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ASTOX Symposium 2017

on

Endocrine Disruptors

and Ecotoxicology

Vienna, 2017, April 20th-21st

Österreichische Gesellschaft für Toxikologie

Austrian Society of Toxicology
ASTOX

Dedicated to Elucidate and Assess the Safety of Chemical Compounds

The **Austrian Society of Toxicology** (ASTOX) is pleased to welcome you at the ASTOX-Symposium 2017 on Endocrine Disruptors and Ecotoxicology.

ASTOX was launched in January 1991. Since that time **ASTOX** is well acknowledged by academia, national and international authorities, and industry as competent partner upholding and promoting the science of toxicology in Austria. **ASTOX** organizes scientific meetings, takes care of education and professional development, is responsible for registration of qualified toxicologists according to EUROTOX and their certification as European Registered Toxicologists (ERT), provides competent advice in matters of public health and stays in touch with other societies of toxicology at the international level.

Fostering and support of the next generation of toxicologists is one of the core missions of **ASTOX**. In this respect, **ASTOX**-symposia are ideal platforms for young scientists to present and discuss their projects. The posters will be displayed throughout the symposium. The presenters will be asked to stay at the poster during the poster viewing session and to provide a short oral overview. The best poster presentation will be granted with the **ASTOX AWARD 2017.**

The symposium will deal with actual topics in toxicology. The first day will comprise lectures on adverse outcome pathways in risk assessment and novel testing strategies, followed by actual topics in ecotoxicology. The second day will focus on endocrine disruptors and actual studies on myco- and phytoestrogens. In addition to the scientific symposium, we will have a public keynote lecture on the impact of pharmaceutical residues on vulture populations. After the public keynote lecture, a **get-together with wine and snacks** will be provided, kindly sponsored by Boehringer Ingelheim. Furthermore, we would like to express our gratitude to the Faculty of Chemistry of the University of Vienna for hosting the symposium.

We hope that you will find the symposium stimulating
On behalf of the organizing committee

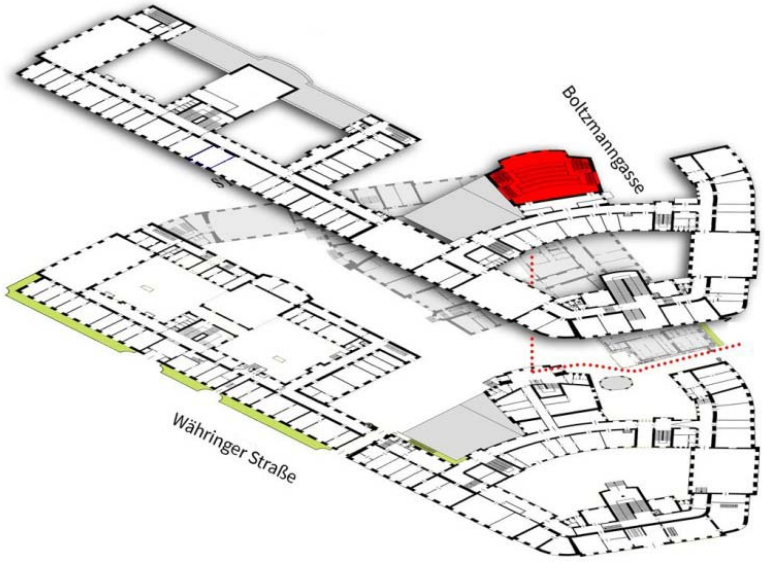
Doris Marko (Chair of ASTOX)

Conference Venue:

University of Vienna, Faculty of Chemistry, Währingerstr. 38,

Carl-Auer-von Welsbach Lecture Hall (Entrance Boltzmanngasse)

A-1090 Vienna, Austria



Programme

Thursday, 20th April, 2017

12:00 Registration and poster placement

13:15 Welcome

Session 1: AOPs and Testing Strategies
Chair:Doris Marko (University of Vienna)

13:30 *Keynote lecture*
AOPs in toxicology and risk assessment
B. Landesmann, Ispra

14:00 **Uncertainties of testing methods: What do we (want to) know about carcinogenicity?**
M. Paparella, A. Colacci, M.N. Jacobs

14:20 **Mesenchyme-derived growth factors: crucial for tumor promotion by non-genotoxic hepatocarcinogens**
B. Grasl-Kraupp, M. Nejabat, T. Riegler, T. Reitinger, W.W. Huber, R. Schulte-Hermann

Short Communications

14:50 **Cellular reaction to VOC exposures**
J.M. Gostner, J. Zeisler, M.T. Alam, S. Martini, P. Fagundes dos Santos, S. Geisler, M. Hermann, F. Ueberall, D. Fuchs

15:05 **Characterization of the toxicity of deoxynivalenol in a mechanically stimulated environment**
G. Del Favero, L. Woelflingseder, S. Seriani, P. Gallina, O. Sbaizero, D. Marko

15:20 **Coffee break and poster viewing part I**
Chair: W. Huber (Medical University Vienna)

Session 2: Actual Aspects in Ecotoxicology
Chair: Barbara Zemann (AGES)

16:40 **Wastewater-Analysis: Determination of Drugs-of-Abuse in municipal waste-waters - Which information do we try to obtain, which one can we actually get?**
R. Schmid (Medical University of Vienna)

17:10 **Phylogenomic concepts in ecotoxicology: Gastropod metallothioneins as a potential lineage-specific biomarker system in marine, freshwater and terrestrial ecosystems**
R. Dallinger (University of Innsbruck)

17:40 **Short break, refreshments**

18:00-19:00	**Public keynote lecture - öffentlich, Eintritt frei** **Akute tödliche Vergiftungen durch Arzneimittelrückstände in Nutztierkadavern und deren Auswirkung auf den weltweiten Geierbestand** *J. Dämmgen*

Geier spielen oder spielten in vielen Regionen der Erde eine gewichtige Rolle. Sie beseitigen und ‚desinfizieren' Kadaver von Wild- und Nutztieren und sie machen die darin enthaltenen Nährstoffe für Pflanzen verfügbar. Besonders prominent war bis in die jüngere Vergangenheit der Beitrag von Geiern im vorderindischen Kulturkreis. Bedingt durch das strikte Verbot des Verzehrs von Rindfleisch durch Hindus und den großen Bedarf an Milch und Milchprodukten, gab es in Indien eine große Population von Rindern, die eines natürlichen Todes starben und traditionell Aasfressern als Nahrung zur Verfügung standen. So konnte der Indische Bengalgeier zum häufigsten Greifvogel der Erde werden. Als in den Neunzigerjahren des vorigen Jahrhunderts die Bestände vorderindischen Geier zusammenbrachen, suchten Forscher aller Disziplinen fieberhaft nach möglichen Ursachen des mysteriösen Geiersterbens. Durch die fruchtbare Zusammenarbeit indischer, pakistanischer, amerikanischer und südafrikanischer Wissenschaftler konnte der Entzündungshemmer „Diclofenac" als wesentlicher Auslöser der Katastrophe identifiziert werden. Inzwischen konnten andere in Indien verbreitete Veterinärarzneispezialitäten als weitere mögliche Gefahren für Geier und andere aasfressende Greifvögel identifiziert werden. Deutlich komplexer sind die Ursachen des derzeit auf dem afrikanischen Kontinent zu beobachtenden Zusammenbruchs fast aller Geierpopulationen. Neben vielen anderen Gefahren spielt in den letzten Jahren das gezielte Ausschalten von Geiern durch Nashorn- und Elfenbeinwilderer durch Pestizide eine entscheidende Rolle. Im Gegensatz zur schwierigen Situation in Indien und Afrika erscheint die Lage in Europa deutlich günstiger: Bis auf den Schmutzgeier sind alle europäischen Geier in einem Aufwärtstrend. Getragen wird diese Entwicklung im Wesentlichen durch Schutzanstrengungen auf der iberischen Halbinsel.

Dr. Dämmgen war von 2001 to 2008 "Global head of Research and Development of Boehringer-Ingelheim Animal Health". Seit seiner Pensionierung im Jahre 2008 ist er Mitglied des "Scientific Advisory Board of the 'Centre de Recerca en Sanitat Animal" in Barcelona, Spain. Sein persönlicher Fokus ist der Erhalt und globale Schutz des Geiers und anderer Greifvögel.

19:00-20:00	**Wein und Knabbereien (sponsored by Boehringer Ingelheim)** *In der Poster viewing Zone*
20:00	**Gemütliches Zusammensein in der Stieglambulanz, Altes AKH** ASTOX-Mitglieder und geladene SprecherInnen sind eingeladen

Friday, 21st April, 2017

Session 3: Endocrine Disruptors, Regulatory Aspects

Chair: Elke Rauscher-Gabernig

9:00	**Defining scientific criteria to identify EDs in the pesticide and biocide areas – a never ending story?** A. Fischer (AGES)
9:20	**Endocrine disruptors and plant protection products – Major characteristics in ecotoxicology** J. Berchtold (AGES)
9:40	**Endocrine disrupters within the context of REACH** A. Losert (Umweltbundesamt)
10:00	**Coffee break and poster session II** Chair: W.W. Huber (Medical University of Vienna)

Session 4: Myco- and Phytoestrogens

Chair: Bettina Grasl-Kraupp (Medical University of Vienna)

11.30	**Metabolism of zearalenone and its major modified forms in pigs** S. Binder, H. Schwartz-Zimmermann, E. Varga, G. Bichl, H. Michlmayr, G. Adam, F. Berthiller (Boku)
12:00	**"Estrogenic cocktails" in our diet: combinatory effects of myco- and phytoestrogens** D. Marko, K. Vejdovszky, K. Hahn, J. Beisl, G. Aichinger (University of Vienna)
12:30	**Biotransformation of soy isoflavones in humans, rats and mice** S. Kulling (Max-Rubner-Institut, Karlsruhe, Germany)

ASTOX Award

13:00	**ASTOX award for the best poster presentation**
13:30	**End of symposium**

13:30-14:00	**Short Break –Refreshments and Snacks**
14:00	**ASTOX Membership Assembly** Carl-Auer-von-Welsbach-Lecture Hall

Invited Speakers

Brigitte LANDESMANN

Brigitte Landesmann is a medical doctor and has worked for many years in clinical medicine. She got the MD from the University of Vienna and the MSc in Public Health from the London University (LSHTM). She joined the European Commission's Joint Research Centre (JRC) in Ispra, Italy in 2002. At the Chemical Safety and Alternative Methods Unit incorporating the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) of Directorate F (Health, Consumers and Reference Materials) she got mainly involved in the generation, dissemination and application of Adverse Outcome Pathway (AOP) knowledge. In the context of the FP7 SEURAT-1 research initiative she has developed an AOP to liver fibrosis which has been endorsed and published by OECD. As a member of the OECD AOP training group she got experience in the organisation and execution of AOP training courses.

Dr. Alexandra Fischer, MSc Toxicology, ERT

Expertise: Studies of Human Biology and Toxicology at the University of Vienna and at the Medical University of Vienna. Five years of experience in cancer research with a main focus on signalling pathways involved in late stage hepatocarcinogenesis. For more than eleven years she has been working as a regulatory toxicologist. First at the department of chemicals at the Environment Agency Austria, where she was employed as toxicology expert for risk assessment of new and existing substances, classification and labelling of hazardous substances, and nanotechnology. Since 2006 she is regulatory toxicologist for pesticides at the Austrian Agency for Health and Food Safety, Business Area Food Security, Institute for Plant Protection Products, Department for Toxicology, where she is responsible for the toxicological evaluation and risk assessment of plant protection products and their active ingredients (national and EU-level). As a Member State expert she represents Austria at the EFSA (PRAS meetings), at the ECHA (ED expert group) and in Brussels.

Mag[a] rer nat. Julitta Berchtold

Expertise: Studies of Biology with focus on Zoology and Ecology at the University of Vienna. Five years of experience in environmental education and environmental protection in course of her employment for WWF Austria and the National Park Neusiedler See – Seewinkel. From 2008 to 2010 she worked as a field assistant conducting ecological studies, researching the effects of plant protection products to the environment. Since 2011 she is regulatory ecotoxicologist for pesticides at the Austrian Agency for Health and food Safety, Division for Food Security, Institute for Plant Protection Products, where she is responsible for the ecotoxicological assessment and risk assessments of plant protection products and their active ingredients (national and EU-level). As a

Member State expert she represents Austria at the EFSA in PRAS meetings and in working groups for the development and revision of guidance documents .

Ass. Prof. Dr. Franz Berthiller

Franz Berthiller is Associate Professor at the University of Natural Resources and Life Sciences, Vienna (BOKU) and Head of the Christian Doppler Laboratory for Mycotoxin Metabolism. He studied chemistry at the University of Vienna and the Vienna University of Technology and completed his PhD thesis on masked mycotoxins in the lab of Rudi Krska at the IFA-Tulln in 2006. His scientific output includes over 130 peer-reviewed publications and he serves on the editorial board of the Journal of Food Protection as well as the World Mycotoxin Journal.

Prof. Dr. Sabine E. Kulling

Sabine Kulling is Head of the Department of Safety and Quality of Fruit and Vegetables at the Max Rubner Institut in Karlsruhe and Honorary Professor at the Karlsruhe Institute of Technology (KIT) since 2010. After studying food chemistry and environmental toxicology at the University of Kaiserslautern, and passing her 2nd state examination at a state food control laboratory, she obtained her PhD in 1996 in Kaiserslautern. A *venia legendi* in food chemistry followed in 2002 at the University of Karlsruhe. From 2004 to 2009 she was Professor of Food Chemistry at the university of Hamburg and Potsdam, Germany.

Abstracts of oral presentations

AOPs in Toxicology and Risk Assessment

Brigitte Landesmann

EUROPEAN COMMISSION
DIRECTORATE-GENERAL JOINT RESEARCH CENTRE
Directorate F - Health, Consumers and Reference Materials
Unit F.3 - Chemical Safety and Alternative Methods, incorporating the
European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM)

Toxicology is currently undergoing a paradigm shift moving away from an observational approach based on animal testing towards an understanding of the underlying mechanisms of toxicity and a knowledge-based safety assessment of chemicals. In this context, the Adverse Outcome Pathway (AOP) framework has been developed to support the collection, organisation, and evaluation of relevant information for use in chemical risk assessment.

AOPs are a conceptual construct that describes a sequential chain of causally linked events starting from an initial interaction of a chemical with a biological target on molecular level leading through different levels of biological organisation to an adverse health or eco-toxicological outcome of regulatory relevance; they are the central element of a toxicological knowledge framework, built to support chemical risk assessment based on mechanistic reasoning.

Besides various applications in scientific context AOP descriptions are useful for regulatory applications like hazard identification, chemical categorization and read across, screening, and prioritization. They facilitate Integrated Approaches to Testing and Assessment (IATA) that combine all types of available relevant data and information for the prediction of adverse outcomes. AOPs also provide a platform for interdisciplinary collaboration.

Uncertainties of testing methods: What do we (want to) know about carcinogenicity?

M. Paparella[1], A. Colacci[2], M. N. Jacobs[3]

[1] Chemicals & Biocides, Environment Agency Austria, Vienna, Austria; [2] Agency for Prevention, Environment and Energy, Emilia-Romagna, Italy; [3] Department of Toxicology, Centre for Radiation, Chemical and Environmental Hazards Public Health England, Chilton, Oxfordshire, UK

Background: An in vitro approach to testing of chemicals for non-genotoxic carcinogenicity is being developed at OECD level (Jacobs et al. 2016)**.** In support of this work an approach for a systematic description of the uncertainties and complexity of potential in vivo reference data for carcinogenicity is explored by using an OECD Guidance document that was originally developed for reporting defined in vitro approaches to testing and assessment (OECD, 2016).

Objectives: This structured approach shall allow 1) fostering interest in developing improved defined in silico and in vitro approaches; 2) the definition of what type of effects should be predicted by the new approach; 3) selection of the most suitable reference data and assessments; 4) definition of the weight that the standard animal reference data should have, compared to human reference data and mechanistic information in the context of assessing the fitness of the new in vitro and in silico approach; 5) definition of a benchmark for the minimum performance of the new approach, based on a conceptual recognition that correlation of alternative assessment results with reference animal results is limited by uncertainties and complexity of the latter.

Methods: Literature review.

Results and Discussion: The format is suitable for this re-purposing and it appears that the potential multitude of approaches for integrating and interpreting data from standard animal testing may ultimately be conceptually similar to the challenge of integrating relevant in vitro and in silico data. A longer term perspective is indicated for evolving the definition of adversity for classification and regulatory purposes. The work is being followed within an OECD Expert group (Paparella et al. 2017).

Grant support: The PARERE and OECD work of Martin Paparella is supported by BMLFUW.

References:

Jacobs et al. 2016. ALTEX 33(4), 2016: 359-392. doi: 10.14573/altex.1601201

OECD 2016. Guidance document on the reporting of defined approaches to be used within integrated approaches to testing and assessment. Series of Testing and Assessment 255. ENV/JM/MONO (2016)28.

Paparella et al. 2017. ALTEX in press, online first:
http://www.altex.ch/resources/epub_Paparella_of_1610241.pdf

Mesenchyme-derived growth factors: crucial for tumor promotion by non-genotoxic hepatocarcinogens

B. Grasl-Kraupp, M. Nejabat, T. Riegler, T. Reitinger, W.W. Huber, R. Schulte-Hermann.

Institute for Cancer Research, Medical University of Vienna, Austria

Background: Unlike genotoxic carcinogens, the mode of action of non-genotoxic carcinogens (NGC) is far from being understood. Steroid hormones act as NGC and account for cancer of the breast, prostate, or other organs. As ligands of nuclear receptors hormones induce growth in target tissues and support the outgrowth of mutated cells to malignancy. An almost identical principle is underlying the action of NGC producing liver tumors in long-term rodent bioassays. This group of compounds comprises drugs, like hypolipidemics, synthetic steroid hormones, or barbiturates. A thorough knowledge of the mode of action of NGC is important to estimate the risk of exposed humans.

Objectives: The action of NGC is considered to be confined largely to parenchymal cells. Here, we elucidate the role of the hepatic mesenchyme for two prototypical NGC, phenobarbital (PB), an anti-epileptic drug, and cyproterone acetate (CPA), a progestin used in contraceptive pills.

Methods: Mesenchymal liver cells (MC) and hepatocytes (HC) were isolated from control and PB/CPA-treated rat livers by collagenase perfusion and subsequent percoll-gradient centrifugation steps. Transcriptomics was performed by oligo-array analyses (Affymetrix). Cytokine levels were determined by ELISA. Nuclear translocation of NFkB was shown by immunoblotts of nuclear extracts and by reporter gene assay

Results and Conclusion: Analyses by oligo-arrays revealed that PB and CPA treatment altered the transcriptome profiles of HC and MC. In PB-treated MC, there were deregulations of pro-inflammatory cytokines and chemokines of the TNF-, CCL- and CXCL-family. PB treatment in vivo or in vitro elevated the production and release of tumor necrosis factor α (TNFα) from MC. The secretome of PB-treated MC induced in HC a pro-inflammatory reaction and nuclear translocation of nuclear factor-kB (NFkB), an effect also observed with recombinant TNFα. Both, TNFα and the secretome of PB-treated MC, protected HC from pro-apoptotic stimuli.

Transcriptome analyses of CPA-treated MC revealed enhanced expression not only of pro-inflammatory cytokines but also of several potent growth factors, such as Hepatocyte Growth Factor. Accordingly, the supernatant of CPA-treated MC enhanced considerably the DNA replication of preneoplastic hepatocytes.

We conclude that PB and CPA appear to alter considerably the function of the hepatic mesenchyme. The resulting release of cytokines induces growth and/or activates anti-apoptotic pathways in HC which may contribute to the tumor promoting activity of these compounds. Thus, epithelial-mesenchymal interactions appear crucial in NGC-driven hepatocarcinogenesis.

Grant support: Project MARCAR, funded by IMI JU under grant agreement Nr 115001.

Cellular reaction to VOC exposures

J.M. Gostner, J. Zeisler, M. T. Alam, S. Martini, P. Fagundes dos Santos, S. Geisler, M. Hermann, F. Ueberall and D. Fuchs

Division of Medical Biochemistry and Division of Biological Chemistry, Biocenter, and Department of Anaesthesiology and Critical Care Medicine, Medical University of Innsbruck, Bioenergy2020+, Graz, Austria; Warwick Medical School, University of Warwick, Coventry, UK (johanna.gostner@i-med.ac.at)

In recent years, special attention has been paid to volatile organic compounds (VOC) present in indoor environments. Associated adverse effects are mostly driven by chronic exposure to low concentrations and include respiratory tract irritation and sensitization leading to the development of allergy and asthma.

To investigate cellular reactions that are initiated by low-dose of volatile compounds in an in vitro approach, we developed an exposure platform prototype for long-term airborne treatments of air-liquid interface (ALI) lung cell cultures with volatile compounds [1].

In a proof of principle study, ALI cultures of A549 lung cells were exposed to formaldehyde at regulatory threshold concentrations over a period of three days.

Despite the low exposure concentrations of 0.1 and 0.5 ppm, differential response patterns emerged in an unbiased functional genomics approach, indicating, e.g., lipid biosynthetic pathways to be affected at lower concentrations, while higher concentrations indicated changes in signaling cascades that are involved in proliferation and differentiation, though cell viability was not yet affected.

A limitation is certainly that molecular interactions, which can be retrieved from databases, are usually not characterized in a low-level perturbation context, where hormetic responses become important. As the reaction of epithelial cells to external stimuli is generally moderate due to their barrier function, further research focused on the integration of different immune cell populations in the cell model. Thereby, the tryptophan breakdown pathway via indoleamine 2,3-dioxygenase (IDO-1) may serve as potential biomarker, as this immunoregulatory enzyme is important for the maintenance of tolerance [2,3].

References:

[1] Gostner JM, Sci Rep 2016 1;6:37842.

[2] Gostner JM, et al. Immunol Lett 2015;168:285-92.

[3] Fallarino F, Cell Cycle 2014;13:2645-6.

Characterization of the toxicity of deoxynivalenol in a mechanically stimulated environment

G. Del Favero[1], L. Woelflingseder[1], S. Seriani[2,3], P. Gallina[2], O. Sbaizero[2], D. Marko[1]

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[2] *Department of Engineering and Architecture, University of Trieste Pl. Europa 1/via A. Valerio 10, 34127 Trieste, Italy*

[3] *Robotik und Mechatronik Zentrum, Deutsches Zentrum für Luft- und Raumfahrt e.V. (DLR), Germany*

Background: The development of new and more efficient methods for *in vitro* toxicity testing is one of the crucial challenges toward the limitation of the use of animals in scientific research. *In vivo*, cells that are challenged by toxicants are concomitantly exposed to biomechanical stimulation and the comprehension of the interplay between these two components is of crucial importance for a proper development of new methods for the determination of the cytotoxic potential of contaminants.

Objectives: In line with the abovementioned approach, we established a new workflow for the characterization of the cytotoxic effects of the food/feed-contaminant mycotoxin Deoxynivalenol (DON, Vomitoxin) in a biomechanically stimulated environment.

Methods: For the purpose of the study A431 cells were incubated with 3 concentrations of DON for 24 h (0.1, 1, 10 µM) and the effects of the toxin were measured in terms of morphological/functional impairment.

Results and Discussion: In our experimental conditions, DON triggered a concentration-dependent effect on cellular cytoskeleton of A431 cells, as well as a clear impact on cell size and volume. These effects were accompanied by the impairment of the capability of A431 cells to respond to biomechanical stimulation (24 h cyclic uniaxial stretching 0.5 Hz, 15 % substrate-deformation), measured as integrity of cellular cytoskeleton and distribution of intra-cellular organelles. In conclusion, the implementation of biomechanical stimulation into cytotoxicity studies proved to be an important parameter for a proper and more comprehensive evaluation of the impairment of the cellular function triggered by the mycotoxin DON.

Wastewater-Analysis: Determination of Drugs-of-Abuse in municipal waste-waters - Which information do we try to obtain, which one can we actually get?

Rainer Schmid,

Scientific Coordinator of the drug-prevention project 'checkit!' of the City of Vienna and Medical University of Vienna - e-mail: rainer.schmid@meduniwien.ac.at.

Since recent years interest has dramatically increased to follow the fate of pharmaceutical substances in the environment after they have 'fulfilled' their therapeutical functions, by checking their content of municipal waste-waters. A similar approach is now followed, when controlled drug substances ('drugs-of-abuse'-DOA) are analyzed in this sample matrix, with the background, to obtain more statistic prevalence data on the (illicit) consumption pattern of certain drugs in the general population. This new trend became (suddenly) possible, because of (dramatic) developments in the performance of modern analytical techniques as liquid-chromatography/mass-fragmentography. As for many pharmaceuticals, also DOA substances (and/or their metabolites!), after elimination from the body in the urine, pass without further modification through the sewage system. By measuring their content in sewage waters in water treatment plants this could allow to back-calculate their amount of consumption in the population living in the area of waste-water inflow system. This can be of especially of interest when following (otherwise undetected objective) trends and changes in drug use. While these ('objective') data look very intriguing, they have to be discussed with a necessary critical distance, especially when considering a number of uncertainties in assumptions in the calculations. Finally these pure statistical drug prevalence date have low social relevance, if not combined with other drug monitoring data and if not discussed in context of actual and effective drug prevention programs.

Example of review study on waste-water analysis:

http://www.sniffer.org.uk/files/9113/4183/7992/ER09_Final_e-version_FINAL_3May101.pdf

Phylogenomic concepts in Ecotoxicology: Gastropod metallothioneins as a potential lineage-specific biomarker system in marine, freshwater and terrestrial ecosystems

R. Dallinger, R. Lackner, M. Dvorak, M. Niederwanger, Ò. Palacios, M. Capdevila

Institute of Zoology, University of Innsbruck, Technikerstrasse 25, 6020 Innsbruck

Background: With about 70 000 species, Gastropoda (snails and slugs) are the largest and most diversified class of Mollusca, often with economic and medical importance, having adapted to nearly all habitats of the world. We have identified and partially characterized about 50 novel Metallothioneins (MTs) from more than 30 species across major gastropod lineages. This enables us to present a lineage-based biomonitoring concept by applying gastropod MTs as biomarkers for stress exposure across all major habitats.

Objectives: To explore the lineage-specific biomarker concept with respect to its predictability and general applicability in biomonitoring.

Methods: We relied on a number of phylogenomic, bioinformatic, molecular, biochemical, microscopic and ecotoxicological methods.

Results and Discussion: The following features make the gastropod MT system a potentially universal biomarker system. 1. Modular protein structures with domain multiplications. 2. Stoichiometry-based metal specificity. 3. Predictability of metal specificity due to primary structure features. 4. Correlation between metal specificity and MT inducibility. 5. Simple quantification methods. 7. Extension of the concept to gastropod species from all major habitats of the world.

Study granted by the Austrian Science Foundation (FWF), DACH project No. I 1482-N28 to R. D.

Defining scientific criteria to identify EDs in the pesticide and biocide areas – a never ending story?

A. Fischer

AGES - Austrian Agency for Health and Food Safety, Institute for Plant Protection Products, Department for Toxicology

In 1999, the European Commission adopted a “Strategy on Endocrine Disruptors”. The revisions of the legislation on chemicals, pesticides and biocides performed during subsequent years took this strategy into consideration. Thus, the Plant Protection Products Regulation (EC) 1107/2009 (PPPR) and the Biocidal Products Regulation (EU) 528/2012 (BPR) already state measures relating to endocrine disruptors.

Currently, under the PPPR and BPR interim criteria are in force to identify endocrine disrupting active substances, which should have been replaced by specific scientific criteria already several years ago. The aim was to determine uniform, horizontal scientific criteria for pesticides and biocides that could be applied also to other chemicals in the future. However, this turned out to be more difficult than initially expected, and the process is still ongoing. Since 15 June 2016, a draft Commission proposal for scientific ED criteria is available, which has been extensively discussed and revised, but still not achieving a qualified majority among the EU Member States.

Endocrine Disruptors and Plant Protection Products – Major Characteristics in Ecotoxicology

J. Berchtold

AGES - Austrian Agency for Health and Food Safety- Department for Ecotoxicology

The European Commission presented on 15 June 2016 scientific criteria for the identification of EDs. These are divided into criteria for (i) EDs relevant to humans and (ii) EDs with ecotoxicological relevance. In this presentation, the major specific characteristics in the ecotoxicological assessment of endocrine disrupting properties are highlighted, which include the limited availability of appropriate testing methods, the issue of population relevance as well as the derogation regarding growth regulators.

Endocrine Disrupters within the Context of REACH

A. Losert

Environment Agency Austria

There is growing concern about negative human health and environmental impacts caused by endocrine disruptors. The EU has introduced specific legislative obligations aimed at phasing out endocrine disruptors in industrial chemicals, plant protection products and biocides.

In REACH, endocrine disrupting chemicals (EDCs) may be of similar regulatory concern as substances of very high concern (SVHC) – which are CMRs, PBTs/vPvBs, respiratory sensitisers and more. It is foreseen to subject such chemicals to authorisation and support their substitution via this procedure. In its SVHC roadmap to 2020 the EU gives a commitment to identify all relevant SVHC substances by 2020 and include them in the candidate list, which is the first requirement for subjecting chemicals to authorisation.

Although the scientific criteria for the identification of EDCs are not yet officially agreed several substances, like alkylphenols, have been identified already as endocrine disrupters under REACH, based on the WHO 2002 definition. It can be anticipated that several more EDCs will be subjected to authorisation or will be regulated under different elements of REACH in the near future.

Metabolism of Zearalenone and Its Major Modified Forms in Pigs

S. Binder, H. Schwartz-Zimmermann, E. Varga, G. Bichl, H. Michlmayr, G. Adam, F. Berthiller

Christian Doppler Laboratory for Mycotoxin Metabolism, Dept. IFA-Tulln, University of Natural Resources and Life Sciences, Vienna (BOKU)

Background: The Fusarium mycotoxin zearalenone (ZEN) can be conjugated with polar molecules, like sugars or sulfates, by plants and fungi. To date, the fate of these modified forms of ZEN has not yet been elucidated in animals.

Objectives: We wanted to investigate whether the plant metabolites ZEN-14-glucoside, ZEN-16-glucoside and the fungal metabolite ZEN-14-sulfate contribute to the total ZEN exposure in pigs.

Methods: ZEN (10 µg/kg b.w.) and equimolar amounts of its metabolites were orally administered at the NOAEL to four pigs as a single bolus using a repeated measures design. The concentrations of ZEN, its modified forms and its mammalian metabolites ZEN-14-glucuronide, □-zearalenol (□-ZEL) and □-ZEL-14-glucuronide in excreta were analysed by HPLC-MS/MS based methods.

Results and Discussion: The biological recovery of ZEN in urine was 26±10%, the total biological recovery in excreta was 40±8%. Intact modified forms were not detected in urine or feces. After ZEN-14-sulfate application, 19±5% of the administered dose was recovered in urine. The total biological recoveries of ZEN-14-glucoside and ZEN-16-glucoside in the form of their metabolites in urine were 19±11% and 13±7%, respectively. The total biological recoveries in urine and feces amounted to 48±7% and 34±3%. An explanation for the low biological recoveries could be extensive metabolization by intestinal bacteria to yet unknown metabolites. In summary, the investigated modified mycotoxins were completely hydrolyzed in the gastrointestinal tract of swine, thus contributing to the overall toxicity of ZEN.

Grant support: We thank the Federal Ministry of Science, Research and Economy, the National Foundation for Research, Technology and Development, BIOMIN and the WWTF for financial support.

“Estrogenic cocktails” in our diet: combinatory effects of myco- and phytoestrogens

D. Marko, K. Vejdovszky, K. Hahn, J. Beisl, G. Aichinger

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Mycotoxins are toxic secondary metabolites formed by various fungal species that are found as natural contaminants in food. This very heterogeneous group of compounds triggers multiple toxic mechanisms, including endocrine disruptive potential. Current risk assessment of mycotoxins, as for most chemical substances, is based on the effects of single compounds. The isoflavone genistein, present in soy-based food and dietary supplements, is also known for its estrogenic potential. In addition to phytoestrogens, food may also contain mycotoxins with estrogenic properties. This includes zearalenone (ZEN) and α-zearalenol (α-ZEL) produced by *Fusarium* fungi and alternariol (AOH), a mycotoxin with comparable weak estrogenic properties formed by *Alternaria* species. For evaluation of effects, estrogen-dependent activation of alkaline phosphatase (AlP) and cell proliferation were tested in the adenocarcinoma cell line Ishikawa. All three mycotoxins were found to act as partial agonists of the estrogen receptor. The majority of binary combinations, even at very low concentrations in case of α-ZEL, showed strong synergism in the AlP assay [1]. Already nanomolar concentrations of AOH were sufficient to enhance the estrogenic effects of ZEN and α-ZEL.

Combinations of genistein with either ZEN or AOH, showed synergism orantagonism in the AlP assay, depending on the combination ratios and the concentration range. For combinations of ZEN with genistein synergistic effects dominated [2].

These potentiating phenomena of estrogenic mixtures highlight the urgent need to incorporate combinatory effects into future risk assessment, especially when endocrine disruptors are involved.

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[2] Vejdovszky, K., Schmidt, V., Warth, B., Marko, D., 2017b. Combinatory estrogenic effects between the isoflavone genistein and the mycotoxins zearalenone and alternariol in vitro. Mol Nutr Food Res 61.

Biotransformation of soy isoflavones in humans, rats and mice

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Background: Soy isoflavones (IF) are in the focus of biomedical research since more than two decades. To assess potential beneficial or adverse effects of IF, rats and mice are used as common models.

Objectives: As the biological activity of IF is affected by their biotransformation, our aim was to comprehensively compare the conjugative and microbial metabolism of daidzein and genistein in adult humans, rats and mice of both sexes.

Methods: Humans, rats and mice were orally exposed to an identical soy extract. IF metabolites were analyzed in the biofluids using a validated LC-MS method.

Results and Discussion: We detected considerable differences between the three species. In rats and mice also sex-specific differences were observed. The major plasma phase II metabolites in humans were the 4'-sulfo-7-glucuronides and, in case of genistein, also the diglucuronide. In mice monosulfates and monoglucuronides predominated. In male rats the disulfates and 4'-sulfo-7-glucuronides were predominant, while in female rats the 7-glucuronides exhibited highest concentrations. The portion of aglycones was low in humans (0.5-1.3 %) and rats (0.5-3.1 %) but comparatively high in mice (3.1-26.0 %), especially in the case of daidzein. Furthermore, substantial differences were observed between daidzein and genistein metabolism. In contrast to humans, all rats and mice were equol producer, independent of their sex.

Conclusion: These marked differences may help to explain inconsistencies in results concerning the physiological effects of IF and should be considered when applying findings of animal studies to humans, e.g., for risk assessment.

Grant support: DFG KU 1079/9-1, DI 716/12-2, and VO 410/12-1 (IsoCross project)

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Antagonistic influence of two dietary polyphenols on the genotoxicity of alternariol

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Background: Alternariol, one of the emerging mycotoxins produced by Alternaria spp., has been reported to act genotoxic due to its abilities to induce oxidative stress and to poison topoisomerase II, a crucial enzyme for DNA maintenance. On the other hand, polyphenols are well-known anti-oxidative agents, and some of them – including the catechol delphinidin – have recently been describe to counteract the effectiveness of chemotherapeutic topoisomerase poisons. Also the soy isoflavone genistein was recently described to inhibit this very enzyme.

Objectives: We assessed the question if delphinidin and genistein is able to protect colon cells from alternariol-induced DNA.

Methods: The influence of polyphenols on the genotoxicity of Alternariol was assessed with comet assays. The elucidation of underlying mechanisms was executed using DCF assays for assessment of effects on oxidative stress and "in vivo complex of enzyme" (ICE) assays to observe interactions at the level of topoisomerase inhibition.

Results and Discussion: Indeed, an antagonistic influence of both polyphenols manifested, with delphinidin being more potent regarding its protective effects against mycotoxin-induced DNA damages. Furthermore, we found delphinidin to suppress the generation of oxidative stress and both polyphenols to antagonize the poisoning of topoisomerase IIβ by Alternariol.

This study shows that dietary polyphenols have potential to temper mycotoxicosis caused by *Alternaria spp*. and constitutes an initial base for further studies on the in vivo situation.

Does consumption of synthetic cannabinoids cause genetic damage in drug users?

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Background: Synthetic cannabinoids (SCs) cause similar psychotropic effects as marihuana and are marketed worldwide in herbal mixtures.

Objectives: We studied for the first time the genotoxic properties of representatives of different groups, namely, aminoalkylindoles (AM-2201 and UR-144), 1-alkylindazoles (5F-AKB-48), a cyclohexylphenol (CP-47,497-C8), an indolmethanone XLR-11 (the most widely marketed SC in the US) and a benzoyl-analogue (RCS-4).

Methods, **Results and Discussion:** None of the substances induced gene mutations in bacterial assays, but all caused DNA migration in comet assays with lymphocytes and TR146. Also in micronucleus assays positive results were obtained with lymphocytes and TR146, indicating that SCs cause chromosomal damage. The effects were seen with most compounds with concentrations between 50 and 100 µM. CP-47,497-C8 was already active at doses ≥10 µM. Addition of liver homogenate or proteins reduced their genotoxic effects, indicating that the drugs are directly active and detoxified by protein binding. Further, mechanistic studies showed that none of them causes oxidation of DNA bases; with one representative drug (CP-47,497-C8) proteome analyses with lymphocytes were conducted which indicate that it reduces the levels of DNA repair enzymes. Finally, experiments were conducted with an air-liquid interphase system which reflects inhalative exposure of epithelial cells with XLR-11 and RCS-4. We found pronounced induction of DNA damage in a lung fibroblast cell line (A-549) and in buccal derived cells (TR146) in comet assays. Our results indicate that consumption of SCs may cause adverse health effects in users due to damage of the genetic material in tissues of the respiratory tract.

Grants: This study was supported by the Austrian Federal Ministry of Health (BMG-237609/0001-I/3/13), by the EU Commission in the frame of the projects “SPICE and synthetic cannabinoids” (JUST/2009/DPIP/AG/0948) and “SPICE II Plus” (JUST/2011/DPIP/AG/3597).

Simultaneous quantification of mycotoxins in human breast milk

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Background: Mycotoxins can contaminate food and feed worldwide. Due to their chronic toxicity they are the most hazardous contaminants of foodstuff worldwide [1]. Human breast milk is the best source of nutrients for newborns and it is reflected to be typically safe. For the first six months exclusive breast feeding is recommended by the WHO [2]. However, the transfer of mycotoxins to breast milk may be possible. Besides of aflatoxins and ochratoxin A, data on mycotoxins in this biofluid are only scarcely available [3].

Objectives: In this study, a high-performance liquid chromatography tandem mass spectrometric method was developed for the simultaneous quantification of 15 mycotoxins including the parent compounds (aflatoxins, ochratoxins and zearalenone) and some selected phase I metabolites (α-zearalenol and β-zearalenol, ochratoxin alpha).

Methods: Sample preparation was performed using an adapted version of the QuEChERS protocol [4]. Additionally, the QuEChERS extraction was coupled to a SPE (Waters Oasis HLB Prime) clean-up procedure. Both sample preparations were evaluated and compared.

Results and Discussion: After the extraction procedure recoveries of spiked breast milk samples at a concentration of 1 ng/mL were in the range of 86 – 116 % with standard deviations below 20% for all analytes.

Besides, recoveries for the additional SPE clean-up were analyte dependent. While all aflatoxins and ochratoxin alpha had recoveries between 74 – 123%, ochratoxin A, B, zeraralenone and its metabolites incurred a major loss during SPE. Matrix-matched calibration resulted in limits of detection of were < 0.2, 0.2, 0.4 and 0.5 ng/mL for aflatoxins, zeralenone, zeralenols and ochratoxins, respectively.

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The metallothionein system of the terrestrial slug (*Arion vulgaris*) as a potential biomarker approach

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Background: Metallothioneins (MTs) are small metal-binding proteins. In terrestrial gastropods they play a role in metal metabolism and detoxification. *Arion vulgaris* (*A.v.*) is a wide-spread slug considered as an invasive pest in European countries. *A.v.* possesses two metal-inducible MT isoforms, whose expression is linked to the accumulation of Cd and Cu (publication pending).

Objectives: To explore whether the induction of the two MT isoforms by Cd and Cu can be applied as a biomarker system for environmental metal pollution.

Methods: After characterization of two novel MT genes from *A.v.*, metal concentrations (Cd, Cu) were detected in organs of exposed and control slugs. Midgut gland homogenate supernatants were separated by chromatography, and metals and MT concentrations quantified in the collected fractions. qRT-PCR was applied to assess the inducibility of the two MT isoform genes.

Results and Discussion: Cd and Cu were accumulated predominantly in the slug midgut gland, being associated with the expressed MT isoforms. Cd exposure induced the expression of a Cd/Cu-specific MT (Cd/CuMT) isoform, whereas Cu exposure induced both, the Cd/CuMT gene and a Cu-specific (CuMT) isoform. It is proposed that the combined inducibility of the two isoform genes may serve as a biomarker system for environmental pollution by these two important trace elements.

The project was funded by the Austrian Science Foundation (FWF) No. I 1482-N28 (DACH) granted to R. D.

FGF5 – a potential angiogenic factor in hepatocarcinogenesis?

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Background: Hepatocellular carcinoma (HCC) is the most common type of liver cancer, with great prevalence worldwide. It is often caused by chemical compounds such ethanol, nitrosamines or aflatoxin B1. In contrast to the etiological factors, the pathogenesis of this disease remains to be elucidated.

Angiogenesis is critical in the progression of HCC, with neoangiogenesis providing new blood vessels for a sufficient supply of oxygen and nutrients in the developing tumor. Furthermore, lymphangiogenesis provides new lymphatic vessels to eliminate toxic metabolic by-products. Members of fibroblast growth factor (FGF) family serve as neoangiogenic factors, such as FGF2 and FGF18. It has not been determined whether FGF5 exerts similar effects.

Objectives: To investigate the impact of FGF5 on the different steps of angiogenesis, i.e. proliferation, tube formation and sprouting of blood and lymphatic endothelial cells.

Methods: FGF5 expression was investigated by qRT-PCR in human liver samples, comprising healthy and cirrhotic tissue as well as HCC samples. Immunostaining was performed on paraffin- embedded tissue sections.

Angiogenesis assays were performed on blood (BEC) and lymphatic (LEC) endothelial cell lines. Vascular endothelial growth factor (vEGF) served as positive control. The impact of FGF5 on DNA replication and growth was determined. For tube formation assays, BEC and LEC were seeded onto angiogenesis µ-slides coated with growth factor-reduced matrigel. For sprouting assays, BEC and LEC spheroids were formed in high viscosity methyl cellulose, embedded in rat collagen. Microscopic images of tubes and sprouts were quantified using ImageJ software.

Results and Conclusion: An increase in FGF5 expression was observed in a subset of HCC cases, when compared to the surrounding tissue. Immunohistochemistry showed FGF5 protein expression in hepatocytes only. FGF5 exhibited minor effects on DNA replication but a strong impact on sprouting and the tube formation of BEC and LEC.

We conclude that FGF5 is expressed in hepatocytes and acts in a paracrine way on blood and lymphatic endothelial cells, thus driving several steps of angiogenesis, namely tube formation and sprouting. The elevated expression of FGF5 in a subset of HCC suggests that it may act as neo-angiogenesis factor in hepatocarcinogenesis.

Increased cholesterol levels are involved in resistance to Destruxins in cancer cells

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Background: Ionophoric Destruxins (Dtx), metabolites of entomopathogenic fungi, were proposed for cancer treatment. Prior to clinical use, possible resistance mechanisms which may occur during therapy need to be investigated.

Objectives: We analyzed resistance mechanisms to DtxA, B, or E in a colon carcinoma cell model (HCT116).

Methods: Sublines were established by exposure selection to increasing concentrations of DtxA, B or E (A-, B-, E-subline) followed by determination of cross-resistance profiles to chemotherapeutics and impact on gene expression patterns. In addition, patch clamp experiments as well as measurement of basic levels and *de novo* synthesis of cholesterol and lanosterol were done in resistant and parental cells.

Results and Discussion: While lack of cross-resistance to standard anti-cancer drugs was detected, A- and B-sublines were cross-resistant to DtxB or A, respectively. The E-subline was hypersensitive to DtxA and B, suggesting similar resistance mechanisms of the A- and B- but not for the E-subline. ABCB1, commonly involved in chemoresistance, was slightly overexpressed in E- but not A- and B-sublines. Expression arrays suggested hyperactivation of cholesterol synthesis in A- and B-resistant cells, in line with higher synthesis rates of lanosterol and cholesterol. The pore forming activity of DtxA and B, but not E, was reduced in the respective resistant cells. Additionally, sensitivity to DtxA and B could be partly restored by Fluvastatin (HMG-CoR inhibitor) and Zometa (mevalonate pathway inhibitor).

Our results indicate Dtx resistance in cancer cells based on increased cholesterol synthesis causing alteration of cell membrane composition and reduction of ionophoric activity of Dtx.

Grant support Austrian Science Fund (FWF) (451-B18), Johanna Malke, geb.-Obermann-Stiftung

Aurofusarin, a secondary metabolite of Fusarium fungi, is cytotoxic, genotoxic and induces oxidative stress in colon carcinoma cells

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Aurofusarin (AURO) is a secondary metabolite produced by *Fusarium spp*. The compound is an ubiquitously occurring contaminant on food and feed, but toxicological data are scarce. Previously, AURO was reported to induce cytotoxicity in colon cells[1]. In order to contribute further to the toxicological characterization of AURO, we investigated potential toxic effects to HT29 colon carcinoma cells. Assessment of the chemical stability with LC-MS/MS showed that AURO is strongly light- and temperature-dependent, with a pronounced disintegration visible already after one day at RT. Nevertheless the secondary metabolite induced significant cytotoxicity in HT29 cells after 24h-72h of incubation. Investigations of the genotoxic impact of AURO revealed the induction of DNA-damage after 1h of incubation, using the comet assay. In the decatenation assay AURO was found to potently inhibit the activity of topoisomerase II (topo), an enzyme crucial for the topology of DNA. In the catalytic cycle of topo different ways of interference and impact on DNA integrity are possible. In the ICE assay AURO did not affect the stability of the covalent DNA-topo-intermediates, formed during the catalytic cycle, thus arguing for a role as a catalytic topo inhibitor. In addition, in the DCF assay significant ROS formation was observed, which could be linked to a potential onset of oxidative stress, visible as a shift of the GSSG/GSH ratio after 3h and 24h. Taken together, the observed effects induced by AURO in HT29 cells suggest that the secondary metabolite might be considered as a mycotoxin.

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Glucose and growth promotion of first stages of hepatocarcinogenesis

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Background: Hepatocellular carcinoma (HCC) is a frequent malignancy with raising incidence in the Western world due to an increasing prevalence of obesity, metabolic syndrome and/or type II diabetes. It has not been elucidated in detail, by which pathogenic mechanisms metabolic alterations trigger the development of HCC. Here we investigate the role of glucose as an anabolic growth stimulating factor for early stages of hepatocarcinogenesis.

In a rat model of hepatocarcinogenesis we investigated the transcriptome pattern of these tumor pre-stages. Next, we cultured initiated hepatocytes and investigated their growth in dependence of the glucose-concentration in the medium. In addition we studied the sensitivity of the initiated cells towards inhibition of hexokinase.

Hexokinase phosphorylates glucose which is rate-limiting for a number of intracellular metabolic processes, such as glycolysis, glycogen synthesis or the formation of ribose-5-phosphate for DNA synthesis. Thus, the uptake and phosphorylation of glucose may be a critical step for the proliferation of cells. Here we applied two glucose analogues, 2-deoxyglucose (2DG) and 5-thio-D-glucose (5TG), both potent inhibitors of hexokinase.

Objectives: (i) to investigate possible alterations of the transcriptome pattern of key enzymes of glucose metabolism in liver preneoplasia; (ii) to study the role of glucose as growth stimulator of initiated hepatocytes (iii) to investigate the sensitivity of initiated liver cells towards inhibition of hexokinase.

Methods: Hepatocarcinogenesis was initiated in rat liver by a single dose of the genotoxic carcinogen N-nitrosomopholine (NNM), followed by tumor promotion by phenobarbital (PB). Cancer pre-stages developed and were identified by the selective expression of placental glutathione-S-transferase (GSTp). Preneoplastic cells were micro-dissected and analyzed by transcriptomics (Affymetrix Rat 230-2.0-Array, Cleveland/OH).

Three weeks after NNM treatment, livers were perfused by collagenase. Unaltered as well as initiated HC were isolated for cultivation. Initiated hepatocytes in culture were identified by their selective expression of GSTp by immunostaining. The incorporation of ^{3}H-thymidine was visualized via autoradiography. The percentage of nuclei with incorporated ^{3}H-thymidine was evaluated separately for unaltered GSTp negative HC and GSTp positive initiated cells. The ATP content was measured indirectly via the luciferase reaction. We also measured LDH release and the metabolic activity of the cells via MTT assay.

Results and Discussion: (i) Analysis of the transcriptome profile revealed 333 up- and 183 downregulated genes in the preneoplastic lesions. Several metabolic pathways were altered, including the carbohydrate metabolism. Upregulation of both oxidative and non-oxidative pentose-phosphate pathway suggests provision of ribose for nucleotide synthesis. Upregulation of hexokinase was evident as well.

(ii) GSTp-negative and GSTp-positive hepatocytes were cultured in medium containing either glucose or not. Under both conditions, GSTp positive hepatocytes displayed a considerably higher DNA replication rate compared to the unaltered cells, indicating an inherent growth advantage of the first stages of hepatocarcinogenesis. Under glucose-depleted conditions the DNA replication was unaffected in GSTp-negative hepatocytes, but was reduced in GSTp-positive initiated hepatocytes.

(iii) To impair the hexokinase activity 2-deoxyglucose (2-DG), a non-competitive inhibitor, and 5-thioglucose (5-TG), a competitive inhibitor, were applied. Both compounds marginally affected the viability and the LDH release to the medium, indicating no unspecific cytotoxicity. 2-DG and 5-TG lowered the ATP content and DNA replication of unaltered GSTp-negative hepatocytes. In GSTp-positive initiated cells the suppression of DNA replication was considerable indicating that the very first tumor pre-stages are sensitive towards a lack of glucose-6-P.

To conclude, early stages of hepatocarcinogenesis exhibit an altered glucose metabolism. Upregulation of hexokinase and key enzymes of the pentose-phosphate pathway are linked to enhanced cell replication. These alterations appear to be evident already in initiated hepatocytes.

Grant support by the European Union Innovative Medicines Initiative (IMI JU) under Grant Agreement no 11 5001 is gratefully acknowledged.

Impact of xanthohumol on DNA stability in humans: Results of a placebo-controlled intervention trial

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Background: Xanthohumol (XN) is a hop flavonoid contained in beers and soft drinks. *In vitro* and animal studies indicated that XN has DNA and cancer protective properties.

Objectives: To find out, if it causes DNA protective effects in humans an intervention trial was conducted in which the participants (n = 22) consumed a XN containing drink (12 mg XN/P/d). We monitored alterations of the DNA stability in single cell gel electrophoresis assays (SCGE). A decrease of oxidatively damaged purines by 33% ($p< 0.001$) and protection of 53% ($p<0.05$) towards ROS induced DNA damage was found after the consumption of the beverage.

Methods, **Results and Discussion:** We monitored prevention of DNA damage induced by representatives of two major groups of dietary carcinogens i.e. benzo(a)pyrene (B(a)P) and the heterocyclic aromatic amine2-amino-3-methylimidazo[4,5-f]quinoline (IQ). Lymphocytes were collected before, during and after the intervention and incubated with the carcinogens and with human liver homogenate (S9). We found significant reduction of B(a)P and IQ ($p<0.001$ for both substances) induced DNA damage after consumption of the beverage. The results of a follow-up trial (n = 10) with XN pills showed that the effects are caused by the flavonoid. To elucidate the underlying mechanisms we measured several parameters of glutathione related detoxification. We found clear induction of α-GST (by 42.8%, $p< 0.05$), but no alterations of π-GST. This observation provides a partial explanation for the DNA protective effects and indicates that XN also protects against other carcinogens which are detoxified by α-GST. Taken together, our findings support the assumption that XN has anti-carcinogenic properties in humans.

Grants: TA-XAN AG provided the experimental beverage and parley the funding

Alternaria toxin contaminations in Austrian food commodities: Development, validation and application of an LC-ESI-MS/MS multi-analyte quantification method

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Alternaria toxins are secondary metabolites produced by black molds belonging to the fungal genus *Alternaria*. Both, pre- and post-harvest infestation of cereals, tomatoes and other fruits by these worldwide occurring plant pathogens can result in considerable economic losses due to crop spoilage. Additionally, food and feed products can be contaminated with *Alternaria* toxins, of which several compounds proved acute toxic, genotoxic, mutagenic and estrogenic effects (1). However, no maximum permitted levels for *Alternaria* toxins have been established to date. In order to evaluate, whether such regulatory guidelines would be necessary, further data on occurrence patterns and the toxicological potential of *Alternaria* toxins are required.

In this study, a new liquid chromatography tandem mass spectrometric method was developed to allow the simultaneous quantification of 17 *Alternaria* toxins in the following food matrices: wheat flour (Type 480), tomato sauce and sunflower seed oil. Analytes included are the most relevant parent toxins (derivatives of tetramic acids, dibenzopyrenes, perylene quinones and others), as well as some selected phase II metabolites of alternariol and its monomethyl ether (sulfates and glucosides).
Chromatographic separation was realized on a reversed phase HPLC column (C18, 2.7 µm) using a binary gradient elution. The mass spectrometer was operated in negative ionization and selected reaction monitoring (SRM) mode, including 2 parent to fragment ion transitions per analyte with optimized ion source and fragmentation parameters. The method validation was based on the artificial fortification of blank matrices, due to the lack of certified reference materials.

A survey on food products purchased from Austrian supermarkets was conducted to prove the method's applicability and give first insights about *Alternaria* toxin contaminations patterns, comparing commodities of different commercial brands, product price ranges and agricultural production types (conventional and organic production).

We acknowledge Prof. Dr. Joachim Podlech (Institut für Organische Chemie, Karlsruher Institut für Technologie, Germany) and Prof. Dr. De Saeger and Dr. Jeoren Walravens (Laboratory of food analysis, Ghent University, Belgium) for providing reference materials and the staff of the Mass Spectrometry Center (Faculty of Chemistry, University of Vienna) for the technical support.

Reference: (1) EFSA, EFSA Journal 2011, 9, 2407

Derivation of tolerance levels for selected endocrine disrupting substances in drinking water

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Background: The substances bisphenol A (BPA), bisphenol S (BPS), triclosan (TCS), perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) may be found in drinking water. They all have the ability to interfere with the endocrine system in the human body.

Objectives: The aim of this study was to set up tolerance levels in drinking water.

Methods: Based on the respective health based guidance valueof the substance and a consumption of 2 L of drinking water per day for an adult with 60 kg body weight, a derived tolerance level for the respective substance was calculated assuming a 20% exhaustion of the health based guidance via drinking water (ADI allocation concept). In addition, these calculations were also carried out for an infant with 5 kg body weight and a consumption of 0.75 L of drinking water.

Results and Discussion: Due to structural similarities, the substances BPA and BPS can be considered as a group and the t-TDI of 4 µg/kg bw/d for BPA was extended to a group t-TDI. For the group of BPA and BPS, tolerance levels of 24 and 5.3 µg/L were calculated for adults and infants, respectively.

For TCS based on an ADI of 0.12 mg/kg bw/d, tolerance levels of 720 and 160 µg/L were calculated for adults and infants, respectively.

For PFOA based on a TDI of of 1.5 µg/kg bw/d, tolerance levels of 9 and 2 µg/L were calculated for adults and infants, respectively.

For PFOS based on a TDI of of 150 ng/kg bw/d, tolerance levels of 0.9 and 0.2 µg/L were calculated for adults and infants, respectively.

If these tolerance levels are exceeded, the sources should be identified and possible minimization measures should be elaborated. They have no direct relationship to a harmful effect of a single sample, which has to be assessed in each case.

The calculations refer only to single substances, except for BPA and BPS, and not to any combination effects.

Grant support: none

Applying metallothionein genes as potential biomarkers in embryos of terrestrial snails

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Background: Embryonic development is an important phase in the life cycle of terrestrial snails because of its high impact on population dynamics. In response to soil metal contamination, snail embryos express different metallothionein (MT) isoforms. The pattern of expression, however, is greatly different from that in adult snails.

Objectives: We test the transcription of two metal-specific MT genes (CdMT and CuMT), and of an unspecific MT gene (Cd/CuMT) in different developmental stages of snail embryos (*Helix pomatia* and *Cantareus aspersus*) depending on the level and duration of metal exposure.

Methods:Snail embryos were exposed to different Cd and Cu concentrations. The expression levels of all three MT isoforms were assessed during exposure by quantitative Real Time PCR (qPCR). Results were compared to the known expression levels of adults.

Results and Discussion: In control embryos, the expression of the Cd/CuMT exceeds that of the two other MT isoforms, which is in contrast to the situation in adults. In Cd-exposed embryos, however, the CdMT is upregulated as it is in adult snails. Whereas the CuMT is not responsive in adults, all three MT isoforms were upregulated in embryos by Cu exposure. Therefore, we want to propose the peculiar transcription patterns of the three MT isoform genes as specific biomarkers of sensitivity to metal pollution in embryonic snails.

Grant support: FWF P23635-B20 to RD

Impact of FGFR4 Gly388Arg single nucleotide polymorphism on malignancy in hepatocarcinogenesis

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Background: Hepatocellular carcinoma (HCC) is characterized by high incidence and mortality. Studies in patient tumor samples revealed a correlation of HCC with dysregulation of fibroblast growth factor receptor 4 (FGFR4) expression. A Gly388Arg single nucleotide polymorphism (SNP) in the transmembrane domain was described and our preliminary investigations associated FGFR4/Arg with increased malignancy of HCC.

Objectives: The role of FGFR4-G388R in malignant behavior of HCC cell lines is investigated as a possible target for new therapeutic approaches.

Methods: Human model cell lines for HCC were used for transient overexpression of FGFR4/Gly or FGFR4/Arg. Additionally, stably overexpressing clones were established for both alleles. Several tests were performed focusing on anchorage-independency (soft agar assay), cell survival at low density (clonogenicity assay), cell-cell and cell-matrix interactions (hanging drop and adhesion assay).

Results and Discussion: Results demonstrate a clear difference between FGFR4 alleles, as FGFR4/Arg expressing cells exhibit enhanced anchorage-independent growth and elevated survival and clonogenic growth. Concerning metastatic capabilities, FGFR4/Arg is involved in reduced cell-matrix adhesion with concomitantly increased cell-cell aggregation.

Based on our findings we postulate that FGFR4/Arg has distinctly different effects on the progression of HCC compared to FGFR4/Gly. In conclusion, further studies on the different mechanistic roles of the Gly388Arg allelic variants in HCC malignancy are required to improve diagnostic and therapeutic possibilities for HCC patients.

Copper-complexing hydrophilic compounds in green tea subfractions identified by a combination of HPLC/MS and EPR

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Background: Plant extracts contain compounds capable of forming strong complexes with various metal ions, thereby posing a risk of transition metal intoxication, but also the possibility of being able to remove toxic metals from the human body.

Objectives: By using hot aqueous extracts of leaves of *Camellia sinensis* as a model system, we have investigated the hydrophilic, low molecular weight compounds that form stable copper complexes at acidic and neutral pH.
Methods: Subfractions of green tea (1g/50ml boiling water/10min/filtered (0.2µm) and lyophilized) were obtained by solid phase extraction (30mg lyophilized powder dissolved in 1ml H_2O) over C18-SPE columns. The first two colourless aqueous fractions were collected, and analysed using a Waters Micromass Quattro micro LC-MS/MS. After addition of Cu(II), a Bruker ESP300E EPR spectrometer was used to investigate its complexes in the pH range 1.7-8 to simulate various regions of the digestive system.

Results and Discussion: The two separated fractions consisted mainly of amino acids and other carboxylic acids (e.g. quinic acid, oxalic acid, malic acid, citric acid and succinic acid). Parameters for the Cu(II) complexes of the above mentioned compounds were estimated from the EPR spectra.

Conclusions: Reaction of Cu(II) with hot aqueous extracts of leaves of *Camellia sinensis* involves the formation of mixed ligand complexes. These exhibit EPR spectra at lower pH values than complexes with known individual ligands. It is likely that similar mixed ligand complexes are responsible for the chelation behaviour of metals and probably also some of the biological effects of extracts from other plant products

NX3-M1: a detoxification product of NX-3? Impact on cytotoxicity and intracellular reactive oxygen species in human intestinal cells

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Background: NX-3 is a recently discovered type A trichothecene produced by certain *Fusarium* strains. Structurally, it shares the major features of the better-known mycotoxin deoxynivalenol (DON) and can be degraded to NX3-M1, which differentiates from NX-3 by an opened epoxide ring. In *in vitro* translation assays NX-3 was found to inhibit protein biosynthesis to almost the same extent as DON, whereas NX3-M1 did not show any inhibitory effect [1]. However, to date no detailed biological characterisation of NX-3 and NX3-M1 in any human cell system has been carried out to evaluate if their entrance into the food chain may pose a risk for consumers.

Objectives: The potency of NX-3 and NX3-M1 to trigger cytotoxicity and oxidative stress in two different colon cell lines was explored in direct comparison to DON.

Methods: The cytotoxic potential was assessed in the SRB and Alamar Blue assay and the effects on oxidative stress were determined by the DCF assay.

Results and Discussion: In HT-29 cells 10 µM NX-3 and DON induced significant cytotoxicity, whereas in HCEC-1CT already 1 µM triggered significant effects. NX3-M1 did not show any cytotoxic potential.

In the DCF assay NX-3 and DON induced in HT-29 cells a fast and transient increase of the intracellular ROS levels, whereas in HCEC-1CT intracellular ROS levels were enhanced to a greater and longer extend; NX3-M1 did not trigger oxidative stress.

In conclusion, these data demonstrate that NX-3 possesses toxicological potency comparable to DON, thus demanding the evaluation of the risk associated to the entrance of this toxin into the food chain. More detailed characterisation of the properties of NX-3 appears to be essential for future risk ssessment. In regard to NX3-M1, these data suggest that it can be considered a detoxification product of its parent compound.

The authors thank the Austrian Science Fund (project SFB Fusarium #F3702-B11, #F3706-B11 and #F3718-B11) for the financial support.

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