blood metabolites, immunoglobulin concentrations, brain neurochemical concentrations and breast meat discoloration were monitored. Laying hens were fed similar diets for three months. Feed consumption, blood metabolites, immune status, egg production and egg and eggshell quality were measured. Starter pigs (initial body weight 10 kg) were fed contaminated diets for 21 days. Growth rates, feed consumption, brain neurochemical concentrations, blood metabolites and immune status were monitored. In an equine study, mature, non-exercising mares were fed contaminated grains in the concentrate portion of the diet for three weeks. Feed intake, body weight and blood parameters were monitored weekly.

The feeding of contaminated grains significantly depressed broiler growth rates and feed intake in the grower phase of production. The growth of more slowly growing birds was reduced only in the finisher phase. The feeding of contaminated grains also elevated blood concentrations of haemoglobin, red blood cells and uric acid. There was also a significant increase in breast meat discoloration. Biliary IgA concentration was significantly reduced. All these changes were prevented by the feeding of 0.2% GMA.

The feeding of contaminated grains to laying hens resulted in significantly less feed consumption, egg production, egg weight and eggshell weight. Blood uric acid concentrations were significantly increased. These effects were prevented by the feeding of 0.2% GMA.

It was observed that starter pigs fed contaminated grains had elevated hypothalamic ratios of 5-hydroxyindoleacetic acid (5-HIAA) : serotonin (5-HT) and reduced concentrations of dopamine. Elevated 5-HIAA : 5-HT and reduced concentrations of norepinephrine were observed in pons. Such changes are associated with reduced feed intake, lethargy and reduced blood pressure. The feeding of GMA prevented the neurochemical changes.

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Horses fed contaminated grains in the concentrate portion of the diet significantly reduced their consumption of concentrate. The inclusion of 0.2% GMA significantly increased concentrate consumption but not to control levels. Blood activities of gamma-glutamylamino transferase were significantly increased after one and two weeks of feeding contaminated grains. Differences were not significant after three weeks of feeding. Enzyme activities in blood from horses fed GMA did not differ from controls.

It was concluded that many of the behavioural and metabolic indices characteristic of *Fusarium* mycotoxicoses in broiler chickens, laying hens, starter pigs and mature horses can be prevented by the appropriate use of a polymeric glucomannan mycotoxin adsorbent. Efficacy will depend on the degree of challenge posed by the contaminated materials and the dietary level of inclusion of the adsorbent.

Neogen Corporation: developing and marketing products dedicated to food and animal safety

Mohamed M. Abouzied* and Pierre Belhadj

Neogen Corporation, USA

Neogen Corporation (located in Lansing, Michigan, USA; www.neogen.com) develops and markets products dedicated to food and animal safety. The company's Food Safety Division markets dehydrated culture media, and diagnostic test kits to detect foodborne bacteria, natural toxins, genetic modifications, food allergens, drug residues, plant diseases and sanitation concerns. Neogen's Animal Safety Division markets a complete line of diagnostics, veterinary instruments, veterinary pharmaceuticals and nutritional supplements.

For natural toxins, Neogen Corporation developed and markets sensitive, easy to use and inexpensive immunoassay tests for the following natural toxins: deoxynivalenol (DON), aflatoxins, ochratoxin, zearalenone, T-2 toxin, fumonisin and histamine. Results can be obtained in less than 5 minutes with detection limits less than 1 part per billion (ppb).

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Charm Sciences aflatoxin M1 and B1 assays: rapid, quantitative, low-cost results

Annie Baumgartner*

Charm Sciences, USA

Charm Sciences has been the leader in food safety technology for 25 years.

The Charm II is a comprehensive system for detecting antibiotics, aflatoxins and other contaminants in food products, with assays for aflatoxin M1 and B1. Aflatoxin B1 can be detected as low as 2 ppb to 40 ppb in feed and grain, or 20 ppb in nuts and legume beans. The Charm II aflatoxin M1 assay for milk detects as low as 25 ppt. The tests are rapid (15-30 minutes), quantitative, and low in cost.

The Charm ROSA (Rapid One Step Assay) tests are lateral flow based strip tests, which use minimal equipment (incubator) and can be read either visually or with a reader. The tests are portable, field applicable, and have detection levels designed to match either European or US action levels. The ROSA tests are at the forefront of aflatoxin testing technology, giving results in 15 minutes. Aflatoxin B1 in feed and grain samples (peanuts, peanut butter, corn, rice, oats and other grains) can be detected as low as 2 ppb with the ROSA test. The ROSA MRL aflatoxin test detects aflatoxin M1 in milk as positive approximately 80% of the time at 50 ppt, or can be as sensitive as 10 ppt with a precolumn and extended assay.

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A new quantitative detection kit to monitor *Fusarium* in cereals

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Fusarium head scab is caused by a complex of species and has devastating effects on yield and quality. We developed a diagnostic multiplex PCR method to detect *Fusarium avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae*, *F. proliferatum*, *Microdochium nivale* var. *majus* and *M. nivale* var. *nivale*, which are among the most common species of *Fusarium* occurring on wheat in Western Europe. We used this qualitative multiplex kit to survey *Fusarium* in 40 wheat fields during 2000 and 2001. Up to 400 *Fusarium* isolates were unambiguously identified resulting in the conclusion that *F. graminearum* is the major constituent of the *Fusarium* species complex on wheat in the Netherlands, which is in sharp contrast with earlier surveys, when *F. culmorum* was the most predominant species. However, drawbacks of a qualitative multiplex kit are the requirement of pure fungal cultures and it cannot be used for monitoring purposes due to the necessary vast numbers of isolates for statistical analyses.

We therefore developed a quantitative *Fusarium* detection kit that gives in to these disadvantages and that can be applied in a high throughput way. The kit is based on the TaqmaN® technology and can be used on randomly sampled plant material throughout the season. We will present data on the application of the kit during the growing season showing the different population dynamics of the constituent species in the *Fusarium* population infecting wheat. The correlation with the expression of the *tri5* gene, encoding trichodiene synthase and DON content in harvested product, as determined by HPLC and ELISA will be also discussed. Hence, using the kit enables monitoring of relative frequencies of *Fusarium* sub-populations in the field, without the cumbersome isolation of hundreds of individual isolates for appropriate statistical analyses. It is therefore a new tool for the evaluation of the effect of crop management strategies on *Fusarium* in the field.

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Analysis of deoxynivalenol by DONprep immunoaffinity column

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Deoxynivalenol, also known as vomitoxin or DON is the most reported mycotoxin after aflatoxin (CAST Mycotoxin report 2003); it occurs at high levels with high incidence in a range of cereals and cereal products. DON is believed to cause acute gastrointestinal illness, vomiting and throat irritation in humans and feed refusal, vomiting and reduced reproductive efficiency in a range of farm animals.

According to the latest CAST mycotoxin survey, the annual cost of DON contamination of cereal crops in the USA alone is over $650M. Guideline levels are in place for food or feed commodities in (amongst others) the US and the EU. In addition Germany has recently proposed national limits for DON in a range of food commodities including infant food. Therefore key commodities of interest are cereals, cereal products, baby food and animal feed.

R-Biopharm Rhône (RBR), part of the R-Biopharm AG group (Darmstadt, Germany), manufactures immunoaffinity columns, card tests and ELISAs for analysis of mycotoxins in food and feed. The most recent development is DONprep, an immunoaffinity column, based on a monoclonal antibody for DON, bound to sepharose beads and contained in a wide bodied 3ml polypropylene column.

RBR have conducted substantial research and development work as well as external customer trials with DONprep on a variety of commodities of interest; with a view to developing suitable methods giving high recovery and reliable statistical performance. Some examples of results achieved using DONprep with “difficult” samples are included in the presentation; for example multiple analysis of a BCR reference material by a customer gave mean recoveries of 92% and a CV of 2.1%. Analysis of poultry feed contaminated at 5 ppm gave mean recoveries of 92% and analysis of a variety of baby food (including those containing chocolate, yoghurt and fruit) contaminated at 50 ppb gave mean recoveries of over 85%. The use of high amounts of monoclonal antibody ensures excellent chromatography; as well as accurate results at high levels of contamination, which are especially common in animal feed.

In conclusion, the use of DONprep immunoafffinity columns gives a number of benefits to the user; high recoveries for a range of relevant commodities - including “difficult” samples, good statistical reliability from external triallists, the possibility to use extraction solvents other than water if required and rapid sample throughput thanks to the large format immunoaffinity column.

More information on DONprep will be presented at this presentation and the product is also the subject of two posters at this Forum.

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Romer Labs® New AgraQuant® ELISA line for the detection of mycotoxins

Johann Binder*

Romer Labs®, Germany

The new AgraQuant® ELISA line further expands Romer Labs®´ expertise in mycotoxin detection. This new AgraQuant® ELISA line completes Romer Labs®´ extensive service and product portfolio for the detection of mycotoxins.

A brief overview providing information about the range of mycotoxins covered, the quantification range and data about cross reactivities will be followed by the presentation of results of a recently finalised comprehensive validation study for total aflatoxin and ochratoxin. Both validation studies followed GIPSA/FDA and/or AOAC standards and will subsequently allow submission for approvals. Excellent data prove the outstanding accuracy, precision, ruggedness and stability of these testkits. The testkits are validated for a broad range of relevant commodities. They were specifically designed to obtain reliable quantitative results. The 15 to 20 min incubation time was selected carefully and does not constitute a compromise between incubation time and quality of quantitative results.

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Recent applications of immunoaffinity columns in the analysis of mycotoxins

Nancy Zabe*

VICAM, USA

Three new VICAM immunoaffinity columns and one new method are available for the determination of mycotoxins. Two of the new immunoaffinity columns are wide bore columns (3 ml total column volume) for the determination of aflatoxin (AflaTest WB) and zearalenone (ZearalaTest WB). The third column is the AflaOchraZea column for the simultaneous determination of aflatoxin, ochratoxin and zearalenone using one sample preparation and one HPLC run. Also, a new HPLC method for the determination of T2 using immunoaffinity column clean-up will be presented.

The AflaTest WB columns have a range of 0 to 100μg/kg total aflatoxins, recoveries between 76% and 121%, limit of detection of 0.03 to 0.11μg/kg for the individual aflatoxins and RSD of less than 7%. The ZearalaTest WB columns have a range of 0 to 5000μg/kg, recoveries between 102 and 121%, limit of detection of 1.9μg/kg and RSD of less than 5%. The T2 HPLC method has a range of 0 to 1.5mg/kg, recoveries between 80 and 99%, limit of detection 0.005mg/kg and RSD of less than 6%. In addition, data will be presented on the AflaOchraZea column.

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P1

A very sensitive (less than 10 ppb) ELISA test for the detection and quantitation of the mycotoxin deoxynivalenol (DON) in food and feed samples

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A very sensitive monoclonal antibody-based enzyme linked immunosorbent assay (ELISA) test to detect and quantitate the trichothecene mycotoxin deoxynivalenol (DON) in food and feed commodities was developed. A novel chemistry procedure was used to prepare DON-protein conjugate to use as immunogen and to prepare DON-horseradish peroxidase (DON-HRP) as the labelled antigen in the ELISA test. The assay is a competitive direct ELISA in a microwell format. Free DON in the sample and controls will be allowed to compete with enzyme-labelled DON for the antibody binding sites. The absorbance signal generated by substrate reaction with the bound enzyme is inversely proportional to DON concentrations. The detection limit of the assay is 0.9 ng/ml of DON (4.5 ppb). Concentration of DON, 3-acetyl-DON, 15-acetyl-DON, fusarenon-x, nivalenol and T-2 toxin that required for 50% binding inhibition is 12, 18, 180, 5500, 650 and 4500 ng/ml, respectively. The antibody used is very specific for DON. The cross-reactivity of 3-acetyl-DON, 15-acetyl-DON, fusarenon-x, nivalenol and T-2 toxin were 67, 7, 0.2, 1.8 and 0.3 %, respectively. Samples are extracted by shaking for 5 minutes with water and the extract filtrate used in the ELISA test. The assay can be used to quantitate DON in corn, wheat, barley, malted barley, rice, baby food and feed. The assay can be used to quantitate DON in samples within 20 minutes (two 10-minute incubation steps). This test is very useful for testing baby food to insure the absence of DON at very low levels that other available methods can not detect.

P2

Performances of a new immunoassay for the detection of deoxynivalenol in cereals and sub-products

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Deoxynivalenol (DON) is a major mycotoxin contaminant found in various cereals all over the world. In order to quickly screen for DON contamination in cereals, we have developed a new method, Transia Plate DON, combining a water-extraction step followed by an ELISA detection. The new immunoassay covers a range from 0.5 to 6 ppm and offers a limit of detection lower than 0.5 ppm. The total assay time is 15 min and the complete analytical time takes less than 30 min. Several cereals or beans (such as corn, wheat, oat, barley, mixture of cereals, horse bean and sunflower) were spiked with amounts of DON between 0.5 and 2 ppm: most of the recoveries were between 80 and 120%, and none false positive result was noted with the matrices not spiked. Beer sample was also analysed with spiking level of 0.5,1 and 2 ppm DON and showed weaker recoveries (from 65 to 74%) compared to cereals.

This new immunoassay represents an efficient semi-quantitative method for a quick and easy screening of cereals and other sub-products.

P3

A sensitive and fast ELISA method for screening for the presence of the aflatoxins B1/B2 in biological matrices

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Aflatoxins are toxic compounds produced by moulds of the *Aspergillus* spp. Aflatoxins occur in food and feed products derived from tropical and sub-tropical areas. The most pronounced contaminations have been encountered in cereals, rice, maize, soy, tree nuts and peanuts. Aflatoxins are related to the occurrence of primary liver carcinomas and particular tumours in lungs, breasts and the gastro-intestinal tract. Aflatoxin B1 and B2 are the most common aflatoxins followed by the aflatoxins G1 and G2. In lactating mammals B1 and B2 can be converted into M1 and M2. The concentration of M1/M2 in milk is approximately 2% of the amount of B1/B2 ingested. If the aflatoxins G1/G2 are present than also the aflatoxins B1/B2 are present, but in a higher concentration. For this reason Euro-Diagnostica has developed and validated a sensitive ELISA method for screening for the presence of aflatoxins B1/B2 in food and feed samples.

Principle

The microtiter plate based ELISA kit consists of 12 strips, each 8 wells, precoated with rabbit antibodies to mouse IgG. Specific antibodies (mouse anti-aflatoxin), horseradish peroxidase labelled aflatoxin (enzyme conjugate) as well as aflatoxin B1 standard solution or samples are pipetted into the precoated wells followed by a single incubation step. The specific antibodies are bound by the immobilised rabbit anti-mouse antibodies and simultaneously free aflatoxins (in the standard solution or in the sample) and enzyme labelled aflatoxin compete for the specific antibody binding sites (competitive enzyme immunoassay). After an incubation time of one hour, the non-bound (enzyme labelled) reagents are removed in a washing step. The amount of bound enzyme conjugate is visualised by the addition of substrate/chromogen (peroxide/tetramethylbenzidine, TMB). During the incubation the colourless chromogen is converted by the enzyme into a blue reaction product. This blue colour is inversely proportional to the amount of bound aflatoxin. The more aflatoxin is present in the standard solution or sample, the less colour is developed. The substrate reaction is stopped by the addition of sulphuric acid. In the acidic environment the blue colour changes into a yellow colour. The colour intensity is measured photometrically at 450 nm.

Sample treatment

Approximately 50-100 g of sample is grinded and pulverised into a fine powder. An amount of 3 g of grinded sample is extracted with 9 ml of 80% methanol in distilled water. After thoroughly shaking at room temperature for several minutes, the sample is centrifuged (10 min, 2000 g) or filtrated. An aliquot of 100:l of the supernatant or filtrate is diluted with 300:l of dilution buffer to obtain a solution containing 20% methanol. An aliquot of 50:l is used in the ELISA test.

Specificity and sensitivity

The aflatoxin B1/B2 ELISA utilises antibodies raised in mouse against protein conjugated aflatoxin. The reactivity pattern of the antibody is: aflatoxin B1, 100%; aflatoxin B2, 58%; aflatoxin G1, < 0.1%; aflatoxin G2, < 0.1%. The calibration curve is virtually linear in the range of 0.03-1 ng/ml. The limit of detection (LOD) is 0.4 ng/ml (Xn ± 3SD; n > 20).

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P4

Evaluation of a new immunoaffinity column for deoxynivalenol

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TNO Nutrition and Food Research, The Netherlands

The mycotoxin deoxynivalenol, also known as DON or vomitoxin is part of a group of related compounds known as the trichothecenes. Trichothecenes are formed by a number of the *Fusarium* genera, including *F. graminearum* and *F. culmorum.* The main commodities affected by DON contamination are cereals. Due to its stability, DON has also been detected in a range of processed cereal products. The formation of DON in growing crops is dependent on climate and varies between geographical regions and years. Ingestion of DON contaminated commodities is known to induce vomiting, feed refusal, weight loss and diarrhoea. Studies also suggest that DON may also have effects on the immune system. In many countries no limits have been set for DON in cereals or only for wheat and wheat products. Until now, many national authorities used a general guideline of 1000 µg/kg for DON in cereals. A draft recommendation of the EC is 500 µg/kg for cereal products and 750 µg/kg for flour used as raw material.

Generally, analytical methods for DON are based on GC separation, followed by ECD, FID or MS detection. Clean-up is done by a cleaning step using charcoal/alumina/celite columns.
R-Biopharm Rhône has developed DONPREP, a new wide format immunoaffinity column. The column contains a highly specific antibody for DON. When the extract passes through the column, the mycotoxin binds to the antibody. After a washing step, the mycotoxin is eluted from the column and a concentration and clean-up step has been obtained. In the study the optimal elution solvent and volume were investigated as well as the optimal pH of the sample extract. The comparative analysis were executed using:

- The TNO in-house method for the analysis of DON, based on extraction with acetonitrile/water, clean-up with Romer Mycosep # 225 columns, evaporation under a stream of nitrogen, tri-methyl-silyl derivatisation, a washing step with phosphate buffer and separation on GC with ECD detection.
- In the same method DONPREP columns were used instead of Mycosep columns after extraction with water.
- At last a HPLC method was used. This method is based on extraction with water, clean-up with immunoaffinity columns, elution with methanol, evaporation under a stream of nitrogen, reconstitution in mobile phase and separation on reversed phase HPLC with PDA detection.

The comparative study was carried out by analysing six times BCR wheat flour Certified Reference Material 379 (containing 670 µg/kg).

In view of the results it could be concluded that the immunoaffinity column works well. No significant difference, both for the mean and variance, was found between the three applied methods. For the matrix wheat flour DONPREP appeared suitable with advantages in terms of time saving and clean chromatography. An advantage of the GC-Mycosep method remains the possibility of simultaneous analysis of other trichothecenes.

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DONPREP, immunoaffinity column for detection of deoxynivalenol in cereals and cereal based products using HPLC. Instructions for use, R-Biopharm Rhône Ltd.

P5

Rapid antibody-based technologies for aflatoxin B1 detection

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Aflatoxins are highly toxic fungal metabolites that contaminate cereals, feedstuffs and milk. The EU and WHO standards are now demanding reductions in aflatoxin levels in food, due to their high cancer-causing effects. This is economically significant for Ireland and other countries given that up to 25 % of all food crops may be contaminated by mycotoxins. We have developed a number of antibodies and novel antibody-derived (single chain Fv) fragments for the detection of aflatoxins with excellent sensitivities. They have been incorporated into a number of assay formats including enzyme-linked immunosorbent assays (ELISA's), lateral flow technology, and also surface plasmon-resonance-based biosensors. These systems can offer sensitive and reproducible detection limits for on-site determination and fast analysis, with ease of use.

P6

Deoxynivalenol - isolation and analytical characterisation for the application as reference material

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Trichothecene mycotoxins are the largest group of toxins produced particularly by moulds belonging to the genus *Fusarium*. Several surveys [1] suggest that one of the most prevalent mycotoxin is the type B-trichothecene deoxynivalenol (DON). It is mainly found as a contaminant on maize, oats and wheat. DON leads to symptoms like vomiting, diarrhoea, weight loss and adversely affects the immune system in humans and animals. In order to be able to monitor suspected commodities in the µg/kg range, laboratories all over the world have established analytical methodologies for DON [2].

The basis of a good analytical method is the availability of appropriate reference standards of defined purity and concentration. Trichothecene standards can be purchased from several commercial sources and are often obtained as crystalline solids in vials at quantities $\leq$ 10 mg. If given at all, the purity of the toxins are often claimed to be > 97%. However, the purity of trichothecene standards is often uncertain and the impurities may constitute 10% or more of the total amount. The results of a recent study showed that there is a variation in purity between DON-standards from different sources and even from different batches [3].

Therefore a strategy for isolation and purification of crystalline DON with a purity of > 99% was developed. DON was produced on rice with a *Fusarium graminearum* strain with an average yield of 1.5 g DON/kg substrate. The toxin was extracted from the culture material and afterwards separated from matrix components employing modern purification techniques including solid phase extraction and liquid chromatography. After final purification of DON (recrystallisation from ethyl acetate) the crystalline substance was subjected to an extensive analytical examination. With the assistance of sophisticated analytical procedures such as GC-MS, NMR- and IR-spectroscopy the structure identity of DON was confirmed. Through GC- and LC-techniques the purity content of the toxin in comparison with a commercially available reference substance was investigated.

All analytical data of the structural characterisation and determination of purity of the two DON standards will be presented [4].

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P7

Type A-trichothecenes - quantitative analysis using LC-MS and occurrence in Austrian maize and oats

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Type A trichothecenes are a group of sesquiterpenes produced by various *Fusarium* species like *F. sporotrichoides, F. poae* or *F. equiseti*. They occur on many different cereals like wheat, oat or maize. The most important structural features of trichothecenes responsible for causing certain symptoms in humans and animals are: the presence of the 12,13-epoxy ring, the presence of hydroxyl or acetyl groups at appropriate positions on the trichothecene nucleus and the structure and position of a side-chain. The strong impact of this group of structurally related mycotoxins on the health of animals fed contaminated material is mainly due to their immunosuppressive effects. Type A trichothecenes are in the focus of interest because many researchers have demonstrated that they are more toxic than the related type B trichothecenes.

Conventional methods for analysis of these mycotoxins have many limitations, which can be overcome by the use of liquid chromatography coupled to a mass spectrometer (LC-MS). Therefore a method for quantitative determination of diacetoxyscirpenol (DAS), T2 toxin and HT2 toxin using T2d3 toxin as an internal standard method was developed.

Sample preparation was performed by extraction with acetonitrile/water (84:16 v/v) and clean up was done by applying Mycosep™-columns (#227) purchased from Romer™ Labs, Inc. (Union, MO). After chromatographic separation the analytes were introduced into the quadrupole detector via electrospray interface. For detection of the analyte two characteristic mass fragments of each analyte and of the internal standard were used. An internal calibration was used for quantifying the amount of type A trichothecenes in the samples. Thus, a sensitive LC-MS method (LC-MS with electro spray) for the detection of these three harmful toxic compounds in maize and oats was developed and validated with limits of detection in the range of 15 to 50 µg/kg using.

Data on the occurrence of DAS, T2 toxin and HT2 toxin in Austrian maize and oat samples harvested in the year 2002 will be presented.

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P8

Rapid screening of fungal infection on corn using mid-infrared attenuated total reflection (IR-ATR) spectroscopy

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Fusarium graminearum is an agriculturally important toxigenic fungus causing great damage in the food and livestock industry. Its most important metabolite is deoxynivalenol (DON), a mycotoxin causing symptoms, which can lead to the death of affected animals [1]. Other symptoms observed include diarrhoea, dermal toxicity and vomiting. Determination of ergosterol, which serves as a parameter for the total fungal biomass [2] and DON itself include labour intensive and time-consuming procedures. After an extraction and a clean-up step determination is usually performed with HPLC-DAD (ergosterol) and GC-ECD (DON) [3].

The application of Fourier Transform mid-infrared spectroscopy with attenuated total reflection (ATR) for the detection of *Fusarium graminearum* as an indicator for the presence of the mycotoxin DON is described. This rapid screening method for the determination of *Fusarium* fungi, which produces DON as its main metabolite, could speed up analysis time, resulting in a higher sample throughput, as conventional determination is a time consuming and tedious task. In this study the fungus itself was determined on maize by pressing the ground sample against a diamond ATR-crystal with 3 internal reflections and recording the mid-infrared absorption spectrum. Reference measurements were performed by determining ergosterol with HPLC/DAD. Obtained concentrations of ergosterol served as a parameter for the total fungal biomass contained in the sample. DON was determined with GC-ECD after extraction with acetonitrile and clean-up with Mycosep™ columns. Principal component analysis (PCA) was used to separate contaminated maize from blank samples using the 1st derivative of spectra. Results showed that, for concentrations greater than 8.23 mg/kg ergosterol and 0.13 mg/kg DON up to 100 % of samples were correctly classified. Concentrations of ergosterol and DON in tested samples ranged from 0.79 to 947 mg/kg (ergosterol) and from 0.13 to 2.59 mg/kg (DON). For PCA these samples were always compared with a blank.

These promising results show the feasibility of the approach described and the capability of the mid-infrared region for the determination of fungi and its metabolites in maize.

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P9

Use of fluorescent derivatisation for toxin determination

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Already used for amino acid [1,2], polyamine, alcohol [3] and phenol [4] derivatisation the fluorescent labelling reagent 6-aminonaphtyl-N-hydroxysuccinimidyl carbamate (SINC) was tested for trichothecene mycotoxins determination. The labelling conditions were optimised for NIV, DON and T-2 toxin. Under very mild reaction conditions (25°C for 1 min) in a borate buffer, the tested mycotoxins were derivatized by the reagent to yield highly stable fluorescent carbamate derivatives. The maximum excitation and emission wavelengths are 290 nm and 365 nm respectively. Both NIV and DON were found to give two closely related stable derivatives. The detection limit (S/N=3) with fluorescence detection is at the low ppb level.

Taking place in an aqueous medium, the reaction leads to a global fluorescent fingerprint that could be even done on the crude extract. All the derivatives are baseline separated and eluted following their chemical functionalities. Until now, this fingerprint was only used as orientation test for derivatized toxin determination and as confirmation for other analytical methods.

Applications in the field of feed and food are described.

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P10

A flow-through enzyme immunoassay for the screening of fumonisin B1 in maize*

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Fumonisins are a group of at least 15 closely related secondary metabolites produced by the moulds *Fusarium moniliforme* and *F. proliferatum*. These mycotoxins, the most important of which is fumonisin B1, have been found worldwide as contaminants, mainly in maize and maize-based products. Fumonisins are neurotoxic, hepatotoxic and are classified as potential carcinogens for humans (class 2B carcinogens) by the IARC. In the EU there is still no legislation on fumonisin B1 levels. Switzerland is the only country in Europe with a maximum tolerated level for fumonisins in maize produced for human consumption (sum of FB1 and FB2 $\leq$ 1000 μg / kg).

The format of the flow-through enzyme immunoassay, developed by De Saeger and Van Peteghem [1] has been optimised to detect fumonisin B1 in maize with a visual detection limit - this is the smallest mycotoxin concentration that results in no colour development - of 1000 μg/kg. The flow-through assay system consists of an Immunodyne ABC membrane, coated with anti-mouse antibodies and anti-HRP antibodies (internal control), and an absorbent material. It is a competitive enzyme immunoassay where each reagent is applied in sequence to the membrane: monoclonal anti-FB1 antibody, washing solution, analyte, FB1-HRP conjugate and finally substrate solution for colour development. Less colour is produced with increasing concentrations of analyte. Results are visually evaluated or measured with a portable colorimeter.

An assay procedure is established using FB1 standard solutions (27% MeOH/ PBS buffer, pH 7.4, v/v) resulting in a total inhibition of colour development at 200 ng FB1/ml. Application of the assay procedure to grounded maize samples spiked with FB1 levels ranging from 0 to 1200 μg FB1/kg shows a visual detection limit of 1000 μg/kg. The extraction method uses a 50% MeOH/H_2O (v/v) extraction solvent and includes shaking with the hand, a dilution and a filtration step. Results of assay validation will be presented in this poster.

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* This work is carried out within the framework of the O.S.T.C.- PADDII- CPAA15 project “Mycotoxin contamination of regular and organic foodstuffs”, which is supported by the Belgian Government, Offices of the prime Minister, Federal office for Scientific, Technical and Cultural Affairs (O.S.T.C.).

P11

Determination of aflatoxin B1 and ochratoxin A in olive oil by immunoaffinity and high pressure liquid chromatography

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A method for the determination of aflatoxin B1 (AFB1) and ochratoxin A (OTA) in olive oil is evaluated. The oil sample was mixed with methanol-water (80-20), shaken and centrifuged. One ml of the supernatant was mixed with 9 ml of water and transferred onto an Aflaprep column. AFB1 was eluted with acetonitrile. Two ml of the supernatant were mixed with 40 ml of PBS and transferred onto an Ochraprep column. OTA was eluted with methanol acetic acid (98-2). Both eluates were evaporated until dryness and AFB1 is derivatized to its hemiacetal form (AFB2a). AFB2a and OTA were quantitated by HPLC using the same C18 (60 A, 4.6x250 mm) column equipped with fluorescence detector. The recovery for AFB1 and OTA were 85% and 107.6% respectively and the detection limits 56 ng/kg and 20 ng/kg respectively. Fifty samples of olive oil of Greek origin were examined for the presence of AFB1 and OTA. Results showed the presence of OTA in 88% of the samples. The range of contamination was 23-810 ng OTA/kg. AFB1 was found only in three samples and the range of contamination was low (60-70 ng AFB1/kg).

P12

Using of 1-anthroylnitrile as a fluorescent labelling reagent for the determination of T-2 toxin in cereals by high-performance liquid chromatography

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T-2 toxin (T-2), a highly toxic trichothecene mainly produced by *Fusarium sporotrichioides*, can occur in several grains such as wheat, corn, oats, barley, rice, sorghum, beans and cereal-based products, especially in north temperate climates. Animal exposure to the toxin causes a variety of adverse effects, including skin vesicles or necrosis, nausea, weight loss, vomiting and diarrhoea, weakness, ataxia, collapse, reduced cardiac output, haemorrhage and death. T-2 has been shown to be the causative agent of alimentary toxic aleukia (ATA), a fatal disease occurred in Russia in 1942-47 in population fed overwintered cereals and, together with other trichothecenes, has been reported as chemical warfare agent in the "yellow rain" attack in Southeast Asia.

Sensitive and accurate methods for the determination of T-2 in cereals are highly desirable in order to fulfil the need to protect consumer health from the risk of exposure to the toxin. Several analytical methods for the determination of T-2, alone or in combination with other trichothecenes, have been reported including thin-layer chromatography (TLC), enzyme-linked immunoassays, gas chromatography (GC) combined with electron capture detector (ECD), flame ionisation detector (FID) or mass spectrometry (MS), high-performance liquid chromatography (HPLC)/MS and supercritical fluid chromatography (SFC)/MS. HPLC with fluorescence detection can be applied to the analysis of trichothecenes after derivatisation with an appropriate labelling reagent.

In this study, 1-anthroylnitrile (1-AN) has been shown to be an efficient labelling reagent for the determination of T-2 by HPLC with fluorescence detector due to its high reactivity towards the T-2 hydroxyl group. The derivatisation conditions have been optimised by investigating different reaction solvents, reagent molar ratios, temperatures and reaction times. T-2 was quantitatively converted under mild conditions into a stable fluorescent ester by reaction with 1-AN in presence of 4-dimethylaminopyridine (DMAP) in toluene. The optimum conditions in terms of sensitivity and repeatability of the derivatisation reaction were found with molar ratios 1:20:40 (T-2:1-AN:DMAP). The optimum reaction temperature and time were 50°C and 15 min, respectively. This derivatisation procedure was used for the HPLC determination of T-2 in wheat, corn, barley, oats, rice and sorghum after extraction with methanol:water (80:20, v/v) and clean-up by immunoaffinity columns containing antibodies specific for T-2 (T-2 TAG™, Vicam LP, USA). The toxin was quantified by reversed-phase HPLC with fluorometric detector ($\lambda_{ex.}$=381 nm, $\lambda_{em.}$=470 nm) after pre-column derivatisation with 1-AN. Recoveries of the analytical method applied to different cereals spiked with T-2 at different levels (from 0.05 µg/g to 1.5 µg/g) ranged from 80.4% to 98.7%, with relative standard deviations lesser than 6%. The limit of detection was 0.005 µg/g, based on a signal-to-noise-ratio of 3:1. The method allows the determination of T-2 at nanograms per gram (ppb) levels in various cereals with good accuracy and precision and is appropriate to quantify the toxin at levels that can occur in naturally contaminated cereal samples.

P13

Simultaneous determination of trichothecenes by HPLC-UV DAD

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Trichothecenes, mycotoxins produced by *Fusarium graminearum* and *F. culmorum*, are known to occur as natural contaminants of cereals worldwide and have shown to cause a variety of toxic effects on humans and animals.

An approach for simultaneous determination of the main type B-trichothecenes by high-performance liquid chromatography-diode array detection (HPLC-DAD) in whole wheat flour samples was developed. Deoxynivalenol (DON), nivalenol (NIV), 15-acetyldeoxynivalenol (15-acDON) and 3-acetyldeoxynivalenol (3-acDON) were separated on a reversed phase C18 column, using a multi-linear gradient. This method involves extraction and purification of toxins by using multifunctional MycoSep Columns.

A series of naturally contaminated wheat samples were analysed both by our developed method and a screening method like enzyme-linked immunosorbent assay (ELISA). The results have shown a good agreement (r^2=0.955) between the two methods.

P14

Development of multi-mycotoxin procedure as screening method

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Many analytical methods have been developed for the determination of mycotoxins including TLC, HPLC, GC and ELISA. Due to the quite different properties of the mycotoxin families, most of the procedures of extraction and clean up were developed for the determination of only one specific mycotoxin.

New mycotoxins and simultaneous contamination of known mycotoxins are being discovered at high rates. Thus, the mycotoxin risk management requires the development of multi-mycotoxin analysis methods able to identify and quantify simultaneously several mycotoxins of different structures or families in cereals and other foodstuffs.

Methods for the determination of low levels of mycotoxins in cereals and animal feeding stuffs are commonly complex, lengthy and costly to carry out. The immunoassays (ELISA, ...) is being increasingly used to develop simple, fast and sensitive methods for individual compounds. However, while the inherent specificity of those immunoassays substantially overcomes problems of interference in detection from co-extracted compounds, this specificity precludes their use to screen foods and feedstuffs for a range of mycotoxins. To investigate cases of suspected mycotoxicosis, studies on the natural occurrence of mycotoxins in materials or for multi-toxin surveillance programmes, reliable, sensitive and proven multi-methods are required.

The aim of our research is to develop a multi-mycotoxin procedure able to determine most of the mycotoxins of interest. The main steps are the extraction, the purification, the analysis and the detection, respectively and must be non-specific. Indeed, a solvent mixture has to be chosen that allows the extraction of a maximum of mycotoxins. The use of anionic exchange columns as purification has to enable the separation between the acidic and neutral mycotoxins. Further, the trapped mycotoxins and the non-retained neutral mycotoxins were purified following their own specific way. Finally, if possible, one unique analytical method should be developed for the determination of the analysed mycotoxins.

This procedure, when completed, will offer a complete screening of mycotoxins likely to be present in a lot of samples.

This poster will present the results of the first tests carried out on samples following the developed procedure. Several extraction solvent mixtures and purification have been tested on whole wheat flour spiked with the most important mycotoxins and on naturally contaminated wheat.

P15

Quantitative analysis of zearalenone and alpha-zearalenol in animal feed and culture media

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A reversed-phase HPLC method with fluorescence detection (λ_{ex} = 274 nm, λ_{em} = 440 nm) was developed for the quantitative determination of zearalenone (ZEA) and alpha-zearalenol (α-ZOL) in animal feed and culture media. A mobile phase of acetonitrile : water (50 : 50 v/v) and a flow-rate of 1.0 ml.min^{-1} resulted in a good separation between ZEA and α-ZOL. Immunoaffinity clean-up was compared to C_{18} and Florisil column clean-up. Using C_{18} and Florisil resulted in too high matrix interferences. Therefore, animal feed samples and *Fusarium graminearum* cultures were analysed using the developed HPLC method coupled to immunoaffinity clean-up. Recoveries for spiked ZEA and α-ZOL feed samples ranged from 89 to 110 % with CVs between 5.2 and 11.2 % (under within-laboratory reproducibility conditions). The linear range was between 25 and 600 µg.kg^{-1} for ZEA and α-ZOL in feed samples.

Naturally contaminated feed samples were analysed for their ZEA and α-ZOL content.*Fusarium graminearum* (IHEM 2994 and 2995) strains were tested for their ability to produce ZEA after culturing on rice grains and on a minimal growth medium.

P16

Rapid detection methods for mycotoxin detection

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There are various platforms and formats for mycotoxin analysis. Methods of analysis for the detection of mycotoxins must be sensitive, rapid and reproducible. Immunochemical assays such as ELISA have been established over the past decade and they are under constant development. Basic commercial immunological techniques for mycotoxin analysis for, e.g., nuts, grains, seeds, milk and feeds are commercially available. Commercial immunological techniques for mycotoxins are based on specific monoclonal and polyclonal antibodies produced against the toxin, and divided broadly into immunoaffinity column-based analysis, membrane-based flow-through immunological assays and ELISAs. Other research techniques for mycotoxins using an immunological approach reported in the literature include optical and acoustic biosensors, and capillary electrophoresis. The major advantages of these new analytical methods are that they are rapid in that a sample can be analysed within 10 minutes, and that multiple samples can be analysed. However, the most important factor in mycotoxin screening today is rapidity, and inexpensiveness. Membrane-based flow-through immunological screening tests and some column-based fluorometric immunological assays are currently in wide use. However, some micro-titre plate ELISAs capable of analysing a few samples are also commercially available.

P17

Improved HPLC methods for the analysis of *Alternaria* toxins in rice and carrots*

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Fungi of the genus *Alternaria* include saprophytic and plant pathogen species responsible for the spoilage of a variety of fruits and vegetables in the field and during transport and storage. *A. alternata* is associated with leaf, stem, fruit and carrot spots, whereas *A. radicina* is pathogenic to carrot seeds and plants causing leaf blight, black rot of roots and damping-off of seedlings. *A. alternata* produces several secondary metabolites recognised as mycotoxins, including tenuazonic acid (TeA), alternariol (AOH), alternariol monomethyl ether (AME), and altertoxin-I (ATX-I), whereas *A. radicina* produces radicinin (RAD) and radicinol (ROH) recognised as phytotoxins. *A. alternata* f. sp. *lycopersici* has been reported to produce AAL-toxins (TA and TB) and small amounts of fumonisins. Improved HPLC methods for the analysis of *Alternaria* toxins both in inoculated rice seeds and in carrots have been developed in the framework of the EU-supported project Safe Organic Vegetables.

For the simultaneous analysis of AAL toxins (TA) and fumonisins (FB1 and FB2) in inoculated rice and carrot materials a suitable HPLC method was developed. An acidified mixture of acetonitrile+water and a polymeric reversed phase column (Oasis® HLB) were used for the extraction and clean-up steps, respectively. The purified extract was derivatised with *o*-phthaldialdehyde reagent and analysed by C18 reversed phase HPLC with a fluorometric detector. A suitable isocratic HPLC mobile phase, consisting of a mixture of acetonitrile+phosphate buffer, was optimised with Drylab® software. Mean recoveries of TA, FB_1 and FB_2 from spiked (0.5-5.0 µg/g) blank rice ranged from 70 to 96% whereas relative standard deviations (RSD_r) ranged from 6 to 29%. A high level of TA toxin (1450 µg/g) was found in a rice culture inoculated with *A. alternata* f. sp. *lycopersici,* whereas fumonisins were not detected.

An acidified mixture of acetonitrile+methanol+water was used for the extraction of RAD, ROH, TeA, ATX-I, AOH and AME from inoculated rice and carrot culture materials. Filtered extracts were diluted with water and analysed by C18 reversed phase HPLC with an ultraviolet diode array detector, which delivered multi-signal chromatograms for the simultaneous detection of the toxins. The HPLC mobile phase, consisting of two consecutive isocratic mixtures of acetonitrile+phosphate buffer, was optimised with Drylab® software. Mean recoveries of RAD, TeA, ATX-I, AOH and AME from spiked (5-100 µg/g) blank rice ranged from 70 to 94%, whereas RSD_r ranged from 3 to 12%. Tenuazonic acid, ATX-I, AOH and AME were found in rice cultures inoculated with *A. alternata* isolated from carrots, whereas only AOH and AME were produced by the same fungus when grown on carrots. High levels of RAD were found in rice cultures inoculated with *A. radicina* isolated from carrots, whereas this fungus produced *epi*-radicinol, but not RAD, when grown on carrots.

For the analysis of TeA, ATX-I and AME in fresh carrot roots two different clean-up procedures were used before HPLC analysis. In particular, the Oasis® column clean-up was used for the analysis of TeA and a silica based C18 column clean-up was used for the analysis of ATX-I and AME. Mean recoveries of TeA, ATX-I and AME from spiked (1.0-3.0 µg/g) blank carrot ranged from 63 to 92% whereas RSD_r ranged from 1 to 25%. Limits of detection for TeA, ATX-I, AME, were 23, 22 and 12 ng/g respectively.

* Acknowledgement: This research is financially supported by the European Commission, Quality of Life and Management of Living Resources Programme (QLK1), Key Action 1 on Food, Nutrition and Health.

P18

Sample preparation: slurry mixing or dry milling?

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Since January 1st 1999, EC Directives for aflatoxins entered into force, which consisted of sampling plans resulting in sample weights of up to 30 kilogram. This raised questions on how these relatively big samples could fulfil the regulations requirement to “finely grind and mix thoroughly each laboratory sample using a process that has been demonstrated to achieve complete homogenisation”. Some members of CEN TC275/WG5 were asked to collect data on this item. These were scarce and it took some effort to carry out new experiments. While working on this subject the Agricultural Research Service of the Western Regional Research Center (ARS-WRRC) of the USDA presented the results of a study on subsample preparation of pistachios.

The choice of matrices was discussed at CEN TC275/WG5 meetings, considering existing and upcoming legislation for different mycotoxins and food types. In practice however it turned out that the availability of contaminated samples that could be used for these experiments was the limiting factor. Therefore experiments consisted of measurements of ochratoxin A in cacao and green coffee beans and of aflatoxins, of which only B1 is useful for this purpose, in almonds, pistachios and a sample of mixed spices.

For each commodity, experiments were carried out by the following procedure:

- sampling according to the EC Directive, resulting in 10 kg sample;
- milling the 10 kg sample by a Romer mill with a split ratio of 10%;
- making slurry of the 90% part by Silverson mixing (sub sample A);
- taking a dry sample out of the 10% part as usual for Romer mill users (sub sample B);
- making slurry of the remaining part of the 10% part of the sample (sub sample C); and
- analysing the three sub samples A, B and C by HPLC methods.

Doing so, the mycotoxins content in the sample can be reconstructed afterwards by calculation.

The analytical results for the A, B and C sub samples, as measured by Inspectorate for Health Protection and Veterinary Public Health, and Wiertz Eggert Jörissen are presented. They reveal that the dry milling process resulted in different mycotoxins content in both samples in which the sample is divided by dry milling. Standard deviation and coefficient of variation are calculated. Results were also evaluated by comparing the acceptance or rejectance of a lot, as this is a critical point on enforcing a directive. At the ARS-WRRC-USDA, particle sizes were measured to calculate theoretical CV’s, which were compared with experimental ones. It showed that slurries contain smaller particles than dry milled pistachio samples. From these data it was concluded that slurrying is the preferred sub sample preparation, because it generates the lowest possible CV values, which indicates that the slurry preparation procedure achieves better homogenisation of a sample.

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P19

Single run LC-MS/MS analysis of mycotoxins subject to actual and upcoming EU legislation in one sample extract

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This contribution describes a method for the determination of 13 mycotoxins in food using Liquid Chromatography-Mass Spectrometry. Aflatoxins and ochratoxin A are subject to EU legislation for some years. 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, T2, HT2, 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 LC-MS 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 LC-MS run by means of multiple reaction monitoring. The LC-separation of the mycotoxins is done using a gradient of water and acetonitrile on a reversed-phase column within 25 minutes. The Mass spectrometer is used with an Electrospray Interface in the positive mode (ES^+).

With the described method it is possible to determine aflatoxins, ochratoxin A, DON, 3-acetyl-deoxynivalenol (3-Ac-DON), fumonisin B1 and B2, diacetoxyscirpenol (DAS), zearalenone (ZON), T2-toxin and HT2-toxin. 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. Therefore validation data are presented for several matrices. If method set-up is completed with validation data on relevant matrices, the result of all these efforts is a multimycotoxin method with which EC regulations can be maintained in a less laborious way. This poster presents the results until now. The matrix effect is shown as well as validation data on some food types.

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P20

On the way to multicomponent determination of mycotoxins: a comparison of multicomponent analytical techniques

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In the food producing industry and controlling organs fast and sensitive analyses are required for contaminant and residue screening. The preferred methods are simple multicomponent analyses in compliance with the legal regulations in terms of limits of detection. A few multicomponent methods have been described for mycotoxin determinations based on either TLC, HPLC or GC separations. Novel analytical methods like the Biacore technology offer attractive alternatives. With the foreseen introduction of the multichannel Biacore TAS system eight parallel analyses will be possible. Probably even more promising is the approach of Luminex: in principle enabling the simultaneous determination of up to 100 components.

In this presentation a critical overview is given on the available mulitcomponent analyses, the required sample preparation methods and financial aspects of the various techniques. The presentation starts with a review of the requirements and available methods. Additionally potentially available novel methods not yet used for mycotoxin analysis will be discussed. Considerations of assay set-up and strategies will be presented including the economical aspects. Results obtained using different analysis methods for food and feed samples are compared and discussed.

P21

Determination of deoxynivalenol (DON) in baby food and cereals with RIDASCREEN® DON screening test and DONPREP + HPLC

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Deoxynivalenol (DON, vomitoxin) belongs to the trichothecenes, a group of mycotoxins produced by the *Fusarium* moulds, which is found in abundance in various cereal crops. Due to its acute toxicity and long-term effects on animal and human health, surveillance surveys have been carried out over several years and the first advisory levels for human consumption have been defined as 500 ppb and 750 ppb. The European commission now plans to set an advisory level of 100 ppb for baby food.

This study assesses two different test systems, the RIDASCREEN® DON ELISA screening test and the DONPREP immunoaffinity columns with HPLC for determination of DON in baby food and relevant cereal samples.

RIDASCREEN®DON is a fast microplate assay (45 min incubation time) with a standard calibration range of 3.7 - 100 ppb. For sample analysis, a measuring range of 18.5 - 500 ppb or 37 - 1000 ppb can be obtained depending on the sample dilution factor. Baby food and cereal samples were analysed after a simple extraction step with water and the majority of the samples analysed were found to be below the limit of detection of the test. Samples spiked with DON at a 100 ppb level were found to give recoveries of >80% using RIDASCREEN®DON.

DONPREP immunoaffinity columns were used for purification of complex samples prior to HPLC. Again sample extraction with water was found to be suitable for the analysis of cereals, whereas water with the addition of polyethylene glycol (PEG) was found to give better recoveries for more complex baby food samples. DONPREP showed recoveries of >80% for cereal and for baby food samples spiked at 50 ppb and 100 ppb. The HPLC traces were found to be clean with little background interference even with highly complex and coloured baby food samples.

In summary, both test systems investigated were found to be highly suitable for analysing a variety of baby food samples and cereals at a low concentration level of 100 ppb, in accordance with the proposed European legislative limits.

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P22

Membrane cards for the screening of aflatoxins and ochratoxin A in a range of commodities

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The introduction of new aflatoxin and ochratoxin A legislation has led to an increase in the demand for fast, accurate methods for screening a range of food and feed commodities. In July 2001 an extension was made to the current EC aflatoxin legislation to incorporate a wide range of spices and in April 2002 legislation for ochratoxin A in cereals, cereal products and dried vine fruit was introduced. Due to the toxicity of aflatoxins and ochratoxin A and the lack of HPLC systems in many food laboratories, accurate, rapid, and easy to use methods for the detection of aflatoxins in a range of commodities is essential. In order to overcome these problems, RBR have developed a range of membrane based screening cards for analysis of aflatoxin B1, total aflatoxins and ochratoxin A.

The method for analysis of aflatoxins involves sample extraction with 80% methanol followed by blending for 2 minutes. The sample is then filtered, diluted and passed through a unique clean up column to remove background interference. The sample is then applied directly to Aflacard membrane. For ochratoxin A analysis, the sample clean up is carried out using ochratoxin immunoaffinity columns before being applied to the Ochracard.

Aflacard B1 and Aflacard Total can detect aflatoxins in line with EC legislation. Methods are available for analysis of aflatoxins at 2 ppb, 4 ppb, 5 ppb, 10 ppb, 20 ppb and 30 ppb and for ochratoxin A at 3 ppb and 5 ppb.

In the study a range of food and feed commodities including cereals, spices and nuts were analysed by Aflacard B1 and Aflacard Total for aflatoxin content and a range of cereals were analysed by Ochracard for ochratoxin A content. The card results were compared to HPLC analysis. The results generated were comparable to analysis by HPLC. All positive samples were correctly identified by the cards. Therefore it can be concluded that Aflacard B1, Aflacard Total and Ochracard are suitable for screening a range of commodities at levels in line with legislation and are ideal for laboratories with limited facilities.

P23

Validation of a new microtiter plate ELISA total aflatoxin test (AgraQuant®)

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An ELISA total aflatoxin test, AgraQuant® was developed to measure total aflatoxins in a range from 4-40 ppb using a monoclonal antibody specific for aflatoxins. The test is performed as a solid phase direct competitive ELISA using a horseradish peroxidase conjugate as the competing, measurable entity. For the test method aflatoxins are extracted from ground samples with 70 % methanol and sample extracts plus conjugate are mixed and then added to the antibody-coated microwells. After the plates incubate for 15 min. at room temperature they are washed and enzyme substrate is added and allowed to incubate for an additional 5 minutes. Stop solution is added and the intensity of the resulting yellow colour is measured optically with a microplate reader at 450 nm. Results obtained from internal validation studies assessing accelerated stability, ruggedness, accuracy, precision and limit of detection in corn and other grains and grain products, comparison of method to HPLC, and ability to detect individual aflatoxins determined this test to be a rugged, sensitive, precise, accurate and effective test comparable to HPLC for measuring total aflatoxins ranging from 4-40 ppb in several commodities.

P24

Application of HACCP and other quality management techniques to reduce the risk of mycotoxin contamination in cereals*

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Quality management techniques currently used in the food industry have evolved from being solely concerned with quality control ('QC' - stopping one's mistakes reaching the customer) to quality assurance ('QA' - preventing the mistake from happening in the first place). In terms of food-safety, a key technique used is 'Hazard Analysis Critical Control Point' (HACCP). Originally applied to food-manufacture, the technique has since been applied to food -distribution and -retail services and is now starting to be applied to agricultural practices. HACCP requires a detailed knowledge of: the nature of the raw materials; the processes that raw materials and finished products undergo and; the hazards, together with the risk of that hazard occurring, presented by both the raw materials and the processes.

For some years CCFRA has been using HACCP, other quality management techniques and developments in information technology to analyse the phenomenon of mycotoxin contamination of cereals. Analyses of the literature, industrial codes of practice, and general commercial practices have made it possible to identify points/risk factors in the commercial flow of grain (from field to fork) critical to the management of mycotoxin contamination. Originally this approach was used to address the question of ochratoxin A in the UK cereal crop (main causative organism *Penicillium verrucosum*) and has since been extended to examine the occurrence of mycotoxins (e.g. deoxynivalenol) produced by the genera *Fusarium* in the European wheat, maize and barley crops.

Mycotoxin contamination of cereals can be broadly divided on the basis of that occurring primarily in the field or in storage. Toxins produced by members of the *Fusarium* genera are produced mainly prior to harvest, while ochratoxin A is produced post harvest [1]. In the case of ochratoxin A, a key factor is moisture control and the need to reduce and keep grain parcels at moisture levels that do not sustain mycotoxin contamination. With regard to *Fusarium* mycotoxins, the phenomenon is more complex and mainly involves preharvest events. These are mainly concerned with agronomic practices, in particular the management of Fusarium Ear Blight.

Identification of these risk factors and the necessary quality control/assurance techniques to manage them is a prerequisite in the development of approaches which can be applied within a commercial context and so reduce the risk of mycotoxin contamination. These approaches can then be adopted into codes of practice and/or appropriate quality assurance schemes.

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* Acknowledgement: The author thanks the UK Food Standards Agency (grant no. C03009) and the European Commission (grant no. QLK1-CT-1999-00996) for financial support.

P25

Natamycin: an effective fungicide to prevent growth of mycotoxin-forming fungi on cheeses and dry cured sausages

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Fungal growth on food and feedstuffs is not only unsavoury and economically unfavourable, but also hazardous due to possible production of mycotoxins. Fungi are found everywhere and good storage practices can not always prevent fungal growth. Products stored in the open air at high relative humidity are particularly sensitive for fungal growth. Examples of such products are cheeses and dry sausages. Under such conditions mould and yeast can easily grow and spoilage may occur. Species associated with cheeses and sausages spoilage are known to produce mycotoxins. Most mycotoxins are chemically stable and can survive storage and processing even when cooked to quite high temperatures. Therefore fungal growth in foods and feeds should be avoided.

Natamycin

Prevention of fungal growth can be effectively achieved with natamycin.
The fungicide natamycin was first isolated in 1955 in the Gist-brocades Research Laboratories. Natamycin, also known as pimaricin, is produced on industrial scale using *Streptomyces natalensis*. In the EU natamycin is permitted as food additive for the surface treatment of cheese and dry cured sausages.

Cheeses and sausages

The major advantages of natamycin are its activity in small quantities against most of the food-borne yeast and moulds and that it affects neither the product quality nor the taste. Moreover in contrast to other preservatives it does not induce development of resistance. Natamycin is an effective fungicide to prevent the growth of toxin-forming fungi associated with cheese and sausage spoilage. Natamycin can be applied on cheeses and sausages through a coating but also by dipping or spraying. For species such as *Aspergillus ochraceus*, *Penicillium expansum* and *Penicillium chrysogenum*, the minimum inhibitory concentration was found below 5µg/ml.

P26

Inhibition of *P. citrinum* by microwave treatment in black olives

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Black olives can be contaminated with moulds during processing transporting, storage and consumption under suitable conditions. Mould growth causes softness of the olive tissue and hence quality losses. Different genera of moulds such as *Penicillium, Aspergillus, Alternaria, Cladosporium* and *Rhizopus* were isolated from the surface of olives during fermentation (Eltem and Öner, 1990). The predominant mould genus was found to be *Penicillium* in the samples obtained from different markets in Istanbul (Erol-Meriç and Heperkan, 2002). Moulds can also produce mycotoxin in olives. Gourama and Bullerman (1988) found that *A. flavus* did not produce aflatoxin, whereas *A. ochraceus* could produce penicillic acid but not ochratoxin, patulin ve citrinin in black olives. Oral and Heperkan (1999) found that *P.citrinum* produced citrinin both in olives and in brine. Moulds and mycotoxin contamination in olives can cause economical losses as well as health risk in Turkey, since the table olive production is approximately 450,000 tonnes per year. Therefore inhibition of *P. citrinum* by microwave treatment in black table olives were studied.

In this study, the olive samples were obtained from the area of Gemlik in January 2001. Olives were inoculated with pure culture of *Penicillium citrinum* provided by CBS, Baarn. After incubation at 25°C for 14 days, samples were treated by microwave and moulds were enumerated using malt extract agar. The inhibition of *P. citrinum* increased with increasing time. After 240 seconds of microwave treatment 6.4 log reduction of *P. citrinum* was obtained in olives with water and 6.4-7.2 log reduction was obtained in olives with different concentration of brine solutions.

Based on the results of this study, it can be concluded that microwave treatment can be used to prevent or reduce contamination of moulds and subsequent mycotoxin production after processing of black olives.

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P27

Determination of the factors affecting the occurrence of aflatoxins in hazelnuts and preventive measures 2002-2004*

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The main objective of the project is determination of the factors affecting possible aflatoxin formation in hazelnut during cultivation, harvesting, and post harvest stages such as drying, processing and storage during three subsequent years. To minimise the aflatoxin formation risk, Good Agricultural Practices in orchards, harvesting, drying techniques and storage conditions will be improved. To disseminate the knowledge, improved techniques, technologies and preventive measures intensive training programmes for producers, traders, processors and exporters will be realised each season in the production regions. At the end of the project, the problem will be pictured and resolved by scientific and technological approaches. To serve this aim, following stages are in the scope of the project: 1) intensive training programmes, posters and brochures about aflatoxins for producers, traders, processors and exporters, 2) investigations on mould flora and aflatoxins in hazelnuts starting from the flowering stage, during maturation and harvesting, correlating the aflatoxin formation with orchard conditions, climatic conditions, agricultural practices applied, 3) determination of the effect of traditional post harvest applications on aflatoxin formation and development of the most suitable alternative technologies, and 4) implementation of the preventive methods to minimise the aflatoxin formation.

Results of the 2002 studies

The training programmes and sampling studies from the totally 72 orchards and postharvest applications have been completed. Approximately 2500 growers, traders and producers have been trained with the co-operation of the Ministry of Agriculture and Rural Affairs. Out of 648 sampling from different regions/orchards/ parts of the trees the incidence of aflatoxin was only 1.5% and the level of aflatoxin content was between 0.77-0.1 ng/g. The aflatoxin was not detected for the other samples where the minimum quantification limit for B1 was 0.04 and 0.1 for total aflatoxin. It was determined that there was a significant difference between the regions and altitudes in terms of their mould load (SPSS 10.1 version, T-test). The level of mould loads was inversely proportional with the altitude. The level of mould loads was the highest at the samples taken from the orchards at 0-250 m. It was also determined that: (i) there was no significant difference between the samples collected from upper and lower branches in terms of their mould loads (at 95% confidence level), (ii) there was a significant difference between soil and leaf samples in terms of their mould loads (at $p<0.10$), (iii) water activity (a_w) of the samples decreased until harvest whereas there was no correlation between a_w and mould loads within the observed a_w range, and (iv) the incidence for aflatoxin detection and the level increased relatively in the periods near to the harvest. 47% of the Mould identification studies has been completed, and out of 1,730 strains identified 87% were *A. flavus* and 13% were *A. parasiticus.*

Totally, 6 different harvesting methods were studied in two regions: Giresun and Akcakoca. The only application where aflatoxin detected was: (i) hazelnuts fallen to the ground and stayed there for 3 days, (ii) the hazelnuts picked up from the ground and put into nylon sacks, (iii) wither the husk on the ground for easy removal without tent, and (iv) the hazelnuts dried on the ground without tent after husk removal. The samples dried with 6 different methods are stored under both controlled (5°C, 65% RH) and uncontrolled conditions and are being followed at one month intervals for aflatoxin formation.

* Sponsored by Hazelnut Promotion Group

P28

Control of mycotoxins in corn: 7 years of research on crop techniques and postharvest treatments

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The occurrence of mycotoxins in corn kernel can be related to unsafe harvest and storage measures, but their production starts in fields, when toxigenic strains of field fungi infected the crop. The Department of Agronomy, Silviculture and Land Management at the University of Turin (North Italy) since 1996 co-ordinate a multidisciplinary research project to evaluate the most important techniques in order to prevent mycotoxins contamination in corn kernel.

At a first stage (1996-99) mycotoxins production during grain conservation was investigated. Through the analysis of 1200 corn samples in 6 drying units, it was observed that mycotoxins did not increase in standard drying processes and when temperature conditions in the silo are properly controlled. Moreover zearalenone and particularly fumonisins were often reduced with a double winnowing before and after drying process. It became evident that mycotoxins contents are more dependent by climatic condition before harvest and crop techniques. Therefore, at the second stage (2000-02) mycotoxins production in field was investigated, comparing crop practices in order to control moulds development and toxin production before harvesting. This research, in progress in West Northern Italy (45° N, 7° E), analysed more than 800 corn grain samples in order to evaluate the following crop techniques: varieties, seeding time, plant density, nitrogen fertilisation, irrigation, harvesting time and European corn borer infection and control. Each sample was analysed with High Performance Liquid Chromatography methods (HPLC) researching the following mycotoxins: aflatoxin B1, B2, G1, G2, ochratoxin A, fumonisin B1, zeralenone, deoxynivalenol.

Data stress the attention to general and diffuse occurrence of *Fusarium* toxins such as fumonisin B1 (86.2%), zearalenone (56.6%) and deoxynivalenol (41.7%) in corn kernel at harvesting, whereas only in few samples aflatoxin B1 (2.5%) and ochratoxin A (5.7%) contents were superior to the instrumental limit. Climatic conditions (rainfall during ripening) influenced zearalenone and deoxynivalenol contents, but they weren't critical for fumonisins, which presence was high in all years. The examined agronomical techniques showed clear effects on the mycotoxin occurrence in corn kernel. Hybrids of 600 and 700 FAO classes had zearalenone and deoxynivalenol contamination levels 3-4 times higher compared to early hybrids (400-500 FAO classes). Instead, fumonisins were correlated mainly to other variety factors as kernel specific weight. March-April seeding times seem to provide the best results, while there is an increase of fumonisins contents when seeding is delayed to may. Also hight plant density (7.5-8 plant/m^2) increase fumonisins contamination comparison with usual density (6.5 plant/m^2). High nitrogen fertilisation (> 400 kg ha^{-1}) increase fumonisins and zearalenone; also N deficiency (as in organic farming) could increase mycotoxin. Fumonisins, but not zearalenone, appear to be correlated with European corn borer infection. Chemical control, against this phytophagous, was successful to control fumonisins, while *Trichogramma maidis* (a corn borer parasite) seems to be still inefficient. Delayed harvesting times caused an increased of fumonisins and particularly of zearalenone contamination in late maturing hybrids (600-700 FAO classes).

Since 2003, major farmer associations will receive guidelines to prevent corn mycotoxin contamination in the field.

P29

Mycotochain: a European initiative on reducing mycotoxins in the cereal chain

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Mycotoxins produced in cereals by the plant pathogen *Fusarium* spp. pose a health risk to consumers of bread and other cereal products. To avoid this risk, much research is necessary. Part of this research is co-ordinated in a concerted action (CA) entitled 'Quality control measures in the production and processing chain to reduce *Fusarium* mycotoxin contamination of food and feed grains (PL 98-4094)'. This CA is funded within the EU-FAIR program and allows 20 European partners spanning the whole cereal chain from research and industry to combine forces. The CA aims to identify critical control points and to design a protocol for reducing risks to a minimum. The focus is on trichothecenes in wheat, especially deoxynivalenol (DON) and fumonisin in maize. The approach includes the breeding of *Fusarium*-resistant cultivars, good agricultural practice, optimal storage and processing and adequate, cheap and effective assessing methods. In regular meetings, partners exchange and disseminate knowledge, explore solutions, identify gaps in knowledge and define fields for further co-operation.

Plant resistance may offer a full solution for the problem. Although various sources of resistance are found, breeding still encounters two major problems. In the first place, the relation between rate of *Fusarium* infection and mycotoxin contamination is not yet established unambiguously. In the second place, resistance seems not to be based on a simple genetic system. Therefore efficient selection methods, for example implying molecular marker assisted techniques, urgently need to be developed.

Guidelines for maximum tolerable levels of mycotoxin contamination are elaborated in view of EU-legislation and EUREPGAP requirements. The methodology for assessing fumonisins in maize is available and accurate, however methods for accurately measuring the content of the DON in wheat still need to be further developed whereas a cheap and reliable method is badly for industrial-scale surveys.

Setting up a system of downgrading wheat and maize lots according to mycotoxin content presents the problem what can still be the use of the rejected lots. Traditionally these are channelled in the feed chain, but evidence is accumulating that growth of fowl and cattle is reduced and health risks to the animals may develop. According to upcoming legislation, blending of seed lots with the object to lower the mycotoxin contamination may not be allowed in the future. Alternative use may have to be sought when mycotoxin concentrations would surpass these limits.

More information can be obtained at: http://www.plant.wageningen-ur.nl/projects/fusarium

P30

Evaluation of the effect of measures taken after a DON outbreak

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In April 1999 an amount of 2600 µg/kg DON was found in a sample breakfast cereals in the Netherlands. This event was the start of a lot of activities, which dealt with the prevention, control, health and consumer aspects of DON in food for human consumption. The Food Inspection Services started a monitoring program to measure DON in cereal products, flour and raw cereals. The National Institute of Public Health and the Environment, another part of the Ministry of Health in the Netherlands, was asked to carry out a risk analysis on DON. This was the basis for the Minister of Health to set an action limit for consumer products. She also informed Brussels and asked for a European limit. The Main Board on Agriculture set out to implement measures to be taken at harvesting, milling and bread baking industry. The Scientific Committee on Food expressed an opinion on DON in December 1999. Worldwide attention was also raised and led to discussion of a DON limit by JECFA in February 2001.

In the period May 1999 until March 2002 a number of more than 1700 samples were analysed on DON. These originated from the cereal harvest of the years 1998 until 2001. The results showed a sharp decrease of DON content in samples of harvest 1999 when compared to 1998. This lower level was maintained in the 2000 and 2001 harvests. Apparently the measures taken to control the DON level succeeded to maintain values below the action limits. Despite these activities a smaller outbreak of DON appeared in pasta products at a lower extent. This indicated that control should be done systematically, not sporadically, and at a European level, which is made possible since EC has set a limit in July 2000. Analytical results of the measurements are presented, together with the chronological order of the associated activities of national, EU and worldwide bodies on human health control. Special attention is paid to DON in bread, related to the level in flour.

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P31

Extracellular enzymes and production of aflatoxins in solid substrate fermentation

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Solid-state fermentation (SSF) is generally defined as the growth of microorganisms on solid substrates such as wheat in the absence or near absence of free water. A variety of micro-organisms, including bacteria, yeast and filamentous fungi, have been reported to produce enzymes. Raw starch-digesting amyloglucosidase reported so far have been produced using submerged fermentation. On the other hand, the use of solid-state fermentation (SSF) has been found to be more advantageous than submerged fermentation and allows the cheaper production of enzymes. Moulds such as *Aspergillus flavus* and *A. parasiticus* growing on grains can be characterised by their high capacity of metabolising carbohydrates. Many toxigenic fungi, which contaminate cereal such as wheat, can produce mycotoxins under certain environmental conditions such as temperature and water activity. The three major genera of mycotoxin production fungi are *Aspergillus*, *Penicillium* and *Fusarium* and are considered to be the most significant toxin producing moulds. Studies have been shown that *Aspergillus* and *Penicilium* spp. differ considerably in their ability to produce different enzymes. The objectives of the studies were to investigate the relationship between production of extracellular enzymes and aflatoxin production by aflatoxigenic *Aspergillus* in a solid substrate fermentation process. Our results indicate that there might be a correlation between the production and levels of aflatoxins (B1, B2, G1 and G2) formation and enzymes activities with *A. flavus*, and *A. parasiticus.*

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P32

Dietary exposure to ochratoxin A in the Netherlands

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Ochratoxin A (OTA), a mycotoxin produced by fungi, can cause an irreversible and fatal kidney disease in humans. OTA is predominantly found in cereals and cereal products, but also in raisins, grape juice, wine, coffee, cocoa and in some pulses and nuts, mainly as a result of poor storage of the commodities. As OTA is very persistent, products such as meat and dairy have been found to contain this contaminant as well. In the present study the human dietary intake of OTA in the Netherlands is assessed by combining data on concentrations in different food products and the consumption rate of these products. The Dutch Inspectorate for Health Protection and Veterinary Public Health provided data on OTA concentrations in grain, coffee, wine, nuts and spices. The Dutch data were combined with data from other countries for white wine, cocoa and nuts, rye, meat, milk and beer reported by the FAO/WHO, the EU and the Dutch State Institute for Quality Control of Agricultural Products. The consumption rate of the products containing OTA was examined with the Dutch National Food Consumption Survey (DNFCS), which describes the consumption pattern of the Dutch population in 1998 and includes information on the daily consumption over two consecutive days and a record of age, sex and body weight of 6250 individuals. Combining the concentration and consumption data results in two daily OTA intakes of all individuals included in the survey. It appears that more than half of the intake of OTA occurs via the consumption of cereals. Together with coffee, red wine and meat, 80% of the total intake of OTA is covered, while milk and beer add another 9%. The total geometric average intake calculated in this manner is 59 ng/day (0.9 ng/kg body weight/day). This is somewhat higher than that of 45 ng/day found in an earlier study in the Netherlands, which is due to higher concentrations in meat, nuts and cocoa used in the present calculation. The geometric mean of 51 ng/day (0.7 ng/kg body weight/day), measured in a duplicate diet study performed at our institute, agrees well with the value determined in the present study. To distinguish the variation between individuals from the daily fluctuations in consumption, we used the STatistical Exposure Model STEM, which is developed at our institute, and estimates the mean dietary intake as a function of age. It combines regression analysis on age by fitting an appropriate curve to the data with a nested analysis of variance. Analysing the data with STEM shows that the relative intake per age class is between 0.5 and 2 ng/kg body weight/day and decreases with age. The maximum value for the 99th percentile (age 1-year) is 5.5 ng/kg body weight/day, which is still lower than the provisional TDI (100 ng/kg body weight/week or 14 ng/kg body weight/day).

P33

Mycotoxins in food: samples analysed by ARPA Emilia Romagna in the years 2000-2001

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The food laboratory of ARPA ER analysed samples for government authority such as customs offices and Health department of Emilia Romagna District. This laboratory is accredited for determination aflatoxins and ochratoxin A in food.

In the years 2000-2001 aflatoxins B1, B2, G1 and G2 were determined in about 252 samples of dried fruits, cereals and legumes; 808 samples of nuts and 64 samples of spices and infusional herbs. About 3% of foodstuff examined present a level of aflatoxins exceeding limits fixed in EU. The kinds of food that present a high contamination were: dried figs, peanuts, pistachio, paprika and pepper.

The laboratory also determined ochratoxin A in coffee and cereals samples. Traces have been detected in some samples.

P34

Patulin in Dutch (infant) food

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Patulin is a mycotoxin produced by the species of *Penicillium, Aspergillus* and *Byssochylamys.* These moulds can be found on fruits (especially apples), grains and vegetables. Patulin has been found to be immunotoxic, neurotoxic and carcinogen in rats. This mycotoxin exhibits antibiotic activity but is too toxic to use in humans. The LD_{50} varies from 8 mg/kg inv-rat to 32 mg/kg orl-ham. Legislation in the European Union will be renewed in the near future. Draft amendments for EU Directive No 466/2001 have already been published. A limit of 50 µg/l resp. µg/kg for fruit juices, ciders, apple wines and solid apple including puree is recommended. For infant food and juices consumed by young children the recommended limit is 25 µg/kg resp. µg/l.

It should be clear that workable methods for analysis are necessary to meet these requirements. Most methods are based on liquid/liquid extraction and evaporation steps. These steps will introduce inefficiency and a decrease of the reproducibility. In order to avoid evaporation and extraction, TNO has developed a new method. This method is based on a clean-up/concentration step using a SPE C18 column, followed by a clean-up step using a silica column (Romer #224). Analysis is performed by using a reversed phase gradient HPLC system with a photo diode array detector. In case of apples an optional depectinase step has to be used. The developed method has good validation characteristics and meets the criteria of CEN CR 13505. Also a good baseline separation between 5-HMF (5-hydroxymethyl furfural) and patulin was achieved, which is a prerequisite because 5-HMF is produced during processing of apples, where heating is applied. During the heating steps carbohydrates are converted into 5-HMF.

In this study, apples, apple puree, apple juice, cider, drinks and baby food samples, both from organic and conventional source, have been analysed and compared. Within the study, only in one (organic) apple juice patulin was found above the limit of quantification (<25 µg/l), the measured concentration was 880 µg/l. Considering the number of samples in this study, no conclusions regarding patulin contamination in organic and conventional samples can be made based on this incident.

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P35

Genotoxicity assessment of some mycotoxins found in food, feed and in the environment by simple *in vitro* bioassays

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Mycotoxins are secondary metabolites of moulds that have adverse effects on humans, animals and crops. Beside important economic losses at a worldwide scale, health impairments seem to be more frequent and diversified than previously supposed. The most frequent toxigenic fungi in Europe are *Aspergillus*, *Penicillium* and *Fusarium* species. They produce aflatoxin B1 as well as ochratoxins, zearalenone, fumonisin B1, patulin, trichotecenes…, which are of increasing concern in animal and human health as they are found in the environment (indoor pollution), and in food and feed where they are not destroyed by normal industrial processing or cooking since they are heat-stable.

Even though the specific modes of action of most of the toxins are not well established, acute and chronic effects on health have been reported. Most of the mycotoxins are indeed genotoxic agents: aflatoxin B1 is a category I known human liver carcinogen and is mutagenic in many genotoxicity tests (chromosomal aberrations, sister chromatid exchange, micronuclei, unscheduled DNA synthesis, DNA strand breaks, DNA adducts in human and rodent cells); ochratoxin A is a potent carcinogen responsible for urinary tract cancer and kidney damage; trichotecenes are highly immunosuppressive; patulin, mainly found in apples and apple products is acutely toxic but also mutagenic and carcinogenic; zearalenone causes oestrogenic effects in animals and man; fumonisins were so far believed to act by disrupting the signal transduction pathway but they recently also appear to have a DNA damaging potential that has probably been underestimated and could be the cause of oesophageal cancer. Considering the adverse effects of the mycotoxins on health and the impossibility to completely eliminate them from the food and from the environment, several analytical methods have been developed to assess their presence. These techniques are very useful but only applicable if one family of toxins or even sometimes one single mycotoxin is suspected to be present. These limitations clearly show that a certain risk thus persists to miss some unsuspected or unknown toxins and highlights the need for global toxicity detection methods, and in particular for biological methods like bioassays that allow an holistic approach in the detection of the toxicity and biological activities of mycotoxins.

In the present work, we show that some very simple, rapid and cost effective bioassays on cells and bacteria allow the genotoxicity assessment of the most common mycotoxins. By performing Comet assays (SCGE), we confirmed the DNA damaging activity of aflatoxins, ochratoxin A and fumonisin B1 on K562 human erythroblasts and HepG2 hepatocytes. The Comet assay also allowed us to easily demonstrate the clastogenic activity of patulin, only shown so far by longer or tedious methods. The VITOTOX™ bacterial test was also used to assess the genotoxic potential of mycotoxins. This assay is performed on genetically modified *Salmonella thyphimurium* that emit bioluminescence each time the SOS response mechanism is triggered, notably by contact with DNA damaging agents. This test allowed us to detect the genotoxic activity of aflatoxins and ochratoxin A at concentrations corresponding to the regulatory limits.

This study demonstrates that some *in vitro* bioassays can provide rapid and crucial information on the genotoxic potential of mycotoxins found in contaminated substrates prior to expensive and long-term animal studies.

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P36

The distribution of trichothecenes between husk and kernel in oats

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In total 39 samples of oats were collected from a mill in Moss, Norway (Regal Mølle) in the period August 2000 - April 2002. This mill produces mainly products for the food industry. All the samples were divided into husk and kernels. The grinded sample (25 g) was extracted with acetonitrile/water. After filtration, the extract was cleaned on a Mycosep #225 column (Romer Labs), evaporated and derivatised before it was analysed on GC-MS.

In the husk, HT-2 toxin was found in 92% and T-2 toxin in 69% of the samples. The mean concentrations of HT-2 and T-2 toxin were 330 and 120 μg/kg, respectively. The highest concentrations of HT-2 and T-2 toxin were 2300 and 890 μg/kg, respectively. Deoxynivalenol (DON) was detected in 74% of the husk samples, with a highest level of 800 μg/kg. Nivalenol (Niv) was detected in 13% of the husk samples, with a highest level of 190 μg/kg. In the kernel samples, trichothecenes were detected (detection limit: 20 μg/kg) in 12-13% of the samples, and the mean concentrations was between 10-15 μg/kg.

Calculating the distribution of trichothecenes between husk and kernel, about 90% of the toxins were found in the husk part of the sample, with an exception for Niv who had 70% in the husk part. The results indicate that the main amount of trichothecenes is connected to the oats husk. As for oats, husk is of minimal use, and mycotoxin contamination in oats, seems to be a smaller problem than first assumed.

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Mycotoxin surveillance and training programmes

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Consumption of foods heavily contaminated with mycotoxins is not likely to occur to any substantial level in most developed countries because of the existence of strict food regulations. Conversely, in developing countries, consumers face the prevalence of chronic exposure to high levels of mycotoxins. Regional and/or international co-operation activities provide opportunities to alleviate mycotoxin problem and ensure a wholesome food supply. This is reflected in actions undertaken by IAEA under the joint FAO/IAEA and IAEA Technical Co-operation programmes since 1998. These programmes aim to help Member countries to acquire appropriate skills and to have access to regulatory principles and guidelines, ensuring compliance to international standards, which would underpin export trade and guarantee consumer safety. These IAEA initiatives will allow the countries to bypass decades of gradual progress made by developed countries and will promote efficient and effective implementation of food quality control and safety systems that benefit domestic consumers and promote food trade.

P38

Detoxification of aflatoxin M1 in milk with lactic acid bacteria and related genera

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Detoxification of aflatoxin contaminated foods has been a continuing challenge for the food industry. Natural contamination of milk with aflatoxin M1 (AFM1) may be encountered in the dairy industry. It is known that milk contaminated with AFM1 may be carried over into milk products such as cheese, cream, butter and other dairy products.

Detoxification of mycotoxins is grouped into three categories namely physical, chemical, and biological methods. Of the biological methods, some strains of lactic acid bacteria and bifidobacteria have been reported to be affective in removing aflatoxin B1 (AFB1). El-Nezami et al. (1998) investigated the ability of dairy strains of lactic acid bacteria to bind food carcinogen, AFB1. Also Haskard et al. (2001) screened the surface binding of AFB1 by lactic acid bacteria. In our study we investigated the ability of dairy strains of lactic acid bacteria and bifidobacteria to detoxify aflatoxin M1 in processed milk under different environmental conditions.

Results showed that certain strains of lactic acid bacteria detoxified AFM1 by more than 70%. The efficacy in detoxification was higher with lactic acid bacteria than bifidobacteria. The ability of *Aspergillus flavus* and *Aspergillus parasiticus* to produce aflatoxin in the presence of lactic acid bacteria is also been investigated.

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P39

Presence of fumonisin in finger millet, kidney beans and peanuts derived from Tanzania

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Finger millet, kidney beans and peanuts were collected from various farmers, transporting lorries and market retailers in Tanzania after harvest (AH) period in July 2001 and six months after harvest (SMAH) in January 2002. The objective was to evaluate the presence of fumonisins in these ingredients, which are used in the formulation of complementary food (CF) for infants in Tanzania.

Fumonisins were extracted from the samples by 70% methanol and quantified by enzyme-linked immunosorbent assay (ELISA). There was significant difference in fumonisin concentration for the samples collected AH and those collected SMAH. Samples collected SMAH were found to have low levels of fumonisins compared to the samples collected AH. All the grains were found to have fumonisin concentration ranging from less than 5 ppb to 440 ppb. Peanuts samples collected AH were found to have comparatively high fumonisin levels (mean 105 ppb), followed by kidney beans (mean 43 ppb) and finger millet (lower than 5 ppb). Samples collected SMAH had fumonisin levels ranging from 0-36 ppb. Variations in fumonisin levels were also observed between samples obtained from farmers, lorries and retailers.

The low fumonisin concentrations found in the AH and SMAH grains suggest that these ingredients are comparatively not susceptible to *Fusarium* attack as it has been found in corn. Further research is required to establish safe and acceptable limits for fumonisins taking into account the potential toxicity of fumonisins and the tender age of infants, who are fed on CF prepared from these ingredients.

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P40

Cocontamination by ochratoxin A and citrinin increases OTA toxicity *in vitro* and *in vivo*

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Ochratoxin A (OTA), a nephrotoxic and carcinogenic mycotoxin is implicated in the aetiology of Balkan endemic nephropathy (BEN), a chronic disease affecting populations in the Balkans. Studies on food contamination conducted in Bulgaria demonstrated that, a higher percentage of the staple food was contaminated by OTA and citrinin in the BEN endemic area than in the non-endemic areas. *In vivo* studies have shown a synergistic effect of these two mycotoxins in several animals. Simultaneous administration of OTA and CIT enhanced the incidence of renal cell tumours in male DDD mice. The aim of this study was to determine the combined effect of citrinin and ochratoxin A on cytotoxicity and genotoxicity, (i) in an *in vitro* system (cells culture) and (ii) *in vivo* on Dark agouty rat fed for 3 weeks with ground wheat enriched with OTA and/or CIT.

Cytotoxic effect of increasing amounts of OTA alone (0.1µM to 100 µM) or citrinin (CIT) alone (0.5 µM to 100µM) or both has been tested on human epithelial bronchial cells and on opossum kidney cells. For low doses of OTA alone or CIT alone (below 2µM), cell proliferation is observed. CIT exhibit a higher proliferative effect than OTA. For the doses above 2µM cytotoxic effects are observed for both mycotoxins. The cytotoxic effect of OTA is higher than that of CIT. When the mycotoxins are simultaneously present, the cytotoxicity is considerably enhanced. In both cases, DNA adducts are formed. The pattern of DNA adduct is specific to each mycotoxin. The formation of DNA adduct is time and concentration dependent. When both mycotoxins are present simultaneously, the OTA and CIT DNA adduct patterns are modified. Some new adducts appeared. Analysis of the metabolites found in cell cultures, indicated the formation of new derivatives when CIT is also present.

We have also analysed and compared kidney DNA adduct from rat fed 3 weeks by food contaminated by OTA alone, CIT alone or by both mycotoxins. DNA adduct patterns are similar to those obtained in cell culture. The main OTA adduct is dramatically increased.

In conclusion, a synergistic effect between OTA and CIT is observed which pinpoints the importance of CIT.

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P41

Fate of deoxynivalenol during brewing and effect of different brewing yeasts

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Fusarium head blight (FHB), which is caused by *Fusarium* species' infection, is an important disease for many cereals including malting barley. *Fusarium* infection is associated with the production of mycotoxins, for example deoxynivalenol (DON). Consequently, beer could be contaminated with DON when contaminated barley is used for its production. DON has been shown to cause a variety of toxic effects in animals, with symptoms such as diarrhoea, and low weight gain. In humans it is immunodepressive.

Several studies have investigated the fate of DON during malting and brewing. Mannio and Enari (1973) studied the detection of *Fusarium* toxins in naturally and artificially contaminated barley. At that time the lack of suitable, sensitive analytical methodology did not allow the detection of mycotoxins in beer. Niesen and Donheuser (1993) observed that the quantity of DON increased by a factor of 3 to 8 during the mashing step of brewing performed at temperatures between 50 and 72 °C. The same authors reported that DON could not be recovered in the spent grains, neither reduced during wort boiling or during fermentation. Scott et al. (1992) and Boswall et al. (1995) showed that DON is stable during beer production. Schwarz et al. (1995) obtained recoveries ranging from 80 to 93% for malts contaminated at levels between 1.8 and 17 µg DON/g. Only 2.7 % of DON was found in the spent grains. Only one study has been performed so far by Scott et al. (1992) to show the stability of DON when added to wort undergoing fermentation with 3 different strains of *Saccharomyces cerevisiae*.

In this poster we present the results obtained from a brewing experiment using naturally contaminated barley. We looked at the effect of eight different commercial yeasts that included four ale strains coming from breweries in England, two lager strains, one from Germany and one from the Czech Republic, and a wheat beer yeast from Germany. The barley used had a DON contamination level of around 3.0 ppm and was harvested in 2001 in North Dakota. Thirteen kilos of this malt were used to produce about 80 litres of beer with an alcohol level of 5%. The fermentations were performed in triplicates in a 3-liter pitching tank for each yeast. The samples analysed for DON included the milled barley, the wort, the spent grains, the yeast cakes for each yeast and the resulting beers. Analysis of these samples for DON involved an extraction step with water for the cereals followed by immunoaffinity column cleanup or direct application for the beer samples, and gas chromatographic separation with mass spectrometry detection.

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Deoxynivalenol (DON) in beer

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DON is a secondary metabolite produced by several fungal genera, but mainly by *Fusarium* species. Growth of *Fusarium* species and toxin production can occur at relatively low temperatures on agricultural commodities in the field or during storage. Therefore, DON is commonly found in cereals from moderate climatic zones. In 1999 high concentrations of DON were found in breakfast cereals sold on the Dutch market (levels up to 2600 μg/kg). A survey was carried out in the Netherlands in the first half of 1999 and 2000 to determine the quantities of DON in (imported) wheat (1998 and 1999 harvests). In about 40% of the wheat samples the temporary DON limit of 500 μg/kg for cleaned wheat that is effective in the Netherlands was exceeded. The presence of DON is however not only limited to wheat and can also be present at high concentrations in other grains. If these grains are used in the brewing process, DON can contaminate the produced beer.

For the determination of DON in beer a method based on capillary gas chromatography (GC) with Flame Ionisation Detection (FID) has been developed and validated. DON is extracted from the sample matrix by ChemElut® extraction columns. Two different Mycosep® clean-up columns are used to purify the extract. Quantification is based on the internal standard α-chloralose. The average recovery for DON is 92% (at a level of 190 μg/l) and the limit of quantification is 25 μg/l at a signal-to-noise ratio of 9.

In 2000 a survey was carried out in the Netherlands to determine the quantities of DON in beers marketed in the Netherlands. In only 3 of the 51 beer samples low quantities of DON were found, ranging from 26 μg/l to 41 μg/l.

P43

Occurrence of ochratoxin A and citrinin in French wheat - is there a relation with some renal tumours?

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During storage of cereals, some fungi grow and elaborate mycotoxins such as ochratoxin A (OTA) or citrinin (CIT), both of which are nephrotoxic. Simultaneous administration of OTA and CIT enhanced the incidence of renal tumours in male mice. Simultaneous presence of OTA and CIT has been observed in Bulgaria in food from families affected by nephropathy and urinary tract tumours. Contamination of cereal by OTA occurs also in other part of Europe and can be responsible for some renal tumours.

The aim of this study was to evaluate the level of contamination of wheat by OTA or CIT in France. Several samples of wheat were collected in various parts of France from co-operatives and farms at different period of the year. OTA and CIT were analysed by HPLC, with fluorimetric detection. More than 50% of the samples contain at least one of these mycotoxins. OTA contamination levels ranged between 0.1 to 68µg/kg. For CIT in some case the levels reached value as high as 520 µg/kg. The highest contaminations irrespective of the mycotoxin were found in wheat from the north of France. A statistical difference is observed between farm samples and the others. Indeed, farm samples are more contaminated. We also observed that contamination increased during time of storage. One third of the sample are simultaneously contaminated by OTA and CIT. Durum wheat is more frequently contaminated and at higher levels than wheat used to make flour. Fifty percent of the OTA-contaminated wheat exceeds 3µg/kg and twenty five % of the 5µg/kg.

In order to establish an eventual implication of OTA in some human urinary tract tumours in France, we have analysed kidney and bladder tumours obtained after surgery from 23 patients. Several DNA adduct were detected in all tumours. DNA adduct patterns were different from one individual to the other, indicating various kind of contaminant. 7/17 kidney tumours and 2/6 bladder tumours exhibited OTA-specific DNA adduct patterns similar to those found in Bulgarian suffering Balkan endemic nephropathy, OTA-treated rodent and OTA-treated pig. OTA has been found only in organs of patients for which OTA-specific DNA adduct were observed. Preliminary re-analysis of the DNA adduct patterns demonstrate possible implication of citrinin.

In conclusion, according to these results, we hypothesise that OTA (and probably CIT) plays a possible role in the development of some human kidney tumours in France.

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P44

A survey of ochratoxin A in wines from South Africa and Italy

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The natural occurrence of the carcinogenic mycotoxin, ochratoxin A, in wines sold in local retail outlets in South Africa and Italy was investigated by HPLC analysis with fluorescence detection following immunoaffinity column purification. All 24 local South African wines tested (15 white, 9 red) were found to contain detectable levels (>0.01 µg/l) of OTA, with a mean of 0.16 µg/l in the white wines and a mean of 0.24 µg/l in the red wines. Results were subsequently confirmed by LC-MS analysis using positive ion electrospray ionisation with selected reaction monitoring of product ions at m/z 358 and m/*z* 386 formed by collision induced dissociation of the protonated molecular ion at m/z 404. Comparison with the fluorescence method gave a significant correlation ($r=0.87$; $p<0.01$). Although OTA contamination was present in all the South African samples analysed, levels were well below the suggested European Union limit of 0.5 ng/g. Of the 8 Italian wines analysed, 2 red wines were contaminated above this level.

P45

The ability of *Fusarium avenaceum* to accumulate moniliformin in Norwegian grain

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The aim of the project was to examine the contamination of Norwegian grain with moniliformin and compare the moniliformin concentrations with the incidence of *Fusarium avenaceum* infested kernels.

Of the 231 analysed samples, 53 were from the 2000 season (21 oats, 19 barley, 13 wheat), 84 were from the 2001 season (26 oats, 23 barley, 35 wheat) and 96 were from the 2002 season (27 oats, 33 barley, 36 wheat). Prior to quantification of moniliformin using ion pairing reversed phase HPLC with UV-detection by a diode array detector, all samples were extracted using 84% acetonitrile and purified using disposable strong anion exchange columns. The limit of detection was 40 µg/kg. Moniliformin was found in 32, 25 and 76% of the samples of oats, barley and wheat, respectively. The highest amount of moniliformin in oats was 210 µg/kg, 380 µg/kg in barley and 950 µg/kg in wheat.

The amount of moniliformin in the grain samples was not correlated to the incidence of kernels infested with the moniliformin-producing fungus *Fusarium avenaceum*, surface disinfection having no impact on the results. This indicates that other factors than level of infection are of importance.

P46

Use of glucomannan to reduce the effects of ochratoxin A in broilers

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Mycotoxins are a group of structurally diverse secondary fungal metabolites that occur as contaminants of grain worldwide. Among the various mycotoxins identified, ochratoxin A being the most potent toxin, is produced by *Aspergillus ochraceus*, a mould that is widely distributed in nature. The presence of ochratoxin is often associated with an unexplained reduction in the performance of poultry and swine. Ingestion of ochratoxin causes severe kidney damage precipitating into conditions like nephritis and gout in field situations. With the advent of biotechnology, glucomannans (Mycosorb^{TM} from Alltech) derived from the inner cell wall of yeast, *Saccharomyces cerevisiae* *1026* has been found to significantly alleviate the adverse effects of mycotoxicosis in poultry [1-4]. In a recent trial, glucomannans adsorbed aflatoxin up to 75-90% and T-2 toxin up to 30-35% in the gastrointestinal tract of broilers [5]. In order to ascertain the range of mycotoxin-adsorptive ability of glucomannans an *in vitro* and an *in vivo* trial were conducted to determine the efficacy of glucomannans in counteracting the toxic effects of ochratoxin A in commercial broilers. Ochratoxin A was produced by using *Aspergillus ochraceus* NRRL 3174 culture and the material was added to the respective dietary treatments at a concentration of 0.5 ppm. Two hundred and eighty-day-old broiler chicks were randomly distributed to four equal groups with five replicates of seventy birds per treatment. The treatments were: 1) control diet, 2) control + GM (0.05%), 3) ochratoxin contaminated diet (0.5 ppm), and 4) ochratoxin contaminated diet (0.5 ppm) + GM (0.05%). Parameters measured were body weight change, kidney lesions, serum proteins and enzyme concentrations and hematocrit values. The effects of dietary treatments on broiler performance are presented in Table 1. Feeding of the ochratoxin contaminated diet resulted in a significant reduction in body weight gain (17.6%), increased gamma glutamyl transferase (GGT) activity (38.5%), and a decrease in total proteins (16.4%) and in hematocrit values (11.3%). Grossly, kidneys from broilers fed ochratoxin contaminated diet were enlarged, friable and congested in appearance. Mortality of 8.57% was observed in ochratoxin fed birds. Supplementation of glucomannans to the ochratoxin contaminated diet resulted in: improved body weight gain (3.6%), total proteins (11%), hematocrit values (4.7%), decreased GGT activity (14.7%), and reduced mortality.Glucomannans, when used in conjunction with other management practices, may prove to be a very useful tool in the management of ochratoxin contaminated feedstuffs in poultry and livestock.

Table 1. The effects of dietary treatments on broiler performance.

Trt	Mycosorb (0.05%)	OA (0.5 ppm)	B.wt (g)	GGT IU/L	Total proteins (g/100ml)	Hematocrit %
T1	-	-	1391^{c}	8.3^{a}	2.62^{c}	36.0^{b}
T2	+	-	1441^{d}	8.6^{a}	2.76^{d}	37.5^{b}
T3	-	+	1146^{a}	11.5^{c}	2.19^{a}	31.9^{a}
T4	+	+	1187^{b}	9.8^{b}	2.43^{b}	33.4^{a}

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Mycotoxins in forage feeds for dairy cattle

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This poster reviews the occurrence of mycotoxins in forage feeds for dairy cows, the impact on animal health and the transfer of mycotoxins from feed to milk. The diet of dairy cows consists of three types of feed: concentrate, by-products and forages. Forages generally represent 50-80% of the diet. Grass and maize are the main forage crops in Europe. Grass is fed fresh (grazed or harvested daily) or after preservation (silage or hay). Maize is fed as silage only, generally whole crop silage.

Occurrence of mycotoxins in forages

Moulds can contaminate forages at different stages. Maize and, to a lesser extent, grass may be infected in the field by moulds, *Fusarium* species in particular. Data from Germany, Austria and The Netherlands indicate that *Fusarium* mycotoxins zearalenone and deoxynivalenol (DON) are frequently found in maize. Concentrations of these mycotoxins in preharvest maize and maize silage are similar, indicating chemical stability during the silage fermentation process. Apart from mould infection in the field, certain moulds may develop during forage preservation and storage. Growth of moulds in silage is associated with exposure to air, for instance due to insufficient sealing or slow feeding of a silage clamp. *Penicillium roqueforti*, an acid-tolerant species capable of forming the mycotoxin roquefortine C, is typically associated with silages. Quantitative data on the occurrence of roquefortine C in silages are lacking. The third group of moulds are the endophytic moulds (e.g. *Neotyphodium*), which live in symbiosis with the plant. These moulds form alkaloid mycotoxins and occur in wild grass populations. Grass cultivars used in Europe for grasslands for cattle are endophyte-free. Extensification of farming systems in Europe may however lead to an increase in the use of endophyte-containing grasslands.

Estimation of daily mycotoxin intake by dairy cattle

The theoretical average and maximum daily intake of DON and zearalenone by dairy cows and the contribution of the different feed categories were calculated using estimates for average and maximum concentrations based on literature data. These calculations showed that maize silage contributed approximately 80% of the daily DON intake and approximately 50% of the daily zearalenone intake, indicating that maize silage is a major source of mycotoxins in the diet of dairy cows.

Impact on animal health and transfer to milk

DON and other trichothecene mycotoxines are degraded and detoxified to a larger extent by the micro-organisms in the cow's rumen. Therefore, ruminants are less sensitive to these mycotoxins than non-ruminants such as pigs and poultry. Zearalenone is also metabolised in the rumen, but the resulting metabolites have a similar or even higher toxicity. High levels of zearalenone have affect on the animal's fertility. Literature data indicate that the transfer zearalenone and DON to milk is extremely low (0.03% or less).

Conclusions

A limited amount of quantitative data is available about the occurrence of mycotoxins in forage feeds. The available data indicate that maize silage potentially is an important source of mycotoxins in dairy cattle diets. However, there is no evidence that mycotoxins in forages constitute a serious risk factor with respect to animal health. Due to the limited transfer to milk, it is generally accepted that mycotoxins in feeds for dairy cattle do not form a significant risk for human health.

P48

Yeast-derived glucomannans decrease oxidative stress caused by T-2 mycotoxicosis in quail

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Antioxidant systems are considered to play an important role in protection against the damaging effects of free radicals and their toxic metabolites. A delicate balance between antioxidants and pro-oxidants, is responsible for regulation of various metabolic pathways leading to maintenance of immunocompetence, growth and development and protection against the stress conditions of commercial poultry production. This balance can be compromised by dietary mycotoxins [1]. The aim of the present work was to assess the efficiency of a yeast-derived glucomannan product (Mycosorb^{TM} from Alltech) to decrease the oxidative stress in quail caused by T-2 toxin. Four groups of 4-day old quail were formed with 20 birds per group. The three treatment diets consisted of the basal diet with T-2 toxin added (final T-2 toxin concentration was 8.1 mg/kg feed), T-2 toxin (8.1 mg/kg) plus zeolite (30 g/kg feed), and T-2 toxin (8.1 mg/kg) plus Mycosorb^{TM}, (1 g/kg feed). After 30 days of feeding (34 days old) all birds were sacrificed and samples for biochemical analyses were collected from five quail in each of the four groups. Antioxidants and lipid peroxidation were analysed by HPLC-based methods and total antioxidant activity (TAA) of plasma was assayed by spectrophotometry.

Inclusion of T-2 toxin in the quail diet only slightly (by 6.1%) decreased feed consumption. However, final body weight was decreased by 14.3%. During the last 2 weeks of the experiment mild signs of toxicity were observed. They included inflammatory response in the mouth, which reached a necrotic status in some birds by the end of experiment. There were also some lesions at the root of the tongue. Our data indicate an oxidative stress caused by T-2 toxin. In particular, tocopherols, carotenoids, ascorbic acid and reduced glutathione concentrations in the liver as well as TAA of plasma were significantly decreased as a result of T-2 toxicosis. Retinol ester concentrations in the liver were also decreased. Inclusion of yeast glucomannans (Mycosorb^{TM}) in T-2 toxin-containing diets fed to quail significantly slowed the depletion of natural antioxidants (alpha- and gamma-tocopherols, ascorbic acid, reduced glutathione, retinol and retinyl esters) in the liver and TAA of plasma and a significant decrease in liver susceptibility to lipid peroxidation in comparison to results for birds fed the diet containing only T-2 toxin. In fact, concentrations of γ-tocopherol and ascorbic acid in the liver were maintained at control values. It is interesting to note that a protective effect of Mycosorb against T-2 toxin was associated with prevention in body weight decrease and the reduction of inflammatory signs of toxicity. However, inclusion of Mycosorb in the quail diet was unable to completely prevent the adverse effects of T-2 toxin on the antioxidant systems of the liver of the growing quail; indicating that not all T-2 toxin was bound. In contrast to Mycosorb, zeolite was not effective in preventing the damaging effects of T-2 toxin on the antioxidant system of quail. These data are in agreement with previous observations indicating low zeolite affinity to T-2 toxin. The principal finding from this research is a protective effect of a yeast cell wall-derived glucomannan product against T-2 toxicosis by preventing toxin damage to the hepatic antioxidant system.

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P49

Physico-chemical properties of a magnesium smectite as a natural and safe mycotoxin adsorbent feed additive

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The objective of the research presented here was to determine the physico-chemical characteristics of a magnesium smectite from Yunclillos (Spain) as compared to aluminium smectites and sepiolite which are commonly used in animal nutrition and to identify the most relevant characteristics regarding aflatoxin B1 (AB1) adsorption.

In vivo AB1 protection was calculated using as indicators changes in cholesterol and albumin concentrations of blood samples from ducklings fed either a control diet or contaminated diet (90 ppb AB1) with and without clay adsorbents (Magnin and Guyonvarch, 2003).

Physico-chemical characteristics of mineral clay adsorbents can be used to predict their in vivo adsorption efficacy. Surface area (SA) and cation exchange capacity (CEC) have been selected as the most relevant characteristics. A quadratic correlation (R^2 = 0,69, P< 0,01) was found between in vivo efficacy per unit of SA and CEC per unit of SA.

Table 1. Selected physico-chemical characteristics of the adsorbents.

Adsorbent	N	CEC [a],meq/100g	SA[b], m^2/g	CEC/SA
Magnesium Smectite	5	46,2±8,5	147 ± 41	0,34 ± 0,14
Aluminium Smectite	9	90,0±18,11	63 ± 14	1,53 ± 0,60
Sepiolite	4	15,9±3,2	268 ± 55	0,06 ± 0,03

[a] Determined using an ammonia electrode according to Miller et al. (1975).
[b] Determined by multilinear Langmuir adsorption of N_2, according to Brunauer et al. (1938), Norm ASTM – D3663-84.

Figure 1. Relation between CEC/SA ratio and AB1 protection per unit of SA

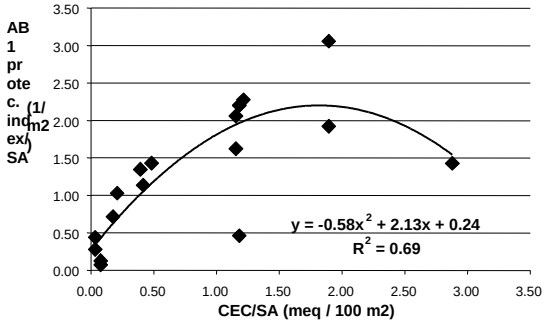
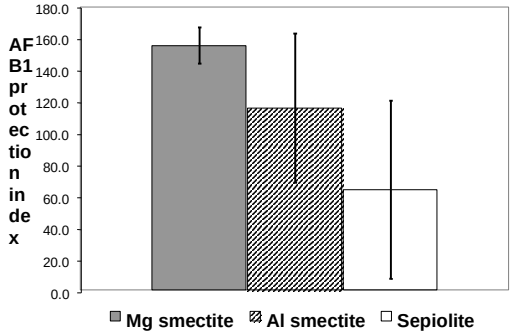


Figure 2. AFB1 protection per unit of adsorbent.



From these results can be concluded that SA and an adequate ratio of CEC to SA are required for effective adsorption of AB1 by mineral adsorbents. This conclusion is coincident with that obtained from similar studies conducted with in vitro trials.

P50

Measurement of elimination of mycotoxins

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In farm animals mycotoxins cause higher mortality, increased medication costs and reduced production. Many methods are considered for detoxification. Two new protocols for the testing of the efficacy of possible toxin eliminators were examined: A gut simulation model and a toxicity test based on specific gene expressions.

Gut simulation model

A large variety of substances are claimed to have toxin binding capacities. Clay minerals are the most widespread products for this purpose. Phillips et al. suggested that the aluminium ions form a complex with the β-carbonyl system of aflatoxins. Other reports find that the size of the cavities in the minerals is important to trap the toxins. Whether the bound toxins stay bound in the entire digestive tract is difficult to assess. There some reports that the adsorption grade was slightly lower at pH 2 then at pH 7. Little has been publicised about the influence of the other aspects of the digestion system. Other types of inactivators become more popular, such as enzymes and fragments from the cell walls of yeasts. These are not much described in the literature about the possible. We screened many potential toxin eliminators in a simulation system of a chicken-gut. The research group of Drs H. Clarijs (HAS, the Netherlands) developed a simulation of the small intestine of chickens for these tests. With relatively simple and constant means the effect of the complete range of factors affecting of toxin binding in this case in the animal was mimicked. There were significant differences between the classical *in vitro* tests and the gut simulation model. With these results a combination product (Elitox) was developed. This product is a combination between a clay mineral, chitosan and enzymes and has a broader effect than the single ingredients.

Elimination of mycotoxins tested with the activation of stress genes

Stress inducers (i.e. toxic substances) will activate specific reactions in cells. These reactions are the results of the induction of specific genes: stress genes. These stress genes generate stress proteins. Most of these stress proteins either neutralise the toxic effects by repairing the damage and/or by inactivating the toxin itself. These genes can be used in laboratory tests to predict either the toxicity of a substance, the mode of action of a toxin, or to identify the toxin in a mixed substance. The cell lines have a modified genome: the genome of each individual cell line contain a construct that is made of a stress promoter combined with detectable reporter gene (e.g. encoding for an enzyme: ß-galactosidase). Activation of the promoter as a result of environmental stress results in a measurable signal (e.g. ß-galactosidase activity) that is indicative for a specific cyto- or genotoxic parameter. In a project with De Nayer Institute and the RUCA University 5 ppm aflatoxin B1 was added with or without Elitox. Aflatoxin activated specifically the UmuDC-gene. This UmuDC gene encodes for the intracellular stress response to DNA damage and blocking of DNA replication and plays a role in subsequent targeted mutagenesis. The sample that was treated with Elitox did not the same induction of the UmuDC gene. The results indicate that Elitox is able to inhibit the toxic effect of aflatoxin B1. The simulation of the gut is an important improvement for determination of the efficacy of mycotoxin eliminators. The screening of a large variety of possible active components resulted in the development of a combined product, Elitox. The detoxification is also confirmed for aflatoxin B1 in a new toxicity test system that used stress gene technology. This system is potentially valuable for testing of contaminations with more than one mycotoxin.

P51

Effect of yeast cell wall based toxin binders on performance and health of gilts fed diets containing zearalenone and DON

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The aim of this trial was to investigate the effect of two yeast cell wall based mycotoxin binders on performance, development of the reproductive organs and bile zearalenone concentrations of growing gilts. The trial was set up as a complete randomised design with 4 treatments and 12 animals per treatment. Animals (German hybrid cross) were kept individually on slated floor pens. Animals were 70 days of age at the beginning of the trial. The trial lasted 160 days. The feed met commercial standards (13.4 MJ ME/kg, 19.7% CP and 1.02% lysine) and was fed as mash. Feed and water were provided *ad libitum.* Treatments are summarised in Table 1. Feed intake, body weight and FCR were recorded weekly. Exterior reproductive organs were evaluated daily. In addition, 4 animals per treatment were sacrificed at 150, 190 and 230 days of age for analyses of reproductive organs. Uterus weight was determined and the developments of the uterus and the ovaries were judged macroscopically. Bile samples were cleaned up with an immunoaffinity column and bile zearalenone concentrations were determined using reverse phase HPLC. All data very subjected to analyses of variance. Due to an outbreak of influenza performance parameters were only compared for 84 days. Table 2 summarises performance data. No macroscopic changes were noticed in the development of the reproductive organs, indicating that the zearalenone challenge applied was too low to lead to changes. Bile zearalenone concentrations did not differ between treatments: control (1.07 ± 0.98 ng/ml), mycotoxin (1.26 ± 1.39 ng/ml), Mycosorb (0.98 ± 0.90 ng/ml), and Zeasorb (1.08 ± 1.23 ng/ml). The lack of difference between the 'control' and the 'mycotoxin' treatment indicates that the method was not suitable to detect differences in zearalenone challenge as used in this trial. The binders tested in this trial did reverse the negative effect of mycotoxins on feed intake and weight gain, indicating a detoxifying effect on DON. However, the concentration of zearalenone applied in this trial was too low to lead to significant changes in the development of the reproductive organs or in bile zearalenone concentrations. Therefore potential effects of the binders on zearalenone could not be evaluated.

Table 1. Mycotoxin and binder concentration of different diets.

Treatment	Description	zearalenone ppb	DON ppb	additive
Control	low mycotoxin challenge	33.7	70	no
Mycotoxin	high mycotoxin challenge, no binder	81.7	560	no
Mycosorb	high mycotoxin challenge, commercial binder	75.3	590	1000 ppm[1]
Zeasorb	high mycotoxin challenge, experimental binder	78.9	610	1000 ppm[2]

[1] Mycosorb™, Alltech, Inc. (commercial product)
[2] Zeasorb™ (experimental product with high capacity to adsorb zearalenone *in vitro*)

Table 2. Daily weight gain, daily feed intake and FCR of gilts challenge with mycotoxins and fed toxin binders.

Treatment	Intake (kg/ head and day)			ADG (g/head and day)			FCR		
	Period 1	Period 2	Overall	Period 1	Period 2	Overall	Period 1	Period 2	Overall
Control	2,09 [a,b*]	2,73	2,25 [a,b]	780 [a,b]	852	798 [a,b]	2,68 [a]	3,24	2,82 [a]
	± 0,18	± 0,29	± 0,16	± 57	± 143	± 54	± 0,17	± 0,33	± 0,12
Mycotoxin	1,98 [a]	2,65	2,15 [a]	743 [a]	785	754 [a]	2,67 [a]	3,42	2,85 [a]
	± 0,17	± 0,30	± 0,10	± 58	± 128	± 33	± 0,10	± 0,36	± 0,12
Mycosorb	2,16 [a,b]	2,86	2,34 [b]	830 [b]	890	845 [b]	2,61 [a]	3,24	2,76 [a]
	± 0,22	± 0,29	± 0,20	± 80	± 129	± 61	± 0,13	± 0,28	± 0,10
Zeasorb	2,21 [b]	2,86	2,37 [b]	775 [a,b]	835	790 [a,b]	2,87 [b]	3,45	3,01 [b]
	± 0,25	± 0,28	± 0,20	± 102	± 108	± 77	± 0,26	± 0,38	± 0,25

[a,b]different letters within one column indicate significant differences

P52

Comparison of commercial mycotoxin adsorbents for their adsorption kinetics with aflatoxin B1 *in vitro*

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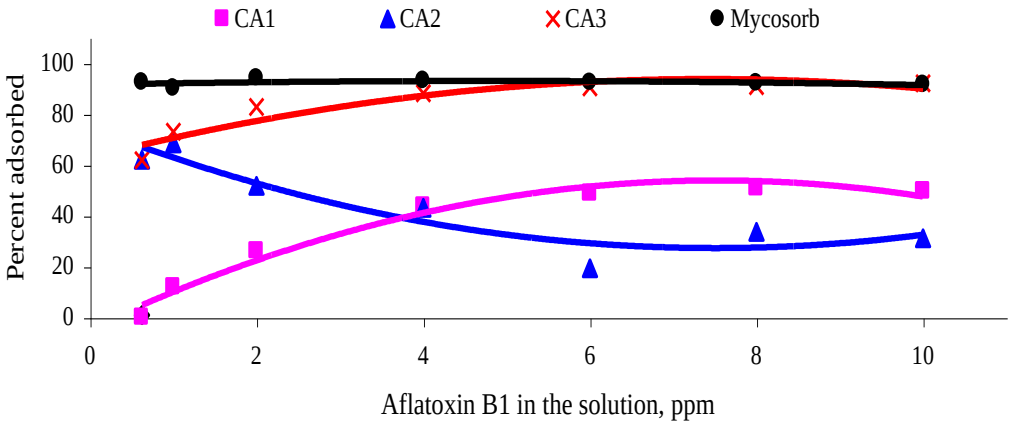
Aflatoxin B1 (AF B1), secondary metabolites produced by *Aspergillus* species of fungi, may contaminate animal feedstuff resulting in decreased performance in livestock. Several mycotoxin adsorbents have been shown to reduce the deleterious effects of AF B1 to varying extent by forming a complex with AF B1 in the gastrointestinal tract and thereby reducing its bioavailibility. In this study, three commercial clay-based adsorbents (CA1, CA2 and CA3) and modified glucan preparation, Mycosorb^{TM} (Alltech, Inc., Nicholasville, KY), were evaluated for their in vitro isothermal adsorption kinetics with AF B1 in water. Adsorbents (1g/L in water) were individually exposed to increasing concentration of AF B1 (0.625, 1, 2, 4, 6, 8 and 10 ppm) for 90 minutes with shaking (150 rpm) at 37 ^{o}C. Incubated samples were centrifuged and the supernatant was analysed for AF B1 concentration using high performance liquid chromatography. The percent toxin adsorbed was calculated and the values were plotted on an isothermal saturation curve to estimate K_d (affinity coefficient) and K_{sat} (saturation point). In all cases, the relative amount of toxin adsorbed was found to be concentration dependent (Figure 1). The adsorption kinetics for CA2 did not show standard saturation characteristics and may reflect a different type of adsorption mechanism. Mycosorb and CA3 exhibited the greatest adsorption capacity while CA1 showed a much lower total capacity. Mycosorb showed more than 90% adsorption at all levels of AF B1 tested, the heighest adsorption of 94.3% was noticed at 2 ppm. The clay-based adsorbent, CA3, exhibited maximum adsorption of 92.2% at 10 ppm AF B1 level. These capacities are reflected in K_{sat} value for Mycosorb, CA1 and CA3 of 0.642, 3.199 and 0.928 ppm, respectively. CA1 and CA2 showed a maximum adsorption of 51.0 and 68.7%, respectively. The lower K_d value of Mycosorb (<0.32 ppm) compared to CA1 (1.851 ppm) and CA3 (0.414 ppm) indicate that Mycosorb had the greatest affinity for AF B1 and was more efficient in adsorbing AF B1 at low toxin concentrations. As a result, Mycosorb exhibited significantly higher adsorption (P < 0.05) at lower concentrations (0.625 and 1 ppm AF B1) when compared to CA1, CA2 and CA3. The relatively high capacity and high affinity of the modified glucan-based adsorbent suggests that in may be useful aflatoxin control strategies when used at low concentrations in animal feed

Figure 1. Percent AF B1 adsorbed by different commercial adsorbents with increasing AF B1 concentration in liquid media.



P53

Use of an organoaluminosilicate to reduce the toxic effect of a mixture of aflatoxin and zearalenone on the egg production of laying hens

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This study was conducted in order to evaluate the efficacy of an organoaluminosilicate for protection against a mixture of aflatoxins and zearalenone in laying hens. For this purpose 96 hens 30 weeks old were used during 10 weeks. They were divided in four groups of three replicates, each of 8 hens. The group 1, control group, was fed with a diet free of mycotoxins and no organoaluminosilicate was used. The group 2 was fed with 3 g of the organoaluminosilicate per kg of diet. The group 3 was fed with the mycotoxins and the group 4 was fed with mycotoxins and 3 g of the organoaluminosilicate per kg of diet. For the first 3 weeks, the hens of groups 3 and 4 were fed only with 1,000 ppb of aflatoxin B1 and no effect was observed. After that period, a mixture of 4,000 ppb of aflatoxin B1 and 4,000 ppb of zearalenone was given to the hens of groups 3 and 4 during 4 weeks. For this level of mycotoxins, the egg production decreased from 93.5% to 75.9% for the hens of group 3. In the case of the group 4, a less severe drop on the egg production was observed, in fact the egg production decreased to 82.7%. The control group at the same period had an egg production higher of 90%. A difference in the egg size was also observed with the group 3 having the lower size. By considering the egg mass, the drop of production for the group 3 was more noticeable. Therefore, the inclusion of 0.3% of the organoaluminosilicate was partially effective in ameliorating the combined toxic effect of aflatoxin B1 and zearalenone present in the rations at the level of 4000 ppb each. The last 3 weeks of the experiment, all the hens were fed with the control diet, and the groups 3 and 4 got the normal egg production.

P54

Experimental aflatoxicosis model in ducklings: interest for the screening of aflatoxin binders

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Among the poultry species, ducks are most sensitive to the toxic effects of several mycotoxins, especially aflatoxin B1. A model of experimental aflatoxicosis was developed with White Pekin Ducklings to investigate different feeding approaches for the prevention of aflatoxicosis. In a first study, a purified preparation of aflatoxin B1 (ref. 227340100 - Acros Organics, France) was used to prepare aflatoxin-contaminated feeds (based on a standard duckling feed formula in mash form). The theoretical range of contamination was 0 - 50-125-250-500 µg/kg. 240 day-old White Pekin ducklings (Grimaud Frères, Roussay, France) were placed in 10 cages (2 replicates of 24 birds for each aflatoxin level) during 21 days. Ducks were observed twice a day and mortality recorded; bodyweight was measured individually for all birds at days 7, 14 and 21. Heart, spleen, gizzard, liver weights (days 7, 14 and 21), blood parameters at day 21 (total proteins, albumin, total cholesterol) were determined individually on 6 birds per cage. Feed consumption was also calculated for each cage. A significant ($p<0.05$) decrease in body weights and feed intake was observed at all ages for the highest aflatoxin dose (500 µg/kg). The mortality during the period 1 to 21 days was significantly increased at 500 µg/kg. Relative weights (ratio organ weight to total body weight) increased significantly for the heart (100 µg/kg: days 14 and 21; 250 µg/kg: day 21; 500 µg/kg: days 14 and 21), the spleen (100 µg/kg: day 14; 250 µg/kg: day 21; 500 µg/kg: days 7, 14 and 21), the proventriculus (500 µg/kg: days 14 and 21), the gizzard (500 µg/kg: day 21). Liver weight was not significantly affected. At day 21, total protein and albumin were significantly lowered for every aflatoxin concentration. Total cholesterol was significantly lowered at 100, 250 and 500 µg/kg. In conclusion, blood parameters (total proteins, albumin and total cholesterol) in ducklings fed aflatoxin-contaminated feeds are significantly lowered. Hence they are valuable indicators to study the prevention of the toxic effects by feed supplementation.

In the second study, naturally contaminated corn (origin: Ivory Coast) was used to prepare an experimental feed containing 90 µg/kg of aflatoxin B1. 13 cages of 10 day-old White Pekin ducklings were used (one cage per treatment): a negative control (aflatoxin-free diet), a positive group (aflatoxin- contaminated diet) and 11 groups receiving the aflatoxin-contaminated feed and a binder. Eleven different treatments have been tested: 6 clays at different inclusion levels and a preparation of yeast cell walls. At day 8, the birds were killed and blood samples analysed for total cholesterol and albumin content. A protection factor was calculated for each treatment, as the following ratio: [(value of treated group - value of positive control) - (value of negative group - value of positive control)] x 100. The results showed that partial recoveries are observed for both total cholesterol and albumin blood content, according to the type of product and the inclusion level. The protection factors calculated for each treatment for the two blood parameters were significantly correlated ($R2 = 0.88$). In this study, the highest protection factors were respectively 89% and 60% for total cholesterol and albumin.

Thus, a simple but sensitive aflatoxicosis model (low level of toxin, few number of birds, short duration test) can be used to screen feed additives (toxin binder and / or other nutritional adaptations) which can improve the resistance of poultry to zootechnical and economical effects of the toxin.

P55

Contamination with zearalenone in sorghum from the United States

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Grain, sorghum and corn are ingredients in the Mexican diets of broilers, layers and pigs. The Mexican animal feed industry included grains as much national as imported. In December 2001, one Mexican company imported two vessels (Melini and Sanmar Pride) containing 50,000 metric tons of sorghum (in bulk) with tolerance 10% more or less, from New Orleans, USA. The quality specification was sorghum US No.2 or better, with 14% of moisture maximum. All grade factors of quality, including moisture, to comply with USDA (U.S. Department of Agriculture) standards on a sublot basis. Sublot size to be a maximum of 2, 000 metric tons. Seller provided an independent laboratory analysis certifying on the sublot basis the following: maximum 10 ppb (μg/kg) aflatoxin, maximum 100 ppb zearalenone, maximum 100 ppb vomitoxin.

Analysis results certified on 36 samples were negative to zearalenone. When the vessels arrived to Tuxpan, Mexico, again an other group of samples were obtained. The sorghum samples of the one vessel were sent to our NUTEK laboratory for zearalenone quantification by HPLC method. The average reported was 2,300 ppb of zearalenone. With these reports the buyer company demanded from the seller company in the U.S. embassy in Mexico City. The U.S. Department of Justice, Civil Division sent the sorghum samples subject to the captioned lawsuit for testing for zearalenone to: Trilogy Analytical Laboratory. The testing was scheduled to begin August 5, 2002. The analytical results certificate from Trilogy showed the contamination with zearalenone in all the samples, only one was reported less than 100 ppb. The average contamination in the Melini vessel was 659 ppb and in Sanmar Pride was 2,326 ppb. The highest level reported was 3,612 ppb. At present the lawsuit is in process.

P56

Consequences of mycotoxins in the Dutch pig pyramid

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Mycotoxins in feed, like deoxynivalenol (DON) and zearalenone (ZEA) may result into acute or subacute intoxications in, amongst others, pigs. Many mycotoxins are immunotoxic. Mycotoxicosis possibly leads to severe health problems and decreasing performance in Dutch pig husbandry. Because of unfamiliarity with the signs of intoxications, more antibiotics are being used without any result. The risk of mycotoxicosis seems to rise since more cereals which are used in feed in the Netherlands are contaminated with *Fusarium* mycotoxins. Unknown is the prevalence of mycotoxins in feed and the costs of it for the pig farmers. Therefore more research is necessary to estimate the risk of mycotoxicosis in the Dutch pig husbandry and to estimate the economic consequences for the Dutch Pig pyramid.

Effects of DON and ZEA in pigs

DON: vomiting; decreased feed intake; decreased growth; decreased feed efficiency; immune defects. ZEA: reduced conception rates; swelling of vulva in new born piglets; reduced embryonic survival; delayed return to estrus post-weaning; enlargements of ovaries and uteris, stillbirths [1].

Recent research

Recently in the Netherlands many research has been carried out on mycotoxins by different institutes and companies. Literature research was carried out on the effects of mycotoxins on ruminants and non-ruminants and on the human risks of mycotoxins in animal products. Food, foodstuff and feed are monitored on mycotoxins, analytical methods and internal quality systems are developed or improved. Also research was carried out for *Fusarium* resistant types of wheat and for toxin absorbents.

Knowledge lacunas

Still little is known about: the effect of mycotoxins on pig health and performance under different practical circumstances; the prevalence and amounts of mycotoxins in different kinds of pig feed (cereals, concentrates, roughage and silage); the extension and severity of the economical damage in the Dutch pig husbandry; increased use of antibiotics due to mycotoxins.

Future research

To contribute to the before mentioned knowledge lacunas practical research is necessary. Therefore, together with the Faculty of Veterinary Medicine, the Research Institute for Animal Husbandry intend to perform: a dose response test under controlled circumstances on a long term; a dose response test at farms with different health status; a monitoring on roughage, straw, cereals, silage and concentrates; a calculation of the economical damage.

Advantages could be achieved not only by the Dutch pig husbandry sector but also by the cereal and feed producers

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P57

Effects on aflatoxin M1 residues in dairy cow milk by addition of some clays to naturally aflatoxin-contaminated diets

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Milking cows fed rations contaminated by aflatoxin B1 (AFB1), excrete the metabolite aflatoxin M1 (AFM1) into the milk: this molecule has shown similar toxic acute effects to AFB1, but is less carcinogenic. AFM1 was categorised by the IARC as a class 2B, possible human carcinogen; the presence of AFM1 in milk and milk products is considered to be undesirable, as the foods are widely used and important in feeding infants. Due to this, in 1998 the Commission of the EC fixed a limit for AFM1 in milk and milk products (50 ng/l for milk). Since 1991, the Commission has set a limit for AFB1 of 5 µg/kg for supplementary feedingstuffs for dairy cattle. Several researches carried out *in vitro* and *in vivo*, revealed that some aluminosilicates can adsorb AFB1 in solution and reduce its bioavailability for gastro-intestinal absorption. Some authors reported that particular clays added to AFB1 contaminated diets for dairy cows are capable of reducing the secretion of AFM1 into milk.
The objective of our research was to test the effectiveness of a bentonite (BCA) and two sepiolites (SAA and SAA plus) to reduce the carry-over of AFB1 into milk at low levels of contamination. These products had shown a greater than 85% adsorption capability in an *in vitro* trial.

Twenty four Holstein cows in mid lactation (average production 29.7 kg of milk) were used in a 4x4 Latin square design with 7-day periods; the animals were fed a total mixed ration consisting of 3.5 kg of compound feed, 6.5 kg of a cereal mixture, 5 kg of hay, 2 kg of cotton seeds and 20 kg of maize silage. The compound feed contained contaminated groundnut and cottonseed meals so as to obtain an AFB1 level of 9 µg/kg of feed; the other components of the ration did not contain AFB1, as shown by analysis. The four dietary treatments were the compound feed without adsorbents and the same feed with 2% of BCA, or 2% of SAA, or 2% of SAA plus. On day 7 of each experimental period, the morning and evening milk samples of each cow were mixed in the proportion of the morning and evening milk production. These samples were again put together in proportion to the daily milk production of each cow in order to constitute a representative bulk milk sample of the cows of each dietary treatment in each period. The contaminated compound feed and milk samples were analysed for AFB1 and AFM1 by HPLC using fluorimetric detection. The results of the experiment are given in Table 1. All the clays reduced the AFM_1 level in the milk; for BCA, a reduction of 61.5% was observed.

Table 1. Aflatoxin M1 in milk (ng/kg).

Treatments	1st week	2nd week	3rd week	4th week	average
Control	39	28	33	30	32.5
Bentonite CA	15	12	10	13	12.5
Sepiolite AA	22	19	18	19	19.5
Sepiolite AA Plus	20	18	12	20	17.5

P58

Glucomannan: a promising solution for the adsorption of T-2 toxin in the biological system of chickens

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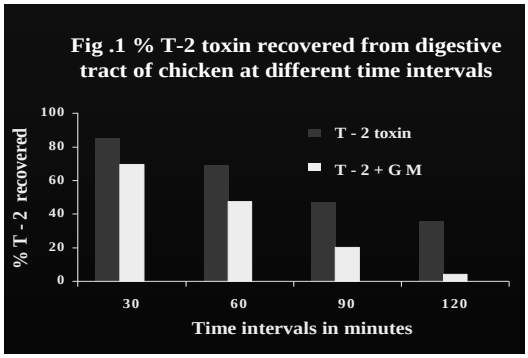
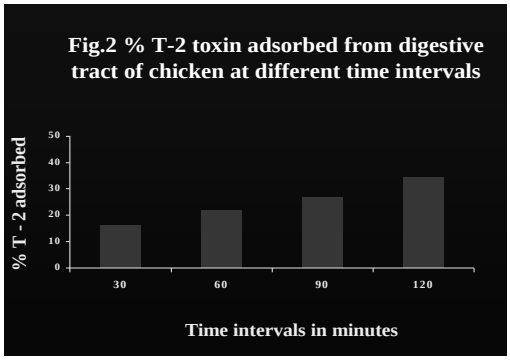
Among various fungal metabolites, aflatoxins, ochratoxins and trichothecenes are of the greatest importance to the poultry industry as they are frequently encountered in feedstuffs and are highly toxic. Among the trichothecenes, T-2 toxin produced by fungi of genus *Fusarium* has been reported to cause several adverse effects in poultry such as; oral lesions; reduced body weight; plasma enzyme changes; regression of ovaries in layers and immunosuppression [1]. Glucomannans (Mycosorb™ from Alltech) derived from the inner cell wall of yeast, *Saccharomyces cerevisiae 1026* has been found to significantly alleviate the adverse effects of mycotoxicosis in poultry [2-5]. A biological trial was conducted to ascertain the T-2 toxin adsorptive-ability of glucomannan in counteracting the toxic effects of T-2 toxin in chickens. *Fusarium sporotrichoides* was propagated on oat meal agar and it was utilised to produce T-2 toxin on whole wheat which was used to formulate the following treatment diets: 1) control diet, 2) control + Mycosorb™ (0.1%), 3) control + 500 ppb T-2 toxin, and 4) control + 500 ppb T-2 toxin + Mycosorb™ (0.1%). Five week old broiler chicken of both sexes were selected and divided into four groups of twenty birds each and were separately fed with above treatment diets in individual cages. Four birds were sacrificed at an interval of 30 minutes that is at 0, 30, 60, 90, and 120 minutes from each experimental group. The whole gut contents of each bird were collected separately, dried and toxin concentration in the samples was determined. The percent toxin recovered is presented in Figure 1. No toxin was detected in T 1 and T 2. In T 3, the toxin recovery reduced in proportion to the increase in time intervals, from consumption of the toxin contaminated diet to the time of sacrifice, due to absorption of toxin from the gut. There was a further significant reduction in the percentage of toxin recovered in the gut samples of Glucomannan treated toxin-contaminated diets T 4 compared to T 3. This is most likely due to the adsorption of the toxin by glucomannan. The percentage of T-2 toxin adsorbed by glucomannan from the digestive tract at different time intervals (Figure 2) is obtained by calculating the difference between the percentage of T-2 toxin recovered in Treatments 3 and 4. Adsorption of T-2 toxin by glucomannan increased along with the increase in time intervals. The adsorptive-ability of glucomannan for T-2 toxin is in the region of 35%. Supplementation of glucomannan at 1kg / ton of feed is beneficial in preventing the absorption of T-2 toxin into the biological system.





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P59

Novel strategy for deactivating ochratoxin A in feed*

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Ochratoxin A (OTA) is a mycotoxin with nephrotoxic-, hepatotoxic-, carcinogenic- and immunosuppressive-properties. It is mainly produced by certain *Penicillium* and *Aspergillus* species during storage of grain but there are also many reports about its occurrence in grapes, coffee beans and other commodities. During animal production OTA causes economic losses mainly through a decrease of weight gain. However, affected productivity, the risk of residues in animal tissue and the possible carry over to humans when contaminated meat is consumed, finally led to an intensified search for respective counteracting methods. It has been proven that adsorptive materials do not work efficiently enough against ochratoxin A, thus a project was realised with the aim of finding microorganisms with the capability to deactivate ochratoxins in the intestinal tract of animals.

Based on results of several studies conducted during the past years, habitats like rumen fluid, intestinal contents and soil were screened for OTA-detoxifying microbes. Although many authors have reported that protozoa are mainly responsible for the detoxification of ochratoxin A in rumen fluid of cattle and sheep it was clearly demonstrated that bacteria play an important role in degrading ochratoxin A (Schatzmayr et al., 2002/1). By applying several enrichment methods and isolation procedures, two bacterium species (related to *Clostridium sporogenes* and *Lactobacillus vitulinus*) could be isolated out of rumen fluid, able to cleave OTA into the non-toxic metabolite ochratoxin α and the amino acid phenylalanine. Moreover segments of pig-intestine were screened for OTA-detoxifying microorganisms. Four bacterial strains were finally obtained that - based on the determination of the partial 16S rDNA - could be related to *Streptococcus pleomorphus*, *Eubacterium callanderi* and *Eubacterium ramulus*. Furthermore several aerobic OTA-transforming bacteria were enriched and isolated out of soil samples. The most active ones belonged to the genera *Sphingomonas*, *Stenotrophomonas*, *Rhodococcus*, *Ralstonia* and *Ochrobactrum* (Schatzmayr et al., 2002/2). Besides bacterial strains, different yeasts were investigated for their ochratoxin A detoxifying activity and active strains were found in the genera *Trichosporon*, *Rhodotorula* and *Cryptococcus*.

All these strains were scientifically investigated for their use in a feed additive. At the end of a very comprehensive selection process a new yeast strain belonging to the genus of *Trichosporon* came out on top. During the selection features like velocity of detoxification, pathogenicity, ability to work under gastrointestinal conditions, possibility of fermentation and stabilisation as well as the requirements for the registration for the European Union were considered. A feeding trial conducted with broiler chickens revealed that the negative influence of high doses of ochratoxin A on the performance of broilers could be neutralised by addition of stabilised *Trichosporon* cells. The final weight of the group receiving 1 ppm OTA and yeast (10^5 CFU per gram feed) was on average 61 grams higher than the positive control group (1 ppm OTA without additive). Thus it could be proven that this *Trichosporon* strain can be used in a feed additive for detoxification of OTA-contaminated feed stuffs.

* Acknowledgements: these studies were supported by grants of the Austrian Industrial Research Promotion Fund (FFF).
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P60

The impact of zearalenone on reproduction parameters of boars*

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Zearalenone has a serious impact on the reproduction of pigs because of its oestrogenic activity. The symptoms of contamination that may be observed in sows are a red, swollen vulva, rectal and vaginal prolapse, enlargement of the teats and mammary glands, atrophy of the ovaria, pseudo pregnancy due to retained corpora lutea, foetal mummification, prenatal mortality, etc. In boars, zearalenone may affect the semen quality [1]. A trial was conducted at the Laboratory of Animal Reproduction of the Lithuanian Institute of Animal Science to evaluate the impact of zearalenone and the detoxifying effect of Toxy-Nil Plus dry on the reproductive performance of boars. The trial involved 10-months old Lithuanian White boars with an average live weight of 150-155 kg. The animals were allotted to three groups: 1) Control group, boars fed a mycotoxin-free feed, 2) Treatment 1, boars fed a feed containing 0,57 mg/kg of zearalenone (from Sigma-Aldrich), and 3) Treatment 2, boars fed a feed containing both 0,57 mg/kg zearalenone and the product Toxy-Nil Plus dry (from INVE Nutri-Ad) at a rate of 1 kg per 1000 kg of feed. During a pre-experimental period of 14 days, all boars were fed the same high quality feed and were trained to give semen by manual method. Subsequently, during the experimental period, which lasted 5 weeks, the different treatments were applied (Control, Treatment 1 and Treatment 2). Finally, a 21-days recovery period was foreseen during which the boars of all treatment groups were given the same mycotoxin-free feed again. Semen was collected manually once a week from the boars in all groups. The parameters that were measured on the semen were volume of ejaculation, spermatozoa count per ejaculation and spermatozoa motility. Already during the first week after the start of the experimental period, supplying the zearalenone-containing feed resulted in Treatment 1 in a decrease of the volume of ejaculation by 40,8 % ($P<0,001$) compared with the Control group and amounted to 141,0±12,6 ml. The volume of ejaculation in Treatment 2 remained similar to that in the Control group. The volume of ejaculation in Treatment 1 was recovered in a week's time when non-contaminated feed was offered to the boars after the experimental phase. During the intoxication period, the spermatozoa count per ejaculation in Treatment 1 was reduced substantially within two weeks compared to the Control and Treatment 2 groups. Here as well, when non-contaminated feed was offered after the experimental period, the total spermatozoa count per ejaculation recovered within a week. The lowest spermatozoa motility in the semen collected during intoxication was determined in the group of boars fed zearalenone-contaminated feed (Treatment 1) and was numerically 3,9±0,4 points ($P<0,001$). As soon as the contaminated feed was replaced by good quality feed, the motility of spermatozoa was recovered within a few days and amounted to 7,0±0,2 points. Lower motility of spermatozoa could be associated with either sperm membrane damage or metabolism disturbances in the cell. During the experimental period, the sperm motility of boars fed Toxy-Nil Plus dry treated feed (Treatment 2) was 7,0±0,2 points and did not differ significantly from the sperm motility of the Control group ($P<0,05$).

This *in vivo* trial clearly indicated that when Toxy-Nil Plus dry was supplied to boars receiving zearalenone-contaminated feed, that the negative effect of zearalenone on the semen quality was neutralised.

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The potential to prevent *Fusarium* mycotoxin-induced immunomodulation through the feeding of a polymeric organic mycotoxin adsorbent

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Feed-borne *Fusarium* mycotoxins, including trichothecenes, fusaric acid and fumonisins, have been shown to alter immunity in a variety of animal and avian species. It is likely that chronic exposure to low doses of *Fusarium* mycotoxins causes greater financial losses to livestock and poultry producers than episodic acute poisonings. The potential for mycotoxin-induced immunosuppression to reduce production efficiency will depend on disease challenge. A series of experiments were conducted to determine the effect of feeding blends of grains naturally-contaminated with *Fusarium* mycotoxins on the immune status of broiler chickens, ducks and starter pigs. The potential efficacy of an organic polymeric mycotoxin adsorbent extracted from yeast cell wall in preventing immunological changes was also determined.

In the first study, a total of 360 male broiler chicks were fed diets containing blends of corn and wheat naturally-contaminated with *Fusarium* mycotoxins with and without a polymeric glucomannan mycotoxin adsorbent (GMA). Diets contained up to 9.7 mg/kg deoxynivalenol, 21.6 mg/kg fusaric acid and 0.8 mg/kg zearalenone with and without 2.0 mg/kg GMA and were fed for eight weeks. There was no effect of diet on blood concentrations of immunoglobulins A (IgA), G(IgG) or M(IgM). Biliary IgA concentrations, however, were significantly decreased with the feeding of contaminated grains. This decrease was prevented by the feeding of GMA.

In the second study, a total of 464 male White Pekin ducklings were again fed blends of contaminated corn and wheat with and without 2.0 mg/kg GMA for six weeks. It was observed that there was a significant decrease in cutaneous basophil hypersensitivity response obtained 24 hours after toe-web inoculation with 100 µg of phytohemagglutinin-P after three weeks of feeding. Supplementation with GMA prevented this effect.

In the third study, a total of 175 starter pigs were again fed combinations of corn and wheat naturally contaminated with *Fusarium* mycotoxins for three weeks. The feeding of contaminated grains significantly increased serum IgM and IgA concentrations while the IgG concentrations were not affected. The feeding of 1.0 mg/kg GMA prevented these changes.

It was concluded that the feeding of blends of grains naturally contaminated with combinations of *Fusarium* mycotoxins can alter the immune status of various animal and avian species. Such changes may be of economic importance under commercial production conditions with disease challenge and environmental stresses. The feeding of GMA can prevent the mycotoxin-induced immunological changes and, therefore, may minimise economic losses.

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Oxidative stress as a potential mechanism of mycotoxin toxicity

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Mycotoxins are considered to be unavoidable contaminants in foods and feeds and are a major problem all over the world. They represent a diverse group of fungal metabolites causing toxicological changes in mammalian and avian species. The most significant mycotoxins in naturally-contaminated foods and feeds are aflatoxins (AF), ochratoxins (OA), zearalenone , T-2 toxin, vomitoxin (DON) and fumonisins (FM). In many cases these mycotoxins can be found in combination in contaminated feed. Acute mycotoxicosis outbreaks are rare events in modern animal production, however, low mycotoxin doses which very often are not detected are responsible for reduced production efficiency and increased susceptibility to infectious diseases.

Due to the diversity in the chemical structures of various mycotoxins, molecular mechanisms of their toxic action also vary substantially, but in many cases their direct or indirect effects on major cellular events including protein, DNA and RNA synthesis are observed [1]. Several lines of evidence from published information and our own results suggest that oxidative stress and lipid peroxidation are involved in the development of toxicity of such mycotoxins as AFB1, OA, T-2, DON, FM and aurofusarin:

- Inclusion of mycotoxins in various diets of avian and mammalian species causes oxidative stress and stimulates lipid peroxidation in target tissues.
- Inclusion of the mycotoxins (AF, OA or zearalenone) into various model systems also stimulates lipid peroxidation.
- Mycotoxins cause apoptosis in various tissues. This also could be mediated via antioxidant/prooxidant balance.
- Oxidative stress in the digestive tract could trigger apoptosis and lead to malabsorption (AFB1, OA, T-2 toxin). It seems likely that in the digestive tract antioxidant-prooxidant balance is an important determinant of many vital physiological events, including nutrient absorption.
- Increased dietary supplementation of natural antioxidants (e.g. vitamin E, vitamin C, carotenoids) or their inclusion in model systems substantially decrease oxidative stress and mycotoxin toxic action.
- Dietary supplementation with effective mycotoxin-binders could also decrease oxidative stress and mycotoxin toxicity. It seems likely that a combination of mycotoxin-binders with increased dietary supplementation of natural antioxidants could be a valuable technological approach to decrease the impact of mycotoxins on animal production and human health.
- More research should be done to further understand molecular mechanisms of mycotoxin toxicity in order to find solutions to the global mycotoxin problem.

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Biological deactivation of fumonisins

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Fumonisins are a group of quite recently found mycotoxins mainly produced by *Fusarium moniliforme* and *Fusarium proliferatum*, which are very common contaminants of cereal grains, especially of maize. Among these the fumonisins B1 and B2 are produced most abundantly in nature and quantitatively may be of greatest toxicological concern [1]. Fumonisins have been shown to produce a wide range of pathological effects in animals, including the economically important disease symptoms of leucoencephalomalacia in horses and pulmonary oedema in swine. In addition, these compounds exhibit toxic effects to turkey poults and broiler chicks and cause nephrotoxicity, hepatotoxicity and hepatocellular carcinoma in rats. Although definite evidence of carcinogenicity in humans is lacking, oesophageal cancer occurs at greater frequency in world regions where corn is the dietary staple and levels of *Fusarium* and fumonisin contaminations are high. Fumonisin contamination of corn and corn-based products is reported frequently and in many countries worldwide. The economic implications of animal feeds contaminated with high levels of fumonisins are significant [2]. Together with the high stability of these mycotoxins during storage of the grain and under processing conditions, this implies the need for appropriate decontamination strategies. Biological detoxification – especially the transformation of fumonisins via microorganisms or specific enzymes – seems to be promising. Enzymatic breakdown of fumonisins into compounds that are no longer toxic would provide a very gentle, effective and environmentally friendly way of deactivating these mycotoxins. Moreover, appropriate physical or chemical decontamination strategies for fumonisins are not available so far. Duvick et al. [3] already described the breakdown of FB1 by the yeast *Exophiala spinifera* ATCC 74269 as well as by an aerobic bacterium in the course of complete metabolisation of the toxin. Based on these facts a project was initiated with the aim to find microorganisms with the capability to deactivate fumonisins through enzymatic transformation. The respective organism is intended to be used as part of a feed additive to ensure detoxification of fumonisins in the intestinal tract of animals during feed digestion. A screening for aerobic and anaerobic microorganisms with FB_1-transforming potential was realised by performing fumonisin-degradation experiments in liquid culture media as well as in buffered systems. Besides testing promising bacterial and yeast strains of culture collections, several different habitats were investigated with regard to the presence of toxin-reducing, microbial activity. Since ruminants appear to be tolerant towards fumonisins, rumen fluid was one of these habitats to investigate under anaerobic conditions, although it is not clear so far whether the ruminants' low sensitivity is due to limited absorption of the mycotoxin or depends on ruminal metabolism [4]. Taken into consideration the possibility of using a living, fumonisin-degrading microorganism in the intestine of animals as a feed additive, intestine segments of pigs were screened for FB1-transforming microbes. Different environmental samples were also under investigation, as for example soil. In one of these soil samples, fumonisin-reducing activity could be detected leading us to trials to isolate the respective microorganisms.

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Effect of T-2 toxin on lipid peroxidation in an *in vitro* model system

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Oxidative damage caused by T-2 toxin may be one of the underlying mechanisms for T-2 toxin-induced cell injury and DNA damage. In particular the presence of T-2 toxin in the diet decreased hepatic or plasma α-tocopherol concentrations in chickens, generates free radicals and increases lipid peroxidation in rat liver, decreasing hepatic glutathione and stimulating DNA fragmentation [1]. However, it is not clear at present if T-2 toxin can directly promote lipid peroxidation, or a compromised antioxidant system is responsible for enhancement of lipid peroxidation in tissues. To address this question we used an in vitro model system to evaluate in vitro effects of T-2 toxin on lipid peroxidation.

The model system was based on the peroxidation of phosphatidylcholine (PC) liposomes. Liposomes were formed with or without T-2 toxin (0.4-2.0 μg/mg PC), in the absence or presence of free radical generator (2,2-Azinobis(2,4-dimethylvaleronitrile); AMVN) and incubated at 37°C with continuous shaking. Aliquot samples were withdrawn at specific time intervals and used for PC hydroperoxide (PC-OOH) analysis by HPLC (Spherisorb column, diode array detector and methanol/water (95:5) as a mobile phase) and malondialdehyde (MDA) was determined after reaction with thiobarbituric acid by HPLC with fluorescent and UV detectors.

The results indicate that, when incorporated into liposomes at levels of 0.4-2.0 μg/mg PC T-2 toxin failed to show any stimulating effect on PC-OOH or MDA formation during incubation up to 48 hours. In fact MDA accumulation was moderate and not dependent on T-2 toxin presence. On the other hand, when free radical generator (AMVN) was incorporated into the liposomes, PC-OOH and MDA accumulation in liposomes were time-dependent. In this case, T-2 toxin incorporation into the liposomes further (significantly, $P<0.01$) increased PC-OOH and MDA accumulation in time-dependant manner. On the other hand, incorporation of alpha-tocopherol in the same liposomes decreased lipid peroxidation, but the vitamin E oxidation was time-dependent and in presence of T-2 toxin this process was more pronounced.

These data have shown for the first time that, when combined with other prooxidants, T-2 toxin is able to increase lipid peroxidation in an *in vitro* model system. This could be relevant to the digestive tract where a combination of prooxidants (peroxidised lipids, mycotoxins, Fe, Cu, etc.) could be found. Therefore, it seems likely that T-2 toxin stimulates lipid peroxidation not directly but rather as a result of compromised antioxidant systems. Based on these results it is suggested that inclusion of mycotoxin-binders and their combinations with antioxidants into the diet could prevent mycotoxins from participating in lipid peroxidation promotion in the digestive tract and decrease oxidative stress and mycotoxin toxicity.

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Efficacy of a natural magnesium smectite against aflatoxin B1 and zearalenone tested in a dynamic *in vitro* model of the gastric and small intestine simulating the pig

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Mycotoxins like aflatoxins, ochratoxins, trichothecenes, zearalenone and fumonisins are presently considered economically and toxicologically important. Since these mycotoxins produce diseases in animals and humans strategies have been undertaken to decontaminate mycotoxin-containing foodstuffs. One of the most recent approaches involves inert binders from different sources, such as hydrated sodium calcium aluminiosilicate (HSCAS), zeolites, smectites, sepiolite and activated carbons. When added to the feed they reduce mycotoxin toxicity in animals and carryover of mycotoxin from contaminated foodstuffs to animal products. Binders act by reducing the bioavailability of mycotoxins by adsorption on their surface. The affinity of the binder to the mycotoxin and the stability of the binder-mycotoxin complex are critical parameters and are preferably studied in *in vitro* systems with a high predictive value for the *in vivo* performance. In this study the efficacy of a natural magnesium smectite (ATOX®) against aflatoxin B1 (AFB1) and zearalenone (ZEA) was demonstrated in a dynamic *in vitro* model simulating the gastric and small intestine of the pig.

The TNO *in vitro* gastro-intestinal models (TIM) are multi-compartimental, dynamic, computer-controlled models closely simulating the *in vivo* conditions in human or animals. The *in vitro* gastric and small intestinal model (TIM-1) used for this study simulates the physiological conditions within the GI tract, such as peristaltics, changes in gastric pH, site specific pH, gastric and intestinal secretion of digestive enzymes and bile, transit time of the feed, removal of digested feed compounds and small complexes in solution, all at body temperature. The feed was contaminated with 100 ppb ZEA (natural contamination) and 20 ppb AFB1 (65% natural contamination). The contaminated feed was tested without the presence of a mycotoxin-binder, and in the presence of 0.2%, 0.5% and 1% of natural magnesium smectite (ATOX®) as binder. During six hours of gastric and small intestinal passage in TIM samples were collected from the jejunal and ileal dialysate. This fraction contains the amount of the ZEA and AFB1 that is potentially available for small intestinal absorption. Samples were analysed for ZEA by HPLC after being concentrated with immunoaffinity columns, and for AFB1 by HPLC.

In the absence of the mycotoxin-binder 47% (±5.7%) of AFB1 and 32% (±3.45) of ZEA were recovered in the jejunal plus ileal dialysates as potentially available for small intestinal absorption. The amount of AFB1 and ZEA available for intestinal absorption was drastically reduced by addition of the binder. Whereas for AFB1, the availability for intestinal absorption clearly depended on dosage of binder, this was not observed for ZEA.

Simulating the dynamic conditions of the pig's GI tract in TIM-1 the mycotoxin-binder showed to be effective by reducing drastically the amount of ZEA and AFB1 potentially available for small intestinal absorption. For AFB1 the efficacy was dosage-dependent.

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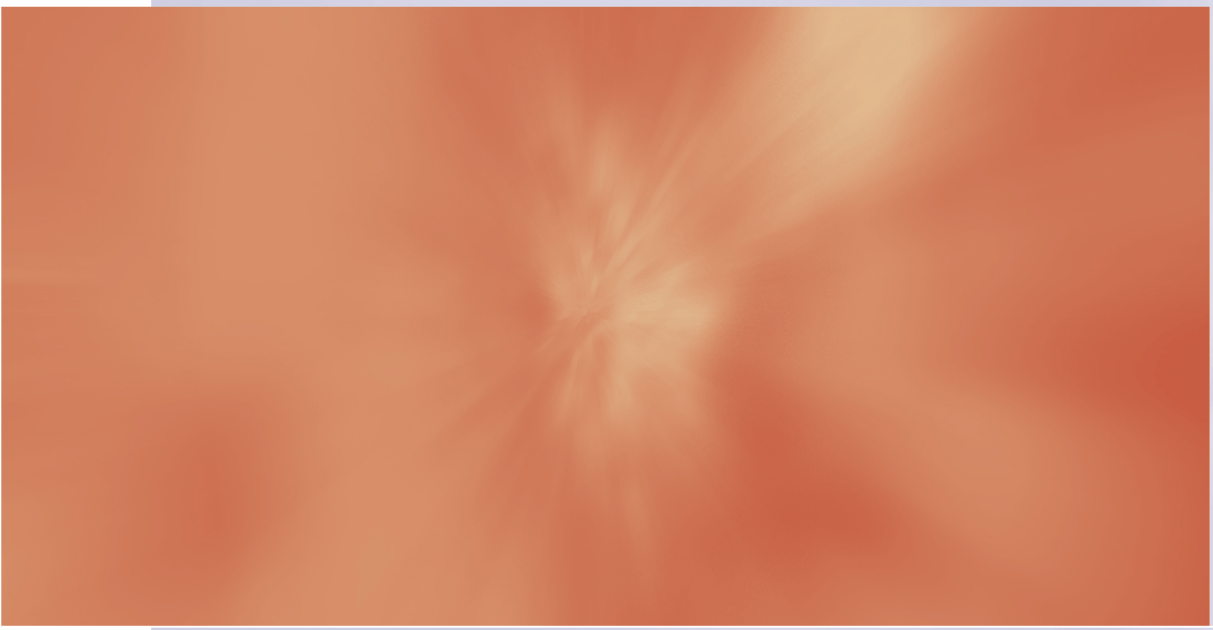

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