interdisciplinary toxicology

Volume 13 | Suppl. 1 | 2020

TOXCON 2020: Programme & Abstracts

AIMS & SCOPE:

Interdisciplinary Toxicology is an international, peer-reviewed interdisciplinary journal covering the fields of Toxicology, Pharmacology, Physiology and related areas such as reproductive medicine, embryology, teratology, molecular biology, biophysics, behavioral pharmacology, biochemistry, neurochemistry, receptor studies, animal breeding, and others.
The Journal publishes Original Papers, Review Articles and Clinical Reports on research relating to the toxicity of drugs and chemicals at molecular, cellular, tissue, target organ and whole body level *in vivo* (by all routes of exposure) and *in vitro*/*ex vivo*. Focus is on teratogenesis, (developmental) reproductive toxicology, carcinogenesis, mutagenesis, pharmacokinetics, toxicogenomics and proteomics, pharmacotoxicological and metabolic mechanisms, risk assessment, environmental toxicology and environmental health as applied to humans (including epidemiological studies), forensic toxicology, military toxicology, environmental chemistry, pesticides, dioxins, regulatory toxicology, occupational toxicology, food toxicology and a broad range of interdisciplines related to toxicology. Papers from both basic research and clinical research will be considered for fast publication.
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Official Journal of

Slovak Toxicology Society SETOX
Institute of Experimental Pharmacology & Toxicology, CEM SASc.

Subscription period: Jan. 1– Dec. 31 (4 issues). For subscription details contact: intertox@setox.eu. Correspondence concerning subscriptions, claims, distribution and advertising should be sent by email to: intertox@setox.eu

Published by
Slovak Toxicology Society SETOX (IČO: 30801729)
Institute of Experimental Pharmacology & Toxicology, CEM SASc. (IČO: 00598453)
Dúbravská cesta 9, SK-841 04 Bratislava, SLOVAKIA
TEL: +421-2-3229 5718 • E-MAIL: info@setox.eu

The journal is registered by the Ministry of Culture of the Slovak Republic as EV 3200/09 and Slovak National ISSN Centre under ISSN 1337-6853 (print) and ISSN 1337-9569 (on-line).

interdisciplinary

*i*toxicology

Vol. 13*(Suppl. 1): 2020*

BRATISLAVA
August 2020

Official journal of

Slovak Toxicology Society SETOX, Bratislava, Slovakia
&
Institute of Experimental Pharmacology & Toxicology,
Centre of Experimental Medicine SASc., Bratislava, Slovakia

Interdisciplinary Toxicology, Volume 13(Suppl. 1), 2020
ISSN 1337-6853 *(print version)* 1337-9569 *(electronic version)*
Published by Slovak Toxicology Society SETOX *(ID Nr. 30801729)*
& Institute of Experimental Pharmacology & Toxicology CEM SASc. *(ID Nr. 00598453)*
Dúbravská cesta 9, SK-841 04 Bratislava, SLOVAKIA
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Additional information: www.degruyter.com/view/j/intox

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TOXCON 2020: 25th Interdisciplinary Toxicology Conference

TOXCON

25th Interdisciplinary Toxicological Conference

Czech Republic

Faculty of Architecture, Czech Technical University in Prague
September 3 – 5, 2020

Programme & Abstracts

Editors

Petr HODEK
Jiří HUDEČEK

ORGANISED BY

Czech Society for Experimental and Clinical Pharmacology and Toxicology, CZ

Czech Society for Biochemistry and Molecular Biology, CZ

Department of Biochemistry, Faculty of Science, Charles University, Prague, CZ

Slovak Toxicology Society SETOX, Bratislava, SK

UNDER PATRONAGE OF

prof. RNDr. Jiří Zima, CSc., the Dean of Faculty of Science, Charles University

TOXCON 2020 is dedicated to the memory of prof. Marie Stiborová

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THURSDAY - September 3, 2020

TOXCON 2020

12:30 – 15:00

REGISTRATION

15:00 – 15:30

Opening Ceremony

15:30

KEYNOTE LECTURE 1

KL01

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Guengerich F.P.

Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146, United States

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Chairpersons: Pavel Anzenbacher, Jan Vondráček

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[1]Institute of Clinical Biochemistry and Diagnostics, University Hospital Hradec Kralove, Czech Republic, [2]Dept. of Cytokinetics, Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i., Brno, CZ, [3]Dept. of Pharmacology, Faculty of Medicine and Dentistry, Palacky University, Olomouc, CZ, [4]Central European Institute of Technology, Masaryk University, Brno, CZ, [5]Center for Toxicology and Health Safety, National Institute of Public Health, Prague, CZ, [6]Dept. of Comprehensive Cancer Care, Masaryk Memorial Cancer Institute, Brno, CZ, [7]Dept. of Surgery, Faculty Hospital Brno, CZ.

L02

METABOLISM OF HELENALIN *IN VITRO* AND ITS INTERACTION WITH HUMAN CYTOCHROME P450 2A13

Šadibolová M.[1], Boušová I.[1], Juvonen R.[2], Auriola S.[2]

[1]Department of Biochemical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Prague, Czech Republic, [2]School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, Kuopio, Finland

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HE COLON MICROENVIROMENT AND CROSS-TALK OF MICROBIAL METABOLITES WITH INTESTINAL EPITHELIAL CELLS IS A MAJOR FACTOR REGULATING BOTH THEIR FUNCTIONS AND XENOBIOTIC METABOLISM

Vondráček J.

Department of Cytokinetics, Institute of Biophysics of the Czech Academy of Sciences, Brno, Czech Republic

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Jourová L.[1], Zemanová N.[1], Lněničková K. [1], Anzenbacher P.[2], Hudcovic T.[3], Anzenbacherová E.[1]
[1]Department of Medical Chemistry and Biochemistry and [2]Department of Pharmacology, Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic, [3]Institute of Microbiology, Academy of Sciences of the Czech Republic, Nový Hrádek, Czech Republic

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Linhart I.[1], Hanzlíková I.[2], Mráz J.[2], Dušková Š.[2], Tvrdíková M.[2], Vachová H.[1]
[1]Department of Organic Chemistry, Faculty of Chemical Technology, University of Chemistry and Technology, Prague, Czech Republic; [2]Centre of Occupational Health, National Institute of Public Health, Prague, Czech Republic

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Hemminki K.[1], Vodickova L.[1,2,3], Vodicka P.[1,2,3], Försti A.[4,5]
[1]Faculty of Medicine and Biomedical Center in Pilsen, Charles University, Pilsen, 30605, Czech Republic; [2]Department of Molecular Biology of Cancer, Institute of Experimental Medicine, The Czech Academy of Sciences, Prague, 14220, Czech Republic; [3]Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University, Prague, 12800, Czech Republic; [4]Hopp Children's Cancer Center (KiTZ), Heidelberg, Germany; [5]Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ), German Cancer Consortium (DKTK), Heidelberg, Germany

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

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Vodicka P.[1,2,3], Vodickova L.[1,2,3], Opattova A.[1,2,3], Vodenkova S.[1], Kroupa M.[1,3], Vymetalkova V.[1,2,3]

[1]Department of Molecular Biology of Cancer, Institute of Experimental Medicine of the Czech Academy of Sciences, Videnska 1083, 142 00 Prague, Czech Republic, [2]Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University, 128 00 Prague, Czech Republic, [3]Faculty of Medicine and Biomedical Center in Pilsen, Charles University in Prague, 30605 Pilsen, Czech Republic

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Kristensen V.N.[1,2], Bjørklund S.S.[1], Nebdal D.[1], Tekpli X.[1,2], Ragle Aure M.[1], Lüders T.[2]

[1]Department of Cancer Genetics, Institute for Cancer Research, Oslo University Hospital, Oslo, [2]Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway

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*[1]Institute of Biotechnology of the Czech Academy of Sciences, BIOCEV Research Center, Vestec, Czech Republic, [2]School of Medical Science, Griffith University, Southport, Qld, Australia, [3]Faculty of Sciences, BIOCEV Research Center, Charles University, Vestec, Czech Republic, [4]Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic, *Current address: Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, USA*

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Hilscherová K., Pípal M., Jonáš A., Priebojová J., Sehnal L., Smutná M.

RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic

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[1]Department of Pharmacology and Toxicology, [2] Department of Pharmaceutical Technology, Faculty of Pharmacy, Charles University, Ak. Heyrovského 1203, Hradec Kralove, 500 05, Czech Republic, [3]Department of Food Safety, German Federal Institute for Risk Assessment, Max-Dohrn-Str. 8-10, 10589, Berlin, Germany, and Department of Toxicology, University of Tübingen, Wilhelmstr. 56, 72074, Tübingen, Germany, [4]Department of Pharmacology, Faculty of Medicine in Hradec Kralove, Charles University, Simkova, Hradec Kralove, Czech Republic.

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Mohelnikova-Duchonova B.
Department of Oncology, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic

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[1]Centre of Toxicology and Health Safety, National Institute of Public Health, Prague, Czech Republic, [2]Department of Medical Biophysics, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University in Olomouc, Czech Republic, [3]Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic, [4]FICAM, Faculty of Medicine and Health Technology, FI-33014 Tampere University, Tampere, Finland

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Chairpersons: Mojmír Mach, Ingrid Brucknerová

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Horackova H., Karahoda R., Abad C., Cerveny L., Vachalova V., Staud F.
Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Kralove, Charles University, Prague, Czech Republic

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Staud F., Horackova H., Karahoda R., Abad C.
Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Kralove, Charles University, Czech Republic

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LUNCH

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[1]Toxicogenomics Unit, National Institute of Public Health, Prague, Czech Republic, [2]Third Faculty of Medicine, Charles University, Prague, Czech Republic, [3]Department of Oncosurgery, MEDICON, Prague, Czech Republic, [4]Department of Oncology, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic, [5]Department of Surgery, EUC Hospital and University of Tomas Bata in Zlin, Zlin, Czech Republic

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[1]Centre of Experimental Medicine SAS, Bratislava, Slovak Republic, [2]Institute of Biochemistry and Microbiology, Faculty of Chemical and Food Technology STU, Bratislava, Slovak Republic, [3]Center for Cardiology, Cardiology I, Medical Center of the Johannes Gutenberg-Uni Mainz, Germany, [4]Dept of Animal Physiology and Ethology, Faculty of Natural Sciences, Bratislava, Slovak Republic

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

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Tuček M.[1], Škerjanc A.[2]
[1]Institute of Hygiene and Epidemiology, First Faculty of Medicine, Charles University, Prague, Czech Republic, [2]University Medical Centre Ljubljana, Clinical Institute for Occupational, Traffic and Sports Medicine, Zaloska cesta 002, 1000 Ljubljana, Slovenia

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Mráz J.[1], Hanzlíková I.[1], Dušková Š.[1], Tvrdíková M.[1], Linhart I.[2]
[1]Centre of Occupational Health, National Institute of Public Health, Prague, Czech Republic; [2]Department of Organic Chemistry, Faculty of Chemical Technology, University of Chemistry and Technology, Prague, Czech Republic

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Dlabková A., Herman D., Žďárová Karasová J.
Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, University of Defence, Hradec Králové, Czech Republic

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Pejchal J.[1], Žďárová J.[1], Lísa M.[2], Andrýs R.[2]
[1]Department of Toxicology, Faculty of Military Health Sciences, University of Defence, Trebesska 1575, 500 01 Hradec Kralove, Czech Republic, [2]Department of Chemistry, Faculty of Science, University of Hradec Kralove, Hradecka 1285, 500 03 Hradec Kralove, Czech Republic

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Kucera T., Fibigar J.
Faculty of Military Health Sciences, University of Defence in Brno, Czech Republic

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Kassa J., Hepnarova V., Hatlapatkova J., Zdarova Karasova J.
Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic

17:00 – 18:00

POSTER SESSION 2

(Posters 53-104)

19:00

GALA-DINNER
(Czechia Boat & Legie Hotel)

SATURDAY - September 5, 2020

8:30

Company-sponsored breakfast

9:00

KEYNOTE LECTURE 3

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Arlt V.M.[1,2]
[1]Toxicology Department, GAB Consulting GmbH, Heidelberg, Germany, [2]Department of Analytical, Environmental and Forensic Sciences, King's College London, London, United Kingdom

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Chairpersons: Eva Kmoníčková, Eva Horvathová

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[1]Department of Pharmacology, Second Faculty of Medicine, Charles University, Czech Republic
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[1]University of Chemistry and Technology Prague, Department of Biochemistry and Microbiology, Prague, The Czech Republic, [2]Faculty of Medicine in Pilsen, Charles University, Department of Toxicology, Pilsen, The Czech Republic, [3]University of Chemistry and Technology Prague, Department of Chemistry of Natural Compounds, Prague, The Czech Republic

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Šindlerová L.[1], Babica P.[2;3], Vašíček O.[1], Adamovský O.[2], Kubala L.[1]
[1]Institute of Biophysics of the Czech Academy of Sciences, The Department of Biophysics of Immune System, Brno, Czech Republic; [2]Masaryk University, Faculty of Science, RECETOX, Brno, Czech Republic, 3Institute of Botany of the Czech Academy of Sciences, The Department of Experimental Phycology and Ecotoxicology, Brno, Czech Republic

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

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11:10 – 12:50

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Chairpersons: Tomáš Cajthaml, Klára Hilscherová

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Jaklová Dytrtová J.[1,2], Jakl M.[3]

[1]Institute of Organic Chemistry and Biochemistry of the CAS, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic, E-mail: dytrtova@uochb.cas.cz, [2]Department of Physiology and Biochemistry, Faculty of Physical Education and Sport, Charles University, José Martího 31, 162 52 Prague 6, Czech Republic, [3]Department of Agro-Environmental Chemistry and Plant Nutrition, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 00 Prague – Suchdol, Czech Republic

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Semerád J.[1;2], Cajthaml T.[1;2]

[1]Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic, [2]Institute for Environmental Studies, Faculty of Science, Charles University, Prague, Czech Republic

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Smrček S., Grasserová A., Krmelová T., Luptáková D.

Department of Organic Chemistry, Charles University, Faculty of Science, Prague

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Ezechiáš M.[1], Cajthaml T.[1,2]

[1]Laboratory of Environmental Biotechnology, Institute of Microbiology of the CAS, v.v.i., Vídeňská 1083, Prague, 142 20, Czech Republic, [2]Institute for Environmental Studies, Faculty of Science, Charles University in Prague, Albertov 6, Prague, 128 43, Czech Republic

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Cajthaml T.[1,2]

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LUNCH

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[1]RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic

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Šimečková P., Procházková J., Pěnčíková K., Machala M.
Department of Pharmacology and Toxicology, Veterinary Research Institute, Brno, Czech Republic

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Kováč O., Pěnčíková K., Slavík J., Procházková J., Machala M.
Department of Pharmacology and Toxicology, Veterinary Research Institute, Brno, Czech Republic

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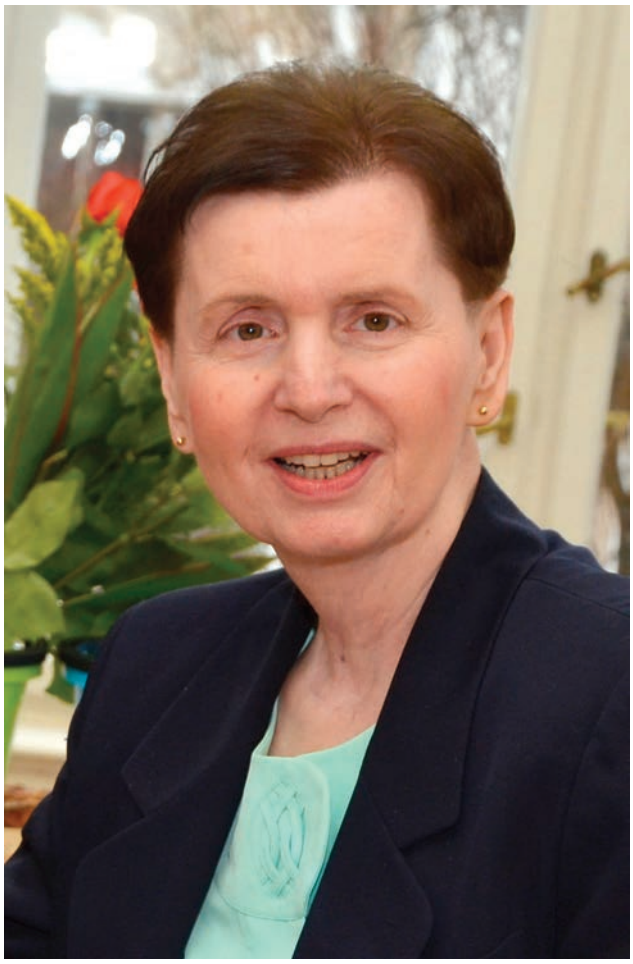
Icha J.
East Port Praha s.r.o, Prague, Czech Republic

15:40 – 16:00
COFFEE BREAK

16:00
CLOSING CEREMONY

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*



Prof. RNDr. Marie Stiborová, DrSc.

February 2. 1950 – February 13, 2020

Marie Stiborová, 70, a professor of medicinal chemistry and biochemistry and former head of the Department of Biochemistry at the Faculty of Science, Charles University of Prague in the Czech Republic, died after a long bout with thyroid cancer on February 13, just days after her birthday.

Born in Modřany, now part of Prague, she earned her degrees in chemistry and biochemistry in the Faculty of Science of the Charles University in Prague. She received her doctorate in Biochemistry in 1978 for her work on plant alcohol dehydrogenases, their structure and properties, and the influences of various environmental factors.

She started her career as an independent researcher in 1978 in the Oncological Institute in Prague, where her interest shifted to interactions of environmental pollutants with nucleic acids, this research to be her main research topic in later years. After a short time, in 1981, she moved as an assistant professor to the Department of Environmental and Landscape Ecology at the Faculty of Science of Charles University, returning to the research of plant enzymes and their interaction with environmental pollutants (as triazines and metal ions). This topic brought her finally back to the Department of Biochemistry at the same faculty in 1987, where she joined the group interested in various aspects of the metabolism of xenobiotics, incl. mechanism, activation, and function of P450s and peroxidases. She then worked till her death in this department, promoted to an associate professor (in 1988), and a full professor (in 2007). She was a very active teacher, reading courses in general biochemistry, xenobiochemistry and chemical carcinogenesis, and a very valued supervisor of students´ work.

The scientific development of professor Stiborova was deeply influenced by the collaboration with the German Cancer Research Center in Heidelberg, which started by a stay here in 1989 and lasted till 2017. Her research interest was focused on aristolochic acids and their role in nephropathies and kidney cancer. At the same time, she developed excellent skills in the ^{32}P-postlabelling method for the study on nucleic acid covalent modifications with carcinogenic compounds. Later, this collaboration also included cancer research laboratories in the UK (London, Dundee). She co-authored six book chapters and about 400 scientific papers in international journals.

Stiborova was a member of the Czech Chemical Society, Czech Medical Association (Section of Experimental Pharmacology and Toxicology), and of the Czech Society for Biochemistry and Molecular Biology, where she served since 2008 as a member of its committee. She actively participated in organizing several international scientific meetings, mostly in Prague (e.g., 2003 Cytochrome P450, 2009, 2018 FEBS Congresses). In the early 1990s, she was also politically active, serving for three legislative periods as a member of the Czech Parliament.

For her achievements, she got several awards, including the Memorial Medal of the Faculty of Science, and the Gold Medal of the Charles University - both of them she, unfortunately, could not accept in person.

Marie Stiborova is survived by her husband, Oldřich, daughter, Martina, and four grandchildren.

ABSTRACT BOOK

25th Interdisciplinary Toxicological Conference TOXCON 2020

Note: The authors are solely responsible for the scientific content and linguistic presentation of the abstracts.

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

KL1
HISTORY OF THE ROLES OF CYTOCHROME P450 ENZYMES IN DRUG TOXICITY

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The history of drug metabolism began in the 19th Century and developed slowly. In the mid-20th Century the relationship between drug metabolism and toxicity became appreciated, and the roles of cytochrome P450 (P450) enzymes began to be defined in the 1960s. Today we understand much about the metabolism of drugs and many aspects of safety assessment in the context of a relatively small number of human P450s. P450s affect drug toxicity mainly by either reducing exposure to the parent molecule or, in some cases, by converting the drug into a toxic entity. Some of the factors involved are enzyme induction, enzyme inhibition (both reversible and irreversible), and pharmacogenetics. Issues related to drug toxicity include drug-drug interactions, drug-food interactions, and the roles of chemical moieties of drug candidates. The maturation of the field of P450 and drug toxicity has been facilitated by advances in analytical chemistry, computational capability, biochemistry and enzymology, and molecular and cell biology. Problems still arise with P450s and drug toxicity in drug discovery and development, and in the pharmaceutical industry the interaction of scientists in medicinal chemistry, drug metabolism, and safety assessment is critical for success.

Supported by U.S. National Institutes of Health Grant R01 GM118122.

KL2
GENOTOXICOLOGY OF CHROMOSOMAL ABERRATIONS

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Many human malignancies exhibit genomic instability, which contributes to malignant transformation. Unrepaired or insufficiently repaired DNA double-strand breaks, as well as telomere shortening, are important contributors in the formation of structural chromosomal aberrations (CAs). Structural CA include both randomly occurring and recurrent types. The randomly occurring CAs have been measured as occupational exposure markers to genotoxicants. The recurring CA have important clinical diagnostic and prognostic implications, particularly in hematological malignancies.

In the present review we discuss potential mechanisms for the formation of CAs and their relation to cancer. Based on our own studies we illustrate how inherited genetic variation may modify frequencies and types of CAs in humans. We review CAs in terms of variants in genes relevant in maintaining genomic integrity, such as those encoding xenobiotic-metabolising enzymes, DNA repair, the tumour suppressor TP53, spindle assembly checkpoint and cyclin D1 (CCND1). While individually genetic variation in these genes exerted small modulating effects, in combination they were associated with CA frequencies in peripheral blood lymphocytes of healthy volunteers. Moreover, we observed opposite associations between the *CCND1* splice site polymorphism rs9344 G870A and the frequency of CAs compared to its association with translocation t(11,14), a recurrent CA in many haematological malignancies.

Our review summarizes the evidence that variants in genes in relevant cellular pathways modulate the frequency of CAs, and discussed the possible mechanisms on how these may associate with telomere length in lymphocytes. More functional/mechanistic studies elucidating these pathways are required. There are several emerging questions, such as the role of CAs in malignancies with respect to a particular phenotype and heterogeneity, the formation of CAs during the process of malignant transformation and formation of CAs in individual types of lymphocytes in relation to the immune response.

KL3
ARISTOLOCHIC ACID-INDUCED UROTHELIAL MALIGNANCY: AN UPDATE ON MOLECULAR MECHANISMS IMPORTANT FOR CARCINOGENESIS

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Exposure to the phytotoxin aristolochic acid (AA) contained in *Aristolochia* species is associated with human nephropathy and urothelial cancer. The plant extract AA which is present in medicinal herbal remedies contains two major components, aristolochic acid I (AAI) and aristolochic acid II (AAII). AA initially was involved in an outbreak of cases of rapidly progressive renal fibrosis reported in Belgium after intake of root extracts of *Aristolochia fangchi* imported from China but aristolochic acid nephropathy (AAN) is now recognised as a global disease. A high prevalence of urothelial malignancy is found in AAN patients but urothelial tumours do not develop in all individuals exposed to AA and thus differences in the activities of enzymes catalysing the metabolism of AAI and AAII might be one of the reasons for an individual's susceptibility. The major activation pathway of AA involves reduction of the

nitro group, primarily catalysed by NAD(P)H:quinone oxidoreductase (NQO1), to an electrophilic cyclic *N*-acylnitrenium ion that reacts preferentially with purine bases to form covalent DNA adducts. Particularly AAI-derived DNA adducts (dA-AAI) have been identified in urothelial tissues from AAN patients and they not only serve as biomarker of prior exposure, but they also trigger urothelial malignancy by inducing specific mutations (AT to TA transversions) in critical genes of carcinogenesis, including the tumour suppressor *TP53*. Recent studies using whole genome sequencing (WGS) also identified a characteristic AA mutational signature dominated by AT to TA transversions in the genome (COSMIC signature 22) and this WGS signature was recapitulated in cultured cells exposed to AAI, but not AAII, indicating that AAI is the critical component causing AAN-associated urothelial malignancy. Other recent studies examined the role of the tumour suppressor p53 in AA-induced nephrotoxicity.

Using *Trp53(+/+)*, *Trp53(+/-)*, and *Trp53(-/-)* mice as a model showed that p53 protects from AAI-induced renal proximal tubular injury, while *Trp53* genotype had no impact on AAI-DNA adduct levels. In contrast, primary mouse embryonic fibroblasts (MEFs) derived from these mice showed that after AAI exposure *in vitro Trp53* genotype impacted on the expression of Nqo1. Nqo1 induction was highest in *Trp53(+/+)* MEFs and lowest in *Trp53(-/-)* MEFs; and it correlated with AAI-DNA adduct formation, with lowest adduct levels being observed in AAI-exposed *Trp53(-/-)* MEFs. Herbal products containing AA are still available in many parts of the world and thus improved regulation is warranted to help eradicate this entirely preventable disease.

The lecture is dedicated to the memory of Professor Marie Stiborová.

L01
REGULATION OF CYP450 EXPRESSION IN HEPATOCELLULAR CARCINOMA

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Hepatocellular carcinoma (HCC) is a highly prevalent primary liver cancer with limited treatment options and poor prognosis, which is reflected in the 6th rank of liver cancer in overall cancer incidence and 4th rank in cancer-related deaths. Genetic alterations in HCC include significant changes in the expression of biotransformation enzymes; pair-wise comparison of tumour tissue and the respective peri-tumour liver tissue reveals a major downregulation of CYP mRNAs and proteins in frequent cases, namely in high-grade HCC tumours. Major CYP forms affected in our cohort of 35 patients were CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2E1, CYP3A4, and CYP3A5. Patients with larger high-grade tumours might be at a higher risk of drug toxicity; on the other hand, CYP-lacking tumours might be more sensitive to targeted therapy by tyrosine kinase inhibitors as these are frequently metabolized by CYPs (predominantly CYP3A4).

We performed a comparative transcriptome analysis of HCC tumours with a strong (CYP-) vs. weak (CYP+) down-regulation of CYPs to search for underlying regulatory mechanisms (genetic and/or epigenetic). Levels of mRNA, lncRNA and miRNA transcripts were compared using Agilent SurePrint G3 8x60k microarrays for mRNAs and lncRNAs, and TaqMan Array Human MicroRNA v3.0 TLDA qPCR panel of 754 human microRNAs in 20 pairs of tumour/peri-tumour tissue. Transcriptome analysis produced a number of differentially expressed mRNAs when comparing groups of tumours to peri-tumour tissue. Subsequent comparison of CYP- to CYP+ groups of tumours has shifted the output towards the non-coding RNAs with 34 lncRNAs and 5 miRNAs to be differentially expressed between CYP- vs. CYP+ group. The potential regulatory effect of the highlighted miRNAs has further been validated *in vitro* using specific miRNA mimics or inhibitors. We newly show that the targeted up/down-regulation of selected miRNAs resulted in significant changes in the mRNA level of several CYP450 family members. Our results suggest potential functional associations between the particular miRNAs and CYP mRNAs in human hepatocytes, and highlight the need to further study potential consequences and relevance in HCC tumours.

Supported by Ministry of Health of the Czech Republic, grant nr. 17-28231A.

L02
METABOLISM OF HELENALIN *IN VITRO* AND ITS INTERACTION WITH HUMAN CYTOCHROME P450 2A13

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Helenalin (HEL) is a sesquiterpene lactone found especially in *Arnica montana* and *Arnica chamissonis* that demonstrates a potent anti-inflammatory activity mediated by direct alkylation of Cys38 within the DNA binding domain of RelA (p65), a key member of the nuclear factor-κB (NF-κB) family of transcription factors. Besides, HEL has been found to exert remarkable

anticancer, antibacterial and antiprotozoal activity. HEL is an active component of herbal arnica preparations that have long been used to reduce muscle and joint pain, post-surgical pain as well as to treat minor sports injuries, bruises and swelling associated with trauma, contusions and sprains. Despite the frequent use of these products neither the fate of HEL in the human organism, nor its interaction with xenobiotic-metabolizing enzymes has been studied so far. To address this issue, we have first investigated the metabolism of HEL and characterized the kinetics of metabolite formation using human and rat liver subcellular fractions, and human recombinant cytochrome P450 (CYP) enzymes. UHPLC-MS/MS technique was employed for this purpose. HEL was oxidized into five metabolites by both, human and rat liver microsomes. Moreover, several human CYP isoforms were found to be involved in the oxidation of HEL, namely CYP2A13, 2B6, 3A4, 3A5 and 3A7. Given the estimated kinetic parameters, the oxidation of HEL was generally far more efficient in rat microsomes compared to human microsomes, except for one metabolite. In the following experiments, we have also tested the inhibitory potential of HEL towards human recombinant CYP enzymes. Out of all tested CYPs, the most effective inhibition (the lowest IC_{50} value) was observed for CYP2A13. In addition, the inhibition of CYP2A13 was NADPH- and time-dependent suggesting that HEL may act as a mechanism-based inhibitor of CYP2A13. HEL also inhibited the activity of CYP3A4, but the inhibition was less effective than that of CYP2A13 and did not appear to be NADPH-dependent.

Supported by the Czech Science Foundation (grant No. 18-09946S) and the Charles University Grant Agency (grant No. GAUK 1302120).

L03
THE COLON MICROENVIROMENT AND CROSS-TALK OF MICROBIAL METABOLITES WITH INTESTINAL EPITHELIAL CELLS IS A MAJOR FACTOR REGULATING BOTH THEIR FUNCTIONS AND XENOBIOTIC METABOLISM

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The gut microbiome has gained an increasing attention as a principal factor contributing to the maintenance of intestinal homeostasis. The commensal microflora, in symbiosis with intestinal epithelial cells, including colon epithelial cells (colonocytes), helps to coordinate absorptive processes, to form intestinal barrier and to regulate mucosal immunity. Nevertheless, the major colon microbiota metabolites, which include short chain fatty acids (SCFAs), products of tryptophan metabolism (such as tryptamine and various indole derivatives), or further compounds, such as secondary bile acids, seem to have a major impact also on activities of transcriptional factors involved in the control of xenobiotic-metabolizing enzymes (XMEs), including the aryl hydrocarbon receptor (AhR). This presentation intends to provide an overview of the recent findings about the possible role of some of these metabolites as important regulators of gut homeostasis (mediated via the AhR activity), and to comment upon our own data indicating that interactions of SCFAs with endogenous/exogenous ligands of the AhR may alter levels/activities of XMEs in colon mucosa. This may have implications not only for the metabolism of dietary contaminants/toxicants, such as carcinogenic polycyclic aromatic hydrocarbons (which are known as efficient AhR ligands), but it can also contribute to regulation of endogenous metabolic functions in colon epithelial cells.

Supported by grant no. 19-00236S from the Czech Science Foundation.

L04
THE EFFECT OF GUT MICROBIOME ON HEPATIC INFLAMMATION AND RELATED DRUG METABOLISM

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The gut microbiome, an aggregate genome of trillions of microorganisms, is now known to play a critical role in human health and predisposition to disease. Wide range of pathological states including inflammatory liver disease were associated with imbalance in the composition of this immense bacterial community. Over the past couple of years, the human gut microbiome has received increasing attention as a potential regulator of the metabolism of drugs. To date, despite numerous examples of the effects of the human gastrointestinal microbiome on drug efficacy and toxicity, there is often an incomplete understanding of the underlying mechanism. The results from a few recent studies indicate that one of the possible mechanisms of action of the gut microbiome in terms of influencing drug bioavailability may be its impact on the expression of hepatic biotransformation enzymes. Cytochromes P450 (CYPs) are key enzymes involved in the initial drug metabolism in human. Enzymes belonging to the families labeled as CYP1, CYP2, and CYP3 are known to metabolize the most of the xenobiotics including 70–80% of all drugs in clinical use. They are responsible for enormous variability in drug response. Studies on germ-free (GF) mice, lacking the intestinal bacteria, have shown that microbiome-derived metabolites may play an important role in regulation of the expression of hepatic biotransformation enzymes - including CYPs. Expression of CYPs is regulated by specific nuclear receptors such as aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR) and pregnane X receptor (PXR). The link between the regulation of CYPs expression and infection and inflammation has been also reported and the nuclear receptors play an important role here. As inflammation plays a central role in many pathologies

and inflammatory signaling and metabolism pathways are intertwined, we suppose that gut microbiota may play an important role in these processes; differently, at healthy or at pathological conditions. For these reasons, the better understanding the ways how gut microbiota may modulate inflammation and influence drug metabolism, could make a significant contribution to improvement of pharmacotherapy.

Supported by the grants 19-08294S from Grant Agency of the Czech Republic and IGA_LF_2020_022 project of Palacký University Olomouc.

L05
AMINOARYLCYSTEINE ADDUCTS IN GLOBIN, A NEW TYPE OF PROSPECTIVE BIOMARKERS OF EXPOSURE TO ARYLAMINES AND NITROARENES

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Both arylamines and nitroarenes are metabolically activated via arylhydroxylamines to reactive electrophilic arylnitrenium ions. These can bind to nucleophilic sites in the DNA leading to DNA adducts, mutations, and ultimately carcinogenesis. While the arylnitrenium DNA adducts have been known for a long time, an evidence of their protein counterparts have not been presented until our current studies. Here we report on the formation of aminoarylcysteine adducts in globin after single *i.p.* dosing of rats with 1- and 2-naphthylamine (1-NA and 2-NA), 1- and 2-nitronaphthalene (1-NN and 2-NN) (all 0.162 mmol/kg b.w.) or 3-nitrobenzanthrone (3-NBA) (3.6 μmol/kg b.w). HPLC-ESI-MS^2 analyses of globin hydrolysates revealed new aminoarylcysteine adducts which were identified and quantified using authentic standards. Both 1-NA and 1-NN gave *S*-(1-amino-2-naphthyl)cysteine (1A2NC) together with *S*-(4-amino-1-naphthyl)cysteine (4A1NC) whereas 2-NA and 2-NN gave *S*-(2-amino-1-naphthyl)cysteine (2A1NC). Similarly, 3-NBA afforded *S*-(3-amino-2-benzanthronyl)cysteine. Haemoglobin binding index (HBI) [(mmol adduct/mol Hb)/(dose in mmol/kg b.w.)] used to describe extent of the adduct formation was 14.0, 4.4, 0.54, 0.41 and 0.21 for 2-NA, 3-NBA, 2-NN, 1-NN and 1-NA, respectively. For comparison, HBI values of known sulphinamide adducts of the same parent compounds, which were also determined in this study, were about one order of magnitude lower (1.04, 0.76, 0.05, 0.05, and 0.08, respectively). These results indicate that aminoarylcysteine adducts can be formed from both carcinogenic and non-carcinogenic arylamines and nitroarenes but the carcinogens (2-NA and 3-NBA) afforded notably higher adduct levels. As these new globin adducts are more abundant and are formed *via* toxicologically relevant arylnitrenium ions and/or their arylhydroxylamine conjugate precursors, they appear to be more valuable biomarkers of cumulative exposure to arylamines, nitroarenes and possibly also to heteroaromatic amines than the corresponding sulphinamide adducts.

The study was funded by the institutional support DRO (National Institute of Public Health - NIPH, IN 75010330) from the Ministry of Health of the Czech Republic, and by the Program of long-term development of the University of Chemistry and Technology, Prague.

L06
DNA DAMAGE RESPONSE (DDR) AS A PLAYER IN SENSITIVITY/RESISTENCE OF SOLID TUMORS TO TOWARDS CHEMOTHERAPEUTICS

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The DNA damage response (DDR) is a complex cellular pathway in the cell that responds to arising DNA damage thus preventing deleterious mutations and chromosomal damage. The pivotal constituent of DDR, DNA repair, ensures genomic integrity. Alterations in DNA repair that occurring in many cancers, contribute to the accumulation of mutations in the genome, resulting in genomic instability and cancer progression. DNA repair also plays a substantial role in response to chemotherapeutics: rapidly dividing colon cancer cells, vulnerable to DNA-damaging agents and overcoming DNA repair, undergo cell death. DNA repair capacity (DRC) represents an integrative marker for functional evaluation of multigene DNA repair processes in cancer onset with future prospects in personalized prevention and/or cancer treatment. The role of DNA repair is entirely different in the cancer onset than in the cancer treatment, despite the whole system is universal for eukaryotes. Since cancer is a heterogeneous complex disease, numerous points have to be considered: a) DNA damage and DRC measured in surrogate/target tissues, b) changes in the levels of DNA damage and DRC may be a cause or a consequence of the disease, c) changes in DRC alter sensitivity of tumour cells to antineoplastic drugs, d) one time point-sampling of patients provides insufficient information on the role of DNA damage and its repair in carcinogenesis. Finally, systemic cancer therapy is targeted at DNA damage and its repair. A proper understanding of these processes is a key precondition for the optimisation of therapy regimens, prediction of therapeutic response and prognosis in cancer patients. As apparent from our studies on colorectal cancer, rather than absolute values of DRC in tumor tissue and adjacent mucosa the ratio in DRC between both is critical. Patients with higher level of DNA damage and lower DRC in tumor tissue along with lower level of DNA damage and higher DRC in adjacent

mucosa had significantly better prognosis than those with DRC higher in tumor tissue.

Here, we discuss the role of DDR and DNA repair in sensitivity of cancer cells/patients towards various chemotherapeutic agents.

The authors thank for support GACR 18-09709S, 19-10543S and AZV NV18-03-00199.

L07
MOLECULAR MECHANISMS OF BREAST CANCER CELLS INSTABILITY

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Genomic instability is a hallmark of malignant tumors, causing disturbed integrity of the genome, numerical alterations, and structural changes. For various cancer types genomic instability has been associated with poor prognosis, suggesting that genomic instability may confer growth advantage of cancer cells. In most cancer types, genomic instability is characterized by copy number alterations, allelic imbalance, or the loss of heterozygosity. The molecular basis of genomic instability remains still unclear; however, mutations in key checkpoint proteins and DNA repair genes are supposed to be involved. Interestingly, the effects of disordered genomic organization may also have an unfavorable effect on the overall viability and fitness of cancer cells. Precise delineation of the negative and positive effects of genomic instability on cancer cells is of potentially great importance for tumor classification, survival prediction, and individualized therapy. Our research is focused on the assessment of genomic instability in a variety of tumor types by applying various methods or the developing novel algorithms. This work includes studies about the complexity and genomic aberrations in breast tumors; integrated omics analysis; efficient algorithms for single- and multi-track copy number segmentation analysis; genomic architecture; comparison of platforms and algorithms for classification of copy number alterations in human breast tumors. In recent study, we analyzed changes in pure ductal carcinomas in situ (DCIS) in comparison with invasive breast cancers (IBC) from different patients. Three levels of genomic data were obtained; gene expression, DNA methylation, and DNA copy number. Subtype stratified analyses identified key differences between DCIS and IBC that suggest subtype specific progression. Prominent differences were found in tumors of the basal-like subtype, *e.g.* basal-like DCIS were less proliferative and showed a higher degree of differentiation than basal-like IBC and exhibited fewer copy number aberrations. Methylome analysis revealed hypermethylation of multiple protocadherin genes in basal-like IBC compared with basal-like DCIS and normal tissue, possibly caused by long range epigenetic silencing. Our work confirms that subtype stratification is essential when studying progression from DCIS to IBC and questions the assumption that basal-like DCIS is a direct precursor of basal-like invasive breast cancer.

Supported by the Horizon 2020 project RESCUER, grant agreement ID:847912.

L08
REPURPOSING AN IRON CHELATOR: MITOCHONDRIALLY-TARGETED DEFEROXAMINE EXHIBITS POTENT CYTOSTATIC, CYTOTOXIC AND MIGRASTATIC ANTI-CANCER PROPERTIES AND INDUCES MITOPHAGY

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Deferoxamine (DFO) represents a widely used iron chelator for treatment of iron overload. Here we describe the use of mitochondrially targeted deferoxamine (mitoDFO) as a novel approach to selectively target cancer cells. The agent shows marked cytostatic, cytotoxic and migrastatic properties *in vitro*, significantly suppressing tumor growth and lung metastasis formation *in vivo*. The underlying molecular mechanisms include (I) impairment of [Fe-S] cluster biogenesis, leading to destabilization and loss of the activity of [Fe-S] cluster containing enzymes, (II) inhibition of mitochondrial respiration leading to mitochondrial ROS production, resulting in dysfunctional mitochondria with markedly reduced supercomplexes, and (III) fragmentation of the mitochondrial network and induction of mitophagy. We show that mitochondrial targeting of DFO represents a way to deprive cancer cells of biologically active iron, which is incompatible with their proliferation and invasion, without disrupting systemic iron metabolism. Our findings highlight the importance of mitochondrial iron metabolism for cancer cells and demonstrate a novel mechanism to induce mitophagy via mitochondrial targeting of deferoxamine.

L09
TERATOGENIC POTENTIAL AND RETINOID-LIKE ACTIVITY OF CYANOBACTERIAL METABOLITES

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Development of cyanobacterial water blooms and associated production of diverse bioactive and toxic compounds have been linked with adverse effects in exposed organisms and potential risk to human health. Cyanobacteria have been recently indicated to produce

retinoid-like compounds, but there is little information on their levels and related potential adverse effects and risks. Our studies employed bioanalytical approaches for the characterization of the retinoid-like potencies of extracts and exudates from laboratory cultured cyanobacteria from different orders and samples of environmental water blooms and their surrounding surface water collected from field studies. The retinoid-like potencies were characterized in reporter gene assays based on the interaction of samples with retinoid acid receptor. The relevance of the detected *in vitro* effects of prioritized samples and compounds for whole organism was evaluated in zFET assay using embryos of zebrafish (*Danio rerio*) and FETAX test with frog embryos (*Xenopus laevis*), for the assessment of developmental toxicity and embryotoxicity. Malformations typical for retinoid signaling disruption were detected after exposure to the cyanobacterial samples. Observed effect phenotypes in both fish and frog embryos and effective concentrations of cyanobacterial samples corresponded to all-trans retinoic acid (ATRA) equivalents, which supports the hypothesis that the teratogenic effects of cyanobacterial samples are probably associated with retinoid-like activity. Sensitive analytical methods (LC-MS-MS) documented production of compounds with this bioactivity into surface waters by various cyanobacterial species and environmental water blooms. In some cases, the level of retinoid-like activity reached values that can cause adverse developmental effects in exposed organisms. We have identified a set of compounds contributing to the detected retinoid-like activity in both laboratory and field samples. These include ATRA, 9/13cis retinoic acid (RA), as well as several novel cyanobacterial metabolites, such as 5,6epoxy-RA or 4keto-ATRA with high retinoid-like potency. Our studies document that the production of retinoids by cyanobacteria into the aquatic environment is a common phenomenon, since retinoid-like activity and presence of individual retinoids have been shown to be associated with cyanobacterial water blooms dominated by many different species.

Supported by the grant 18-15199S from the Grant Agency of the Czech Republic.

L10
STILBENE COMPOUND TRANS-3,4,5,4´-TETRAMETHOXYSTILBENE IS A CONSTITUTIVE ANDROSTANE RECEPTOR (CAR) AGONIST WITHOUT PROLIFERATIVE ACTIVITY THAT VIOLATES A PARADIGMA FOR NON-GENOTOXIC CARCINOGENS

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The constitutive androstane receptor (CAR, NR1I3) is a ligand-activated transcription factor belonging to the nuclear receptor subfamily NR1 together with Pregnane X receptor (PXR). Both receptors are recognized as xenobiotic-sensing nuclear receptors that transcriptionally regulate the expression of numerous detoxification enzymes and transporters. Recent studies also suggest that mouse CAR plays important roles in the metabolism of glucose, lipids, fatty acids, bile acids, bilirubin and thyroid hormones.

Rodent CAR activation is connected with mitogenic effects, liver hypertrophy and hyperplasia and rodent CAR ligands are known as non-genotoxic mouse liver carcinogens.

We identified TMS as a novel moderate murine CAR agonist. TMS significantly up-regulated *Cyp2b10* and *Cyp2c55* mRNAs, typical murine CAR target genes, but down-regulated expression of genes involved in gluconeogenesis and lipogenesis such as *Pck1*, *G6pc*, *Scd1, Acaca* and *Fasn* as was observed for TCPOBOP, a prototype mouse CAR ligand. However, TMS did not display hypertrophic or hyperplastic effects in mouse liver.

We also observed that TMS did not promote EdU incorporation in AML12 cells, did not increase liver weigh and had no statistically significant effect on Ki67 and Pcna labeling indices in mouse liver *in vivo*. At the same time, TMS did not induce genes involved in liver proliferation or apoptosis such as *Mki67*, *Foxm1*, *Myc*, *Mcl1, Pcna, Bcl2, Bax* or *Mdm2* in the mouse liver.

We conclude that TMS is a novel mouse CAR ligand with limited effects on liver hypertrophy and hyperplasia. Explanation of the observation will be discussed in the presentation.

Funded by GAČR 19-14497S to P.P. and S.M and by OPVVV project InoMed (CZ.02.1.01/0.0/0.0/18_069/0010046) to J.D. and P.P.

L11
THE ROLE OF CYTOTOXICITY IN CLINICAL ONCOLOGY

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While mortality rates of many cancer types are declining in last decade pancreatic cancer is one of the exceptions. Only 10-20% patients are diagnosed in the stage of resectable disease. The prognosis of locally advanced or metastatic disease remains very poor despite new advances in diagnostics and treatment. Chemotherapy is the primary and usual still the only treatment modality for patients with locally advanced and metastatic pancreatic cancer. The first anticancer drug which significantly improved survival in this setting was gemcitabine. It has become standard of care since the 1990s. Many cytotoxic or targeted agents alone or in combination with gemcitabine were tested in clinical trials and failed to improve survival of the pancreatic cancer patients during two decades. Nevertheless, most

recently new chemotherapy regimens as FOLFIRINOX and nab-paklitaxel demonstrated the superiority over gemcitabine alone. PARP-inhibitor olaparib is the new and only targeted agent that showed benefit for PDAC patients and has been approved as the treatment option for metastatic PADC patients this year.

The aim of this *in vitro* study was to evaluate the chemosensitivity of human PDAC cell lines to PARP-inhibitors, and its combination with various cytostatic agents with regard to their expression profiles.

Human adherent pancreatic cell lines BxPC-3, MiaPaCa-2, PaCa-44 and BRCA2 deficient Capan-1 were treated with gemcitabine, cisplatin, paclitaxel, doxorubicin, cyclophosphamide, 5-fluorouracil and olaparib. Combinations of those cytostatic agents with olaparib were also tested. IC50 values were determined and cross comparison of sensitivity/resistance to tested cytostatic agents and combinations was made. Expression profiles of drug-able genes and key membrane transporters were analyzed.

PDAC human pancreatic cell lines showed highly resistant expression profiles when compared to other cancer types *e.g.* colorectal cancer. Significant differences in chemosensitivity and gene expression profiles were observed concerning tested cell lines.

Generally, the use of chemotherapy to treat cancers is limited by the inter-individual variability in drug response and by the development of resistance. From this point of view it seems obvious that biomarkers enabling prediction of optimal type, combination and dose of drugs for each patient may exist and their use for individualization of therapy is envisaged. Such individualization would be highly cost-effective and socially favorable due to the prolonged survival and improved quality of life of large number of cancer patients.

This work was supported by the Ministry of Health of the Czech Republic grant no. NV-19-03-00097 and student grant IGA_LF_2020_005 from Palacky University.

L12
IN VITRO METHODS USED FOR EVALUATION OF MUTAGENICITY/GENOTOXICITY OF SELECTED PARABENS

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Growing worldwide effort has been made to replace (reduce) animal testing and to improve alternative *in vitro* tests which may be more efficient in terms of both time and cost. Regulatory acceptance of alternative methods is lagging behind the technological progress, however, genotoxicity and mutagenicity endpoints have already been addressed by legislation (*e.g.* OECD TG 473, 487, 471). Parabens are phenolic derivates of benzoic acid widely used as preservatives in cosmetic, pharmaceutical and food industries due to their unique properties. Many toxicological studies on parabens have been already published, frequently with ambiguous or warning conclusions. The aim of this study was to summarize the existing information on parabens obtained with the use of *in vitro* toxicological methods. Individual method limits, advantages and possible combinations to achieve greater sensitivity towards the *in vivo* data is discussed. Experimental data on 7 parabens (Methyl, Ethyl, Propyl, Isopropyl, Butyl, Isobuthyl, Benzyl), generated by *in vitro* mammalian chromosomal aberration test (OECD TG 473) using human peripheral lymphocytes and by the *in vitro* Comet assay performed on 2 non-tumor cell lines derived from human keratinocytes (HaCat, SVK14), will be presented. The Comet assay identified Ethylparaben and Benzylparaben as potentially genotoxic. The chromosomal aberration test revealed weak genotoxic potential in case of Ethylparaben and positive genotoxicity in case of Butylparaben, Propylparaben and Isopropylparaben. The results confirmed that the Comet assay should serve as a screening test and should not be used as a stand-alone method for classification of genotoxicity. This test evaluates the induction of single and double strand breaks of DNA, while the chromosomal aberration test indentifies additional structural chromosomal aberrations (deletions, rearrangements) and aneuploidy with / without metabolic activation. Due to specific genotoxic mechanisms and the diversity in test systems, the *in vitro* / *in vivo* results generally exhibit high variability. The weight of evidence approach in risk assessment should be supported with data generated with the use of human relevant *in vitro* methods based on cells / tissues of human origin.

The study was supported by the ERDF/ESF project „International competitiveness of NIPH in research, development and education in alternative toxicological methods" (No. CZ.02.1.01/0.0/0.0/16_019/0000860).

L13
EFFECT OF ANTIDEPRESSANTS ON PLACENTAL SEROTONIN HOMEOSTASIS; IMPORTANCE OF FETAL SEX

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It has been reported that up to 25% of pregnant women are affected by depression and approximately 10% are prescribed antidepressant drugs (ADs). However, safety of this treatment is still unclear, since poor pregnancy outcomes such as organ malformations, neurological disorders and preeclampsia have been reported in pregnant woman using ADs. Nowadays, most frequently

prescribed ADs in pregnancy are selective serotonin reuptake inhibitors (SSRIs) and selective serotonin and noradrenalin reuptake inhibitors (SNRIs). Nevertheless, interaction with placental serotonin homeostasis has not been properly investigated to date. We have recently characterized serotonin transporter (SERT), organic cation transporter 3 (OCT3) and monoamine oxidase (MAO-A) as crucial components of serotonin uptake and metabolism in the trophoblast. In the current study, we investigated the effect of several ADs on serotonin placental uptake via SERT and/or OCT3 from maternal and fetal circulation, respectively. In both human and rat placenta, we observed dose-dependent inhibitory effect of all tested drugs on placental handling of serotonin. Interestingly, our data indicate the role of fetal sex in inhibition of OCT3 in rat placenta. This phenomenon was shown to be independent of OCT3 transcript and protein levels. We suggest, that use of ADs during the pregnancy affect serotonin homeostasis in placenta and may alter fetal development.

Supported by the grant GAUK 1464119/C/2019, SVV 2019/260414, GACR 20-13017S

L14
MONOAMINE CLEARANCE BY THE PLACENTA; FETAL POINT OF VIEW

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Prenatal period is the foundation for later life outcomes and the placenta is the key organ to maintain optimal *in utero* conditions for proper fetal development and programming. In particular, placental homeostasis of bioactive amines, serotonin (5HT), dopamine (DA), and norepinephrine (NE) is of crucial importance for fetal brain development and 'wiring'. Therefore, their concentrations must be tightly regulated in the feto-placental unit during the whole period of gestation. Interestingly, despite being a non-neuronal tissue, trophoblast cells of the placenta express a machinery of enzymes and transporters similar, if not identical, to the central nervous system. Subsequently, placenta is capable of synthesizing, metabolizing and transporting all bioactive amines. Using a battery of experimental approaches, including perfused rat placenta and vesicles prepared from the apical and basal membranes of human placenta, we have investigated expression and function of key transporters and enzymes responsible for placental homeostasis of 5HT, DA and NE. We show that in the apical, mother facing membrane of the placenta, selective, high-affinity but low-capacity transporters mediate uptake of 5HT, DA and NE from the maternal circulation. In addition, we are the first to describe that clearance of all monoamines from the fetal circulation is controlled by a single transporter, organic cation transporter 3 (OCT3/SLC22A3). This is a polyspecific, low-affinity but high-capacity transporter abundantly expressed in the basal membrane of syncytiotrophoblast. OCT3-mediated uptake of monoamines from the fetal circulation is concentration-dependent, inhibitable by both endogenous (corticosteroids) and exogenous (pharmaceuticals) molecules and in the case of 5HT transport, it is affected by fetal sex. We conclude that the placenta plays a protective role against excessive levels of free monoamines in the fetal circulation by taking them up into trophoblast cells via OCT3 for subsequent metabolism by monoamine oxidase. Importantly, various drugs administered during pregnancy (*e.g.* antidepressants, antidiabetics, antiretrovirals) or elevated cortisol levels (due to maternal stress) may compromise this protective role of the placenta and hence affect fetal development or programming.

Supported by GACR 20-13017S and EFSA-CDN (No. CZ.02.1.01/0.0/0.0/16_019/0000841).

L15
PHARMACOGENOMICS OF BREAST CANCER

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Breast cancer is the most frequent cancer in women globally. Despite progress in diagnostics and therapeutics over the last decades, inactivation of anticancer drugs by biotransformation enzymes, dysregulated uptake and efflux of drugs, changes in cell-cycle checkpoints, increased DNA repair or repressed cell death machinery and altered cellular compartmentalization may contribute to the development of tumor resistance to therapy. However, comprehensive germline genetic variability screen enabling prediction of chemoresistance is virtually missing. The aim of our study was to explore germline genetic variability of panel consisting of drug resistance relevant genes and evaluate their predictive and prognostic value in breast cancer patients. All exons with short overlaps into intronic sequences in both directions of 509 genes were sequenced using massive parallel sequencing in blood DNA from 105 breast cancer patients in the testing phase. Variants with minor allele frequency over 5% were further prioritized for validation phase using newly developed bioinformatics pipeline for functional predictions and associations with response to cytotoxic therapy or disease-free survival of patients. Variants prioritized in the testing phase were used for validation in 805 patients with clinical follow up using KASP™ technology. In total more than 18 thousands variants have been identified of which 2,565 were novel variants in the testing phase. Functionally relevant variants with major allele frequency over 0.05

and associations with response to cytotoxic therapy or disease-free survival of patients were further prioritized for validation phase. Out of 55 variants prioritized in the testing phase, associations of rs10868138, rs2227291, rs2293194, and rs4376673 located in *SLC28A3, ATP7A, KCNAB1*, and *DFFB* genes, respectively with response to neoadjuvant cytotoxic therapy and rs1801160 in *DPYD* with disease-free survival of patients adjuvantly treated with cytotoxic drugs were confirmed and should be further functionally characterized. In conclusion, germline variability within a panel consisting of drug resistance relevant genes was assessed by massive parallel sequencing in breast cancer patients of Czech origin for the first time and associations of several variants with prognosis or therapeutic response were replicated in large cohort of patients.

Supported by the grant 17-28470A from Czech Medical Council, project GAUK 1776218 from Charles University and The National Centre for Medical Genomics project no. CZ.02.1.01/0.0/0.0/16_013/0001634.

L16
THE EFFECTS OF PPAR GAMMA AND NRF2 ACTIVATION ACTING ON ADJUSTMENT OF HYPERTENSION

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PPAR gamma - peroxisome proliferator activated receptor (PPAR gamma) is a nuclear receptor that has potential parts in cell signaling and serves as a possible target for metabolic syndrome, cardiovascular diseases and hypertension. Activation of PPAR gamma, including agonists (pioglitazone-PIO, tideglusibe-TDG), is possible with the Nrf2 transcription factor of redox regulation producing antioxidant and detoxification output under oxidative stress. In an experimental study, we monitored the results of PPAR gamma agonists PIO and TDG on the development of hypertension, antioxidants Nrf2, and detoxifying effects in age-dependent BHR and SHR.

Blood pressure in the animals was detected by plethysmography. Cell signaling was monitored by gene expression, including qPCR, and NO-synthase and SOD activity were monitored by radioactive detection and UV VIS spectrophotometry.

In renal signaling detection, we found links between PPAR gamma and Nrf2 and antioxidant responses to superoxide dismutases (SOD1-3) responses. in young animals. Nrf2 also activated detoxification outputs (NQO1, HO-1), which improved in young hypertensive BHR. PIO treatment in adult BHR, SHR caused no detectable Nrf2 outputs. The main effects were observed in the adjustment of blood pressure and NO-synthases of young BHR and SHR hyperthensive animals. Blood pressure and NO-synthases and Nrf2 were also adjusted in adult SHR when TDG was administered.

From the experimental results it was found that PPAR agonists act to improve blood pressure through redox regulation though Nrf2 and NO - biological viability.

Grant projects: VEGA 2/0148/17, VEGA 2/0158/20, VEGA 2/0160/18, APVV-18-0548.

L17
ALCOHOL, DRUGS AND PSYCHOTROPIC MEDICATION AT WORK: GUIDELINES FOR MEDICAL FITNESS

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Alcohol, illicit drugs and psychotropic substances either stimulate or inhibit the central nervous system or cause hallucinogenic effects. Their use in the workplace is a relatively widespread but still insufficiently recognised phenomenon. The reliability of judgement and performance of workers especially undertaking safety-critical tasks must not be influenced by physical, mental and/or behavioral disturbances. It is the responsibility of the company to define and implement a proper policy for managing safety risks related to the influence of alcohol, drugs and/or psychotropic medication. Occupational physicians are already involved in the process of prevention in these matters. Authors describe basic recommendations of two biggest international associations of occupational physicians on this field (UEMS, European Union of Medical Specialists and UIMC, the International Union of Railway Medical Services), because workers may not perform safety-critical tasks under the influence of psychotropic substances such as alcohol, illicit drugs and psychotropic medication. It is recommended to systematically screen in the urine the following substances (cut-off values): Amphetamine (AMP) (500 ng/mL of d-Amphetamine), Cocaine (COC) (150 ng/mL of Benzoylecgonine), Cannabis (THC) (50 ng/mL of 11-nor-delta9-THC-9 COOH), Methamphetamine (MET) (500 ng/mL of d-Methamphetamine) and Morphine (MOP) (300 ng/mL of Morphine). It is recommended to respect zero alcohol tolerance because of individual susceptibility. The procedure for collection and detection of illicit drugs in the saliva in comparison with other collection methods is accepted as a more convenient procedure to the donor´s privacy. Confirmatory testing procedures using blood of tested persons should be provided exclusively by specialized laboratories of toxicology or forensic medicine.

L18
N,N-DIMETHYLFORMAMIDE ADDUCTS WITH BLOOD PROTEINS IN HUMAN VOLUNTEERS: EXCRETION OF CLEAVAGE PRODUCTS IN THE URINE

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The industrial solvent *N,N*-dimethylformamide (DMF) undergoes metabolism to a toxic reactive intermediate, *N*-methylisocyanate, that binds to blood protein globin to produce stable adducts *N*-methylcarbamoylvaline (MVU) and N_{ε}-methylcarbamoyllysine (MLU). Earlier we described in rats physiological cleavage of the above adducts which results in the excretion of free MVU and N_{α}-acetyl-MLU (MLU-Ac) in the urine. Here we studied formation and elimination of blood protein adducts and the respective urinary cleavage products following ingestion of 500 mg DMF in human volunteers (n=7). Multiple blood and spot urine samples were collected over 5 months for analyses of target compounds in globin (G), plasma (P), and urine (U). In the blood, toxicokinetics of MVU-G and MLU-G was very similar. Both adducts attained their maximum levels (17.5±3.7 nmol/g globin and 15.7±3.5 nmol/g globin, respectively) 7 days post-exposure and then depleted following a sub-linear kinetics for 130 - 140 days reflecting life span of human erythrocytes. MLU-P peaked 3 to 7 d post-exposure (3.9±0.9 nmol/ml plasma) but then followed an exponential decay reflecting half-life of plasmatic proteins (ca. 25 days). In the urine, MVU-U and MLU-Ac-U peaked 3 to 5 days post-exposure, and their maximal levels (21±6 μg/g creatinine and 145±50 μg/g creatinine, respectively) as well as shape of their elimination curves (MVU: almost stable level for ca. 100 days followed by a decline; MLU-Ac-U: exponential decay) differed significantly. This reflects that MVU-U is a globin-specific cleavage product whereas MLU-Ac-U comes from many different proteins containing MLU. Notably, unlike the smooth course of elimination of MVU-G and MLU-G, the values of MVU-U and MLU-Ac-U in some urines were distinctly deviated. This might indicate occurence of fluctuations in the rate of adduct removal that are sensitively detected by analysis of the short-term spot urine samples. The study was the first to characterize elimination kinetics of urinary cleavage products from blood protein adducts in humans. MVU-U and MLU-Ac-U are proposed as novel, non-invasively accessible biomarkers of cumulative exposure to DMF.

The study was funded by grant NV19-09-00378 and the institutional support DRO (National Institute of Public Health - NIPH, IN 75010330) from the Ministry of Health of the Czech Republic.

L19
LC-MS/MS DETERMINATION OF BZ AGENT IN BIOMATRICES

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Agent BZ (3-quinuclidinyl benzilate) is a centrally acting synthetic anticholinergic agent, considered as a potential military incapacitating chemical warfare agent. Despite its significance as a model compound in pharmacological research and the potential misuse in chemical attacks, no HPLC method for BZ determination in biological samples has been published. The goal of the presented work is to develop sensitive and rapid HPLC-MS/MS method for the agent BZ determination in rat plasma, urine, bile and selected organs to clarify its fate in the organism.

The sample preparation of biological fluids was based on the solid-phase extraction on C-18 cartridges, organs were homogenized a then processed by protein precipitation without extraction. The reversed-phase HPLC coupled with the mass spectrometer with electrospray ionization in the positive ion-selective reaction monitoring mode was employed in the BZ analysis. The atropine was used as an internal standard.

The presented method is selective, accurate, precise, linear (r^2 = 0.9947) in a concentration range from 0.5 ng/ml to 1 000 ng/mL and sensitive enough (lower limit of quantification 0.5 ng/mL) to determine the agent in rats injected with 2 mg/kg and 10 mg/kg of BZ. The toxicokinetic profile of the BZ agent in plasma, brain, liver, and kidney of laboratory rats was described. The BZ level reached the maximum values in plasma (204.5 ± 55.4 ng/ml and 2185.5 ± 465.4 ng/ml, resp.) at the time 3 minutes after the intramuscular injection. The measured BZ concentration in brain was higher (c_{max} = 301.4 ± 41.6 ng/g and 2684.7 ± 396.6 ng/g) and more stable, suggesting good ability of the agent to penetrate the blood-brain barrier, accumulation in brain tissue and quite slow elimination. The agent was partially excreted in an unchanged form by urine, a biliary excretion played only a minority role.

Supported by the Ministry of Defense of the Czech Republic, Long-term organization development plan 1011.

L20
BENEFITS AND RISKS OF CUCURBITURIL TREATMENT OF ORGANOPHOSPHATE POISONING

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Cucurbit[n]urils (CB[n]) are macrocycles formed by condensation of glycoluril and formaldehyde. From the

CB[n] family, the cucurbit[7]uril (CB[7]) binds a variety of positively charged aromatic compounds, certain metal complexes, and platinum-based drugs with organic ligands. This study aimed to evaluate the benefits and risks of CB[7] after a single-dose administration and its efficacy against sarin and paraoxon poisoning when administered with atropine and oximes. Additionally, toxicity and accumulation of CB[7] after repeated administered were tested.

In all experiments, Balb/c mice were used. Firstly, mice were administered with different doses of CB[7] to determine maximally tolerated doses (MTD) after *p.o.*, *i.m.* and *i.p.* administration. At MTD, plasma and kidney concentration profiles were measured. Secondly, we determined plasma and brain concentration profiles of HI-6 (5% LD50) and HI-6@CB[7] (at equimolar dose, both *i.m.*). In sarin experiments, mice were exposed to 1 LD_{50} and treated either with atropine, atropine and HI-6 or atropine, HI-6 and CB[7] (all *i.m.*). After paraoxon intoxication (1 LD_{50}), mice were treated either with atropine, atropine and K027, atropine and CB[7], or atropine, K027 and CB[7] (all *i.m.*). In both experiments, we evaluated acetylcholinesterase activity 60 min after the poisoning and functional observatory battery (FOB) at 2 and 24 h. Finally, CB[7] was administered at 50% MTD (*i.p.*) once daily for 7 days. Accumulation of CB[7] and liver and kidney damage were assessed.

MTDs were determined at 500, 150 and 100 mg/kg after *p.o.*, *i.m.* and *i.p.* administration, respectively. After *i.p.* and *i.m.* administration, CB[7] accumulated in kidneys, whereas it showed limited absorption from the gastrointestinal tract. When administered equimolarly with HI-6, CB[7] changes plasma and brain concentration profiles of HI-6. After sarin and paraoxon poisoning, CB[7] increases acetylcholinesterase activity in the brain. In blood, it decreases acetylcholinesterase activity in mice treated with HI-6. FOB corresponded with acetylcholinesterase activity measurements. Results of the repeated administration of CB[7] will be presented at the conference.

In conclusion, single-dose administration of CB[7] seems promising in the treatment of organophosphate poisoning.

L21
SOFTWARE FOR BIOLOGICAL EFFECTS PREDICTION

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The aim of this work is to create an algorithm and software solution for in silico prediction of possible biological effects of newly developed or discovered chemical substances. The method is based on computational predictions of interactions between biomacromolecules (receptores) and small molecules (ligands). The designed software uses molecular docking in two separated steps. Nowadays, it uses the database scPDB including more than 16,000 protein targets. In the first step, the set of ligands (about 10 to 100 small molecules) is tested over the whole database of receptores by semi-flexible molecular docking and about 100 potential targets are pre-selected. The ligands and their decoys are docked into the pre-selected targets by the semi-flexible molecular docking in the second phase. The gained docking scores of ligands are benchmarked with the scores of decoys. The result is a list of probable biological targets for the set of small molecules and a score assessing the targets. The benefits of this method are quickness, cost-effective and safety compared to classical biochemical approaches. We can test compounds before their synthesis or isolation. It is useful in the field of toxicology as well as of drug development. We are going to provide the software solution to huge public.

The work was supported by the Ministry of Education, Youth and Sports of the Czech Republic (project SV/FVZ202005).

L22
A COMPARISON OF THE REACTIVATING, THERAPEUTIC AND NEUROPROTECTIVE EFFICACY OF A NEWLY DEVELOPED OXIME K870 WITH PRALIDOXIME AND THE OXIME HI-6 IN TABUN-POISONED RATS AND MICE

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The ability of one original chlorinated bispyridinium oxime K870 and two currently available oximes (pralidoxime, HI-6) to reactivate tabun-inhibited acetylcholinesterase and reduce acute toxicity of tabun including neurotoxic signs and symptoms was evaluated in tabun-poisoned rats and mice. The oxime-induced reactivation of tabun-inhibited acetylcholinesterase was measured in whole blood, diaphragm and brain of tabun-poisoned rats. The results showed that the reactivating efficacy of recently developed oxime K870 was significantly higher than pralidoxime and the oxime HI-6 in the whole blood and slightly higher than pralidoxime in diaphragm. On the other hand, the reactivating efficacy of all oximes studied in the brain was negligible. The therapeutic efficacy of all oximes studied roughly corresponds to their reactivating efficacy. While the oxime K870 and pralidoxime were able to reduce acute toxicity of tabun less than 1.6 fold, the oxime HI-6 reduced acute toxicity of tabun more than 1.9 fold. Thus, the differences between therapeutic efficacy of all oximes studied were not significant. All oximes studied combined with atropine were able to markedly decrease tabun-induced neurotoxicity in the case of sublethal poisoning although they did not eliminate all tabun-induced acute neurotoxic signs and symptoms. The ability of K870 to decrease tabun-induced acute neurotoxicity roughly corresponds to the oxime HI-6 and its neuroprotective effect was slightly higher compared to pralidoxime. In

conclusion, the reactivating, therapeutic and neuroprotective efficacy of a newly developed oxime K870 does not markedly prevail the effectiveness of the oxime HI-6 and pralidoxime and, therefore, it is not suitable for its replacement of commonly used oximes for the antidotal treatment of acute tabun poisoning.

Supported by the grant of Ministry of Defence – „Long-term organization development plan – Medical Aspects of Weapons of Mass Destruction".

L23
GANODERMA LUCIDUM INDUCES OXIDATIVE DNA DAMAGE AND ENHANCES THE EFFECT OF 5-FLUOROURACIL IN COLORECTAL CANCER *IN VITRO* AND *IN VIVO*

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The first-line chemotherapy of colorectal cancer (CRC), besides surgery, comprises administration of 5-Fluorouracil (5FU). Apart from cytotoxic effect on cancer cells, 5FU may also cause adverse side effects. *Ganoderma Lucidum* (GLC) is a mushroom used in Traditional Eastern Medicine. We propose that natural compounds, particularly GLC extracts, may sensitize cancer cells to conventional chemotherapeutics. This combination therapy could lead to more selective cancer cell-death and may improve the response to the therapy and diminish the adverse effects of anticancer drugs.

In present study we demonstrate that GLC induced oxidative DNA damage selectively in colorectal cancer cell lines ($p<0.05$), whereas it protected non-malignant cells from the accumulation of reactive oxygen species. Accumulation of DNA damage caused sensitization of cancer cells to 5FU resulting in improved anticancer effect of 5FU. The results obtained in colorectal cell lines were confirmed in *in vivo* study: GLC co-treatment with 5FU increased the survival of treated mice and reduced the tumor volume in comparison with group treated with 5FU alone ($p<0.05$).

Combination of conventional chemotherapeutics and natural compounds is a promising approach, which may reduce the effective curative dose of anticancer drugs, suppress their adverse effects and ultimately lead to better quality of life of CRC patients.

L24
THE THERAPEUTIC EFFECTS OF *AGRIMONIA EUPATORIA* L.

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Common agrimony (*Agrimonia eupatoria* L.) is an herb with time-tested beneficial effects used in folk medicine. Its species name is said to come from King Mithridates Eupator VI of Pontus (132–63 BC) who was reportedly the first to use this herb to treat liver problems. Agrimony´s therapeutic effects were praised by Pallas Athena. Pliny, Galen, Avicenna and the plant was also mentioned in the Old English Herbarium dating back to the 10th century. Its aqueous solutions (infusions and decoctions) are used to treat lung inflammation, intestinal and bladder atony, bleeding disorders, liver diseases and skin defects. Agrimony was listed in the Czechoslovak and German pharmacopoeias. In its documents, the European Medicine Agency (EMA) refers to agrimony as a plant with time-tested use and effects not yet verified by clinical studies. Agrimony is believed to have antimicrobial action and improve digestion.

Phytochemical analyses have identified a variety of bioactive compounds including tannins, flavonoids, phenolic acids, triterpenoids and volatile oils exerting antioxidant, immunomodulatory and antimicrobial effects.

The authors have reviewed the available literature sources examining and explaining the therapeutic and pharmacological effects of *Agrimonia eupatoria* L. at the molecular level *in vitro* and *in vivo* and present results of their research focused on the effect of infusions and decoctions on keratinocytes.

L25
BIOLOGICAL PROPERTIES OF NOVEL PHOTOACTIVABLE BODIPY-LABELLED COLCHICINE DERIVATIVES

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Cancer is one of the greatest challenges of the modern medicine. Although a big effort has been made in development of novel therapeutics, it still remains one of the

most common causes of death in the developed world. Modern cancer research focuses on deeply targeted therapy seeking for minimization of side effects, which are often caused by commonly used chemotherapeutics. Photodynamic therapy (PDT) belongs to such targeted therapies. It uses three separately non-toxic components: a photosensitizer (PS), light, and molecular oxygen. PS is able to generate radicals after light irradiation. Then, these radicals react with the molecular oxygen resulting in accumulation of reactive oxygen species, which cause cellular damage. Cytotoxicity occurs only on the illuminated site of a tissue eliminating undesired side effects. The aim of this work was to investigate biological properties of four newly synthesised colchicine derivatives labelled with fluorescent BODIPY molecules. Colchicine is a natural microtubule binding agent with anticancer properties. Two derivatives were iodinated, which makes these compounds photoactive and, therefore, well applicable in PDT. In addition, fluorescent properties can be used for diagnostic imaging of tumours. Biological properties were evaluated using three cancer cell lines derived from breast carcinoma (MCF-7), lung carcinoma (A549), and osteosarcoma (U2-OS), also one primary cell line of lung fibroblasts (MRC-5) was used for comparison. Using fluorescent markers of cellular compartments, we confirmed localization of the colchicine derivatives in the endoplasmic reticulum of cancer cells. Cytotoxicity and phototoxicity were determined by WST-1 agent. The most toxic compound was colchicine with iodo-BODIPY bound on the cycloheptane ring, while the second iodine derivative was significantly selective for cancer cells. Additionally, effects of derivatives on cell cycle and mechanism of cell death have been investigated. Further research on the iodine-BODIPY derivatives of colchicine is desirable, according to their great toxicity and selectivity for cancer cells, which, together with their photoactive properties, makes them potential cancer therapeutic candidates.

The authors gratefully acknowledge the financial support of this work by "P" 2020/00262, Martina Roeselová Foundation, Czech Republic and by L'Oréal-UNESCO for Women in Science 2019.

L26
CYANOBACTERIAL WATER BLOOM TOXINS ACTIVATE PRO-INFLAMMATORY EFFECTS IN GASTROINTESTINAL TRACT AND INNATE IMMUNE CELLS

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Occurrence of cyanobacterial water blooms (cyanoWB) is increasing worldwide and represents an important environmental issue and also human health hazard. CyanoWB can produce high amounts of diverse toxins. CyanoWB can produce high amounts of diverse toxins, which are mostly secondary metabolites (*e.g.* microcystin LR (MC), cylindrospermopsin (CYN), puwainaphycin F (PUW), and minutissamides (MIN)). Also, cyanobacterial lipopolysaccharide (cyanoLPS), a major component of the cellular wall, is present in higher quantities and released into the water during cyanoWB. Despite the fact, that gastrointestinal tract (GIT) and the mucosal innate immune system are one of the major targets of the exposure to contaminated water, little attention has been paid to study effects of cyanotoxins on GIT and immune cells. To describe effects of MC, CYN, and some cyanoLPS effects on innate immune cells, mouse macrophage cell line RAW 264.7 was used. Our results brought the novel insight into the disruptive mechanism of cyanobacterial toxins and indicate that MC may activate the immune cells via Toll-like receptor 4. Other toxin, CYN, potentiates the effect of cyanoLPS indicating potential increase of the overall toxicity of environmental mixtures. CyanoLPS vary in their structures and also composition in environmental samples, nevertheless our results show significant pro-inflammatory effects of some samples isolated from water bloom as well as from some pure cyanobacterial strains. Moreover, the pro-inflammatory effects were proved also using polymorphonuclear leukocytes isolated from human peripheral blood. To study effects on human intestinal epithelium, differentiated Caco-2 cells were used as an *in vitro* system. Recently, pro-inflammatory effects of PUW and MIN were shown by our group. They induced production of interleukin 8 in non-toxic concentrations and changed expression of tight junction proteins. Similarly, pro-inflammatory effects of cyanoLPS on intestinal Caco-2 and HT-29 cells were observed. Taken together, different types of cyanobacterial toxins activate innate immune cells and also intestinal epithelial cells *in vitro*. In environmental mixtures, their effects can be mutually reinforcing and influencing.

Supported by the Czech Science Foundation project No. GA19-09980S.

L27
THE IMPACT OF TRIAZOLE FUNGICIDES ON NON-TARGET SPECIES

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Triazoles (*e.g.*, tebuconazole, penconazole, propiconazole and cyproconazole) are frequently used agrochemicals against fungal pathogens. Primarily they were designed to block 14alpha-demethylase of fungal pathogens. However, they affect also aromatase and possibly other

enzymes of the broad family of cytochrome P450 enzymes of non-target organisms in soil as well as plants. The effect of triazolic fungicide on the environmental system in the meaning of the soil-plant system is not well understood. As was found previously by our group, the interactions in cocktails with other biologically active chemicals are highly expected. We have to consider also the impact of triazoles in their mixtures because of their joint application or consecutively we have to consider also the group effects of triazoles. This contribution summarized almost 10-years research of triazoles and their impact on the environmental and biochemical systems.

J. Jaklová Dytrtová thanks to the Czech Science Foundation (project no. 18#01710S) for the financial support.

L28
THE SOURCES OF PER- AND POLYFLUORINATED COMPOUNDS IN THE ENVIRONMENT AND POTENTIAL HUMAN EXPOSURE PATHWAYS: 3 CASE STUDIES FROM THE CZECH REPUBLIC

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Ubiquitous contamination of the environment by per- and polyfluoroalkyl substances (PFAS) increases the risks for exposed organisms, including humans. Despite the persistence, bioaccumulation, and increasing knowledge about their adverse effects, PFAS environmental concentrations, fate, and potential pathways of human exposure remain largely unknown. The aforementioned aspects were intensively investigated during 3 case studies in the Czech Republic. Using newly developed methods for PFAS analysis in different environmental matrices, the contamination of fish, wastewater sludge, and drinking water was determined to estimate the human intake of PFAS. First of all, a large screening of PFAS in sludges originating from wastewater treatment plants revealed high PFAS contamination prior to their application in agriculture. Moreover, the results of a sludge screening together with the bioconcentration factors of PFAS in different vegetables were used to evaluate the real risk of human exposure through agricultural products fertilized by sludge. Similar results were obtained by monitoring fish species in two larges rivers in the Czech Republic, where the increasing level of PFAS in fish in the catchment area of the Elbe River reflected the contamination of surface water by anthropogenic processes. Moreover, the relationship found between the trophic position and the level of contamination could help to minimalize potential risks connected to fish consumption. Finally, the findings of several representatives of PFAS in drinking water from different regions of the Czech Republic demonstrate and confirm the widespread contamination and direct human exposure.

The work was supported by the Ministry of Agriculture of the Czech Republic [grant number QK1910095] and by the Center for Geosphere Dynamics (UNCE/SCI/006).

L29
PHARMACEUTICALS IN THE ENVIRONMENT – POTENTIAL CONTAMINANTS OF FOOD CHAINS

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Pharmaceuticals are monitored as environmental contaminants in the last few decades. Over 3000 pharmacologically active substances such as analgesics, antibiotics, antidiabetics, contraceptives, antidepressants, lipid-regulators, beta-blockers are used in human and veterinary medicine, in the European Union. Drugs and their metabolites are preferably excreted from patients via urine and contaminate municipal wastewaters. Wastewater treatment plants are only able to remove this pollution to a limited extent and residues of drugs contaminate the environment. The surface waters are influenced primarily, but the subsequent penetration of contaminants into the terrestrial ecosystem can affect all living organisms. The concentrations of drugs in the environment are much lower than therapeutic and acute toxicity data are not relevant to the evaluation of effects. Unfortunately, the chronic toxicity potential subtle effects are only marginally known. The uptake of drugs and their metabolites by plants can cause food chain contamination. Plants can bind drug contaminants from water and soil as it was recently demonstrated. Consequently, phytoremediation methods are widely used as an attractive approach for environmental decontamination. However, plants can be simultaneously contaminated by drug residues or by their metabolites. Therefore, the interactions of plants with drugs assessing possible risks of food chain contamination preventing potential health effects represent an important field of modern research.

The model experiments of plant drug uptake were performed using *in vitro* cultivated whole-plants. The cultivation medium was supplemented with defined amount of tested drugs from various pharmacological groups (*e.g.* ibuprofen, diclofenac, bromazepam, fluoxetine, carbamazepine, trazodone). The uptake ability, translocation, and extractable residues were evaluated using HPLC/UV analyses. We found that all tested plants (Zea mays, Helianthus annuus, Pisum sativum, Hordeum vulgare) can extract drugs from the liquid medium and at least partially translocate them into aboveground parts. The extraction efficiency depends on the chemical structure of the drug and is usually 30-90%. The amount of extractable residues reaches concentrations μg/g of plant fresh weight.

L30
THE NOVEL MATHEMATICAL MODEL FOR ASSESSING AGONISTIC AND ANTAGONISTIC PROPERTIES OF CHEMICAL MIXTURES

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People are exposed permanently to various chemical compounds. Some of them are administered willingly as drugs or other personal products and some are administered via environmental exposures. Evaluation and prediction of toxic effects of these mixtures is a tough challenge, especially for the compounds that are partial agonists and have both agonistic and antagonistic effects in the assay. Classical Concentration addition (CA) concept cannot handle these problems with partial agonists in the mixtures and in this way to calculate correct toxic outcomes. We created a new mathematical model which can evaluate and predict additive effects of two or more compounds. This model can utilize the partial agonists in the mixtures and their antagonistic effect and as a result provide dose-response curves of mixtures that are also partial agonistic. Our model uses only the parameters from the individual logistic curves of the compounds as the initial values and predicts the proper mixture effect in cases where other models predict false synergism or antagonism. Furthermore, this novel mathematical model enhances our understanding of the antagonistic effect of compounds and extends mathematical equations for Schild and Cheng-Prusoff methods for competitive antagonist characterization. The application of these methods often leads to different results for the same compound. However, using our mathematical approach with extended Gaddum equation, both methods yield similar values of the equilibrium dissociation constants of the competitive antagonists. This is highly relevant not only for environmental contaminants but also for many therapeutic drugs because they usually act as a competitive antagonist for a respective biological receptor. In conclusion, our novel mathematical model provides more accurate predictions for overall mixture effects and enhances our understanding of the theory of receptor-ligand interactions, which is essential for the planning, execution, analysis, and interpretation of toxicology experiments.

Supported by the grant 20-14318Y from Grant Agency of the Czech Republic.

L31
TOXICITY AND ECOTOXICITY OF PER- AND POLYFLUOROALKYL SUBSTANCES

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Per- and polyfluoroalkyl substances (PFAS) represent a group of anthropogenic chemicals that includes perfluorooctanonic acid (PFOA), perfluorooctanesulfonic acid (PFOS) hexafluoropropylene oxide dimer acid (HFPO-DA, GenX), and numerous other chemical individuals (approx. 4,700). PFAS have been produced and employed in various applications from the 1940s. PFOA and PFOS have been the most extensively produced as well as studied representatives of these chemicals. Generally, PFAS are very persistent chemical compounds and the due to the properties of carbon-fluorine bond, there is no natural degradation mechanism biotic, nor abiotic. Moreover, PFAS accumulate in biota and their bioaccumulation and biomagnification properties have been also documented. There are strong evidences of the toxic effects of PFAS and exposure to PFAS can lead to adverse human health effects. The main and well described effects are hypercholesterolemia, ulcerative colitis, thyroid disease, cancer, and pregnancy-induced hypertension and pre-eclampsia. The compounds (mainly (PFOS and PFOA) are hepatotoxic, nephrotoxic, neurotoxic, carcinogenic and cytotoxic. Moreover, various adverse effects were also observed toward population of aquatic organism, and even plants. Despite PFOS and PFOA have been involved among Persistent Organic Pollutants according to the Stockholm conventions, there are replacements as GenX that are suspected to cause similar health problems due to similar toxic mechanisms. This contribution will provide an overview of known toxic mode of actions of PFAS representatives as well as an insight into molecular level mechanism of the toxic effects and other aspects related to the properties of PFAS.

L32
RECENT DEVELOPMENTS IN SCREENING OF THYROID DISRUPTION WITH THE USE OF *IN VITRO* TOXICOLOGICAL METHODS

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Toxicological research has been focused primarily on disruption of steroid hormone signaling. Most recently, the greatest attention is paid to thyroid disruption. Steroid and thyroid signaling represent complex systems of cooperating interactions. Naturally crossing signal pathways and the influence of internal and external factors are involved, affecting the disposition to the development of systemic adverse effects, caused not only by a sole chemical, but additionally by unique chemical mixtures including active metabolites. Medical preparations or chemicals of synthetic or natural origin may act by various mechanisms, *e.g.* through regulatory

receptors, metabolic systems (enzymes, cofactors), ion or transport channels, regulatory proteins, *etc.* Multivariable and overlapping influences of chemicals not only on the endocrine system, but also involving the immune and Phase I and Phase II biotransformation systems, call for embracing integration of new approach methods. With regard to the diversity of multiple functions, real-life exposure variability and individual adaptive mechanisms, it is problematic to identify the causation with a specific apical endpoint or response *in vivo*. Non-animal models suitable for identification of cross-linked mechanisms at cellular, molecular, and epigenetic levels would represent a valuable contribution to this complex issue. Combined toxicological approaches, which include *in silico* and *in vitro* bioassays and chemical analyses, seem to be promising and helpful in identification of molecular initiating events and key events sequenced within the concept of adverse outcome pathways in order to describe the mode of action. EURL ECVAM, which is an integral part of the Joint Research Centre (JRC), the science and knowledge service of the European Commission, is coordinating a validation study of *in vitro* methods to detect thyroid disruptors. This work aims to explain the physiological connections in thyroid disruption and to present the most recent improvements of testing strategies based on recently published *in vitro* data. Pilot experimental data from the development of one *in vitro* method focused on detection of thyroid disruptors potentially interacting with the human TSH receptor will be presented.

The study was supported by the ERDF/ESF project „International competitiveness of NIPH in research, development and education in alternative toxicological methods" (No. CZ.02.1.01/0.0/0.0/16_019/0000860) and coordinated by EURL-ECVAM.

L33
AIR AND DUST INDOOR SAMPLES – ENDOCRINE DISRUPTIVE POTENTIAL OF ORGANIC POLLUTANT MIXTURES ASSESSED BY *IN VITRO* BIOASSAYS AND EFFECT MODELING

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The indoor environment where people spend most of their time contains complex mixtures of organic chemicals that could affect their endocrine system. Their effect is hypothesized to be connected with diverse adverse effects such as impaired neurodevelopment or carcinogenesis. To describe the potential effects of these mixtures, we have collected gaseous and particulate phases of indoor air and the dust from several different environments such as offices, lecture rooms and houses. The sample extracts were assessed for endocrine disruptive potential with a set of *in vitro* bioassays addressing the anti-/estrogenicity, anti-/androgenicity, aryl hydrocarbon receptor-, and thyroid receptor-mediated activities. The bioassays were based on human cell lines with a reporter gene under transcription control by a receptor of interest. The dust sample extracts were analyzed for 177 chemicals and their levels were used for modeling of their contribution to the assessed biological effects. The significant toxic potentials were detected for all assessed endpoints with patterns differing among the studied matrices. The differences indicated the differences in the pollutant mixture composition. Chemical analyses confirmed relatively high levels of phthalates, their emerging alternatives and PAHs in the dust samples. However, despite the large number of analyzed chemicals, the explicability of effects by levels of detected chemicals was rather negligible for most of the activities. Only in the case of estrogenicity in two from three house dust samples, phthalate levels explained 16 and 12% of the effect. This could be explained either by the lack of reliable toxicity characteristics for the analyzed chemicals or the analyzed chemicals did not cover the main toxicity effect drivers. Anyway, the bioassays have confirmed that the indoor samples contain chemicals with endocrine disruptive potential that could have adverse effects on human health.

Supported by the grant 19-20479S from Grant Agency of the Czech Republic.

L34
CELLULAR STRESS RESPONSES AS NOVEL *IN VITRO* TOXICITY MARKERS OF SELECTED POLYCYCLIC AROMATIC HYDROCARBONS

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We established a novel complex *in vitro* system for testing of acute toxicity responses induced by various xenobiotics. The system is based on qRT-PCR analysis of expression profiles of genes that are involved in cellular stress responses, including: early stress response; heat shock protein (HSP) response; DNA damage response; unfolded protein response (UPR; related to endoplasmic reticulum (ER) stress); and immunotoxicity. The cellular stress markers represent integral parameters affected by various intracellular pathways and they are complementary to *e.g.* luciferase reporter gene (CALUX) assays detecting specific nuclear receptor activationUbiquitous environmental pollutants, polycyclic aromatic hydrocarbons (PAHs) represent a diverse group of organic substances exerting different types of toxicities, such as activation of the aryl hydrocarbon receptor (AhR) and/or genotoxicity. In this study, we screened cellular stress markers, in order to detect non-conventional modes of action of PAHs, and compare them with their other well-recognized toxic effects. Six individual PAHs with unique characteristics were selected for the study: fluoranthene (Fla), pyrene (Pyr), chrysene (Chry), benzo[a]anthracene (BaA), benzo[a]pyrene (BaP), and benzo[k]fluoranthene (BkF). Differentiated human liver HepaRG cells were exposed to the selected PAHs for 24 h, and changes of mRNA levels of genes involved in early stress

(EGR1, ATF3, GDF15), DNA damage (CDKN1A), HSP response (HSP70), ER stress (HSPA, DDIT3) and immunotoxicity (interleukin 8) were detected. The results were analysed with respect to the effects of model substances, *e.g.* thapsigargin, ER-stress inducer, or lipopolysaccharide. We found that CDKN1A and early stress genes ATF3 and EGR1 were induced by BaP and BkF. Moreover, GDF15 mRNA was increased by Chry, BaA, BaP and BkF. Expression of other cellular stress markers was not affected by carcinogenic PAHs. Low-molecular-weight PAHs, Fla and Pyr, did not induce cellular stress markers.

Determination of stress markers in the cells exposed to xenobiotics such as environmental contaminants or their mixtures is a novel approach suitable for *in vitro* toxicity profiling. The system represents a sensitive first-line set of parameters suitable for identification of *in vitro* toxicity, complementing the general cytotoxicity assays.

This project was supported by the Czech Ministry of Education, Youth and Sports OPVVV PO1 project "FIT" (Pharmacology, Immunotherapy, nanoToxicology) no. CZ.02.1.01/0.0/0.0/15_003/0000495.

L35
MODULATIONS OF SPHINGOLIPID METABOLISM AND GENE EXPRESSION IN UNDIFFERENTIATED AND DIFFERENTIATED HEPARG CELLS EXPOSED TO TCDD

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Sphingolipids are bioactive lipids, important structural components of cellular membranes, lipid rafts and also significant modulators of intracellular signal pathways. Their crucial role consist in cell survival, signal transduction and cell to cell communications. However, modulation of sphingolipid metabolism, especially by organic xenobiotics, is still poorly understood. In this study, we used differentiated and undifferentiated HepaRG cell line, as a model of human-liver progenitor cells and exposed them to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). We had determined possible impact of this compound on sphingolipid metabolism and on expression of genes linked to sphingolipid metabolism. TCDD is persistent enviromental pollutant and human carcinogen which toxicity is induced via activation of aryl hydrocarbon receptor (AhR). HepaRG cells exposed to 1nM or 10nM TCDD showed increased production of glycosphingolipids (especially glucoceramide) and dihydroceramides (undifferentiated cells) in comparison to the untreated control group. Gene expresion analysis showed deregulated mRNA expresions of specific genes of sphingolipid metabolism. For example, upregulated expresion of UGCG gene coincided with observed enhancement of glucoceramide synthesis.

L36
PROMEGA SOLUTIONS FOR CYTOTOXICITY MEASUREMENT AND ADME-TOX ASSAYS

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There are many strategies to measure cytotoxic effects of compounds on cells. I will present an overview of Promega cell viability and cytotoxicity assays based on luminescence readout that test several cellular parameters, such as ATP content and membrane integrity. These assays are designed for multi-well plate format with easy "add-mix-measure" protocols. Luminescence detection modality shows excellent sensitivity, low background signal and a wide dynamic range, thus these assays are suitable for experiments in low volumes of precious samples and for screening applications. Additionally, assays relevant to ADME screening of drug candidates will be introduced. These luminescence-based assays measure the activities of different variants of cytochrome P450, p-glycoprotein and monoamine oxidase, and total glutathione as an indicator of oxidative stress. This talk will also summarize key factors to consider when optimizing luminescence cell-based assays, so that you collect high quality, reproducible data.

Molecular spectroscopy

FT-IR spectrometer Nicolet iS50 (Thermo Scientific) and Raman spectrometers (BWTek)

Nicolet CZ s.r.o. supplies Raman and FT-IR spectrometers and microscopes from Thermo Scientific, portable and hand-held Raman dispersive, UV-VIS-NIR and LIBS spectrometers from BWTek, scientific Raman spectrometers from S&I, scientific infrared SNOM microscopes from Neaspec, and their accessories. We also offer custom-made analytical methods, instrument servicing, individual and group trainings, and each year we organize several Raman and Infrared spectroscopy courses, some of which are held in collaboration with Ioannes Marcus Marci Spectroscopic Society.

IR-SNOM (Neaspec), Raman microscope DXR3xi and FT-IR spektrometer Summit (Thermo Scientific)

Molecular spectroscopy methods are suitable for the analysis of samples of all forms and states: from intermediate products of industrial production, biological samples, food and its components, up to drugs and toxins and many others. FT-IR and Raman spectroscopy also offer the possibility to perform time-resolved measurements. It's an elegant solution in combination with other analytical methods such as MS, TGA or GC etc.

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P001
DIFFERENTIAL EFFECT OF METFORMIN ON BILE FORMATION IN HEALTHY AND CHOLESTATIC MICE

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Metformin is widely used oral antidiabetic drug that is also considered in therapy of gestation diabetes in pregnancy. However, pregnancy as well as metformin treatment may induce intrahepatic cholestasis (IC) and liver injury. The present study therefore aims at evaluation of metformin's effects on bile flow, bile acid (BA) biochemistry and corresponding molecular pathways using mouse model of IC. The cholestasis was induced by repeated administration of ethinylestradiol (EE, 10 mg/kg BW s.c.) followed by metformin (150 mg/kg BW orally) over 5 consecutive days. Metformin significantly increased rate of bile flow in control animals by increasing biliary secretion of BA via induced expression of Bsep ($p<0.05$), the rate limiting transporter for biliary secretion of BA. The fecal excretion of BA, and their plasma concentrations were unchanged by metformin when compared with untreated control group. Ethinylestradiol produced expected cholestasis by significant downregulation of BA transporting proteins, especially Bsep, and Ntcp, major uptake transporter for BA in hepatocytes. Metformin further significantly worsened EE-induced impairment of biliary BA secretion and related bile formation via downregulation of Bsep, and Ntcp. The plasma concentrations of BA were significantly ($p<0.01$) increased in metformin treated EE group. Together, our data indicate positive reinforcement of bile secretion by metformin in intact liver, but this drug may have deleterious on BA biliary secretion in estrogen-induced cholestasis.

The project was supported by grants Progres Q40/05, SVV 260397/2017, GAUK 5562/18, GACR 19-14497S, and by the ERDF-Project PERSONMED No. CZ.02.1.01/0.0/0.0/16_048/0007441.

P002
SEMISYNTHETIC PRENYLFLAVONOIDS, THEIR EFFECT ON CANCER CELL LINES *IN VITRO*

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Flavonoids are secondary plant metabolites with broad range of biological activities, which include antioxidant effect, modulation of various enzymatic activities and inhibition of cell proliferation. Main limitation of the use of natural flavonoids is their low biological availability and rapid elimination from the organism. Modification of a flavonoid backbone by attaching prenyl group(s) enhances the biological activities of prenylflavonoids and tissue bioaccumulation compared to their unprenylated counterparts. In this study, anticancer effects of four semisynthetic prenylated derivatives of flavanone naringenin were studied. Based on the performed bioinformatic analysis, the oestrogen receptors (ER) α and β and enzymes participating in cell cycle regulation were identified as potential targets of tested prenylflavonoids. Therefore, ER-positive MCF-7 and ER-negative MDA-MB-231 breast cancer cell lines were chosen as suitable model systems. The ER-positive MCF-7 cells were, compared to ER-negative MDA-MB-231 cells, more sensitive to the antiproliferative effect of tested compounds. All the tested prenylflavonoids exerted stronger antiproliferative effect compared to the unprenylated counterpart naringenin. Possibility of ER involvement in the antiproliferative activity of tested prenylflavonoids will be further studied. Shifts in the cell cycle induced by prenylflavonoids were analysed using flow cytometry in propidium iodide stained cells. However, no significant changes in the cell cycle, which would be induced by prenylflavonoids, were detected. Further, quantitation of apoptotic and necrotic cells using flow cytometry was performed. Tested compounds showed the ability to affect viability and proliferation of breast cancer cells, however, further study revealing the mechanism of their action is necessary.

Supported by the Charles University (Research Project SVV 260 550).

P003
NOVEL DERIVATIVES OF CARDIAC GLYCOSIDES WITH IMPROVED CANCER CELL SELECTIVITY

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Cardiac glycosides (CGs) are well established therapeutics for treatment of cardiac insufficiencies and arrhythmias. In the last few years, CGs have been subjected to drug repositioning due to their anticancer potential. However, because of their side toxicity, their usage has been limited, so far. This issue could be circumvented by increasing the selectivity towards cancer cells. Possible approach is to conjugate CGs with compounds, for which increased accumulation in tumors has already been described. In addition, their selectivity can be further augmented with pH-sensitive conjugation, after which the resulting derivative is not able to bind to its molecular target (Na^+/K^+-ATPase) at physiological pH due to steric hindrance. Based on these strategies, the goal of this work was to design CG derivatives with potentially enhanced accumulation of active

compounds in various types of solid tumors and to use *in silico* methods (molecular docking and molecular dynamics) to study behavior of CG derivatives with their molecular targets. By molecular docking, we confirmed that the designed CG derivatives were not able to bind to Na^+/K^+-ATPase. In the next part of our study, we will investigate the behavior of complexes by molecular dynamics and we will also evaluate cytotoxicity of the designed derivatives in various tumor cells to deepen the understanding of their biological properties. We believe that structural modification of CGs will lead to their enhancement of selectivity towards cancer cells and that they will have a potential to be used in a cancer therapy.

This work was supported from the grant of Specific university research A2_FPBT_2020_015.

P004
INHIBITORY EFFECTS OF PARACETAMOL ON PLANT GERMINATION

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Pharmaceuticals enter the environment mainly through wastewater from both medical facilities and municipal waste. The use of wastewater to irrigate crops and sewage sludge as a source of organic matter, essential nutrients and trace elements increases the risk of bioavailability of drugs for terrestrial plants. Paracetamol (PAR) is a widely used non-steroidal anti-inflammatory drug. The aim of this work was to monitor the inhibitory effects of PAR on the germination of barley kernels (*Hordeum vulgare*). The endpoints monitored were: 72hIC50 (concentration at which germination changes reaches 50% of the control), *germinatory energy* – GE (%), *germination rate* – GR (%), *mean germination time* – (MGT) and *germination index* – (GI). *H. vulgare* was incubated in boxes of 10 x 5 kernels (n=200) on cellulose wadding moistened with tap water at 20±2 °C. Prior to use in the test, the kernels were sterilized in 5% sodium hypochlorite solution and then washed in sterile water. Concentrations (0, 250, 500, 1 000, 2 000, 4 000, 6 000, 8 000, 10 000, 12 000, 14 000 µM) were chosen to determine the inhibitory effects (72hIC50) of PAR.

The inhibitory effects of PAR were evaluated based on germination of kernels. The value of the PAR concentration causing a 50% reduction in germination (72hIC50) was determined to be 8070 µM by probit analysis (CI 7183-8957); r 0.9975. The germination intensity, based on the GR parameter, was most affected by the concentration range from 8000 to 14000 µM, when the germination intensity was by 85.8% lower than in the control. As with the GR, another monitored parameter (MGT) was most affected by the highest concentrations. Barley kernels exposed to PAR at concentrations of 8 000–14 000 µM germinated by 13% longer than the control kernels. The MGT in concentrations (250–6 000 µM) was only by 2% longer than in the control. The GE was most affected by concentrations of 10 000–14 000 µM, when the GE values did not reach 10%.

The first study was conducted to examine in detail the effect of PAR on barley germination. Significant inhibition of germination was found to occur from 8000 µM exposure.

Supported by the grant FVHE/Pikula/ITA2020

P005
FRUIT AND VEGETABLE JUICES: IMPACT ON METABOLIC ENZYMES

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Drinking fruit and vegetable juices represents an effective way of intake of polyphenols, vitamins, and minerals. Polyphenols in the diet have been showed to positively affect the metabolic syndrome which includes blood dyslipidemia, hyperglycemia and insulin resistance, high blood pressure, oxidative stress, and inflammation. These compounds can act by multiple mechanisms, such as inhibition of saccharide digestion or glucose transport, increase in insulin secretion and improvement of insulin sensitivity, decrease of lipid absorption, or affect signaling pathways.

In this research, we evaluated the effect of 36 juices on the activities of α-amylase, lipase, and trypsin *in vitro*. The highest inhibition effect on α-amylase was determined for red currant, persimmon, and galangal juices. The activity of lipase was significantly decreased by spinach, lemon, lime, and apricot; and increased by peach and raspberry juices. The most of juices showed no effect on trypsin, except for spinach, onion, and lime juices which significantly inhibited its activity.

Some fruit juices were reported to have adverse effects on metabolism of different drugs. The juice-drug interactions were generally mainly studied for the grapefruit juice and its inhibitory effect on CYP3A4. We determined *in vitro* that the inhibition effect of red currant, galangal, and spinach juices was even 40% higher than of grapefruit. On the other hand, kohlrabi and radish juices increased CYP3A4 activity two to three times.

We also performed a screening of bioactive compounds from the phenolic range in fruit and vegetable juices by mass spectrometry.

This work was supported by the Charles University projects SVV260427/2020 and GA UK No. 1304119.

P006
EMBRYOTOXICITY OF ANTIDEPRESSANT FLUOXETINE HYDROCHLORIDE – COMPARISON BETWEEN ZEBRAFISH (*DANIO RERIO*) AND AFRICAN CLAWED FROG (*XENOPOUS LAEVIS*)

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Since 2000, antidepressant consumption continues to increase in most European countries. There is a significant variation in consumption between countries depending on the prevalence of depression in each country. After application, antidepressants like other pharmaceuticals are excreted in their native form or as metabolites and enter aquatic environment. They are dispersed to surface waters largely via waste water sources, typically at very low concentrations (tens or hundreds of ng/l). One of the frequently detected antidepressant found in surface waters is fluoxetine, a selective serotonin reuptake inhibitor, which is often used to treat depression and obsessive compulsive disorder and bulimia. The aim of our study was to assess embryotoxicity of fluoxetine hydrochloride on early life stages of zebrafish (*Danio rerio*) and African clawed frog (Xenopus laevis). The use of embryos is considered to be a viable alternative that allows to evaluate toxicity since these development stages are not legislatively protected (Directive 2010/63/EU). At first, eggs were visually selected using binocular microscope in order to select only fertilized eggs without obvious irregularities during cleavag for the test. Fertilized eggs were distributed into microwell plates with 48 wells on each plate, while one egg was placed in each well. Embryos were exposed to various concentrations of fluoxetine hydrochloride for the period of 96 hours. The range of concentrations used in the test included environmentally relevant levels and multiples of the lowest tested one in order to determine the dose-response effect. The control group was exposed only to fluoxetine hydrochloride free dilution water and was carried out for each test organism. Temperature during the test was 26 °C and 23 °C for zebrafish and African clawed frog, respectively. Embryos were daily observed for mortality, hatching rate, and occurrence of lethal and sublethal endpoints.

This research was supported by FVHE/VEČEREK/ITA 2020.

P007
PET AND PVC MICROPARTICLES: *IN VITRO* TOXICITY ASSESSMENT USING FISH CELL LINES

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During the last decades, plastics have become a major environmental issue. Due to the mismanagement of waste, a large amount of plastics is entering the environment where it does not degrade but decays to smaller particles called microplastics. An increasing number of studies report that these particles pose a significant risk to living organisms and the environment. Although polyethylene terephthalate (PET) and polyvinyl chloride (PVC) are among the six most produced plastic polymers worldwide, there is only limited information on how microplastics from these two polymers affect living organisms. Therefore, irregularly shaped PET and PVC microparticles were prepared to mimic the particles occurring in natural environment. The toxicity of two different size fractions with an average diameter of 25 and 90 µm was investigated. To assess the *in vitro* toxicity, three different cell lines from rainbow trout *(Oncorhynchus mykiss)* were used, namely RTL-W1 (liver), RTgill-W1 (gill) and RTG-2 (gonads). Multiple assays were used to determine the impact of microplastics on cellular metabolism. The effects on cell viability were explored using three assays based on changes in membrane integrity of the exposed cells AlamarBlue (AB), 5-carboxyfluorescein diacetate, acetoxymethyl ester (CFDA-AM) and neutral red uptake (NRU) assay. The level of oxidative stress was studied using the 2′, 7′-dichlorofluorescein diacetate (DCFH-DA) assay and the effect of microparticles on xenobiotic metabolism was evaluated using the EROD assay, which is based on measuring the cytochrome P450 1A induction. Among the highlighted results was the ability of PVC to increase oxidative stress in all exposed cell lines and also its negative effect on cell viability. In the case of viability, liver cells proved to be the most sensitive. Even though the 90 µm particles induced certain negative effects, the 25 µm particles definitely pose a bigger risk to cells and potentially to the environment.

Supported by the grant 20-29315S from the Czech Science Foundation.

P008
IMPACT OF DIHYDROMYRICETIN ON ALCOHOL METABOLISM

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Dihydromyricetin (DHM) is a natural flavonoid compound with positive effects during severe alcohol intoxication. It is tested in the treatment of habitual alcohol consumption. By affecting the benzodiazepine site of GABA receptors it reduces alcohol craving. The present research is focused on alcohol elimination in the liver on a molecular level. After experiments with primary hepatocytes of rats *in vivo* experiments were performed. Rats were separated into two groups. First group received a

30% ethanol (EtOH) orally while the second group was pre-medicated with a 30% EtOH containing 2.5 mM DHM. Within 60 minutes from the animal treatment each 15 minutes the blood was collected from the eye of the rats. The amount of EtOH and acetaldehyde in the blood was determined. Data show that DHM does not significantly affect the EtOH metabolism rate. Next, the effect of DHM on repeated application of EtOH were tested. After 24 hours, both groups were administered with 30% EtOH. In collected blood the amount of EtOH and acetaldehyde was measured. Amount of EtOH was greater in the blood of rats that were pre-medicated with DHM on the first day compared to rats that were administered with EtOH only. These findings are in contradiction with the suggested effect of DHM on the EtOH metabolism rate. After repeated application of EtOH, liver was removed from the rats and used for preparation of microsomal and cytosolic fractions. Cytosolic fractions were examined for alcoholdehydrogenase (ADH) induction by Western blot technique. Likewise, no effect of DHM on ADH expression was found. It can be concluded that elimination rate of EtOH is not affected by DHM under experimental conditions used. Some effects of DHM on metabolism of EtOH in the liver can be possibly attributed to antioxidant and hepatoprotective properties of DHM.

P009
MONOCYCLIC AND BICYCLIC MONOTERPENES: MODULATION OF ANTIOXIDANT ENZYMES IN HUMAN LIVER

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Monoterpenes, main components of the plant-derived essential oils, are inherent ingredients of human food and they are used in folk medicines, pharmaceutical industry and cosmetics. Due to their pleasant flavor and/or odor, they represent important component of spices, traditional delicacies and beverages. Most of terpenoids easily enter the human body by oral absorption, skin penetration or inhalation leading to measurable blood concentrations. Numerous biological activities, including antitumor activity, of monoterpenes have been reported. On the other hand, some monoterpenes were reported to exhibit toxic effects in various organs of human organism, mostly in liver. The monoterpenes are also able to modulate activity and/or expression of some drug-metabolizing enzymes. Present research was carried out in order to evaluate the effect of five monocyclic and five bicyclic monoterpenes on the activity and mRNA expression of the main antioxidant enzymes in human liver. Firstly, the effect of monoterpenes (100 μM) on the catalytic activities of the antioxidant enzymes was assessed in subcellular fractions obtained from human liver tissue of six volunteers. Among studied monoterpenes, piperitone and α-thujone showed the most pronounced effect on the catalytic activity of glutathione S-transferase in the cytosol. Based on the obtained results, the modulatory effect of these two monoterpenes (10 μM and 50 μM) on the catalytic activity and mRNA expression of antioxidant enzymes was further studied in the human precision-cut liver slices (PCLS) obtained from three volunteers. In human PCLS, significant increase in the mRNA levels of GSTP1/2 and glutathione peroxidase 1 (GPx1) were observed in the case of both compounds (10 μM), while piperitone 50 μM caused significant reduction in GPx2/3 mRNA levels.

Supported by the grant 18-09946S from Grant Agency of the Czech Republic.

P010
RARE CAUSES OF RESPIRATORY INSUFFICIENCY IN NEWBORNS

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Developmental anomalies of the respiratory system, congenital lung masses (CLM), belong into a rare group of causes responsible for acute respiratory insufficiency in newborns. Congenital lung masses include congenital pulmonary airway malformation (CPAM), congenital overinflation, bronchopulmonary sequestration, and bronchial atresia.

The presenting group of patients consists of 13 newborns who were admitted to the Neonatal Department of Intensive Medicine during January 1st 2015 – December 31st 2019 (8 males, 5 females; 2 premature newborns, 11 term newborns; spontaneous delivery: 2, caesarean section: 11) due to the positive prenatal diagnosis of CPAM in all cases (foetal sonography, magnetic resonance imaging, MRI). In 2 cases prenatal intervention was performed (1 patient: drainage of the amniotic fluid; 1 patient: attempt of thoracentesis). Signs of acute respiratory insufficiency immediately after delivery had 5 newborns and 8 newborns were without signs of respiratory distress during the immediate postpartum period. Postnatal echocardiographic investigation confirmed the presence of increased pulmonary pressure in 8 patients, no patient had congenital heart abnormality. A thorax x-ray with the confirmation of the presence of CLM was positive also in asymptomatic patients. Computed tomography in patients brought detailed information about the position, size and character of CPAM. Six patients underwent surgery. In 15.4 % right lungs were affected by cystic malformation (1

patient. complete lobectomy of the right lower lung lobe; 1 patient. complete lobectomy of the right upper lung lobe). In 23 % left lungs were affected (2 patients: non-anatomical resection of the left upper lung lobe; 1 patient: complete lobectomy of the left lower lung lobe). The surgical procedure was contraindicated due to haemodynamic instability and the need of permanent resuscitation in one patient (7.8 %). No patient did not have associated malformations. A final diagnosis of CPAM was confirmed in 5 patients using histopathologic evaluation. Improvement of prenatal sonographic and foetal MRI help to identify the presence of CLM as well as improves evaluation of pulmonary anatomy. The goal of prenatal therapy is to improve foetal haemodynamic and prevent lung hypoplasia. Multidisciplinary cooperation during prenatal as well as postnatal period is necessary.

Supported by the grant 2/0166/20 from Grant Agency of the Slovak Republic.

P011
EFFECT OF BISPHENOL DERIVATIVES ON VIABILITY AND SELECTED TRANSCRIPTS IN HUMAN OVARIAN GRANULOSA TUMOR CELL LINE COV434

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Endocrine disrupting chemicals (EDCs) are substances with various structures capable of interfering with the endocrine functions. Bisphenols are chemical plasticizers and constituents of polycarbonate plastics and epoxy resins. Bisphenol A (BPA), a well-known EDC, was shown to impair functions of the reproductive organs, and consequently has been linked to female reproductive disorders and tumors. Due to these negative health effects, the use of BPA has become reduced. Instead, other bisphenol derivatives have been introduced as replacements for BPA usage in many applications. However, little is known about their biological actions or the similarities or difference with the actions of the prototype BPA. In the present study, we used the human ovarian granulosa tumor cell line COV434 to investigate whether the bisphenols are able to influence cell viability and the mRNA expression of selected transcripts related to epithelial-to-mesenchymal transition involved in cancer progression (*VIM*, *SNAIL*, *MMP9*). The agents were tested in several concentrations (1 nM–100 µM for cytotoxicity, 1 nM–10 µM for mRNA expression), and the cells were treated for 24 and 48 h. We did not observe significant negative effects of either BPA or its analogs on COV434 cell viability using MTS assay, except the highest concentrations, with BPAF exerting the most profound cytotoxic effect. Bisphenol analogs AF and S were able to upregulate *MMP9* mRNA expression at the highest concentration tested. The results indicate that bisphenols might alter certain pathophysiological processes, but further detailed studies are needed for elucidating the consequent effects.

Supported by the Slovak Research and Development Agency under the contract No. APVV-18-0150 and VEGA grant 2/0074/18.

P012
THE POSSIBILITY TO REDUCE RISK OF EXPOSURE TO ACRYLAMIDE.

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Acrylamide is a toxic compound that can be found in both occupational and non-occupational environments. This compound is widely used in constructions for waterproof materials to seal and ground foundations of dams. Presence of acrylamide in the environment is associated with the degradation of polyacrylamide *e.g.* building materials. Acrylamide is also used as flocculant during cleaning processes of wastewater treatment and preparation of drinking water. Because of the potential risk of environmental contamination, the usage of acrylamide in cosmetics and drinking water preparation is regulated. However, acrylamide was identified in food products as a result of Maillard reaction. Acrylamide is monitored in relation to damage to human health. Furthermore, neurotoxicological effects of acrylamide in exposed workers were reported by many authors. IARC classified acrylamide as probably carcinogenic to human. A lot of animal studies have also showed its genotoxicity and teratogenicity. Worldwide, the effects of ACR have been intensively studied for a long period.

Our study is focused on acrylamide resources and possibilities how to reduce the risk of exposure for population.

This work was supported by the Charles University in Prague, First Faculty of Medicine by grant PROGRES Q25/LF1.

P013
COMPARISON OF THE EFFECTS OF TITANIUM DIOXIDE AND MAGNÉLI PHASE TITANIUM SUBOXIDE ON RAINBOW TROUT *(ONCORHYNCHUS MYKISS)*

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Magnéli phase titanium suboxides are novel materials with unique chemical, physical, and optical properties, high electrical conductivity, as well as chemical inertness in oxidizing environments. Those properties differ from traditionally used titanium oxides with anatase, rutile, and brookite crystalline structures. Magnéli phase titanium suboxides have a great potential for industrial applications and thus, an increase of the volume produced in the future is to be expected. They are also incidentally

released into the environment during coal combustion, due to heating of titanium oxides naturally present in coal. To date, there is a lack of knowledge about their effects on aquatic organisms, especially during long term exposure. Therefore, an experiment will be performed following the Fish Juvenile Growth Test (OECD 215) guideline, exposing juvenile trouts to both TiO_2 and Magnéli phase titanium suboxides incorporated in their feed. Four experimental groups, as well as the control group, will be tested. Fish will be fed commercial pellets with added concentrations of 0.1% and 0.01% of both substances. The duration of the experiment will be as follows: two weeks of the acclimatization period, followed by six weeks of the experimental period, finished with two weeks of the depuration period. At the end of both the experimental and the depuration period, samples for the hematological, biochemical, histological, and immunological examination will be collected and evaluated.

Supported by the grant IGA VFU 217/2020/FVHE.

P014
INTERACTION OF 2,6,7-TRIHYDROXY-XANTHENE-3-ONES WITH IRON AND COPPER IN CANCER CELLS

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The need for novel iron or copper chelators is largely driven by side effects, low efficacy or unsuitable pharmacokinetics of the current chelators. Their application is reasonable in the treatment of metal overload conditions (*e.g.* frequent blood transfusion, Wilson disease), but their possible indications seem to be much larger. Both iron and copper are trace elements necessary for cancer growth, and the application of chelators can be used also as potential anti-cancer agents. In this study, a series of 2,6,7-trihydroxy-xanthene-3-ones were tested in order to find a novel potent iron or copper chelator by using a competitive spectrophotometric approach. In addition, cell experiments (breast adenocarcinoma cell lines (MCF7 and its fulvestrant resistant derivate), and erythrocytes were included to evaluate the potential effect and toxicity of the most active compound alone, and in combination with metals. In general, the differences concerning various substitutions on a benzene ring in position 9 of the xanthone molecule had a relatively low effect on chelation. Only the trifluoromethyl substitution led to a stronger chelation activity, probably through a positive effect on solvation. All compounds were able to chelate iron, but their copper chelating effect was only minimal, since it was no longer present under highly competitive conditions. Interestingly, all compounds reduced both iron and copper. Further experiments have shown that the trifluoromethyl derivative protected erythrocytes and cancer cells against copper toxicity. In conclusion, the tested compounds are iron chelators, but they can also reduce iron/copper and copper reducing effect is not associated with increased copper toxicity.

Financial support for this work was provided by project the program Progres Q42.

P015
BIODEGRADABLE POLYURETHANE MICROPLASTICS RAPIDLY SORB POLYCYCLIC AROMATIC HYDROCARBONS FROM CONTAMINATED SOIL

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Generally, personal care products and the degradation of plastics are considered as the main source of microplastics (MPs) in the environment. The fate of MPs is studied merely in aquatic ecosystems all over the world; however, MPs concentration in terrestrial ecosystems is estimated to be higher, even by an order of magnitude. In this study, we focused on the interaction of soil contaminated by polycyclic aromatic hydrocarbons (PAHs) and polyurethane foams, which are used for scent fences along roads and crop fields. Biodegradable and conventional MPs in aged and unaged form were exposed to PAH-contaminated soil for 28 days. Compared to conventional MPs, biodegradable fragments of MPs rapidly sorbed PAHs and their concentration on these fragments was up to 70 x higher than in the soil after 28 days. With respect to statistical analysis, time was determined as a factor significantly influencing PAHs sorption on biodegradable MPs. Analysis of variance and a further post-hoc test showed a significant increase between weekly samplings, except between the 7^{th} and 14^{th} day. The ageing increased the MP surface roughly twice but no changes in sorption after ageing and between MP types were observed; therefore, adsorption was considered as a minor participating process. Determined glass transition temperatures (negative values for biodegradable MPs and positive values for conventional MPs) displayed a glassy state of the conventional MP network and a rubbery state of the biodegradable MP network. These observations suggest that the sorption was driven by MP rigidity.

This study was supported by the Czech Science Foundation (grant number 20-29315S).

P016
THE EFFECT OF COFACTORS AND PH ON THE METABOLISM OF TYROSINE KINASE INHIBITOR VANDETANIB BY CYTOCHROMES P450

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Cancer is the second major cause of death after heart-attack in the world. In recent years, research has focused on tyrosine kinase inhibitors (TKIs) as part of targeted chemotherapeutic treatment. Vandetanib is a TKI affecting epidermal growth factor receptor (EGFR), rearrangement during transfection (RET) and vascular endothelial growth factor receptor 2 (VEGFR2). It is primary used for treatment of medullary thyroid cancer. Vandetanib is biotransformed by cytochromes P450 and flavin monooxygenases in human organism. Cytochromes P450 (CYPs) oxidize vandetanib to only one metabolite, N-desmethyl vandetanib, which exhibits similar efficiency as parental molecule. NADPH is the major cofactor of reaction cycle of CYPs.

The effect of various types of cofactors and pH on oxidation of vandetanib by selected human recombinant cytochromes P450, namely CYP2C8 coexpressed with cyt b5, CYP2D6, CYP3A4 and CYP3A4 coexpressed with cyt b5, was studied. Here, we investigate the effect of cofactors NADPH, NADH and their mixture in a 1:1 ratio on the amount of N-desmethyl vandetanib formed during the biotransformation of vandetanib. The effect of pH on the oxidation of vandetanib by CYP 3A4 and CYP 3A4 + b5 was also analysed. We analysed the amount of the metabolite formed at the pH range 7 to 8.5 and we try to find if the pH optimum of vandetanib biotransformation by selected CYPs is located in this pH range.

The results demonstrate that the most effective cofactor for CYP 2C8 + b5, 2D6 and 3A4 + b5 is NADPH. Other types of cofactors were also able to oxidize vandetanib to its metabolite, but not as effective as NADPH. However, the highest amount of N-desmethyl vandetanib was generated by CYP3A4 in the presence of NADPH-NADH mixture. Stimulation of CYP3A4 by cytochrome b5 resulted in an increase in formation of N-desmethyl vandetanib. Throughout the examination of pH effect, the trend of increasing biotransformation efficiency with increasing pH was observed. No pH optimum of vandetanib oxidation was found in chosen pH range (7 to 8.5).

Supported by the grant GACR 18-10251S.

P017
ARISTOLOCHIC ACID II INCREASES THE FORMATION OF DNA ADDUCTS OF CARCINOGENIC ARISTOLOCHIC ACID I IN VIVO AND *IN VITRO*

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Aristolochic acid nephropathy (AAN) and Balkan endemic nephropathy (BEN) are chronic tubulointerstitial nephropathies caused by a herbal drug aristolochic acid (AA) and their gradual progression to the end-stage leads to formation of upper urothelial carcinomas (UUC). The genotoxicity of AA results from nitroreduction during Phase I biotransformation leading to AA-DNA adduct formation. The most abundant AA-DNA adduct detected in AAN/BEN patients is 7-(deoxyadenosin-N6-yl)-aristolactam I (dA-AAI) which causes characteristic AT→TA transversion mutations (*e.g.* in TP53 gene) and exhibits a long-term persistence in renal tissue. In this study, we investigated the effect of aristolochic acid II (AAII) on reductive metabolism of aristolochic acid I (AAI) in rats in vivo. Hence, the rats were exposed both to AAI and AAII itself, and to their mixture (1:1). Using the method 32P-postlabelling, we determined the levels of AA-DNA adduct, particularly dA-AAI, in liver and kidney of the rats treated with AAI and/or AAII. The results show that dA-AAI adduct formation after treatment with AAI/AAII mixture is 3.6- and 4.5-fold higher in liver and kidney, respectively, compared to the rats treated only with AAI. The AA-DNA adduct formation *in vitro* was studied using hepatic and renal microsomes and cytosols isolated from control (non treated), AAI- and AAI/AAII-exposed rats. The higher levels of dA-AAI adducts were detected in hepatic and renal microsomes, whereas in renal cytosols were observed 0.66-fold lower levels. These results were in accordance with determined enzymatic activities and protein expression of NAD(P)H:quinone oxidoreductase (NQO1), the major enzyme reducing AAI, whose elevated levels after combined exposure to AAI and AAII were observed only in liver, whereas no such effect was detected in kidney. It seems that the reduction of AAI in kidney is mediated rather by cytochromes P450 (CYP) and NADPH:CYP reductase, since CYPs, predominantly detoxifying enzymes, can also participate in activation reactions and their enzymatic activities were also elevated in the treated rats. The results found in this study demonstrate the potentiating effect of AAII on dA-AAI adduct formation, however the significant role plays not only affecting of enzymes reducing AAI, but also AA biodistribution in the rat body.

Supported by GACR (grant 17-12816S).

P018
CHORIOALLANTOIC MEMBRANE AS A MODEL SYSTEM FOR STUDYING BIOCOMPATIBILITY

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The chorioallantoic membrane (CAM) is an extraembryonic membrane that is commonly used to study angiogenesis, and its inhibition in response to tissues, cells, or soluble factors. In the present research, we studied the angiogenic response of quail CAM (*Coturnix coturnix japonica*) to the biopolymer composites with specific physicochemical characteristics and surface bioactivity, as a necessary prerequisite for determining their biocompatibility. We observed differences in the angiogenic response of CAM after implantation biopolymer composites prepared on the base of polyhydroxybutyrate and chitosan (PHB/CHIT, MARSH) depending on the porosity of the material and the addition of vascular endothelial growth factor (VEGF). On embryonic day 6 (ED6), the tested biomaterials (PHB/CHIT, MARSH) were gently placed on the CAM without the addition of VEGF and with the addition of VEGF at an application dose of 1 μg and 25 ng. Placing a piece of the biopolymer on the CAM resulted in a vascular reaction documented visually and by stereomicroscopy on ED9. We observed vessels lead to and from the implant on the surface of the CAM. In terms of the increase of blood vessels, we evaluated the highest angiogenic potential in MARSH (66.50%), whose angiogenic potential was supported by the angiogenic effect of the VEGF at an application dose 25 ng (65.48%). The addition of VEGF at an application dose of 1 μg to MARSH (46.48%) and PHB/CHIT (40.69%) had an anti-angiogenic to cytotoxic effect. We observed the difference in angiogenic response of CAM in relation to pores size of biopolymer composites. In PHB/CHIT, 90% of pores do not reach more than 30 μm. The same percentage of all pores of MARSH reach a size up to 80 μm and 5% of pores size up to 130 μm. The tested biomaterials were biocompatible. The angiogenic potential was supported by a higher porosity of the material and lower application dose of VEGF. Detection of changes in the process of angiogenesis makes this alternative animal model a suitable system for rapid screening of porous biomaterials and their biocompatibility especially in the field of tissue engineering.

Supported by the grant APVV-17-0110 and VEGA 1/0050/19.

P019
ASSESSMENT OF TOXICOLOGICAL EFFECTS OF AG NANOPARTICLES IN ZEBRAFISH BY MALDI-TOF MASS SPECTROMETRY IMAGING

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In recent years, inorganic nanoparticles (NPs) have been used in various applications such as water remediation, textiles, food storage containers, cosmetics, electronic devices or imaging/drug delivery in medicine. Use of NPs is growing exponentially, which increases the risk of NPs' entering into our environmental or human system. Therefore, a knowledge of physicochemical properties, toxicity and the overall quality of a final product made of nanomaterial is required. However, negative effects of the accumulation of NPs in the environment are not explored enough. One of the most used nanoparticles are silver (Ag) nanoparticles. Different techniques can be used for determining the fate of nanoparticles in the environment. In this study, we have focused on optimization of the matrix assisted laser desorption/ionization time-of-flight mass spectrometry imaging (MALDI-TOF MSI) method for determining spatial distribution of reduced and oxidized glutathione (GSH and GSSG), as one of the stress markers, in cryosections of Danio rerio individuals (one year old) affected by Ag nanoparticles dispersed in the fish tank. The size of used Ag nanoparticles was 2 nm and 20 nm in concentration 5 μg/L and 50 μg/L. The time of exposure was 48 hours. We have found, that the MALDI-TOF MSI method is suitable for the detection of spatial distribution of GSH/GSSG in cryosections of Danio rerio individuals. Moreover, to the best of our knowledge, this was probably the first use of mass spectrometry imaging method for mapping GSH/GSSG spatial distribution in Danio rerio sections.

Supported by the project CEITEC 2020 (LQ1601) with financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II, and by EFRR "Multidisciplinary research to increase application potential of nanomaterials in agricultural" (No. CZ.02.1.01/0.0/0.0/16_025/0007314).

P020
EFFECT OF ANTIDEPRESSANT CITALOPRAM HYDROBROMIDE ON EARLY LIFE STAGES OF ZEBRAFISH (*DANIO RERIO*) AND AFRICAN CLAWED FROG (*XENOPOUS LAEVIS*)

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The consumption of antidepressants is increasing each year for the last twenty years. The selective serotonin reuptake inhibitors are used to therapy of depression very often. Subsequently, these medications are detected in the wastewater and surface waters as well. Citalopram – widely used antidepressant in Europe and its metabolite desmethyl citalopram are usually found in the surface waters at low concentrations oftens to hundreds ng/l, Exceptionally higher concentrations reaching as much as thousands ng/l can be detected in some localities. The aim of our study was to assess embryotoxicity of citalopram hydrobromide on early life stages of African clawed frog (Xenopus laevis) and zebrafish (Danio rerio). Eggs were visually selected using binocular microscope in order to select only fertilized eggs without obvious irregularities during cleavage for the test. Fertilized eggs of zebrafish were distributed into microwell plates with 48 wells on each plate and fertilized eggs of clawed frog were distributed into plates with 24 wells on each plate, while one egg was placed in each well. Embryos were exposed to range of citalopram hydrobromide concentrations (0.01–100 000 µg/l) for the period of 96 hours. Tested concentrations included environmentally relevant levels and multiples of the lowest tested one in order to determine the dose-response effect. The control group was exposed only to citalopram hydrobromide free dilution water and was carried out for each test organism. Temperature during the test was 26 °C and 23 °C for zebrafish and African clawed frog, respectively. Embryos were daily observed for mortality, hatching rate, and occurrence of various morphological changes.

This research was supported by FVHE/VEČEREK/ITA 2020

P021
TYROSINE KINASE INHIBITORS AND ANTICANCER DRUGS VANDETANIB, LENVATINIB AND CABOZANTINIB AFFECT THE EXPRESSION OF CYTOCHROMES P450 1A1, 1A2 AND 1B1 IN RATS *IN VIVO*

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In recent years, tyrosine kinase inhibitors have become the effective tool in targeted cancer therapy due to their ability to disrupt intracellular signalling pathways associated with the development and growth of tumours. The tyrosine kinase (TK) inhibitors vandetanib, lenvatinib and cabozantinib are orally administered anticancer drugs used mainly for the treatment of thyroid cancer. To date, very little is known about their impact on the expression and the activity of biotransformation enzymes responsible for metabolism of xenobiotics. Our aim was to investigate the effect of vandetanib, lenvatinib or cabozantinib on the gene and the protein expression and the enzyme activity of cytochrome P450 isoforms, which are crucial for metabolism of drugs, in rat livers and kidneys *in vivo*. For that, Wistar rats were exposed to the single dose 30 mg/kg of these TK inhibitors.

The relative gene expression was evaluated using quantitative PCR and the amount of protein was determined by Western blotting with consecutive immunodetection. In comparison to control rats, all three compounds increased the mRNA as well as protein level of CYP1A1 in rat livers and kidneys and those of CYP1B1 in the kidneys. Moreover, the exposure of rats to cabozantinib led to the enhanced gene and protein expression of hepatic CYP1A2. Subsequently, the enzyme activity of CYP1A1/2 in rat liver microsomes was studied using their marker reactions. Application of the studied compounds led to the changes in enzyme activities similar to the trend corresponding to the expression of CYP1A1/2. So far, the results showed that vandetanib, lenvatinib and cabozantinib are weak inducers of CYP1A1, 1A2 and/or 1B1.

Because combination of drugs is often used in the anticancer treatment and drug-drug interactions may play an important role in therapeutic effect of the administrated drugs, the effect of the studied TK inhibitors on the expression of CYP1A1/2 and 1B1 could result in a modulation of metabolism and changes in the efficacy of the co-administered drugs, which undergo biotransformation by this enzyme, and cause adverse effects on the organism.

Supported by GACR (grant 18-10251S)

P022
EFFECT OF SELECTED COSMETIC PRODUCTS ON ESTROGEN FORMATION

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Estrogens are steroid hormones whose physiologic functions include development of secondary sexual characteristicts, maintenance of bone mass or regulation of gonadotropin secretion. They are produced mainly in the follicular cells of ovaries, placenta and adipose tissue in women and to a lesser extent in the testes in men. Estrogens are formed in the final step of steroid hormone biosynthesis by the aromatization of androgens. This conversion is catalyzed by cytochrome P450 19, aromatase, a member of the cytochrome P450 family. The activity of this enzyme plays a key role in maintaining estrogen levels in the organism. Hormonal imbalance due to endocrine disruptors can result in *e.g.* development of certain cancers or impaired reproduction. Compounds with these effects include, in addition to environmetal pollutants, some drugs and cosmetic additives which people are exposed to on a daily basis. In the present study, the effect of selected perfumes and antiperspirants on the metabolic conversion of testosterone into estradiol, catalyzed by aromatase, was examined using TLC chromatography. The pilot experiments showed that selected antiperspirants do not affect

aromatase activity. Likewise, the most of perfumes tested showed a very low aromatase inhibition. Only one perfume showed some extent of aromatase inhibition. Based on the comparision of perfume compositions we can predict potential aromatase inhibitors, *e.g.* farnesol, coumarin or cinnamal present in the sample.

P023
TREATMENT OF MATERNAL DEPRESSION: OPTIONS AND DILEMMAS

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Depressive disorder is a serious mental illness that affects about 20% of population. Due to activation of neuroendocrine system and significant hormonal changes during pregnancy and lactation, maternal depression is frequent with its prevalence around 12%. Both untreated and treated maternal depression could result in a threaten of maternal health (even life) and development of fetus and neonate. To treat or not to treat maternal depression is a main concern and dilemma for many gynecologists and obstetricians. Consequences of untreated maternal depression involve permanent stress with high levels of stress hormones, maternal morbidity, pre-eclampsia, eclampsia, suicide, homicide, pre-term birth, low birth weight, birth complications, irritability and sleep disorders in neonate. On the other hand, pharmacotherapy of maternal depression may lead to spontaneous abortion, pre-term birth, low birth weight, persistent pulmonary hypertension, neonatal adaptation difficulties or cardiac malformations. However, results of many epidemiological studies are contradictory. Therefore, relevant and well-controlled studies are needed to get knowledge on advantages and disadvantages of pharmacotherapy. Experimental studies using appropriate animal models of maternal depression and treatment regime are necessary to elucidate origins and mechanisms which can finally lead to pathologies in mothers and their offspring. Our results showed that consequences of untreated depression in animals are worse then in those treated with antidepressants. Only subtle behavioral alterations can be observed in offspring of mothers treated with tested antidepressants. This contributes to clinical practice in the sense that it is better and more convenient to treat maternal depression then not to treat it. Up-to-date knowledge on treatment of maternal depression and recent focus of our scientific team will be presented.

This study was supported by the grant VEGA 2/0124/19.

P024
3D *IN VITRO* MODELS FOR HEPATOTOXICITY ASSESSMENT OF ENDOCRINE DISRUPTORS AND CHRONIC LIVER DISEASES.

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Nonalcoholic fatty liver disease (NAFLD) is a common clinicopathological condition characterized by significant lipid deposition in the parenchymal hepatocytes. Subsets of NAFLD, which progress to steatohepatitis and eventually cirrhosis, are recognized as a major cause of liver-related morbidity and mortality. There is increasing evidence suggesting links between NAFLD and chemical exposures, namely exposures to endocrine disruptors (EDs). EDs, through inappropriate environmental exposure, interfere with hormonal control. However, current validated *in vitro* testing methods have limited ability to assess steatogenic- and metabolism-disrupting potential of EDs and related health hazards.

Experimental *in vitro* and *in silico* models are becoming increasingly used in toxicological research as a worldwide effort to minimize the use of experimental animals and to provide more accurate information about chemically-induced disturbances of key biological processes leading to specific adverse health outcomes. Methods of 3-dimensional (3D) cell cultures provide a more relevant *in vitro* model for identifying new chemical hazards or studies of chemopreventive pharmaceutical agents, and for further characterizing their mechanisms of action.

In the present study, we optimized a 3D *in vitro* model based on the HepG2 cell line to evaluate the steatogenic activity of selected EDs *e.g.* bisphenols (BPA, BPS, BPF), phthalates (DEHP, DBP), fluorinated fatty acids (PFOA, PFOS), cadmium, DDE, butyl-paraben. Mature HepG2-derived spheroids showed increased expression of key hepatospecific genes. Spheroid cultures were adapted for 96-well microplate format and multiparametric evaluation of spheroid growth, morphology, viability/metabolic activity, membrane integrity, and ATP content. These assays were complemented by high content fluorescence microscopy of cellular processes involved in liver steatosis development, such as lipid accumulation. This setup was compatible with (semi-)automated workflow and suitable for (semi-)high throughput screening. Furthermore, the effects of selected EDs on the expression of candidate genes involved in lipid metabolism (fatty acid and lipid synthesis, fatty acid oxidation, fatty acid uptake and efflux) were evaluated by qPCR, along with the molecular, biochemical and immunochemical assessment of additional events central to hepatic steatosis.

3D hepatic spheroids represent a perspective model for *in vitro* assessment of key molecular and cellular events of AOPs for hepatic metabolic diseases and evaluation of the steatogenic potential of EDs.

This work was funded by the European Union's Horizon 2020 research and innovation program under grant agreement no 825712 - OBERON project (https://oberon-4eu.com), that is part of the European EURION cluster (http://eurion-cluster.eu/).

P025
REGULATION OF GLUTATHIONE PEROXIDASE 7 BY MICRORNAS AND THE POTENTIAL LINK TO OBESITY

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Aerobic reactions in the organism lead to the formation of reactive oxygen species (ROS). These compounds are necessary for physiological processes and for normal cell function. However, the excessive accumulation of ROS cause cell damage and may contribute to the development of various diseases including obesity. Organisms have developed enzymatic and non-enzymatic systems to detoxify these compounds. We focused on the glutathione peroxidase 7 (GPx7), one of the eight members of GPx family. GPx7 is a monomeric enzyme and is present mainly in the lumen of the endoplasmic reticulum. GPx7 reduces the accumulation of ROS in cells, hence is involved in the maintenance of redox homeostasis, in addition it participates in the correct protein folding. GPx7 is highly expressed in preadipocytes, but not in mature adipocytes. The deficiency of GPx7 promotes adipocyte differentiation and leads to both adipocyte hypertrophy and hyperplasia. microRNAs (miRNAs), single-stranded non-coding RNAs of about 22 nucleotides, are responsible for post-transcriptional gene repression and are present nearly in all eukaryotes. In the last few decades, their role in the organism development as well as pathology has proved to be irreplaceable, and their biogenesis itself is very complex and convoluted. miRNAs are tissue specific and their expression profile is often altered in many diseases. miRNAs can be used as early and specific biomarkers, but also as targets for the treatment of diseases. In this project, we used the bioinformatics program TargetScan to predict miRNAs that have their binding site on the 3´UTR region of GPx7 and may potentially affect the expression of this gene. In addition, these miRNAs have been shown to be associated with obesity and related pathologies. To verify the interaction, we have used luciferase gene reporter assay to demonstrate the effect of predicted miRNAs on gpx7 expression. We have constructed the plasmid pmiR-GLO with inserted 3´UTR region of GPx7 and in parallel also its four mutants of the predicted miRNA binding sites. Upon co-transfection of miRNAs and the constructs we have assessed the function of selected miRNAs as regulators of the GPx7.

P026
HPLC-ESI-MS2 DETERMINATION OF *N*-(2-HYDROXYETHYL)-L-VALYL-L-LEUCINE (HEVL) IN HUMAN URINE: METHOD VALIDATION

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Biomonitoring of ethylene oxide (EO), a carcinogenic industrial intermediate and sterilant, has been based on determination of its adduct with *N*-terminal valine [*N*-(2-hydroxyethyl)-L-valine, HEV] in blood protein globin. However, as sampling of blood is inconvenient in routine occupational health practice, a non-invasive alternative to globin analysis has been searched for. As a result, *N*-(2-hydroxyethyl)-L-valyl-L-leucine (HEVL) was identified as ultimate cleavage product of EO-adducted globin in the rat and human urine. As HEVL is currently being investigated as a promising human biomarker, reliability of the analytical method for its determination has to be adequately validated. The procedure includes clean-up of urine samples (2 ml) on a solid-phase extraction column Strata X-C. The column is loaded with pH-adjusted urine spiked with HEVL-d_4 as an internal standard, washed with water, 80% methanol, and finally with 5% NH_4OH in methanol. After evaporation of the solvent, the residue is dissolved in mobile phase and analyzed by HPLC-ESI-high resolution MS^2 at a transition of m/z 275.1965 → 116.1070. The key elements of this method were now validated according to Guideline on bioanalytical method validation of the European Medicines Agency. They included: limit of quantitation, selectivity, linearity of the calibration curve, repeatibility and reproducibility, stability of the analyte in human urine matrix and in stock and working solutions, freeze-and-thaw stability, and matrix effect. Where applicable, all tests were performed with urine samples containing both low and higher levels of HEVL, typical for unexposed controls (≤1 µg/g creatinine) and occupationally exposed subjects (up to 10 µg/g creatinine), respectively. In conclusion, the method met successfully all criteria requested by the above Guideline and can be recommended for reliable determination of HEVL for both routine and research purposes.

The study was funded by grant NV19-09-00378 from the Ministry of Health of the Czech Republic.

P027
THE EFFECT OF PARACETAMOL ON THE LEVEL OF THIOL COMPOUNDS IN *DAPHNIA MAGNA*

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Paracetamol (PAR) is a widely used non-steroidal anti-inflammatory drug. PAR enters the aquatic environment with wastewater and thus affects aquatic organisms. There is little information on the effect of PAR on detoxification processes. PAR induces oxidative stress which can manifest in endocrine disruption. Thiol

compounds represent an important group of detoxification molecules. The aim of this work was to monitor the acute toxicity of PAR to *D. magna* and SH, GSH, GSSG, GST and MT*D. magna* was bred in an environment according to the OECD 202 methodology, 10 individuals were evaluated for one analysis. PAR (0, 1, 10, 20, 50, 75, 100 mg/L) were used for acute toxicity testing. At the end of the test, *D. magna* was washed in distilled water and frozen at –20 °C. Homogenized in 1 ml of water. PAR concentrations were determined photometrically at 700 nm. Total SH groups (Ellman's method at 405 nm), GSH, GSSG (at 450 nm), GST (at 480 nm) and MT electrochemically by the Brdička method. Total protein levels were determined by pyrogal, biuret and Bradford. Zinc and iron levels were determined.

The EC50 for PAR in *D. magna* were determined at 24 and 48 h (45.23, CI 36.08–56.82 mg/L). In our experimental model, the acute toxicity of PAR was performed in three independent experiments (n=3).

This is the first study was conducted to examine in detail the effect of PAR on the levels of selected thiols.

Supported by the grant FVHE/Pikula/ITA2020

P028
ROLE OF SLC TRANSPORTERS IN CYTOTOXIC THERAPY OUTCOME OF BREAST CARCINOMA PATIENTS – FOCUS ON GENETIC VARIABILITY

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Breast cancer (BC) is the most common cancer in women worldwide. Therapy efficacy is dependent upon a plethora of factors. Among them, multidrug resistance is one of the most common obstacles to successful treatment. Apart from well-known mechanism of P-glycoprotein overexpression, deeply understudied solute carrier (SLC) transporters can import cytotoxic drugs into tumor cells and may cause drug resistance too. We aimed to explore the SLC gene expression variability in tumor tissues among 33 BC patients after receiving 5-fluorouracil, anthracyclines, cyclophosphamide and/or taxanes based preoperative therapy. We compared transcript levels with prognostic clinical data, chemotherapy response and disease-free survival and validated the results in 57 chemotherapy naïve patients. SLC46A1 and SLCO1A2 were selected as most promising candidates. We explored their genetic variability in 112 blood samples by next-generation sequencing using GS Junior (Roche) and MiSeq (Illumina) platforms. Overall, 100 variants were found in *SLCO1A2* and 36 in *SLC46A1* genes. Five of them (three in *SLCO1A2* and two in *SLC46A1*) were found in coding regions. One missense variant in SLCO1A2 and four noncoding in SLC46A1 were predicted pathogenic by *in silico* tools. These putatively pathogenic variants were selected for the validation on a cohort of 815 BC patients. A haplotype block in 3'UTR of *SLC46A1* composed of polymorphisms rs2239911-rs2239910-rs8079943 was significantly associated with HER2 expression and disease-free survival of the patients. In conclusion, we showed that gene expression and genetic germline variability in SLC transporters might play a role in cancer prognosis. Haplotype block in noncoding region of SLC46A1 was associated with prognosis of BC patients and will be further followed.

Supported by the Czech Health Research Council, project no. NV19-08-00113, Czech Science Foundation project no. 19-03063S and Charles University project no. UNCE/MED/006.

P029
EFFECTS OF LONG-TERM EXPOSURE TO SILVER NANOPARTICLES ON SURVIVAL AND FERTILITY IN *DAPHNIA MAGNA*

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Antibacterial properties of silver nanoparticles (AgNPs) have led to extensive application in various areas of human activity. However, the increasing production and usage of AgNPs raises concerns about the potential risk to the environment. Experimental studies clarifying the long-term effects of AgNPs on representatives of invertebrate organisms are necessary to assess for the possible adverse effects on the aquatic ecosystem. In this study we focused on acute (48h) and chronic (21d) toxicity of AgNPs to *Daphnia magna*. *Daphnia magna* is a zooplanktonic crustacean found in freshwater environments and is a standard model organism from the *Cladocera* order. Particle suspension employed in toxicity testing was well-characterized. Experiments were carried out methodologically in accordance with the following standards: Guidelines OECD 202 and OECD 211. The acute toxicity (48h EC50) of AgNPs for *Daphnia magna* caused concentration <23.5 $\mu g.L^{-1}$ and for silver nitrate ($AgNO_3$) <2 $\mu g.L^{-1}$. The chronic toxicity (21d LC50) of AgNPs for *Daphnia magna* caused concentration <0.5 $\mu g.L^{-1}$ and hatching inhibition (21d EC50) caused concentration <0.4 $\mu g.L^{-1}$. In the determination of chronic toxicity in the parental generation (F0) was beside the EC50 value also monitored the effect on the hatching rate and survival of the juveniles (F1). Comparative exposure in the acute toxicity test revealed higher toxicity of ionic form of silver ($AgNO_3$) compared to AgNPs. This study contributes to clarification of the potential risk of AgNPs for crustacean *Daphnia magna*.

However, for better understanding of the impact of AgNPs on the entire aquatic ecosystem, it would be useful to extend the study to long-term effects on representatives of other trophic levels of aquatic biocenosis.

Supported by the grant FVHE/Pikula/ITA2020.

P030
COULD THE MUSK COMPOUND TONALIDE AFFECT THE RAINBOW TROUT IN ENVIRONMENTALLY-RELEVANT CONCENTRATION?

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In this study, the polycyclic musk compound tonalide was tested in two different concentrations. One was chosen to be environmentally-relevant based on the literature sources. For recognizing whether there is also the dose-dependent effect of this compound, the second tested concentration was implied as 10× higher dosage. As the model organism, the rainbow trout (*Oncorhynchus mykiss*) served. At the beginning, the total of 65 individuals was divided into three groups. The two-week lasting acclimatization period enabled fish to adapt to laboratory conditions while the fish were fed with commercials pellets only. After that, the tonalide was tested after oral submission; the commercial feed pellets were continuously given to the control group. The feed pellets of the experimental groups were enriched with specific tonalide concentration: for group E1 (854 µg/kg) and E2 (8,699 µg/kg). Fish were fed in amount 1% of their wet body weight twice a day. The sampling was made after six weeks. The results of haematological profile showed the increase of haematocrit in both tested tonalide groups, while the biochemical analysis did not revealed any changes. As the markers of oxidative stress, the ceruloplasmin and ferric reducing antioxidant power were analysed, however their results did not showed any statistically-significant changes. Additionally, the vitellogenin concentration was measured in plasma samples with no specific indicators that tonalide is responsible for endocrine disruption. In conclusion, the demonstrable negative effect of tonalide in both tested concentrations was not confirmed for rainbow trout.

Authors would like to thank the project ERDF/ESF „PROFISH“ CZ.02.1.01/0.0/0.0/16_019/0000869] for financial support.

P031
DO POLYETHYLENE MICROPARTICLES AFFECT THE RAINBOW TROUT'S HEALTH INDICES AFTER ORAL SUBMISSION?

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Study was focused on polyethylene microparticles (PEMs) effect on 160 rainbow trout (*Oncorhynchus mykiss*) individuals when applied orally. In accordance to the Fish Juvenile Growth Test (OECD 215), the PEMs were incorporated into the commercial feed pellets of experimental fish in three concentrations: 0.5%, 2% and 5% of the feed/day. The homogenous size of the PEMs (40–48 µm) was verified using the scanning electron microscope. The fish were fed twice a day with the amount 1.5 % of their wet weight during the six-week lasting experimental period. There were made three samplings in two-week intervals for time impact observing. Based on haematological indices, the total leukocyte count was significantly decreased not only in time-dependent manner but also with increasing tested PEMs concentration. Haematocrit decreased in time-dependent manner among the same concentration groups. In contrast, the total erythrocyte count and haemoglobin did not reveal any significant changes between control and experimental groups. According to biochemical indices, the ammonia and total phosphorus decreased in time and concentration-manner; in contrast, the glucose levels increased with longer period of the trial. During this experiment, samples for oxidative stress evaluation, gene expression analysis of selected cytokines involved in inflammation and samples of tissues for histological evaluation were also gained; these data are currently analysed and will be commented hereafter. However, even with analysis made to this date, there are evident some negative effects of PEMs on rainbow trout health. Nevertheless, the mechanism of PEMs action was not still fully understood, and it would deserve further analysing.

Authors would like to thank the projects VFU IGA 212/2019/FVHE and ERDF/ESF "PROFISH" [CZ.02.1.01/0.0/0.0/16_019/0000869] for financial support.

P032
THE EFFECT OF ORAL ADMINISTRATION OF MICROPLASTICS WITH AN EMPHASIS ON RAINBOW TROUT INTESTINAL MICROBIOTA

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The production of plastics is increasing dramatically throughout the world and is considered to be a serious threat to the aquatic environment. Microplastics are particles with a size between 20 µm – 5 mm, able to permeate the intestinal endothelium and reach the tissues. Our study is focused on polystyrene microparticles and their impact on rainbow trout (*Oncorhynchus mykiss*) organism. The experiment will be performed in accordance with the Fish Juvenile Growth Test (OECD 215) and the microparticles will be incorporated into commercial pellets for experimental fish. The fish will be fed twice a day 1.5% of their wet weight. In total, three experimental groups will be tested – microplastics at concentrations of 0.5%, 2% and 5% in comparison with the control group without addition of microplastics. Each tested group will consist of 20 fish and the experiment will be performed in duplicate. The whole experiment will be carried out during 8 weeks with a 14-day period of acclimatization and 6 weeks of the experimental phase. At the end of the experimental phase, the effect on hematological, biochemical, histological and immunological indices in a time and dose-dependent manner will be evaluated. The main task of our study will be analysis of intestinal microbiota with an emphasis on *Lactobacillus spp.* and *Brevinema* mutual ratio after microplastics exposure where the DNA will be isolated and submitted to qRT-PCR.

The project was funded by the VFU IGA 212/2020/FVHE and by the ERDF/ESF "PROFISH" [no. CZ.02.1.01/0.0/0.0/16_019/0000869].

P033
DEVELOPMENT OF RESISTANT CELL LINE-BASED MURINE XENOGRAFT OVARIAN CANCER MODEL AND EFFICACY TESTING OF EXPERIMENTAL TAXANE SBT-121605

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A persistent problem for taxane therapy in ovarian carcinoma (OvC) is acquired multidrug resistance. Novel experimental synthetic taxanes (Stony Brook Taxanes – SBT) offer a promising way of overcoming this chemoresistance. After we have proven a new SBT to be efficacious *in vitro*, testing *in vivo* in mice is required. Here, we report successful establishment of an experimental model of OvC in xenograft immunosuppressed NU/NU Nude mice. Initially, 8 mice were subcutaneously injected with the taxane-resistant cell line NCI/ADR-RES (2, 3 and 3 mice injected with 0.5×10^6, 1×10^6 and 2×10^6 cells, respectively) under isoflurane sedation. In 2–5 weeks, depending on dose, all mice developed subcutaneous tumors. Paclitaxel (PCT) was applied twice a week and growth of tumors monitored to confirm non-response. Next, after initial dosage was determined in Balb/c mice, we used the NU/NU xenograft model to test the efficacy and toxicity of SBT-121605, a taxane previously selected for efficacy *in vitro*. NU/NU mice with tumors > 100 mm^3 (n=25) were divided into groups (5x5) and were then intraperitoneally administered either PCT (10 mg/kg) alone, SBT 1 mg/kg + PCT 9 mg/kg, SBT 3 mg/kg + PCT 7 mg/kg, SBT 5 mg/kg + PCT 5 mg/kg, or water as control, twice a week for 4 weeks. Compared to PCT alone, SBT121605 slowed or stopped tumor growth in all concentrations, but was severely toxic at 3 mg/kg and 5 mg/kg, where the experiment was stopped after 5 and 3 doses, respectively. Immediately after a mouse was sacrificed, samples of tumor, liver, kidneys, intestine and blood were taken for further RNA, DNA and protein profile analyses (not presented). SBT121605 has shown efficacy for OvC *in vivo*. Tests in models based on different cell lines, as well as of other promising taxanes, will follow in the future.

Supported by grants from the Czech Ministry of Education, Youth and Sports, programme INTER-ACTION no. LTAUSA19032, and Grant Agency of Charles University project no. UNCE/MED/006.

P034
DOES VARIOUS STRUCTURE DERIVATION OF SALIDROSIDE MOLECULE AFFECT THE ACTIVITY OF DERIVATIVES IN CELL-FREE TESTS AND ON HUMAN CELLS CUTURED *IN VITRO*?

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The protective potential study of phytochemicals applicable in civilisation diseases prevention and health protection is of great importance. Various structures of these compounds and a wide range of their biological activities have inspired organic chemists to sythesize their active derivatives in order to further increase their effectiveness.

The objectives of our study were (i) to prepare salidroside (SAL – tyrosol β-D-glucopyranoside) and a set of its derivatives using less conventional enzymatic

procedures or „green chemistry" approaches: CAFSAL – 6-O-caffeoyl-salidroside, FERSAL – 6-O-feruloyl-salidroside, HOSAL – hydroxysalidroside; (ii) to determine their reducing power, DPPH radical scavenging, chelation and DNA-protective capacity (DNA topology assay) using cell-free approaches and (iii) to evaluate their cytotoxicity (MTT test) and protective potential against hydrogen peroxide (H_2O_2; comet assay) in experiments utilizing *in vitro* cultured human hepatoma HepG2 cells.

We found out a spectrum of different activities for synthesized derivatives in cell-free as well as cellular assays; from HOSAL, the best performing antioxidant and protectant of plasmid DNA and HepG2 cells against Fe^{2+} ions- and H_2O_2-induced damage, to SAL showing the protective effects on HepG2 cells treated whith H_2O_2 even though it displayed almost no or very low antioxidant activity.

Diverse spectrum in effectiveness of the salidroside derivatives observed in this study revealed that their structure in terms of aglycone combined with sugar moiety as well as derivatizing groups affect and contribute to their activities.

Supported by VEGA grant 2/0126/19 from the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences, APVV-17-0384 grant from the Slovak Research and Development Agency and the project implementations: TRANSMED, ITMS: 26240120008 and ITMS: 26240220071 supported by the Research & Development Operational Programme funded by the ERDF.

P035
DOES MYRICETIN AFFECT INTESTINAL BACTERIAL MICROBIOTA OF PATIENTS WITH CROHN'S DISEASE

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The intestinal microbiota contains a large number of microorganisms that interact with each other and also affect the host. For instance, they contributes to immune system function. Attention is being directed towards the influence of foreign substances on the intestinal microbiota composition and subsequent their metabolism. Crohn's disease patients are found to have lower bacterial representation of beneficial bacteria. It is important to examine the interactions between intestinal microbiota and the patient in view of the disease progression and medication used. Plant flavonoid, myricetin, has been tested for its potential effect on a health promoting shift in microbiota of Crohn's disease patients. At first, patient faecal samples (A, B, C) were incubated for 6 hours with myricetin in either McDougall buffer or BHI medium and the reaction mixtures were analyzed on RP-HPLC. It was found that myricetin was degraded fast in both media. Expected reductive metabolite, dihydromyricetin, was not found in any of the samples analysed. Next, faecal samples in McDougall's buffer or BHI medium were incubated up to 72 hours with or without myricetin. The impact of the flavonoid on microbiota composition was determined using a rapid fingerprint analysis of bacterial 16S rRNA gene amplicons by denaturing gradient gel electrophoresis (DGGE). For more precise analysis the new generation sequencing method (NGS) was used. Regardless the faecal sample, significant changes in bacteria composition were observed between the cultivation media used. Among the faecal samples incubated in the McDougall's buffer no marked changes in bacteria composition were observed on PCR-DGGE. Faecal samples incubated in BHI medium differed only marginally. With the PCR-DGGE method and subsequent sequencing, *Faecalibacterium prausnitzi* was found to be present in the faecal sample A and C as well as *Prevotella* bacteria. In conclusion, despite the effective degradation of myricetin in all faecal samples this flavonoid did not affect microbiota diversity and their amounts.

P036
ANTIOXIDANTS AND METALS IN FRUIT AND VEGETABLE JUICES

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Fruit and vegetable juices contain a variety of polyphenols, vitamins, and minerals; many of them being important and key nutrients. Increased consumption has been associated with a reduced risk of cardiovascular diseases. The main mechanism of action of juices includes antioxidant effects of polyphenols – inhibiting lipid, protein and nucleic acid oxidation, improvement of endothelial function by especially anthocyanins, and inhibitory effects on platelet aggregation by flavonoids. In this research, we determined the content of polyphenols and flavonoids and estimated the antioxidant capacity of 36 different juices. Avocado and cherry juices showed highest polyphenols and antioxidants figures. Citrus fruits (*e.g.* lime, lemon, grapefruit) were richest in the flavonoid content.

In the diet, the optimal intake of minerals is of great importance since they are essential for biological processes and metabolic functions. The increased intake of Ca, Mg and K and decreased consumption of Na is correlated with decrease of elevated blood pressure. The most promising values were found in the root vegetables (*e.g.* carrot, kohlrabi, parsley). Trace metals have been found to play both positive and negative roles in human health. They can be classified as essential (Cu, Zn, Fe, Mn, Se, and Co), probably essential (Cr, V), and toxic (As, Cd, Pb, Hg, Ni, *etc.*) metals. Therefore, we focused on determination of some of these metals. Relative

high contents of Cu, Zn and Mn were found in ginger, galangal, curcuma, and parsley.

This work was supported by the Charles University projects SVV260427/2020 and GA UK No. 1304119.

P037
AHR-DEPENDENT REGULATION OF TRANSCRIPTOME AND PHENOTYPIC MANIFESTATION OF HUMAN BRONCHIAL EPITHELIAL CELLS DURING CHEMICAL TRANSFORMATION

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Polycyclic aromatic hydrocarbons (PAHs) and their derivatives belong among principle air pollutants, which elicit both genotoxic and non-genotoxic effects. One of the most studied carcinogenic PAHs is benzo[a]pyrene (BaP), a potent agonist of the aryl hydrocarbon receptor (AhR), which plays an important role in many processes associated with cancer promotion and/or progression. In our study, functional annotation of microarray datasets analyzed in normal human bronchial epithelial HBEC-12KT cells before and after their continuous exposure to BaP (1 mM) for 12 weeks (HBEC-12KT-B1 cell line) was performed to determine significant changes in pathways/biological processes induced during the chemical cell transformation. Moreover, both HBEC-12KT and HBEC-12KT-B1 cells were exposed to BaP and prototypical AhR agonist 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; 10 nM) for short (24–72 hours) and prolonged (2 weeks) time intervals to compare responses of the non-transformed and transformed epithelial airway cells. Both transcriptome analysis and phenotypic changes occurring during epithelial-to-mesenchymal transition (EMT) or cell transformation process, including cell morphology, cell cycle distribution, cell proliferation and migration potential, were investigated during further experiments. Two-week exposure to BaP or TGF-b1 (used as positive control for EMT induction; 1 ng/ml) led to a significant inhibition of both HBEC-12KT and HBEC-12KT-B1 cell proliferation connected with cell cycle arrest, while TCDD had no effect. Next, we evaluated the effects of two-week exposure to BaP, TCDD or TGF-b1 on induction of EMT-like phenotype. While, HBEC-12KT cells exposed to TCDD exhibited epithelial morphology, the cells exposed to BaP or TGF-b1 acquired an elongated, fibroblast-like shape, similar to HBEC-12KT-B1 cell morphology. However, the migration ability of the non-transformed cells did not change significantly after the two-week exposure. On the other hand, prolonged exposure to BaP and TGF-b1 enhanced motility of transformed HBEC-12KT-B1 cells, which correspond to our recent study with lung adenocarcinoma A549 cells.

This study was supported by Czech Science Foundation project No. 19-25365S.

P038
IDENTIFICATION OF ENZYMES OXIDIZING THE TYROSINE KINASE INHIBITOR CABOZANTINIB

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Cabozantinib acts as tyrosine kinase inhibitor (TKI) affecting vascular endothelial growth factor receptor 2 (VEGFR-2) and hepatocyte growth factor receptor. It also displays inhibition of several other kinases, including KIT and AXL. Therefore, cabozantinib is considered a multiple TKI affecting tumor angiogenesis, invasion and metastasis. Because only limited information is known about efficiencies of individual cabozantinib metabolites and available data indicate their lower efficiency, the knowledge of cabozantinib metabolic pathway is crucial for improvement of treatment and prognoses. In the present study we investigated the *in vitro* metabolism of cabozantinib in cell-free systems. We used hepatic subcellular systems (microsomes) isolated from human livers, which are responsible for first pass metabolism of drugs. In addition, individual recombinant human cytochromes P450 (CYPs), flavin-containing monooxygenases (FMOs) and aldehyde oxidase (AO) were employed to identify enzymes capable of metabolize this drug.

First, we investigated the function of human hepatic microsomal system, containing biotransformation enzymes, from individual donors to catalyze the oxidation of cabozantinib. All hepatic microsomes oxidized cabozantinib to three metabolites that were separated by HPLC and identified by mass spectroscopy. The formation of cabozantinib metabolites was dependent on NADPH and without it no oxidation of cabozantinib was detected. The predominant metabolite was identified as cabozantinib N-oxide. The correlation analysis was done and the highest correlation for all three metabolites was found with testosterone-6β-hydroxylation (a marker for CYP3A4). The use of recombinant CYP enzymes expressed in Supersomes™ was another approach to examine activities of individual human CYP enzymes to oxidize cabozantinib. Of several tested CYPs four cytochromes P450 were capable to oxidize cabozantinib under the experimental conditions. The CYP3A4 exhibits the highest efficiency. The potential of aldehyde oxidase and flavin-containing monooxygenases to oxidize cabozantinib was studied with human recombinant enzymes. Aldehyde oxidase and all three tested flavin-containing monooxygenases (FMO 1, 3 and 5) were ineffective in cabozantinib oxidation.

Supported by GACR 18-10251S.

P039
CYTOTOXICITY AND GENOTOXICITY HAZARD OF HOSPITAL WASTEWATERS EVALUATED BY ALTERNATIVE *IN VITRO* ASSAYS

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Hospitals and medical facilities use a wide range of different chemicals and preparations for therapeutic purposes, diagnosis, research, disinfection and daily operations, which are discharged into the sewer (sewage) system, either after pretreatment or without treatment. Some chemicals are not effectively removed in wastewater treatment plants and can therefore be a source of pollution for surface water and groundwater, thus posing an increased risk to human health and environment. Many scientific studies have confirmed that wastewaters (WWs) from medical facilities are often cytotoxic, genotoxic, or both. However, the evaluation of the cyto/genotoxic effects of given compounds in WWs is not a simple matter, mainly due to the variable characteristics of the WWs which depend on the type of hospital activity. We monitored the cyto/genotoxic potential of WWs from 5 hospitals in the Czech Republic and the evaluation was performed by means of various *in vitro* methods, *i.e.* single cell gel electrophoresis assay (Comet assay), bacterial reverse mutation test (Ames test), chicken egg genotoxicity asssay, *Allium cepa* test, cell transformation assay, and hen's egg test for micronucleus induction. Our study confirmed that all tested WWs samples can be assessed as potentially genotoxic or mutagenic regarding the results of the specific *in vitro* tests, with the exception of the Ames test. The evaluation of samples in the Ames test might be affected by adjustment of the samples before testing, particularly sample filtration or sterilization which may lead to the removal of genotoxicants. Contamination by microbiological agents of chlorinated WWs appears to be a key phenomenon causing significant cellular and nuclear damage recorded in this study.

Supported by ERDF/ESF project „International competitiveness of NIPH in research, development and education in alternative toxicological methods" (No. CZ.02.1.01/0.0/0.0/16_019/0000860) and by Ministry of Health, Czech Republic - conceptual development of research organization („National Institute of Public Health - NIPH, IN: 75010330").

P040
VARIOUS METHODS FOR IDENTIFICATION OF PATHOLOGICAL MICRO-ORGANISMS UTILIZED TO AVOID ANTIBIOTIC ABUSE AND OVERDOSE

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The main topic of global medical research in recent decades has been cancer. Today, however, an increasing part of the scientific community is switching its focus to the issue of growing bacterial resistance, which is behind the increasing frequency of failed antibiotic treatment. Lower respiratory tract infections, such as pneumonia, are a leading cause of death especially in children below the age of 5 years. Low and middle-income countries suffer the highest burden of childhood pneumonia. Lower respiratory tract infections are caused mostly by viruses. Discrimination between viral and bacterial causes is frequently impossible in low and middle-income countries due to lack of diagnostics. Therefore, most cases are treated empirically with antibiotics leading to overuse and misuse of antibiotics, which is an important driver of the global epidemic of antimicrobial resistance. We are working on a fast, accurate and reliable method of recognizing specific pathogens, which will be an alternative to standard methods used to identify causative agents using microbiological techniques or polymerase chain reactions. A new approach utilizing the loop-mediated isothermal amplification method can detect certain DNA with high specificity and speed in a single test tube. The method requires no complex equipment or complicated detection of resulting products. The current standard process for identifying pathogens takes many hours to days, while the newly developed methodology will shorten the process to minutes. This will also help combat resistant bacteria, because we could treat patients almost immediately and would not have to take the risk of "blindly" administering antibiotics before knowing the results of pathogen identification using current standard methods. Such a procedure does not unnecessarily expose the patient to the side effects of ineffective antibiotics or delay the start of effective treatment, thus reducing the likelihood of spreading the resistant bacteria.

Supported by the grant 8F20011 from The Ministry of Education, Youth and Sports.

P041
CYTOTOXIC AND IMMUNOMODULATORY ASSESSMENT OF PHENOLIC COMPOUNDS ISOLATED FROM *ZINGIBER OFFICINALE* IN MACROPHAGE CELL LINES

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Zingiber officinale Roscoe is commonly used in food and pharmaceutical products. Currently, more than 80 types of phenolic components, *i.e.* gingerols, have been isolated and their pharmacological activities are studied intensively. Our previous studies showed different biological and immunomodulatory effects of individual derivatives in rodent macrophages. Therefore, the aim of this study was to investigate cytotoxicity and immunomodulatory activity of four phenolic derivatives, 6-, 8- and 10-gingerols and 6-shogaol in human macrophage cell line. Cytotoxicity of compounds (Sigma-Merck) in model of THP-1 cell line (ATCC) was evaluated by WST-1 test (0.25×10^6 cells/ml) and by LDH activity (LDH; 0.1×10^6 cells/ml). Cells were stimulated by lipopolysaccharide (LPS; 0.01–1 μg/ml) for determination of immunomodulatory effect. Secretions of cytokines TNF-α and IL-6 were analysed by ELISA kits or by multi-plex array (Quansys Bioscience, USA). Cellular antioxidant defense system was evaluated by glutathione peroxidase assay using 96-well microplate format. Under 24-h *in vitro* cultivation of THP-1 macrophages, significantly cytotoxic substances were found 6-shogaol and 10-gingerol followed by 8-gingerol. On the other hand, the viability of 6-gingerol was not changed up to tested 100 μM concentration. Biological potential of 6-gingerol was in our study examined to immunosuppressive effect and anti-oxidative activity. Dose-dependent reduction of secretion of cytokines TNF-α, IL-6, IL-1β was observed in macrophages stimulated by LPS. Also mild changes in secretion of chemokines (MCP-1, MIP-1α) were detected. Pilot results indicate anti-oxidative potential of 6-gingerol. Our data point out different biological effects of individual gingerols in human macrophages. Rhizomes of this plant thus represent an interesting tool of compounds for further medical application and food industry.

Supported by the grant „P-projekt“ 2019 and SVV 260 540 (2020).

P042
EFFECTS OF METAL CHELATORS ON ACUTE COBALT TOXICITY TO THE H9C2 CARDIOMYOBLAST CELL LINE

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Cobalt is an essential trace element important for blood cells maturation and normal nervous system function. Physiologically, cobalt is found solely in vitamin B12, whose body storage is tightly regulated. In cobalt excess, substantial unbound cobalt ions can occur and exhibit toxic effects manifested mainly as central nervous system disturbances and cardiomyopathy with high short-term mortality. Cobalt chloride is occasionally used by homeopathic doctors, however, high doses of $CoCl_2$ had been previously used for its stimulatory effect on hematopoiesis in clinics and as a blood doping in humans and racehorses. Alternative sources of cobalt intoxication are metal ion traces released from cobalt-chrome implants used for manufacturing of dental or orthodontic implants. Treatment of cobalt intoxication is difficult and long-term due to its accumulation in body. Therefore, effective cobalt chelation seems to be beneficial and promising treatment strategy, but there is lack of effective and specific cobalt chelators. Hence, the aim of this pilot study was to test cobalt-chelation ability of five selected metal chelators, and to further test their ability to protect rat cardiomyoblast H9c2 cell line against toxicity of $CoCl_2$. Cobalt chloride showed dose-dependent toxicity to H9c2 cardiomyoblasts after 24-hour-exposition, as was determined by MTT assay. Pilot data of protective effects of two of the tested metal chelators, ethylenediaminetetraacetic acid (EDTA) and chloroxine, showed a trend to protect the studied cell line against cobalt-induced toxicity. Toxicity of both chelators was also tested to choose the most suitable concentration for protective study. Further investigation is needed to verify our pilot data and to elucidate molecular mechanism(s) involved.

Supported by the Charles University Research Program PROGRES [No. Q42].

P043
EVALUATION OF XENOBIOTIC METABOLISM IN 3D CULTIVATION MODEL OF IMMORTALIZED HUMAN MIHA HEPATOCYTES

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The presently used *in vitro* liver cell models often suffer from various limitations, as the isolated primary hepatocytes may lose their metabolic capacity, when cultivated as conventional *in vitro* cultures, and various liver-derived cancer cell lines exhibit phenotypes distinct from the mature hepatocytes. The three-dimensional (3D) cultivation of liver cell spheroids may improve the hepatocyte-like phenotype of hepatoma-derived cells, including increased expression of receptors and enzymes involved in both Phase I and II of biotransformation. These enzymes play key roles in the metabolism of drugs and other xenobiotics, but also of other, endogenous, compounds. In this study, we used MIHA cell line, immortalized human hepatocyte-derived cell line, cultivated in 3D conditions. Following

3D cultivation, MIHA cells increased production of albumin, one of the major sign of mature hepatocytes. In MIHA cells cultivated in 3D conditions, we also found higher levels of constitutive androstane receptor (CAR) mRNA, a principal regulator of drug metabolism, as compared with their 2D counterparts. Importantly, we also observed an improved inducibility of cytochrome P450 (CYP) enzymes relevant for the metabolism of pharmaceuticals, such as CYP3A4. The 3D cultivation of MIHA cell spheroids might offer a promising model for evaluation of mechanisms regulating expression and activity of enzymes contributing to metabolic activities of liver cells. We are currently working to optimize cultivation of MIHA cell spheroids, in order to apply them in toxicity studies or screening of enzymatic activities.

P044
IN VITRO THREE-DIMENSIONAL RECONSTRUCTED HUMAN TISSUE MODELS IN THE BIOCOMPATIBILITY ASSESSMENT OF MEDICAL DEVICES WITH INTENDED USE IN THE ORAL CAVITY: LAUNCH OF THE INTERNATIONAL PROJECT TRAIN-SAFEMDS

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Medical devices (MDs) have an irreplaceable role in modern healthcare. The term 'medical device' covers a broad spectrum of products that are crucial in diagnosis and treatment, disease prevention and improving the quality of life of people suffering from disabilities or injuries. MDs used in the oral cavity are usually those helping in the treatment of aphthae or canker sores irritations and lesions of the oral mucosa by forming a barrier that adheres to the oral mucosa and promotes healing. Dental materials and dental prosthetic devices are also an important group of MDs with apparent contact with oral mucosa.

Most of the MDs bio-compatibility assessments are still conducted in animals, however, thanks to the advances in the tissue engineering and accelerated progress in the validation of alternative methods, the MD regulations are also implementing advanced *in vitro* models. This has been demonstrated recently by the adoption of the *in vitro* reconstructed human epidermis model and test for intra-cutaneous testing into the ISO standard 10993-23. Biocompatibility testing of MDs is based on the toxicity assessment of extracts from MDs, that are, in fact, highly diluted solutions of potential irritants and their mixtures. Therefore, an already elsewhere validated and accepted *in vitro* test (*e.g.* by OECD) must be fine-tuned to achieve higher levels of sensitivity for these specific types of materials.

The international project Train-SafeMDs, launched in March 2020 between Austria, Czech Republic and Slovakia, uses commercial as well as in-house prepared 3D reconstructed tissues of oral/buccal epithelia and cell cultures with the origin in the oral cavity to develop a highly sensitive testing strategy for *in vitro* biocompatibility testing of MDs. The project predominantly aims into: 1.) training of students and early career scientist in the construction and use of *in vitro* engineered epithelial tissues for the safety assessment of MDs without the use of experimental animals, 2.) standardisation of the selected *in vitro* 3D tissue models of the oral cavity and 3) generation of datasets with selected positive and negative reference MD materials as a part of the preparation for a broader international collaborative project.

Supported by the APVV grant DS-FR-19-0048 (Slovak Republic), MTMŠ grant 8X20026 (Czech Republic) and OEAD grant MULT 06/2020 (Austria).

P045
BIO-COMPATIBILITY ASSESSMENT OF MEDICAL DEVICES USING RECONSTRUCTED *IN VITRO* 3D HUMAN CORNEA-LIKE TISSUE MODEL

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Medical devices are increasingly used in the population to cope with the degenerative diseases,to treat traumatic injuries or in personal and dental care. Each medical device used in a patient/consumer must be subjected to a structured biological evaluation program within a risk management process described by ISO standards.

In the recent years, the ISO standard implemented series of highly sensitive *in vitro* methods that help to the safety assessors in the screening of potential health hazards of medical devices even before conducting animal studies or clinical trials. Significant success has been achieved with development and validation of *in vitro* protocol for sub-cutaneous irritation testing of medical devices. The outcome led to the development of a new ISO standard 10993-23. Building on the experience obtained in that project, an *in vitro* protocol for ocular irritation testing has been developed using *in vitro* 3D reconstructed cornea-like tissue model.

Benchmark materials, representing standard biocompatible polymers as well as impurities from the production process of medical devices, have been tested to evaluate the sensitivity and specificity of the proposed *in vitro* test.

The accuracy of the test towards these materials was 100%. In the next phase of the project, materials representing medical devices used in ophthalmology will be tested to challenge the *in vitro* predictions and protocol. Development of a protocol for early detection of toxic materials and impurities posing a health risk will help to increase the safety of medical devices for patients. At

the same time, *in vitro* methods will help to reduce or even eliminate the need for follow-up tests on animals in selected medical devices categories.

Supported by the VEGA grant 2/0153/20: Bio-compatibility assessment of medical devices and novel medical device materials using in vitro methods based on 3D reconstructed human tissue models, and APVV grant APVV-19-0591: In vitro bio-compatibility testing of medical devices (MDs) and new generation bio-materials for MDs (Slovak Republic).

P046
THE IMPACT OF AHR INHIBITION IN CANCER CELL MODELS ON CELLULAR LIPID METABOLISM AND CELL PROLIFERATION

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The aryl hydrocarbon receptor (AhR) is a well-known cellular sensor of xenobiotics and major transcription regulator of xenobiotics-metabolizing enzymes. Nevertheless, several studies have indicated that the AhR could play a physiological role in regulation of energy balance and that it may affect cellular metabolism of lipids and fatty acids (FA). Increased protein levels of the AhR that have been found in cancer cells seem to suggest that the AhR might contribute to tumor cell survival and cancer progression. However, the impact of the AhR on lipid metabolism in cancer cells remains largely unknown. In this study, we analyzed possible role of the AhR in lipid metabolic network(s) in tumor cell models, with a particular focus on regulation of synthesis of FA in colon cancer cells. We used chemical inhibitor of the AhR CH-223191, as well as the AhR knockout (AhR KO) variants of human cancer cell lines (human colon adenocarcinoma HCT-116 and HT-29 cells; breast carcinoma MCF7 cells). We evaluated the impact of loss/inhibition of the AhR on cell proliferation, mRNA levels of key enzymes of FA-biosynthetic pathway (ACLY, ACC, FASN, SCD1), or, the levels of specific FAs in human cancer cells. We found that chemical inhibition as well as AhR knockout decreased proliferation rate and expression of some FA-synthesis enzymes. Interestingly, induction of the AhR signaling pathway by strong agonists (TCDD, BaP) increases expression of SCD (stearoyl-CoA desaturase), which is often overexpressed in tumors. The loss of AhR was further associated with alterations in expression/activation of components of PI3K/Akt signaling pathway, major regulator of cellular energy metabolism, as well as with some cell-specific alterations of FA composition. Together, these data seem to indicate that the AhR action might partially contribute to deregulation of lipid metabolism, which is frequently observed in cancer cells.

Supported by grant no. 9-00236S from the Czech Science Foundation.

P047
HORSERADISH PEROXIDASE CATALYSES DIMERIZATION OF NOVEL ANTI-CANCER DRUG VANDETANIB

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Vandetanib is a member of novel targeted anti-cancer drugs, tyrosine kinase inhibitors (TKIs). Mechanism of anti-cancer activity of TKIs is based on alteration of cell signalling pathways by inhibition of tyrosine phosphorylation. Vandetanib inhibits epidermal growth factor receptor (EGFR), rearrangement during transfection (RET) and vascular endothelial growth factor receptor 2 (VEGFR2). Thus, vandetanib is considered multiple TKIs. Vandetanib was approved for treatment of symptomatic or progressive medullary thyroid carcinoma in patients with unresectable locally advanced or metastatic disease. Peroxidases are enzymes that play key role in metabolism of xenobiotics. The metabolism of vandetanib by liver enzymes like cytochromes P450 (CYP450) and Flavin monooxygenases (FMOs) was in detailed investigated in previous studies. Although targeted tissues of thyroid gland contain thyroid peroxidase (TPO), the potential of peroxidases participate on vandetanib metabolism is mysterious. Here, we studied the ability of peroxidases to metabolise vandetanib *in vitro*. Horseradish peroxidase (HRP) was chosen as the model peroxidase. Vandetanib was oxidized in presence of hydrogen peroxide by HRP to its dimer. Metabolite was separated and identified by LC/MS, although the exact structure of dimer is still enigmatic. The formation of vandetanib dimer was linear up to 40 minutes. Optimal pH for vandetanib oxidation to vandetanib dimer by HRP is 6.0. Other pH optimum is 8.5 however efficacy of oxidation at this pH is lower in comparison to pH 6.0.

Supported by the grant 18-10251S.

P048
IN VITRO STUDY ON METABOLIC SULFATION OF SILYMARIN CONSTITUENTS

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Silymarin, a standardized extract from the fruits of milk thistle (*Silybum marianum*), is a well-known herbal remedy with wide spread application area, but mainly utilized as a hepatoprotective agent in the liver damages of various etiologies. The main bioactive constituents of

silymarin include the flavonolignans silychristin, silydianin, silybin and isosilybin. Due to several hydroxyl groups available on the skeleton of these flavonolignans, the phase I of xenobiotic metabolism can be skipped and these compounds can directly undergo the phase II biotransformation processes. In humans, glucuronidation and sulfation were identified as the main types of metabolic conversions. The glucuronidation of the flavonolignans was previously characterized using human hepatocytes, liver microsomal fraction and recombinant enzymes (Vrba et al., J. Pharm. Biomed. Anal. 178 (2020) 112972). In this study, we investigated the sulfation of silychristin A/B, silydianin, silybin A, silybin B, isosilybin A and isosilybin B. Using ultra-performance liquid chromatography coupled with tandem mass spectrometry, we found that individual tested compounds can be sulfated by human liver and human intestine cytosols. Moreover, after incubation of silymarin with the hepatic and intestinal cytosolic fractions, we detected three and five peaks (m/z 561.069) corresponding to monosulfated flavonolignans, respectively. These results demonstrate that both the intestine and the liver can play a role in the sulfation of silymarin flavonolignans. Next effort will be focused on the identification of human sulfotransferases involved in silymarin metabolism.

This research was supported by the grant No. 18-00132S from the Czech Science Foundation.

P049
EFFICACY AND MOLECULAR MECHANISM OF ACTION OF STONY BROOK TAXANES IN RESISTANT *IN VITRO* MODELS OF OVARIAN CANCER CELLS

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Taxanes are successfully used in therapy of different carcinomas, especially breast and ovarian carcinomas. One of the main problems with lower efficiency of conventional taxanes (paclitaxel, docetaxel) is the development of multidrug resistance. This resistance is a multifactorial process related to drug transport, metabolism or alterations in signaling pathways or in apoptosis induction by taxanes. Newly synthesized experimental taxanes (Stony Brook taxanes; SB-T-taxanes) are potential drugs against solid tumors with resistant phenotype. The aim of our study was to compare the efficiency, cell cycle modulation ability and transport of conventional (paclitaxel) and new experimental taxanes (SB-T-1214, SB-T-121402, SB-T-121405, SB-T-121406, SB-T-1216, SB-T-121602, SB-T-121605 and SB-T-121606) in highly resistant ovarian carcinoma cells (NCI/ADR-RES) and select the most efficient taxanes for *in vivo* experiments. In addition, changes in mRNA and miRNA profiles were measured using Affymetrix arrays after the treatment of paclitaxel and taxane SB-T-1216.

The efficiency of new experimental taxanes (namely SB-T-121605, SB-T-121606) were up to 1000 times higher compared to paclitaxel in the *in vitro* model of resistant ovarian carcinoma NCI/ADR-RES cells. Significant changes in cell cycle after exposing the model to different concentration of new taxanes were also observed. Cell cycle arrest at the G2/M phase was induced by 10-times lower concentrations of new taxanes compared to paclitaxel. In addition, uptake of new taxanes was also higher (6.8~15.5-times) as compared to paclitaxel. The most efficicacious new taxane SB-T-121605 was selected for experiments in *in vivo* mice xenografts, where conventional taxane, paclitaxel alone, was not effective. In the frame of molecular mechanisms of taxanes, we have found significant differences in mRNA and miRNA profiles caused by the action of novel taxanes and identified key pathways and genes for molecular mechanism of taxane action (*e.g.*, NOTCH and AhR signaling and Hedgehog pathways). These plausible pathways are currently investigated as potential therapeutic targets of human cancer.

Supported by grant GACR 19-03063S, by projects of the Czech Ministry of Education, Youth and Sports INTER-COST no. LTC19020 and INTER-ACTION no. LTAUSA19032, Grant Agency of Charles University project no. UNCE/MED/006, and a grant from the National Cancer Institute, NIH, U.S.A. CA 103314 (to I.O.).

P050
THE ROLE OF NANOPARTICLES IN THE ENVIRONMENT POLLUTION

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Micropollutants such as pesticides, industrial chemicals, pharmaceuticals and hormones are present in the aquatic environment in very low concentrations, but they can adversely affect aquatic ecosystems. The best known representatives in this group include tonalide (musk compound), bisphenol-A (plastic component), triclosan (antibacterial and antifungal agent), metolachlor (leading pesticide), ketoprofen (anti-inflammatory drug) or estriol (estrogen hormon). Particles that have at least one dimension less than 100 nm are defined as nanoparticles. This group include for example TiO_2, used for its antibacterial effect, or ZnO, used in the electronic industry, in special organic reactions as a catalyst or as an UV absorbing additive in cosmetic products. Many of these substances have toxic effects on aquatic organisms. Water organisms are exposed to these substances and their mixtures for a long time, which may increase their effect. In addition, many studies documented that micropollutants exposure could pose a high risk

to human health. Some of micropollutants are found in water because of their low or zero biodegradability. Conventional wastewater treatment plants are not able to 100% remove all pollutants from the water. Activated carbon, ozone, UV, membrane technology or coagulants (chitosan) are used to purify the water. New methods used to remove micropollutants from water include the use of nanoparticles. For example magnetic nanoparticles – attached fluorographene – based sorbent, or polyvinylpyrrolidone – coated magnetite nanoparticles are tested for the possibility of using their properties to treat drinking water. In addition to the effectiveness of these and other developed methods, the possibility of their re-use, ie the ecological aspect, must be considered. A wide range of pollutants and wide range of their effects in water means that it is constantly a need to improve technologies that are able to repurify water.

Supported by IGA VFU 217/2020/FVHE.

P051
DEREGULATION OF SIGNALING PATHWAYS CONTROLLING CELL SURVIVAL AND PROLIFERATION IN CANCER CELLS ALTERS INDUCIBILITY OF CYTOCHROME P450 1A1 (CYP1A1)

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Deregulation of various CYP enzymes might affect development, progression, or the outcome of cancer treatment in various cancer types. Proliferative behavior, cell density, or presence of the growth factors may alter CYP1A1 inducibility. Therefore, the activity of signaling pathways controlling cell proliferation, which are often aberrantly activated in tumor cells, could have a major impact on expression of CYP enzymes. In this study, we examined the functional role(s) of proliferative/pro-survival signaling (deregulated in tumor cells), and altered recruitment of the p300/CBP histone acetyl transferase (transcriptional co-activator) to the CYP1A1 enhancer region, in modulation of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced up-regulation of CYP1A1. We examined levels of CYP1A1 mRNA (qRT-PCR), protein (Western blotting), as well as the binding of p300/CBP to *CYP1A1* enhancer region (by chromatin immunoprecipitation), using siRNA- or pharmacological inhibition-based approaches in two cell models derived from human liver (HepaRG cells) and colon carcinoma (HCT-116 cells) tumors. We observed that TCDD-induced CYP1A1 mRNA/protein levels were lower in exponentially growing cells, as compared to their non-dividing counterparts. This is in line with the evidence suggesting that p300/CBP utilization differs between proliferating/exponentially growing and non-dividing/confluent cells. Similar to that, we demonstrated that siRNA-mediated inhibition of proliferation pathways (silencing of β-catenin and/or Hippo pathway effectors YAP1/TAZ) resulted in an increase in TCDD-induced CYP1A1 mRNA levels in HepaRG and/or HCT-116 cells. Both effects could be linked to p300 being preferentially utilized for induction of expression of genes contributing to cell proliferation/survival, since a pharmacological inhibition of p300 (and CBP), using C646 inhibitor, resulted in marked decrease of TCDD-induced CYP1A1 mRNA induction in both cell models. We then found that both expression of CYP1A1 and p300 binding to regulatory CYP1A1 gene regions could be altered by manipulation of Wnt/b-catenin signaling, which seems to support our hypothesis. The activities of signaling pathways contributing to cancer cell proliferation/survival (including Wnt/b-catenin signaling or Hippo signaling modules), may thus substantially alter CYP1A1 inducibility, with possible further impact(s) on tumor cells.

Supported by Ministry of Health of the Czech Republic, grant nr. 17-28231A, and the Czech Science Foundation (grant No. 18-00145S).

P053
MODULATION OF ANTHRACYCLINE CYTOTOXICITY TOWARDS CARDIAC AND CANCER CELLS BY DIFFERENT TOPOISOMERASE II INHIBITORS

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Anthracyclines (ANTs) remain essential in a range of cancer treatment regimens. Use of all drugs of this class, however, is limited by the risk of severe cardiotoxicity. To date, dexrazoxane (DEX, ICRF-187) has been the only cardioprotective agent approved for clinical use. This compound, therefore, represents the main lead in the search for effective cardioprotective agents. The focus regarding its cardioprotective mechanism has recently shifted from metal chelation to topoisomerase II (TOP2) modulation.

Thus, the aim of this work was to evaluate the cardioprotective efficiency of different TOP2 inhibitors against ANT cardiotoxicity. We started with a screening of a series of structurally different compounds described in literature as topoisomerase II inhibitors for their potential cardioprotective effects using our well-established *in vitro* model which was proven to have very good predictive value in terms of *in vivo* conditions. Using primary cultures of rat neonatal ventricular cardiomyocytes (NVCM) and clinically relevant concentrations of daunorubicin (DAU), we systematically assessed the effects of diverse TOP2 inhibitors on DAU cardiotoxicity and their inherent toxicities and compared the effects with DEX. Additionally, since the prevention of undesired

cardiotoxic side effects of ANTs would lose meaning if it also hindered their main anticancer effects, the interference of these compounds with antiproliferative efficacy of DAU on HL-60 was evaluated as well. To address also the chelation of iron ions as traditionally proposed putative mechanism of DEX cardioprotection, ability of the compounds of interest to displace iron from its complex with ANT was measured.

The work on elucidation of mechanisms involved in development of ANT cardiotoxicity is still in progress, yet our current results assembled together strongly indicate that TOP2 inhibition and/or depletion is essential for effective cardioprotection that can be achieved without compromising of anticancer effects of ANTs.

This study was supported by the Charles University (project 246219) and project InoMed (NO.CZ.02.1.01/0.0/0.0/18_069/0010046).

P054
THE EFFECT OF ANTHELMINTIC DRUGS METABOLIZED BY LIVESTOCK ON FODDER PLANTS

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The anthelmintic drugs such as albendazole (ABZ), ivermectin (IVM) or monepantel (MOP) are regularly administered to livestock to control nematodes caused infections. The parent drugs as well as the metabolites generated in the gastrointestinal tract of treated animals are released to the environment and this contamination might have a negative influence on non-target soil invertebrates or fodder plants growing on grazing lands. In our previous study, we found several metabolites of ABZ and MOP, converted mostly via S-oxidation. Our research investigates possible phytotoxicity in higher plants, caused by the uptake of anthelmintic metabolites generated in sheep. Time dependent study was performed in *Trifolium pratense* grown in greenhouse conditions with excrements of animals treated with ABZ, or IVM, or MOP. In order to monitor response of plants exposed to ABZ, IVM or MOP metabolites, we evaluated stress markers such as proline accumulation, protein content, changes in lipid peroxidation and the activity of oxidative enzymes.

Monitored stress markers revealed only mild or acute toxicity. No response was observed in protein content. Lipid peroxidation was suppressed after 3 days in clover plants cultivated with manure from IVM and MOP treated sheep but after 3 weeks from ABZ treated sheep. Insignificant changes were observed in H_2O_2 production. On the other hand, proline accumulation increased twice after 3 weeks when cultivated with manure from IVM treated sheep and 1.5 times from MOP treated sheep. After 6 weeks, the proline accumulation was stabilized. Acute stress was expressed by increased activity of superoxide dismutase after 3 days of cultivation with all tested anthelmintics and the activity decreased gradually until no changes were observed after 6 weeks. In overall, no chronic stress was observed.

The fodder plants can be effective in detoxification of grazing land, however the risk of increased resistance of parasites in grazing livestock should be in concern.

Supported by the grant 18-07724S Grant Agency of the Czech Republic, and by the project "Centre for Experimental Plant Biology" [CZ.02.1.01 /0.0/0.0/16_019/0000738], co-funded by ERDF.

P055
ATORVASTATIN IMPAIRS BILE FLOW IN HEALTHY MICE BUT NOT IN MICE WITH DIET-INDUCED STEATOHEPATITIS

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Bile acids (BA) play significant role in pathophysiology of non-alcoholic steatohepatitis (NASH). The present study therefore aims to evaluate modulation of bile acid homeostasis by atorvastatin, a cholesterol lowering agent that is commonly used for treatment of cardiovascular complications accompanying NASH. NASH was induced in mice by continuous 24-week Western diet (WD). At week 21, individual groups of animals were dosed with atorvastatin (20 mg/kg/day, p.o.) for 3 weeks. Atorvastatin reduced plasma concentrations of BA in chow diet fed animals together with reduced BA biliary secretion by down-regulation of the rate limiting transporter, Bsep. These changes resulted from increased fecal excretion of BA. Biochemical and histological analysis confirmed the effectiveness of WD in inducing NASH. These animals had significantly reduced bile flow because of impaired biliary secretion of BA via downregulated Bsep. Fecal excretion of BA was significantly increased in WD animals. Atorvastatin reduced liver steatosis and inflammation in NASH animals consistent with reduction of crucial fatty acid synthetic enzyme Stearoyl-CoA desaturase-1 and NF-κB proinflammatory signaling. In this group, atorvastatin did not change kinetics of total BA, but it significantly increased biliary and fecal excretion of deoxycholic acid. In conclusion, atorvastatin reduces Bsep-mediated BA biliary secretion in mice with intact liver. However, atorvastatin alleviated liver injury in NASH mice. Only minor changes in BA homeostasis in these animals indicate that protective mechanisms of atorvastatin in NASH pathology are independent of BA turnover.

The project was supported by grants Progres Q40/05, SVV 260543/2020, GAUK 3462/18, GACR 19-14497S, and by the Project INOMED CZ.02. 1.01/0.0/0.0/18_069/0010046.

P056
KEY PARAMETER EVALUATION OF ENDOCRINE-DISRUPTION BIOASSAY USING T47D CELL LINES (CXCL-TEST)

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The development of sensitive analytical methods is essential to monitor increasing anthropogenic pollution. While analytical methods usually determine the content of several target chemicals, bioassays evaluate the toxicity of the whole sample. Although the environmental concentrations of the pollutants are often very low (ng/L), they may have some toxic effects that manifest *via* several cell alterations. Among them, hormonal disruption is one of these issues. Up to now, more than 100 bioassays have been developed for the endocrine-disrupting effects of chemicals evaluation and quantification. Apart from the intrinsic advantages and drawbacks of each bioassay, these assays also often differ in sensitivity. Since the bioassays are based on a variable biological material, interlaboratory adaptation of the methods usually does not lead to satisfactory sensitivity of the test, and the assay is therefore sometimes abandoned. Parameters, such as cell line, medium, serum, and cell density are established in the original protocol; however, optimization of these key parameters is essential in order to reach the proper performance of the test in another laboratory. One of the most sensitive bioassays determining estrogenic properties of tested molecules, known as CXCL-test, was developed as an alternative method to the classical proliferation tests. It is based on the cytokine CXCL12 secretion in breast cancer cell line quantification. In the present study, we assessed the effect of key parameters on CXCL12 secretion in T47D originated from three different sources. The linear mixed model (LMM) was used for the fast evaluation of the parameters' involvement in the cell answer. It was revealed that all the tested parameters significantly modified the cell's ability to answer to estrogenic stimulation and affected both sensitivity and detection limit. This work can be used for method adaptation troubleshooting or as a methodology outline for bioassay optimization, as the optimization is crucial to achieve highly sensitive testing of estrogenic compounds.

P057
DEVELOPMENT OF BATTERY OF *IN VITRO* BIOASSAYS FOR SCREENING OF THYROID HORMONE DISRUPTIVE POTENTIAL OF CHEMICALS

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Thyroid Disrupting Chemicals (TDCs) can induce disruption of thyroid hormone (TH) signaling in human, which is proposed to be linked with many human health problems such as neurodevelopmental disorders (*e.g.* autism, attention deficit hyperactivity disorder (ADHD) and learning disabilities) in children, thyroid neoplasms, thyroid autoimmune disorders and increased cardiovascular risk due to altered lipid metabolism in adults. In the EU H2020 ERGO project, we are designing a battery of *in vitro* assays for evaluating thyroid disrupting chemicals. The design is based on adverse outcome pathway concept (AOP) addressing priority molecular initiation events (MIEs) in the thyroid hormone regulation. In this study, we have been developing bioassays for the assessment of the interference of chemicals with the prioritized MIEs. The assay set covers MIEs in TH synthesis, transport, signaling and metabolism. Thyroid peroxidase (TPO) inhibition assay was performed to evaluate the interaction of TDCs with the activity of TPO enzyme that has a strong relation to the TH synthesis. Thyroxine-transthyretin (T4-TTR) binding assay was performed to evaluate effects of TDCs on TH transport, since transthyretin is an important carrier of TH in the blood. Interaction of TDCs on TH receptor level was assessed using human cell line stably transfected with reporter gene (PZ-TR) under control of the receptor activity. For TDCs effect on metabolization of TH, aryl hydrocarbon receptor (AhR), which regulates genes implicated in chemical metabolism, was assessed using another human cell-based reporter gene assay (AZ-AhR). We are also characterizing the effects of 20 prioritized human exposure relevant model chemicals including environmental pollutants, natural compounds and pharmaceuticals on the established *in vitro* assays. A strong inhibition could be found for many chemicals in both TPO and TTR assay while some compounds significantly activated TH receptor. On the other hand, no effect of the model compounds was observed on AhR activation. Thus, the effectiveness of the three assays in the battery was shown and after further validation they could serve for hazard assessment of human exposure relevant chemicals.

The project has received funding from the EU H2020 research and innovation programme project ERGO (grant agreement No.825753).

P058
HEPATOTOXICITY OF FERN EXTRACTS

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Public interests of western countries in natural therapies and healthier lifestyle including herbs and alternative nutrient source has significantly increased over the past decades. In developing countries, fern species are used as traditional medicinal plants to treat hepatitis,

skin diseases, colds, kidney stones, intestinal parasites *etc.* Recent archeological evidence of ferns in dental calculus of the skeleton from medieval necropolis in Spain revealed the use of ferns as remedy also in Europe. However, consumption of natural products considered by many as safer than evidence-based medicine because they come from nature, can lead to fatal results. Therefore, we have explored the hepatotoxicity of twenty fern extracts commonly grown in Europe. We have used nondifferentiated and differentiated HepaRG cells for assessment of the extracts' hepatotoxicity. Five of the tested extracts proved to be toxic only in high concentrations (*e.g.* 100μg/ml) in cancerous-like non-differentiated HepaRG and three in differentiated HepaRG that resemble human hepatocytes. Furthermore, we have explored the pro-oxidant potential of selected extracts using dichlorofluorescein diacetate probe to detect any reactive oxygen species (ROS) as a potential cause of the toxicity of selected extracts. However, we found rather antioxidant properties, as only one extract caused ROS generation. Therefore, in summary our results proved fern extracts rather harmless and non-toxic with antioxidant properties warrant further investigation.

This work was supported by Ministry of Education, Youth, and Sports of the Czech Republic, project LTC17035 Inter-COST. Plant material was collected in Botanical Garden Charles University of Prague (the director Ing. Ladislav Pavlata) and in the private fern collection garden of RNDr. Libor Ekrt, Ph.D. (University of South Bohemia).

P059
EFFECTS OF PHARMACEUTICALS WITH DIFFERENT MECHANISMS OF ACTION ON FISH EMBRYOS

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The occurrence of pharmacologically active substance residues in the aquatic environment has been highly actual and commonly discussed topic over the past decades. One of the main cause of the occurrence of these substances in environment is increasing consumption along with low efficiency of their removal in surface wastewater treatment plants. Antidepressant, antibiotic, and analgetic residues are not only found in the surface waters, but their concentrations are also growing in sediments and water organism tissues. Even though are the concentrations in water environment low, their long-term effect on aquatic biota might be tremendous.

The aim of this study was to assess the impact of pharmacologically active substance residues (in particular nortriptiline, tramadol, enrofloxacin) on embryonic stages of various fish species – zebrafish (*Danio rerio*), common carp (*Cyprinus carpio*), European catfish (*Silurus glanis*), and tench (*Tinca tinca*). The experiment was carried out according to the fish embryo acute toxicity test (OECD 236) over a period of 96 hours with five different concentrations of each substance being tested. In order to evaluate the toxic effects, coagulation of fertilized eggs, lack of somite formation, lack of detachment of the tail-bud from the yolk sack, lack of heartbeat and various malformations were observed.

This research was supported by project IGA VFU Brno 208/2020/FVHE.

P060
EFFECT OF FLUCONAZOLE ON MIXED CULTURES OF PATHOGENIC YEASTS *CANDIDA ALBICANS* AND *CANDIDA GUILLIERMONDII*

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Pathogenic yeasts of the genus *Candida* pose a threat to immunocompromised individuals. In healthy people, these yeasts can be part of the normal microflora, but weakening the body can lead to endogenous or exogenous infection. Infections caused by the yeast *C. albicans* are more common but can be better treated with common antifungals. In contrast, infections caused by *C. guillermondii* do not commonly occur, but if this infection occurs, it is difficult to eliminate with a common antifungal agent, such as fluconazole. This is because it is less sensitive to this antifungal. Mixed cultures of *C. albicans* and *C. guillermondii* are rarely mentioned in the literature. This can be caused not only by their rare occurrence, but also by difficult diagnostics and difficult distinction of individual species in the microbial community. For this purpose, we designed species-specific primers that helped us to distinguish the yeast from each other by quantitative PCR (qPCR). We used the method for the determination of *C. albicans* and *C. gulliermondii* in mixed samples for the analysis of artificial evolution of their cocultures. We passaged these cocultures for 12 rounds under defined conditions, either alone or in the presence of subinhibitory concentrations of the antifungal fluconazole. After this passage, we quantified both yeasts by qPCR with species-specific primers. We found that the individual yeasts were not evenly represented in the resulting samples. *C. albicans* predominated in cultures that did not contain fluconazole. In contrast, *C. guillermondii* predominated in fluconazole samples. These results indicate a lower sensitivity of *C. guillermondii* to fluconazole compared to *C. albicans*.

This work was created with the financial support of the Faculty of Chemical Technology, University of Pardubice, student project SGS_2019_007.

P061
BUNDLES FORMED FROM SELF-ORGANIZED 1-D ANODIC TiO_2 NANOTUBES LAYERS: ASSESSMENT OF NANOTOXICITY USING HUMAN EPITHELIAL CELLS

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In recent years, society has witnessed unparalleled growth of research of various nanomaterials that has resulted in a broad spectrum of their innovative applications in various fields of medicine and industry. Current statistics suggest that there are more than 1000 products available worldwide that take advantage of nanotechnology. Titanium dioxide (TiO_2) nanoparticle is an important product for nanotechnology because of its high stability, anticorrosion and photocatalysis. It is frequently used in the cosmetics, pharmaceutical, paint, and paper industries. TiO_2 has been previously classified as biologically inert; however, our recent study revealed that small (diameter of ~6 nm) TiO_2 nanoparticles exhibit inherent cytotoxicity for mammalian cells. It has been shown that high aspect ratio (HAR) materials have a dramatic impact when in contact with living organism as compared to their 0-D forms. The present study reports on a comprehensive investigation of mechanisms of *in vitro* cytotoxicity of high aspect ratio (HAR) bundles formed from TiO_2 nanotube (TNT) layers prepared via electrochemical anodization of Ti foils. Comparative cytotoxicity studies were performed using two types of HAR TNTs with similar inner diameter of ~110 nm, differing in initial thickness of the nanotubular layer (~35 µm for TNTs-1 vs. ~10 µm for TNTs-2). Using two types of epithelial cell lines (MDA-MB-231, HEK-293), it was found that nanotoxicity is highly cell-type dependent and plausibly associates with higher membrane fluidity and decreased rigidity of cancer (MDA-MB-231) cells enabling simpler penetration of TNTs to the cell membrane towards disruption of membrane integrity and reorganization of F-actin cytoskeletal network. Upon penetration, TNTs dysregulated redox homeostasis followed by DNA fragmentation and apoptotic/necrotic cell death. In addition, both TNTs exhibited haemolytic activity and rapidly activated polarization of RAW 264.7 macrophages. The clarification of the fundamental TNTs cytotoxicity paradigm in cancer cells provide additional opportunities for a future engineering (tuning of size or surface chemistry) of highly sophisticated rational cancer nanomedicines exploiting differences in cell responses to TNTs

The financial support from ERDF "Multidisciplinary research to increase application potential of nanomaterials in agricultural practice" and Ministry of Education, Youth and Sports of the Czech Republic

P062
DOES THE ELBE RIVER CONTAMINATION BY MERCURY STILL REPRESENT A SERIOUS ENVIRONMENTAL PROBLEM?

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The aim of our research was to evaluate spatial and temporal trends in the Elbe River contamination by mercury, a toxic heavy metal representing a potential threat for aquatic organisms and humans. To do so, we analyzed available scientific data for the period of 1991–2016 about total concentrations of this element in dorsal muscle of two omnivorous fish species commonly inhabiting the stream, namely chub (*Squalius cephalus,* L.) and bream (*Abramis brama,* L.). There were significant differences among 11 sampling sites investigated with the highest total mercury concentrations observed near Čelákovice and Neratovice, that both had potential sources of contamination (nonferrous Kovohutě metalworks, and a chemical plant Spolana, respectively). Data analysis revealed, that in chub, the age of sampled fish might also contribute to them, however that was not a case of bream. Total mercury concentrations detected in chub from the heavily contaminated site downstream of Spolana Neratovice plant significantly decreased in time with 1.65 mg L^{-1} found in fish sampled in 2003 and only 0.22 mg L^{-1} observed in ones sampled 8 years later. Again, among other factors, the influence of the age of fish (which varied among relevant studies) must also be considered, but despite of that, it seems to be clear that the extensive measures given by legislation play a crucial role here. Indeed, anthropogenic emissions of mercury were previously markedly reduced by Minamata convention as well as other legal acts. In addition to that, we also calculated hazard indices (HIs) to evaluate health risks from the consumption of fish from the Elbe River. Considering obtained HI values that were far below 1, we do not expect any adverse health effects on consumers from general Czech population. As the mercury concentrations recently detected in fish have been low, even anglers who usually consume much more fish then general population seem to be safe.

Supported by the institutional research fund of the University of Veterinary and Pharmaceutical Sciences Brno (project FVHE/Večerek/ITA2020). Further support was provided by the RECETOX research infrastructure (the Czech Ministry of Education, Youth and Sports; LM2018121) and CETOCOEN EXCELLENCE Teaming 2 project (Horizon 2020; 857560 and the Czech Ministry of Education, Youth and Sports; 02.1.01/0.0/0.0 /18_046/0015975).

P063
MELOXICAM, ITS TOXICITY AND SAFETY. AN ANALYSIS OF ADVERSE REACTIONS REGISTERED IN THE 2015–2018 PERIOD

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Meloxicam is a non-steroidal anti-inflammatory drug (NSAID) used in human and veterinary medicine. When registered since 1995, it was indicated for the treatment of pain or inflammation in rheumatoid arthritis and osteoarthritis in adults and for the treatment of juvenile idiopathic arthritis. Our aim was to review the available information on the safety profile of meloxicam, to critically evaluate the data obtained, to define its position among NSAIDs and to analyze its side effects reported to the Czech State Institute for Drug Control (SÚKL) in the period between 1 January 2015 and 1 January 2019.

Over the above period, a total of 20 reports of adverse reactions related to meloxicam were registered by SÚKL, 14 of which were clearly related to its use whereas, in another 6 cases, other drugs were administered concomitantly. Of the 14 reports, the incidence of skin reactions following meloxicam administration is particularly noteworthy: 9 (64.3%) cases. Meloxicam has shown lower gastrointestinal toxicity in a number of studies compared to traditional NSAIDs. In the evaluated period, SÚKL registered 2 cases of gastric ulcer (out of a total of 20), each time associated with the simultaneous use of ibuprofen and paracetamol. In general, meloxicam is a potent NSAID with a good clinical effect. If the principles of safe pharmacotherapy are observed and potential risks taken into account, patients can make the most of its therapeutic effects to achieve pain relief.

P064
THE EFFECTS OF CYCLOSPORINE AND SCORPION VENOM (*MESOBUTHUS*: *BUTHIDAE*,SCORPION) ON SOME PATHOLOGICAL AND BIOCHEMICAL PARAMETERS

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Mesobuthus eupeus venom is known as buthidae family entered into the blood circulation and could effect on body's organs. In this study cyclosporine (as an anti-inflammatory agent) was selected to suppress inflammatory effects of the venom. *Mesobuthus* venom and cyclosporine in three doses of 10, 20 and 30 mg were selected. Biochemical features plus nitric oxide and IL-2 cytokine were measured in all groups. Also, pathological examination of liver and kidney was performed.

All the studied biochemical and also IL-2 levels were elevated remarkably after *Mesobuthus* venom injection. On the other hand, rat groups which received cyclosporine showed a significant decrease in all the studied parameters. Cyclosporine in dose of 30 mg is able to decrease inflammatory responses and it could be a suitable therapeutic drug to patients who were bitten by scorpion sting.

P065
ENVIRONMENTAL CIRCULATION AND TRANSFORMATION OF VETERINARY DRUG ALBENDAZOLE IN REAL FARM CONDITIONS

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Pharmaceuticals are nowadays considered to be hazardous environmental microcontaminants. Anthelmintics, drugs frequently used in livestock against parasitic worms (helminths), enter the environment directly via excrements in the pastures or via dung from the treated animals. However, the information about subsequent fate of anthelmintics is limited. Present study was designed to follow up the transformation and possible circulation of common anthelmintic albendazole (ABZ) in real conditions of farm and field. The sheep were treated with recommended dose of ABZ. Collected faeces were used for fertilization of field with fodder plants (clover and alfalfa), which served as a feed for sheep from different farm. Using ultrasensitive mass spectrometry, the amount of ABZ and its metabolites were analysed in all samples (dung, soil, fodder plants, ovine plasma, rumen content and faeces) collected during the experiment. Two main ABZ metabolites, ABZ-sulfoxide (anthelmintically active) and ABZ-sulfone (inactive) were detected in all samples in surprisingly high concentrations. Our results showed for the first time an undesirable permeation of ABZ metabolites from sheep excrement to the soil, thence to plants (used as feed) and subsequently to other sheep in real agricultural conditions. Because of this circulation, ecosystems can be permanently exposed to drugs, which could increase its negative impact. In addition, long-term exposure of sheep to traces of anthelmintics may lead to adaptation of helminths. For this reason, dung from treated animals (both in pastures and fields with fodder plants) causes the undesirable circulation of anthelmintics in the environment and can, *inter alia*, promote the development of drug resistance in helminths.

Supported by the Grant Agency of the Czech Republic (grant 18-07714S) and Grant Agency of Charles University (grant 1136120).

P066
THE EFFECT OF ANTHELMINTICS ON RIBWORT PLANTAIN (*PLANTAGO LANCEOLATA* L.)

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Although the usefulness and necessity of anthelmintics in veterinary medicine is unquestionable, their widespread use leads to environmental contamination and may have harmful effects on non-target species due to the abundant excretion of the parent compound and metabolites. The ability of plants to uptake, accumulate and biotransform various xenobiotics, including drugs, has been intensively studied in recent decades.

Ribwort plantain is a common meadow flower that is known for its healing effects. Certain bioactive substances – products of the secondary metabolism of this plant – are responsible for this ability. It is known that the secondary metabolism of plants varies depending on ambient conditions such as temperature or light, and also biotic or abiotic stress cause large changes. In our previous projects, we have established a biotransformation pathway for anthelmintics: albendazole, flubendazole and fenbendazole in ribwort plantain. *In vitro* cultured cells and seedlings were used for this purpose. The results showed that some enzymes important for the formation of secondary metabolites are involved in the biotransformation of anthelmintics. In our new project, we grew plantain in a greenhouse in soil with regular irrigation with a solution of fenbendazole and ivermectin at a concentration of 1 or 10 μM in order to monitor whether anthelmintics stress plants and cause changes in the production of secondary metabolites. Changes in chlorophyll content and increased intensity of lipid peroxidation in plant leaves were measured as stress markers. The concentrations of bioactive compounds: acteoside, lutein, lutein glycoside, catalpol, apigenin and aucubin were determined by HPLC.

This work was supported by Czech Science Foundation, grant No. 18-08452S

P067
EXPLORING THE ADVERSE OUTCOME PATHWAYS (AOP) BEYOND THE METABOLIC AND ENDOCRINE DISRUPTIVE EFFECTS OF NOVEL FLAME RETARDANTS

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Flame retardants are anthropogenic chemicals or mixtures of chemicals used ubiquitously in several commercial or consumer products. Following the ban of long-used polybrominated diphenyl ethers (PBDE), a wide range of novel flame retardants (nFRs) are used as a replacement. Although it has been suggested that nFRs are less persistent and less bioaccumulative than the legacy flame retardants such as PBDE, nonetheless, they have been consistently detected in increasing concentration in various environmental matrixes, human blood, urine, and breast milk. Accumulating evidence suggests that nFRs exposure may be associated with disruption of the endocrine system which has been linked with the etiology of various metabolic disorders including non-alcoholic fatty liver disease (NAFLD). NAFLD is a leading cause of chronic liver disease and has become a major concern worldwide affecting 25% of the global population. It is being characterized by hepatic steatosis, a state of uncontrolled accumulation of fats in the hepatocytes. NAFLD is a multifactorial disease and involves multiple-hit pathogenesis, including exposure to occupational and environmental chemicals.

For steatosis, AOP(s) and AOP network linking several molecular initiating events (MIEs) have been suggested. In this study, we employed an *in vitro* bioassay toolbox to assess key events in the recently proposed AOP(s) for hepatic steatosis. We screened 9 flame retardants using the model human hepatoma cell line HepG2 and assessed lipid accumulation by fluorescent neutral lipid dye (BODIPY 493/503) and expression of steatosis-associated genes by RT-qPCR. Our findings suggest that nFRs contribute to the induction of steatosis *in vitro* even at sub cytotoxic concentrations. Additional endpoints such as activation of a set of nuclear receptors and expression of associated downstream proteins are currently being examined to better understand the molecular mechanism and provide quantitative data for the follow-up development of quantitative AOP (qAOP) using computer simulations through Artificial Intelligence-based tools.

Supported by funding from the European Unions´ Horizon 2020 research and innovation Programme under grant agreements No 733032 (HBM4EU) and No 859891 (PRORISK - Marie Skłodowska-Curie European Training Network), and by the RECETOX Research Infrastructure (LM2015051 and CZ.02.1.01/0.0/0.0/16_013/0001761).

P068
AEROBIC MAMMALIAN CELL CYTOTOXICITY OF HETEROAROMATIC *N*-OXIDES: ROLE OF NAD(P)H:QUINONE OXIDOREDUCTASE (NQO1)

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Derivatives of 3-amino-1,2,4-benzotriazine-1,4-dioxide (tirapazamine) and other heteroaromatic *N*-oxides (ArN→O) are used as potential hypoxia-selective antitumor agents. Although their aerobic cytotoxicity is typically lower than under hypoxia, it may be important in the treatment of oxic tumors, and in the development of new antibacterial, antifungal and antiprotozoan agents. Aerobic cytotoxicity of ArN→O is attributed

to their redox cycling and oxidative stress, however, the roles and mechanisms of enzymatic reduction of ArN→O are poorly understood. We have shown that the cytotoxicity of ArN→O (n=9) in murine hepatoma MH22a cells increased with their single-electron reduction potential or with their reactivity (log k_{cat}/K_m) towards NADPH:cytochrome P-450 reductase (P-450R) (A. Nemeikaitė-Čėnienė *et al.* (2019) *Int. J. Molec. Sci.* 20, 4602). In order to characterize the previously observed parallel involvement of NAD(P)H:quinone oxidoreductase (NQO1) in these processes, we examined its reactions with 18 diverse ArN→O. Under aerobiosis, the formation of stable reduction products was accompanied by the oxidation of excess NADPH and formation of superoxide, which points to atypical single-electron reduction of ArN→O by NQO1 and redox cycling. The k_{cat}/K_m of compounds ranged from 500 $M^{-1}s^{-1}$ to 2.4×10^4 $M^{-1}s^{-1}$ and did not correlate with their reactivity towards P-450R and another single-electron transferring enzyme, ferredoxin reductase. The cytotoxicity of several randomly selected ArN→O was decreased by the antioxidants or dicoumarol. The cytotoxicity of ArN→O in MH22a cells was described by a multiparameter linear regression (r^2=0.829), unequivocally pointing to the involvement of NQO1 ($\Delta\log cL_{50}/\Delta\log k_{cat}/K_m$ (NQO1)=−0.608±0.246, where cL_{50} is compound concentration for 50% cell survival). Another important cytotoxicity factor is the reactivity of ArN→O towards single-electron transferring flavoenzymes like P-450R. However, there is no relationship between the cytotoxicity of ArN→O and their lipophilicity (log *D*).

Supported by the European Social Fund (Measure No. 09.33-LMT-K-712, grant No. DOTSUT-34/09.3.3-LMT-K712-01-0058).

P069
DICLOFENAC-INDUCED CYTOTOXICITY IN CARP LEUKOCYTE CULTURE

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Medicinal products enter waste- and surface-waters on an every day basis. Fish as a part of the aquatic environment are permanently exposed to these chemicals dissolved in water. The most commonly detected compounds in waters include diclofenac and other non-steroidal antiinflammatory drugs. To simulate variable environmental conditions, the aim of our study was to examine cytotoxicity of diclofenac under different temperatures of cell incubation. Destructive and cytostatic effects of diclofenac sodium salt in concentrations 0.001 µg/ml, 0.01 µg/ml, 0.1 µg/ml, 1 µg/ml, 10 µg/ml, 100 µg/ml for the carp (*Cyprinus carpio*) leukocytes in cell culture were quantified using detection of lactate dehydrogenase released from damaged cells. Leukocyte viability is essential for immune functions and any change can lead to reduction of resistance against pathogens, mainly in cold year seasons, when the immune system is naturally supressed. Because ambient temperatures significantly influence physiological processes, including the immune system, effects of diclofenac on experimental cultures were observed under different incubation temperatures (24; 27; 30 °C). After short-term exposure (12 h), diclofenac stimulates the response of leukocytes, leukocyte numbers slightly increase and the percentage of damaged cells does not exceed 6 %. Nevertheless, after medium-term exposure (24 h), the percentage of damaged cells increased from 2 to 26 % and was influenced by a combination of temperature and drug concentration conditions. After long-term exposure (48 h), mainly the cytostatic effect was evident and the percentage of leukocyte numbers decreased to 37 % in comparison with control (non-exposed) cells in dependence on the diclofenac concentration. In conclusion, diclofenac significantly reduces the number of leukocytes in culture. The most effective leukocyte resistance against cytotoxic effects was observed at the medium temperature (27°C), which is recognised as optimal cultivation temperature for carp cells. Therefore, we assume, the fluctuation of environmental temperatures from „fish-comfort temperature" could intensify diclofenac cytotoxicity effects. Our results are part of the study of multistressors such as chemicals, bacterial and/or viral agents on fish population.

P070
MERCURY IN FISH FROM RESERVOIRS IN THE MORAVA RIVER BASIN

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The World Health Organization ranks mercury among the top 10 pollutants which has negative effect on public health. The content of mercury in fish is very variable and depends on many factors. The important role has played kind of fish and its position in the food chain. Content of mercury in fish also reflected contamination of the aquatic environment because of the ability of total mercury to accumulating into living organisms. The aim of this study was to evaluated total mercury (THg) content in the muscle of three kind of fish (common bream-*Abramis brama* L.; roach-*Rutilus rutilus;* perch-*Perca fluviatilis*) which were taken from the following Czech drinking water reservoirs of Morava River Basin: Bojkovice, Boskovice, Hubenov, Karolinka, Landstejn, Ludkovice and Nova Rise. A total of 296 fish were caught during the year 2016–2017 and measured by atomic absorption spectrometry on an AMA-254 analyser. The THg in bream varied from 0.043 to 0.440 $mg.kg^{-1}$ and the highest content was observed in fish from Bojkovice, the lowest in Hubenov and it differed significantly

between sampling sites. The content of THg in muscle of roach varied from 0.025 to 0.584 mg.kg^{-1}. The highest value was determined in Landstejn and the lowest in Ludkovice. In perch was found minimal value of THg 0.073 and varied to maximum value 0.824 mg.kg^{-1}. Differences in THg content in fish muscle between sampling sites were analyzed using analysis of covariance (ANCOVA), including the sampling sites as a categorical factor and fish age as a continuous predictor. The highest content was observed in perch form Landstejn and Karolinka, the lowest in Hubenov. Total mercury content correlated positively with fish age and weight too at almost all water tanks. The correlation between age and weight was found in all individual fish species and reservoirs separately except in roach from Bojkovice and Nova Rise and perch from Hubenov. At Bojkovice, Nova Rise and Landstejn, the increased of total mercury content with age differed significantly between species and was the highest in perch.

This study was supported by ITA VFU Brno (Project No. FVHE/Vecerek/ITA2020).

P071
INHIBITORY EFFECT OF THE ENDOCRINE DISRUPTOR 17α-ETHINYLESTRADIOL ON THE ENZYME ACTIVITY OF HUMAN AND RAT CYTOCHROMES P450

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17α-ethinylestradiol (EE2) is a synthetic derivative of the endogenous estrogen 17β-estradiol. It is one of the most prescribed drugs in the world because it is found in almost all combined hormonal contraceptives, in hormone replacement therapy, in breast and prostate cancer treatment and in agricultural products for increase of livestock productivity. Due to its high estrogenic potential together with its resistance to the chemical degradation and tendency to bioaccumulate in environment, it is considered an important persistent organic pollutant.

In this study, we observed the effect of EE2 on the enzyme activities of various human cytochrome P450 isoforms and their rat orthologs by measuring the rates of specific marker reactions in respective hepatic microsomes. Using recombinant CYP isoforms expressed in Supersomes™, EE2 concentrations causing 50% inhibition (IC_{50}) of CYP activities were determined as well. The activity of all studied CYP isoforms except CYP1A2 was inhibited in the presence of EE2. In rat liver microsomes, at a concentration corresponding to the substrate concentration, EE2 caused the greatest decrease in enzyme activity of CYP2C6 (to 36%). A similar result was observed in human liver microsomes, in which EE2 at a concentration of the reaction substrate also inhibited orthologous CYP2C9 the most (to 54%). In case of rat recombinant CYP2C6, 25μM EE2 led to the undetectable enzyme activity, while IC_{50} for human recombinant CYP2C9 was approximately 40 μM. Other studied rat recombinant CYPs were also inhibited by EE2 and the IC_{50} were very similar under the experimental conditions used – for rCYP1A1 7,9 μM, rCYP2B1 9,6 μM and rCYP3A1 8,3 μM. Human rCYP1A1 and rCYP2B were less affected by EE2 than rat rCYPs and the IC_{50} were higher – for rCYP1A1 25,6 μM and rCYP2B6 60 μM. Human rCYP3A4 was inhibited the most of all isoforms and IC_{50} for EE2 was 4,5 μM at a reaction substrate concentration of 50 μM.

All CYPs in this study belong to the major biotransformation enzymes highly responsible for the metabolism of drugs and other xenobiotics, so the changes in their enzyme activities caused by EE2 might modulate the fate and the action of CYP substrates in the organisms.

P072
COLCHICINE-BODIPY CONJUGATES AS A TOOL FOR MULTIMODAL THERAPY

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A combination of treatment methods with a different mechanism of action is often used to treat a variety of diseases such as cancer. In this study, we have employed the principle of multimodal therapy approach in the development of a new series of red- and green-emitting conjugates of a photosensitizer (PS) with a cytotoxic plant alkaloid colchicine. Colchicine is an inhibitor of microtubule polymerization leading to the cell cycle arrest in the G2/M phase followed by apoptosis. As a PS, iodo-BODIPYs lately proven as very potent agents in photodynamic therapy (PDT) were used. PDT is a non-invasive method for cancer treatment based on light activation of a PS in the presence of the molecular oxygen, which generates highly reactive oxygen species, leading to cell death. Using live-cell fluorescence microscopy, we found that the newly synthesized conjugates were uptaken by MCF-7 and PC-3 cancer cells and localized in the endoplasmic reticulum. Further, we determined cytotoxicity and phototoxicity of the conjugates as well as of colchicine itself in a panel of human cancer cell lines. Any of the conjugates did not exhibit significant toxicity without illumination in cancer cells of HeLa, U-2 OS, PC-3, LNCaP, and MiaPaCa-2 up to 200 nM concentration. Nevertheless, after photoactivation, a colchicine conjugate with a red iodo-BODIPY resulted in a 50% decrease in cell proliferation already at 5 nM concentration for HeLa cells and 10 nM for U-2 OS and PC-3 cells. This derivative was also able to arrest the cell cycle in G2/M phase in HeLa cells. Such extraordinary phototoxicity and negligible dark toxicity make the newly prepared colchicine-iodo-BODIPY conjugate a suitable candidate for use in PDT and an effective tool for multimodal therapy.

The authors gratefully acknowledge the financial support of this work by MSMT No 20-SVV/2020 and by L'Oréal-UNESCO for Women in Science 2019.

P073
ASSESSMENT OF XENOBIOTIC AND NUCLEAR RECEPTOR MEDIATED ACTIVITIES OF MODEL POLYCYCLIC AROMATIC HYDROCARBONS IN HUMAN *IN VITRO* MODELS

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Polycyclic aromatic hydrocarbons (PAHs) are widely distributed environmental contaminants, which metabolized to via a set of xenobiotic-metabolizing enzymes. These enzymes are induced through activation of xenobiotic receptors, such as aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR) and pregnane X receptor (PXR). Both parent PAHs and their phase I metabolites may modulate activities of nuclear receptors linked to steroid and thyroid hormone signaling through estrogen (ER), androgen (AR), glucocorticoid (GR) and thyroid hormone (TR) receptors, and they may alter metabolism of both xenobiotics and endogenous compounds, including steroids and glucose, through CAR and PXR.

For this study, we selected six PAHs that are abundant in the environment, which can activate the AhR with an increasing efficiency in the following order: fluoranthene (Fla), pyrene (Pyr), benz[a]anthracene (BaA), benzo[a]pyrene (BaP), chrysene (Chry) and benzo[k] fluoranthene (BkF). For the measurement of activities of selected receptors, we then used a battery of luciferase reporter gene assays or, when not available, qRT-PCR detection of endogenously expressed genes.

All selected compounds supressed the AR-mediated activity, with an exception of Chry and Pyr, and they modulated the ERalpha-mediated activity. Significant effects on TRalpha and GR activities were not observed; however, some of the PAHs slightly enhanced activity of these receptors, when cells were co-treated with their respective model agonists, triiodothyronine (all compounds except for BkF) or dexamethasone (BaA, BaP and Chry). Fla, Pyr, and partially also BaP, increased the CAR-dependent CYP2B6 expression. In contrast, Chry, BaA, BaP and BkF suppressed the basal PXR-dependent CYP3A4 mRNA levels; this suppression could be linked to the AhR activation.

In conclusion, abundant environmental PAHs elicited multiple effect on nuclear receptors, which could be potentially linked with endocrine disruption, regulation of xenobiotic metabolism, or, indirectly via CAR/PXR modulations, connected with glucose and lipid metabolism

This study was supported by the Czech Science Foundation, grant no. 19-25365S.

P074
INTERSPECIES DIFFERENCES OF LIVER MICROSOMAL CYP ACTIVITIES IN ANIMALS FED BY ANTHOCYANIN RICH WHEATS

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Anthocyanins, subclass of flavonoids, are natural antioxidants causing red or even purple colour of fruits, vegetables and cereals. It is presumed that intake of anthocyanins can alleviate aging, inflammation, reduce obesity and improve eyesight. Cytochromes P450 (CYP) belong to family of monooxygenases and contribute to better excretion of xenobiotic substances from the body by making them more polar. Effects of anthocyanin rich wheats (ARW) on CYP activity were studied on three animal models: rats, rabbits and poultry. Rats and rabbits are frequent pharmacological/toxicological animal models used for prediction of human CYP activity; poultry is important source of meat as well as eggs. Liver microsomal CYP activity of these three animal models was determined with four different substrates: testosterone, chlorzoxazone, bufuralol and ethoxyresorufin. These substrates correspond to subfamily of human CYP3A, CYP2E, CYP2D and CYP1A, respectively. Content of anthocyanins in each wheat was determined by extraction of milled wheats into 1% hydrochloric acid in methanol and spectrophotometric measurement at 520 nm standardized to cyanidin 3-glucoside. Anthocyanin content in each ARW: Skorpion with blue aleurone (harvest 2016) 68,9 mg/kg, Karkulka (harvest 2016) 30,01 mg/kg and Arasajta RU 687-12 (harvest 2015) 36,66 mg/kg both with purple pericarp. Rat CYP3A activity was significantly decreased in Arasajta group. Rabbits had lowered activity of CYP3A enzyme(s) metabolizing testosterone to 70 % of control in group fed by Karkulka wheat. Poultry activity to metabolize testosterone was the highest in Karkulka group (of all studied animal groups). Chlorzoxazone metabolism was the highest in poultry fed by Karkulka wheat. The metabolism of bufuralol was in rats (all groups) 10 and 15 times higher than in rabbits and poultry, respectively. Ethoxyresorufin was metabolized by a similar rate in rats and rabbits but poultry showed less than half activity of corresponding CYP enzyme. Although the differences in CYP activities in liver microsomal samples of these models are interesting, their impact on health and quality of rat, rabbit and poultry well-being is most probably not physiologically significant.

Supported by the Internal Grant Agency of Palacký University IGA_LF_2020_022.

P075
EXPLORATION OF THE STEATOGENIC POTENTIAL OF MICROCYSTIN-LR IN 3D *IN VITRO* LIVER MODEL

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Over the past few years, there has been an increased prevalence of chronic liver diseases especially Non alcoholic Fatty Liver Disease (NAFLD) in European countries including Czech Republic. The hepatotoxic cyanobacterial toxin, Microcystin-LR (MC-LR) has been involved not only in acute human poisonings, but also implicated in the development of chronic liver diseases. Toxicity of MC-LR is dependant on its uptake by organic anion transporting polypeptides (OATPs, chiefly, OATP1B1 and OATP1B3 in liver) and NAFLD has been also linked to alteration in the expression of hepatic OATPs. The depth study of steatogenic potential of MC-LR using 2-dimensional (2D) *in vitro* cultures is difficult, since they usually lack expression of OATPs required for cellular uptake of MC-LR. Here, we used a 3D scaffold-free spheroid culture system of Human Hepatocellular Carcinoma cell line HepG2 to study the alterations in the key cellular and molecular events caused by MC-LR exposure linked to the development of liver steatosis. 2-weeks old HepG2 spheroids showed improved hepatocyte characteristic and functions (*e.g.* albumin secretion) including higher expression of OATP1B1 /1B3. Although MC-LR (≤10 µM, 48 h exposure) was not causing detectable cytotoxic effects (resazurin, ATP levels), expression of OATP1B1/1B3 was significantly reduced by 1–100 nM MC-LR, and nearly absent after 1–10 µM MC-LR treatment. This was accompanied by decrease of other hepatocyte markers (albumin, connexin32) and increased expression of billiary duct/progenitor cell marker connexin43. It suggests possible selective elimination of OATP-expressing hepatocytes by MC-LR and a major shift in the cell population towards less differentiated and/or non-parenchymal cells. This indicates a disruption of liver tissue homeostasis, contributing to the development of chronic liver diseases, such as NAFLD. As such, further to have an in-depth understanding on the development of steatosis due to MC-LR, the study will involve investigation of the key metabolic gene expression alterations, lipid accumulation and other key altered functionalities (proinflammatory cytokine analysis) linked to liver steatosis/ NAFLD development. Thus, 3D culturing techniques show a promising approach to study cell type- and tissuespecific hepatotoxic events including the underlying mechanisms linked to the development of metabolic disorders induced by environmentally-relevant toxicants, such as MC-LR.

Research is supported by the Czech Science Foundation project No. GA19-19143S.

P076
ADVANCED EXTRACTION TECHNIQUES FOR CONTAMINANTS DETERMINATION BASED ON NANOFIBERS COUPLED WITH LIQUID CHROMATOGRAPHY

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Nanofiber polymers are known to offer many attractive properties in various areas of research, industry, medicine, environmental protection, and biotechnologies. These outstanding characteristics also make them promising candidates as the extraction sorbents for sample clean-up before analysis in different areas of analytical chemistry. Sample extraction is an essential pretreatment step in many analytical procedures enabling removal of undesirable interferences, preconcentration of the analytes, decrease in the matrix effects, and particularly protecting the costly analytical devices. Nanofibrous polymers feature a good loading capacity and enhanced kinetics of the adsorption resulting from the high surface to volume ratio.

In our contribution, we will present the advancements in application of electrospun polymers for extraction of contaminants (mycotoxins, endocrine disruptors, pesticides, pharmaceutical residues) from various samples – food, soft drinks, wine, beer, milk, human serum and plasma, and river waters. Pros and cons of extraction techniques based on nanofibers coupled to liquid chromatography systems will be discussed and presented. Preparation of new composite materials consisting of polymer fibers comprising different chemistries specifically developed for extraction, coating, and functionalization of nanofibers will be introduced. Nanofibers with restricted access material functionality for direct extraction of contaminants and pharmaceuticals from proteinaceous matrix – human serum and bovine milk will be presented too.

Supported by the grant project no. 20-19297S from Grant Agency of the Czech Republic.

P077
PITFALLS IN BIOCOMPATIBILITY TESTING OF NANOMATERIALS

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Nowadays, nanomaterials are very often used not only in science but also in everyday life. Thus, people are increasingly coming into contact with nanoparticles in various fields and applications, whether as consumers, patients or workers in the factories that produce these materials.

It is therefore necessary to have reliable cytotoxicity and genotoxicity tests to verify the biocompatibility of the materials used. To this extent, we focused on very frequently used tests *in vitro*, with the emphasis on WST-1 metabolic activity test. Most cytology laboratories use it routinely and no longer think about individual steps of its technical procedure. However, in this respect there are minor nuances that may affect the test result. Especially in relation to the cultivation of cell lines. Scientific teams focusing on this issue are divided into two groups according to available scientific publications. Some teams use medium with 10 % foetal bovine serum (FBS) for cell cultivation which reflects more accurately a real biological system. Others employ FBS-free medium for cell cultivation. The reason for the use of serum-free medium is the fact that nanoparticles in the serum can be coated with proteins. This can cause a change in their interaction pattern with cell lines. In contrast, in the serum-free medium, the nanoparticles are not dispersed; they aggregate, and settle to the bottom of the culture vessel, being in more contact with the exposed cells surface. The lung carcinoma-derived cell line A549 which represents an often used model of the inhalation gateway to the organism, was selected for testing. TiO_2, with a declared particle size of 25 nm, was chosen as a reference nanomaterial for the optimization of the used method. The rate of entry of TiO_2 nanoparticles into the cells was monitored by transmission electron microscopy (TEM) in parallel with cytotoxicity assays. The study demonstrated the fundamental influence of FBS in testing the biocompatibility of TiO_2 nanoparticles. The WST-1 method gives completely different results when using both types of media with respect to ultimate test performance. Some common assays used to monitor cytotoxicity (eg. NRU) are not at all suitable for testing the biocompatibility of nanoparticles because the nanoparticles are not a solution but a suspension. It is therefore necessary to consider which tests to use when verifying the biocompatibility of nanomaterials or whether to choose serum medium, especially when some serum tests cannot be performed.

The work was supported from ERDF-Project „NANOBIO" No. CZ.02.1.01/0.0/0.0/17_ 048/0007421 and from the program PROGRESS Q40 01 of Charles University, Faculty of Medicine in Hradec Králové.

P078
SIZE AND SURFACE MODIFICATION INFLUENCE CYTOTOXICITY OF SILVER NANOPARTICLES IN GONADAL CELLS

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Despite undoubted benefits of silver nanoparticles (Ag NPs) in biomedical and pharmaceutical applications, the experimental findings have demonstrated that NPs may pose adverse effects on animal and human health, including reproductive functions. There is still lack of studies investigating the direct effects of NPs on gonadal cells. The present study aimed to investigate how size and surface modification of Ag NPs may influence their cytotoxic action in gonadal cells. The immortalized human granulosa cell line COV434 and mouse somatic Leydig TM3 cells were cultured in the presence of Ag NPs (0.1–10 μg/ml) of different size (10, 20, 100 nm) and surface modification (100 nm; polyethylene glycol, PEG; lipoic acid, LA) under basal conditions for different time periods (24, 48, 72 h). Cell viability was assessed by tetrazolium dye MTT and CytoTox-ONE Homogenous Membrane Integrity (lactate dehydrogenase, LDH) assay. Levels of reactive oxygen species (ROS) were measured using fluorescent probe 2' 7'-dichlorodihydrofluorescein diacetate (DCDHF). Exposure of COV434 and TM3 cells to Ag NPs induced a significant concentration- and time-dependent inhibition of cell viability, increased cell cytotoxicity, and elevated ROS levels. In both cell lines, smaller NPs significantly reduced cell viability even at lower concentrations and at the highest tested concentration their inhibitory effects were more pronounced than for larger NPs. The value IC50 was about 1 μg/ml in COV434 cells independent of NP size and time of exposure. On the contrary, IC50 ranged from 3 to 12 μg/ml in TM3 cells depending on NP size and time of exposure. The effect of surface modification of Ag NPs was cell-specific. In COV434 cells, surface-modified Ag NPs exhibited decreased cytotoxic potential, the IC50 ranged from 5 to 16 μg/ml depending on exposure time. In TM3 cells, surface-modified Ag NPs induced more profound inhibitory effects on cell viability compared to NPs of the same size without surface treatment, the IC50 value was about 4 μg/ml. The obtained results point to the necessity to consider the physical and chemical properties of NPs and the used experimental model when evaluating potential toxicity of NPs.

Supported by the Slovak Research and Development Agency under the contract No APVV-15-0296 and VEGA Grant 2/0162/20.

P079
LONG NON-CODING RNA (LNCRNA) KNOCKDOWN: METHODOLOGY FOR *IN-VITRO* FUNCTIONAL STUDIES OF LNCRNAS IN MULTIDRUG RESISTANCE

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Multidrug resistance (MDR) is one the major obstacles in the cancer treatment. There are many processes behind the development of MDR, like changes in drug transport, aberrant metabolism of drugs or enhanced DNA repair. Long non-coding RNAs (lncRNAs) are

transcripts longer than 200 nucleotides that are not translated into proteins. LncRNAs are important regulators in many processes, mainly in gene expression, post-transcriptional processing and epigenetic regulation. For determination a role of lncRNAs in MDR, we choose NCI/ADR-RES cell line, model of multidrug resistant ovarian cancer. Gene expression knockdown was selected for studying of possible function of lncRNA in cellular processes. Introduction of this method in our lab was performed on the lncRNA MALAT1. In determination of the most efficient gene expression knockdown of MALAT1, we tried several combinations of transfection agent (2.5 µl – 4 µl – 7 µl), antisense LNA GapmeRs (10 nM – 30 nM – 50 nM) and incubation time (48 h – 72 h). Efficiency of knockdown was subsequently determined by RT-PCR. In addition, proliferation capacity of the cells after knockdown of MALAT1 was measured.

The most efficient gene expression modulation was observed for combination of 50nM GapmeR, 4 µl or 7 µl of transfection agent and 72 h incubation time. The success of the transfection was checked by positive control. In all cases, the transfection was successful. The least effective combination was 10nM GapmeR with 2.5 µl – 4 µl – 7 µl of transfection agents at both incubation times. Efficiency of 30nM GapmeR in all tested combination was comparable. GapmeR in 30nM concentration was enough efficient in gene expression modulation compared to negative control. Cell proliferation monitoring by xCELLigence system showed as the most efficient combination 30nM GapmeR concentration with 0,6 µl of transfection agent, but efficiency of combination 30nM GapmeR with 0,4 µl was only slightly lower.

From our pilot experiments, the most cost-effective combination (30nM GapmeR concentration with 4 µl or 0,4 µl of transfection agent with 72 h incubation) was selected for further analysis of the role of candidate lncRNAs (*e.g.* MALAT1) in MDR.

Supported by the grant INTER-COST n. LTC19020, GACR n. 19-10543S, GAUK n.1074120.

P080
USE OF ALTERNATIVE METHODS IN TESTING THE TOXIC EFFECTS OF SUBSTANCES

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Animal tests are one of the tools used for assessing the environmental hazard of industrial chemicals, plant protection products, biocides, feed additives, and pharmaceuticals. However, such testing must be done with respect to the reduction, refinement, and replacement principle. According to Directive 2010/63/EU, member states of the European Union shall ensure that, wherever possible, a scientifically satisfactory method or testing strategy different from the use of animals for experiments, should be preferred. The directive protects the following animals and their developmental stages: live non-human vertebrates including independently feeding larval forms, foetal forms of mammals from the last third of their normal development, and live cephalopods. Should the earlier developmental stages used in the tests be allowed to live beyond the stage of unprotected developmental stage, the same conditions as for the above mentioned protected animals shall be applied.

One of the common methods used for substance toxicity testing is the fish embryo toxicity test. Using fish embryos is considered to be a replacement or refinement method since these developmental stages are not legislatively protected and are likely to experience less pain or suffering. Similarly, tests on amphibians embryos might be used as an alternative method in case the experiment is terminated before they become independently feeding tadpoles.

In order to get a comprehensive assessment of drug and chemical residue effects in the water environment, various representants of the aquatic food web should be used for toxicity testing. Therefore, the aim of this paper is to summarize the most common and important alternative methods that might be used for the assessment of drug and chemical substance residues in the aquatic environment.

P081
HEME-BASED OXYGEN SENSORS AS A TARGET FIGHTING AGAINST THE ANTIBIOTIC RESISTANCE AND ABUSE

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Oxygen sensors detect changes in bacterium surroundings and enable it to survive in oxygen-deficient environments. Given that the human body detects oxygen using a completely different mechanism than bacteria, the bacterial sensor system represents a very useful therapeutic target to fight bacterial resistance and the growing ineffectiveness of current antibiotics. It is the reason why the mechanism of oxygen detection by heme-based sensor proteins in bacteria has been recently intensively studied. If we can determine the exact mechanism of their function, we will be able to decommission these structures leading to bacteria disorientation to such an extent it would be easier to subsequently destroy them. Bacterial heme-based oxygen sensors are composed of at least two domains – a sensing domain (with an oxygen binding site) and a functional domain (with transcriptional or enzymatic activity). The activity of these proteins depends on whether an oxygen molecule is bound to the heme or not. Hem thus indirectly, through the gas binding, regulates many physiological functions. We have selected three model heme-based oxygen sensors for detail study of their oxygen sensing mechanisms,

namely (i) a globin-coupled histidine kinase from *Anaeromyxobacter* sp. Fw109-5 (*Af*GcHK), (ii) a heme-based oxygen sensor with diguanylate cyclase activity from *Escherichia coli* (YddV) and (iii) a direct oxygen sensor with phosphodiesterase activity from *Escherichia coli* (*Ec*DOS). Our recent results obtained by hydrogen-deuterium exchange analysis for active and inactive forms of *Af*GcHK protein suggest that the structure of the protein performs a scissor-like motion during the transition from active to inactive state (and *vice versa*). Moreover, *Af*GcHK sensing domain forms a dimer structure and the dimerization interface is necessary for the signal transduction from the sensing into the function domain. Moreover, the suggested mechanism of signal transduction depending on the sensing domain dimerization interface seems to be more general among the model heme-based oxygen sensors.

Supported by the grant 8F20011 from The Ministry of Education, Youth and Sports.

P082
PNEUMONIA AS A SIDE EFFECT ACCOMPANYING THERAPY WITH INHALED CORTICOSTEROIDS IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE – SHOULD IT REALLY BE ALWAYS A CONCERN?

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Chronic obstructive pulmonary disease (COPD) belongs the most common chronic lung diseases affecting up to 8% of the adult population. One of the main drug classes in the treatment of COPD are inhaled corticosteroids (ICSs). However, as noted in the report of the European Medicine Agency´s Pharmacovigilance Risk Assessment Committee, issued in early 2016, ICS-treated COPD patients are at increased risk for developing pneumonia.

Our research therefore focused on results of studies and meta-analyses of ICS-related toxicity, and the risk for developing pneumonia in particular, published in the last 5 years.

Recent papers confirmed the conclusions of earlier studies, particularly TORCH and INSPIRE, showing that ICS-treated patients with COPD are indeed at an increased risk for developing pneumonia. However, the individual ICSs differ in the extent of development of this particular side effect, depending as it is not only on the ICS type (fluticasone >> budesonide) but, also, on its dose (the risk increasing with increasing dose). A dose considered safe is one lower than 400 µg/day of budesonide or its equivalent.

These conclusions are supported by our analysis of ICS side effects reported to the (Czech) State Institute for Drug Control over the 2015–2018 period, with the institute receiving a total of 25 reports of side effects related to the use of 3 ICSs; specifically budesonide (14), fluticasone (10), and beclomethasone (1). While none of these reports listed pneumonia as a side effect, 5 reports related to fluticasone and 4 reports related to budesonide included, in specific patients, some of the presentations suggestive of ongoing lower airway and lung infection such as markedly worsened dyspnea (1 vs. 1) and cough (3 vs. 2) or chest pain or a burning sensation in the chest (3 vs.1).

Besides the ICS type and its dose, other factors play a role in the potential of the risk of developing ICS-induced pneumonia such as therapy duration, inhalation technique, associated diseases and related co-pharmacotherapy. As a result, ICS-treated COPD patients require active screening and adjustment of their therapy if needed.

P083
RESIDUES OF SELECTED SULPHONAMIDES, NONSTEROIDAL ANTI-INFLAMMATORY DRUGS AND ANALGESICS-ANTIPYRETICS IN SURFACE WATER OF THE ELBE RIVER BASIN (CZECH REPUBLIC)

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Occurrence of human as well as veterinary drug residues in surface water is caused by insufficient removal ability from wastewater. Drug residues disturb natural balance of water ecosystem, have a negative effects on non-target organisms and pose a significant risk for human health. The main aim of this study was to determinate a concentration of residues of 8 drugs from the sulphonamides group (sulphthiazole, sulphadiazine, sulphadimidine, sulphamethoxazole, sulphadimethoxine, sulphadoxine, sulphamerazine, sulphachlorpyridazine), 4 drugs from nonsteroidal anti-inflammatory drugs group (ibuprofen, ketoprofen, naproxen, diclofenac) and one of analgesics-antipyretics group (paracetamol /acetaminophen/) in surface water of the Elbe river basin. In total 65 samples of surface water from Elbe river basin was taken during August 2018 when the weather was constant without significant fluctuations. The analysis was performed via liquid chromatography with tandem mass spectrometry (LC-MS/MS). The results have shown numerous occurrence of sulphamethoxazole, ibuprofen, naproxen, diclofenac and paracetamol (acetaminophen). Statistically significant negative correlation between river flow rate in the monitored localities and residue concentration was found for ibuprofen, naproxen, diclofenac and paracetamol (acetaminophen).

Supported by the grant FVHE/Večerek/ITA 2020 and FVL/Illek/ITA 2020.

P084
PREVENTING THE CYTOTOXIC EFFECTS OF TYROSINE KINASE INHIBITORS ON HEALTHY BREAST CELLS THROUGH LOADING INTO THE FERRITIN NANOCAGES

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This study is focused on the antiproliferative effects of tyrosine kinase inhibitors (TKIs) loaded into the protein cage ferritin. Since many TKIs show high cytotoxicity not only towards their target, cancer cells, but also towards healthy surrounding tissue, the prevention of these adverse effects is of utmost importance. Development of nanocarriers with loaded active agents has been proven to help minimize this off-target cytotoxicity. It is also very suitable for poorly water soluble pharmaceuticals. In this study, we focused on targeting TKIs, insoluble agents, which target vascular endothelial growth factor. Preserved cytotoxic effect of chemotherapeutics after their loading to nanocarriers is one of the crucial point of the loading process. During nanoconstruct synthesis, effective loading of TKIs (lenvatinib, vandetanib) to the protein nanocage ferritin was essential. The aim was to prevent cytotoxicity towards healthy cells; nevertheless, loss of cytotoxicity towards cancer cells had to be prevented. Therefore, after successful loading process of TKIs to the ferritin it was necessary to verify maintaining the level of their cytotoxicity towards the breast cancer cell lines T-47D (PR+, ER+, HER2-, FR+, MCF-7 (PR+, ER+, HER2-, FR-), as well as healthy breast cell line HBL-100 and blood elements. For this purpose, *in vitro* tests of cytotoxicity were performed, quantifying the antiproliferative effect as well as level of apoptosis after treatment of cell lines. For evaluation of nanoconstruct internalization and their colocalization inside the cell, confocal laser scanning microscopy was used. Antiproliferative effect of nanoconstructs were tested via clonogenic assay and inhibition of cell migration via wound healing assay. The synthesized targeted ferritin nanoconstructs were able to prevent the cytotoxicity of TKIs to healthy cells while maintaining the cytotoxicity to cancer cells.

The authors gratefully acknowledge financial support from the Grant Agency of the Czech Republic (18-10251S)

P085
PERTURBATIONS OF SPHINGOLIPID METABOLISM INDUCED BY ENVIRONMENTAL CHEMICALS IN *IN VITRO* MODELS OF NEURAL CELLS

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Sphingolipids (SL) are bioactive molecules with multiple structural and functional roles in neural cells. We therefore decided to describe perturbations of SL metabolism induced by model environmental neurotoxicants (ENTs) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 3,3´-dichlorobiphenyl (PCB11) and 2,2´,4,4´,5,5´-hexachlorobiphenyl (PCB153). Changes in SL metabolism were analysed in 3 functionally distinct *in vitro* systems of neural cells: mouse neuroectodermal progenitors NE4C, human neuroblastoma cells SK-N-SH and human glial (oligodendrocytic) cells MO3.13. *In vitro* cell systems were exposed to model ENTs for 24h and their effects on mitochondrial activity (WST-1 assay), SL species composition (LC-MS/MS; 50 species) and on gene expression associated with SL metabolism (Custom PCR array) were analysed.

WST-1 assay confirmed generally low potential of tested ENTs to induce acute changes in viability of neural cell lines. On the other hand, analysis of SL species revealed differences in sensitivity of tested cell lines to model ENTs and in spectrum of deregulated SLs. In SK-N-SH cells, mainly PCB153 triggered numerous alternations of SL ratios *e.g.* increase of LacCer/HexCer or decrease of HexCer/Cer. NE4C neural progenitor were the most sensitive cell line to tested ENTs, with PCB11 inducing the majority of detected alternation of SL ratios in these cells. Additionally, all tested compounds induced remarkable and uniform decrease in ceramide-1-phosphate levels in NE4C. Alternations of SL composition in oligodendrocytic precursors MO3.13 were observed preferentially for PCB153. Custom universal probe library (UPL) PCR array (120 genes) analysis of gene expression associated with SL metabolism revealed, that changes in SL composition are only partially reflected in SL-related transcriptome of exposed cells and correspond rather inversely with observed alterations of SL species levels, probably as a result of adaptive feedback loop to SL imbalance.

Study supported by the Czech Ministry of Education, Youth and Sports, project „FIT“, no.CZ.02.1.01/0.0/15_003/0000495.

P086
ADVERSE DEVELOPMENTAL EFFECTS OF RETINOID COMPOUNDS COMMONLY PRODUCED BY CYANOBACTERIA

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Cyanobacterial water blooms and their production of diverse bioactive compounds have been linked with adverse effects on exposed organisms and potential health risks. Among detected compounds produced by cyanobacteria belong retinoid-like compounds, but there is little information on their production, levels in the environment or potential effects and risks associated with their occurrence. Retinoids detected in the environment include all-trans retinoic acid (ATRA),

9/13-cis retinoic acid (RA), 4OH-RA, retinal (RAL) or 4keto-RAL as well as some novel metabolites, such as 5,6epoxy-RA or 4keto-ATRA. A few of these are known as strong teratogens. Disruption of the retinoid signalling pathway can have fatal consequences as it regulates crucial processes during the early development of vertebrates such as formation of nervous system. Our studies employed luciferase reporter cell lines for the characterization of the retinoid and thyroid receptor disrupting potencies of the individualretinoids that were identified in field studies. *In vitro* potencies were compared with their potency to cause teratogenicity in vivo using zebrafish (Danio rerio) embryo toxicity test (zFET). The results document the ability and potency of the compounds produced by cyanobacteria and commonly detected in the environment to interfere with retinoid signalling and provide a unique comparison between the responses in individual specific cell-lines and apical effects in vivo. Effective in vivo concentrations for single compounds are in nM range, which is lower than the levels of individual retinoids detected in the environment. Relative potencies of the retinoids determined in the *in vitro* assays showed relatively good predictability towards their in vivo potency and their ability to disrupt the retinoid signalling pathway and early development at very low concentrations.

This work was supported by the Czech Science Foundation project No.18-15199S.

P087
MODULATION OF CELL VIABILITY AND MIGRATION OF BREAST CARCINOMA CELL LINES BY 7-KETOCHOLESTEROL

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Breast cancer is the most abundant cancerous disease among women worldwide. Patients with estrogen-receptor positive tumors are usually treated with hormonal therapy, one of the most prescribed drugs is tamoxifen. However, *de novo* or acquired resistance to tamoxifen remains a big complication of this treatment. Oxysterols are oxidized derivatives of cholesterol, which are intensively studied in connection with many pathological conditions, including cancer. They are interesting targets for cancer research, since they play roles in cell proliferation, apoptosis, migration or carcinogenesis. Several *in vitro* studies also showed that the presence of different oxysterols may modulate cellular response to various chemotherapeutics, such as doxorubicin, 5-fluorouracil, or cisplatin.

In the present study, we analyzed the role of 7-ketocholesterol in breast carcinoma cell lines. According to our previous results, plasma level of 7-ketocholesterol was higher in breast carcinoma patients after surgery and beginning of the therapy. Therefore, we aimed to examine the role of 7-ketocholesterol in cell viability modulation and the potential role in cellular response to tamoxifen treatment. For these analyses, we used both estrogen-receptor positive (MCF7 and T47D) and negative (BT-20) breast carcinoma cell lines. All lines were incubated with tamoxifen, 7-ketocholesterol or their combination and the viability of cells was analyzed by CellTiter-Blue® Cell Viability Assay. The IC_{50} values were determined after 72 hours of incubation. Co-incubation of cells with tamoxifen and 7-ketocholesterol caused changes in IC_{50} value for tamoxifen in two cell lines – IC_{50} value was higher in MCF7 cells and oppositely, the value was lower in estrogen-receptor negative line BT-20. The difference in T47D cells was not statistically significant. Next, we examined if the presence of 7-ketocholesterol modulates the migration potential of breast cancer lines. For this purpose, the xCELLigence system and CIM-plates 16 were used. In this analysis, we found higher migration potential in cells in the presence of 7-ketocholesterol.

Taken together, 7-ketocholesterol was shown as important player in modulation of breast cancer cell behavior, which makes 7-ketocholesterol an interesting candidate for future studies.

Supported by the Czech Health Research Council, project no. 17-28470A, Czech Science Foundation project no. 19-03063S and Charles University project no. UNCE/MED/006.

P088
INTERACTION OF SOME CYCLIN-DEPENDENT KINASE INHIBITORS – BPA-302, BP-21 AND BP-117 WITH HUMAN LIVER MICROSOMAL CYPS

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Cytochromes P450 (CYPs) are major enzymes of phase I metabolism of xenobiotics. These hemoproteins primarily convert wide range of substrates to more polar products which are easier excreted from the body. Blocking the activity of these enzymes can cause unwanted side effects due to accumulation of non-metabolised drugs. Cytokinins are group of phytohormones that are involved in many processes in plants. These processes including *e.g.* growthing, differentiation and leaf senescence. However, they also have various activities in animals and humans. Three cytokinin derivates, resp. cyclin-dependent kinase inhibitors (CDKi), namely BPA-302, BP-21 and BP-117, were tested for their potential to inhibit activities of human liver microsomal cytochromes P450 (CYP) *in vitro*. All activities (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4) were determined according to established protocols. The most affected

enzyme was CYP2C19. Its activity dropped to 22 % of its original value by BPA-302, to 13 % by BP-21 and to 6 % by BP-117 at the highest concentration (250 µmol/l). The results suggest that the metabolism of concomitantly administered drugs should not be significantly affected at lower doses. On the other hand, a possibility of this effect should be considered when higher doses and concentrations are used.

Supported by the grant IGA UPOL (IGA_LF_2018_011, IGA_LF_2019_011, IGA_LF_2020_009).

P089
COMPARISON OF COMMON ASSAYS FOR THE EVALUATION OF THE TOXICITY OF *MICROCYSTIS AERUGINOSA*

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The proliferation of cyanobacteria and algae in reservoirs leads to the formation of algal blooms, which have negative impacts on the ecosystem. One of the issues is the production of toxic secondary metabolites by these organisms. Cyanobacteria alone produce hundreds of different compounds, rendering the analytical screenings of individual metabolites infeasible. When assessing the risks of cyanobacterial species or algal blooms, toxicological assays are therefore preferred. In this work, common bioassays were compared in order to select the most sensitive and most suitable assay for such determinations. The assays were evaluated using a sample of cellular algal organic matter of *Microcystis aeruginosa*, one of the most common toxin producers in fresh waters. The results were expressed per mg of dissolved organic carbon (DOC) in the organic matter. The most sensitive assay was cytotoxicity determination using the rainbow trout cell lines RTgill-W1, RTL-W1, and RTG-2 with EC_{50} values of 0.48 ± 0.02, 0.80 ± 0.07, and 1.2 ± 0.1 mg_{DOC}/L, respectively, as determined by the cells' uptake of Neutral Red. Toxicity was also recorded after the exposure of the freshwater crustacean *Thamnocephalus platyurus* (LC_{50}=20 ± 1 mg_{DOC}/L) and the vascular plant *Lepidium sativum* (IC_{50}=241 ± 13 mg_{DOC}/L). Conversely, the growth of the alga *Desmodesmus subspicatus* was stimulated in the presence of the organic matter of *M. aeruginosa*. No effect was observed in bacteria or yeasts. Furthermore, the concentration of the most common cyanotoxins (six microcystins, anatoxin-a, cylindrospermopsin, and nodularin) in the organic matter was determined by liquid chromatography - tandem mass spectrometry. A mixture of the identified cyanotoxins was prepared in corresponding concentrations; however, the toxins did not induce response in the rainbow trout cell lines or in *T. platyurus*, thus confirming the necessity of performing ecotoxicological assays in addition to analytical screenings. The unknown compounds that are the cause of the toxicity of *M. aeruginosa* should be studied.

This study was supported by the Czech Science Foundation [Grant No. GA18-14445S].

P090
BIOTRANSFORMATION OF IVERMECTIN AND FENBENDAZOLE IN SOYBEAN (*GLYCINE MAX*)

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Various drugs have become one of the most common types of contaminants. Veterinary drugs regularly used in livestock enter the environment directly via excrements on the pastures or via manure from treated animals applied to the fields. Plants could uptake, translocate and metabolize pharmaceuticals and/or accumulate them or their metabolites in different tissues, including fruits. This accumulation poses a potential risk to human health. Anthelmintic drugs are widely used to treat parasitic diseases in livestock. The macrocyclic lactone ivermectin (IVM) and a broad-spectrum benzimidazole drug fenbendazole (FBZ) are frequently used in animal therapy. Both anthelmintics had significant phytotoxic effects; IVM phytotoxicity to *Sinapis alba* was revealed, FBZ increased the concentration of the plant stress marker proline in *Plantago lanceolata*. However, the study of the presence and effects of anti-parasitic drugs and their metabolites in crop plants has been largely neglected. In our study, the uptake, distribution, and metabolism of IVM and FBZ in soybean (*Glycine max*), plants which are grown and consumed world-wide, was studied. The soybean plants cultivated in a greenhouse were used for this purpose. The metabolites were detected and identified using the UHPLC-MS/MS technique. Our results showed the uptake of IVM by roots and its translocation to leaves. Four IVM metabolites were detected in the roots and one in the leaves of soybean plants. Uptake of FBZ to all tissues (roots, leaves, pods and seeds) was proved. FBZ was extensively metabolized in soybean, 26 metabolites were found in the plant, 13 of them in beans. As FBZ and its metabolites were found in beans, the content of individual isoflavones in beans, the food relevant part of the soybean plant, was also studied. FBZ significantly decreased the content of glycosides of isoflavones (daidzin, glycitin, and genistin) but not the aglycones (daidzein, glycitein, and genistein). In conclusion, manure containing anthelmintics and their metabolites poses a significant risk because these drugs can enter food consumed by humans. In addition, the presence of these drugs in plants can affect plant metabolism,

including the production of isoflavones, which represent pharmaceutically important compounds.

This work was supported by the Czech Science Foundation, grant No. 18-08452S.

P091
BIOANALYTICAL ASSESSMENTS OF RIVER WATER QUALITY IN AN AREA AFFECTED BY UNTREATED WASTEWATER USING PASSIVE SAMPLING AND A BATTERY OF *IN VITRO* BIOASSAYS

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Complex mixtures of contaminants from multiple sources, including agriculture, industry or wastewater enter aquatic environments and might pose hazards or risks to humans or wildlife. Targeted chemical analyses of a few priority substances provide only limited information about the toxicological profile of the examined water sample. In this study, effect-based approach with focus on endocrine disruptive potencies was applied to assess water quality in the River Bosna, in Bosnia and Herzegovina, which is affected by un- or purely treated wastewater. River water samples were collected at 10 sites along the Bosna River by use of passive sampling. The combination of semipermeable membrane devices (SPMDs) and polar organic chemical integrative samplers (POCIS) enabled sampling of a broad range of contaminants from non-polar (polycyclic aromatic hydrocarbons, polychlorinated biphenyls and organochlorine pesticides) to polar compounds (pesticides, pharmaceuticals and hormones), which were determined by use of GC-MS and LC-MS (MS). *In vitro*, cell-based bioassays aimed at the (anti)androgenic, estrogenic and dioxin-like potencies of extracts of the samplers. Cumulative concentrations of organic micropollutants decreased downstream from the city of Sarajevo, which was identified as the major source of organic pollution in the area. Responses in bioassays were observed for samples from all sites whereas dioxin-like and estrogenic potencies were the most frequently detected effects in SPMD and POCIS extracts, respectively. In general, estrogenicity could be well explained by analysis of target estrogens, while the drivers of the other observed effects remained largely unknown. Profiling of hazard quotients based on observed endocrine potencies identified two sites downstream of Sarajevo as hotspots while sites further downstream showed fewer and lower effects due to gradual dilution of polar organic micropollutants. Risk assessment of detected compounds revealed, that 7 compounds (diazinon, diclofenac, 17β-estradiol, estrone, benzo[k]fluoranthene, fluoranthene and benzo[k]fluoranthene) might pose risks to aquatic biota in the Bosna River as their concentrations exceeded the Predicted no effect concentration (PNEC) values. The study brings unique results of bioanalytical assessment of water quality in a region with an insufficient wastewater treatment infrastructure

The study was supported by the EDA-EMERGE project (FP7-PEOPLE-2011-ITN, grant agreement number 290100), SOLUTIONS project funded by the European Union Seventh Framework Programme (FP7, grant agreement no. 603437) and EXPRO Project (GAČR GX20-04676X).

P092
AZURIN: A MODEL PROTEIN TO STUDY ELECTRON TRANSFER PROCESSES

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Electron transfer (ET) is a crucial process for every living organism and occurs in many biochemical pathways. The ET processes are usually utilized during the first step of xenobiotic metabolism resulting in redox reactions which introduce/alter a functional group in xenobiotic skeleton. Study of ET is also a crucial field of modern structural and functional proteomics, and model proteins like azurin from *P. aeruginosa* are utilized in mentioned experiments. This small cupredoxine exhibits absorbance maximum at 630 nm (A_{630}) in 2+ redox state of the central Cu atom. During its reduction to 1+ state the A_{630} value decreases allowing UV-VIS detection of ET reaction.

To study ET processes in proteins during the first phase of xenobiotics metabolism (*e.g.* P450 or cytochrome b_5), we have employed azurin as a model to prove our experimental approach. We have introduced a structural photoinducible analogue of canonical amino acid Met – L-2-amino-5,5-azi-hexanoic acid (photo-Met) – into its sequence. Using previously optimized protocols for recombinant expression in *E. coli* B834 we have inserted photo-Met into two types of azurin: wild-type (WT) azurin and Az2W mutant where two adjacent W residues with confirmed role in electron hopping across protein-protein interface are present. The incorporation percentage of photo-Met in analyzed samples was determined *via* MALDI-TOF MS and reached values in range of 10–20% for Az2W mutant and 40–50% for WT, respectively. Four different concentrations of protein (in range of 5–180 μM) were analyzed and after exposure to intense UV light the results were evaluated *via* UV-VIS spectroscopy and SDS-PAGE. The UV-VIS revealed that A_{630} value decreased for both studied proteins and an additional protein band was observed on SDS-PAGE corresponding to covalent dimer.

We have observed higher dimerization yield in Az2W mutant compared to wild-type azurin and our findings support the role of two additional W residues on the interacting surface (formed by a β-sheet close to

$Cu^{1+/2+}$ center) not only during ET, but also in azurin oligomerization.

The project was supported by Charles University (GAUK n. 1538119) and Grant agency of Czech Republic (20-28126S).

P093
DO BISPHENOLS INFLUENCE BLOOD PRESSURE AFTER ACUTE EXPOSURE?

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Bisphenol A and its structural analogues are environmental contaminants found in daily use products. These compounds can interfere with the physiological role of endocrine receptors and hence cause harmful effects on endocrine and subsequently cardiovascular systems. Recent epidemiological studies have shown that higher urinary BPA concentration in humans is associated with higher incidence of several cardiovascular diseases. Nevertheless, studies describing acute effect of bisphenols on blood pressure are ambiguous. Therefore, in this experimental study bisphenol A and its three structural analogues (bisphenol F, bisphenol S and bisphenol AF) were tested for their potential effect on arterial blood pressure and hemodynamics *ex vivo* and *in vivo*. *Ex vivo* vasodilation/constriction experiments were performed on isolated aorta rings from Wistar Han. *In vivo* experiments analysed arterial blood pressure and parameters of cardiac contractility/relaxation by insertion of a pressure-volume catheter into left ventricle and blood pressure transducer into the *arteria iliaca com. sin.* in Wistar Han rats under urethan anesthesia. Three *i.v.* doses were selected. The first two (0.005 and 0.05 mg/kg) represented a real exposition and were applied as solution in saline. The highest administered dose (2.5 mg/kg) accounting for a toxic dose, was dissolved in solution containing 65% saline, 30% PEG 300 and 5% DMSO. *Ex vivo* experiments showed ability to cause the relaxation of blood vessels in the case of three compounds (bisphenol A, F and AF) with bisphenol AF being the most active. *In vivo* experiments did not fully confirm the results obtained from ex vivo experiments. Nevertheless, some impact was found also *in vivo* after the bisphenol administration. Although some bisphenols were able to potently relax isolated rat vessels, their impact *in vivo* on arterial blood pressure was not confirmed or was relatively low

Supported by the grant 1484119 C from the Grant agency of the Charles University.

P094
THE ROLE OF INTESTINAL MICROBIOME METABOLITES IN REGULATION OF LIPID AND XENOBIOTIC METABOLISM IN COLON CELL MODEL

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Short-chain fatty acids (acetate, propionate, butyrate), secondary bile acids or tryptophan degradation products (indoles) are among the intestinal microbiome metabolites, which are known to play various roles in the control of fate and functions of intestinal epithelial cells. Recently, some of these metabolites have been shown to act as ligands of the aryl hydrocarbon receptor (AhR). The AhR has been for a long time considered to be an important xenobiotic sensor, which regulates the expression of xenobiotic metabolizing enzymes. However, the AhR plays also multiple physiological roles unrelated to drug metabolism, including regulation of immune responses, organ development, metabolic disease, *etc.* Presently, little is known about the impact of endogenous AhR ligands on lipid metabolism within host cells, although several recent studies suggest that AhR or its toxic metabolites may regulate expression of enzymes and transporters involved in fatty acid metabolism. Although xenobiotics, and some short chain fatty acids, have been reported to modulate intestinal lipid metabolism, no data are currently available for the effects of indole-based compounds, which are major products of gut microbiome. In this study, we employed HT-29 human colon adenocarcinoma cells, with both wild-type and AhR knockout phenotype, in order to analyze the impact of selected microbiota metabolites on activation of the AhR- dependent signaling and the potential contribution of the AhR to the regulation of lipid metabolism. We combined butyrate, the most effective short chain fatty acid, with five different tryptophan metabolism products – indole, tryptamine, indole-3-acetamide, indole-3-pyruvate and indole-3-acrylate, which have been identified as the AhR ligands. Our data show that the tested tryptophan metabolites, in particular when combined with butyrate, may have a significant impact not only on the AhR-dependent signaling, but also on several lipid metabolism related genes, in a compound-specific manner. This indicates that combinatory effects of microbial metabolites could have a significant impact on both xenobiotic and lipid metabolism in colon epithelial cells.

Supported by the grant 19-00236S from the Czech Science Foundation.

P095
LC-MS/MS ANALYSIS OF THE SERTRALINE RESIDUES CONTAINED IN THE TISSUES OF RAINBOW TROUT REARED IN MODEL EXPERIMENTAL CONDITIONS

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The growing consumption of pharmaceuticals in the human population and concurrently the insufficient efficiency of their elimination in waste water has a long-term negative impact on the environment of aquatic ecosystems, including the organisms that inhabit them. A significant contributor is the consumption of antidepressants from the SSRI group, which corresponds to their increasing concentration in the environment. The aim of this work was to determine if antidepressant sertraline is able to be stored in fish organisms and to evaluate the content of residues in various body tissues. Rainbow trout (*Oncorhynchuss mykkis)* was selected as the test organism and was artificially exposed to the antidepressant for 1 month (concentrations 0; 4.2; 44 and 400 ng/g sertraline in the feed). Liver, kidney, brain and muscle tissue biopsies samples were taken for analysis. High resolution (HR) LC-MS / MS was used for the analysis. From the collected data, a statistically significant amount of residues was found in the liver, which is the primary site of metabolism. In contrast, the incidence of residues in muscle tissue was low, which is favorable from the point of view of fish meat consumption by humans.

This work was funded by the ERDF/ESF "Profish" [no. CZ.02.1.01/0.0/16_019/0000869, Czech Republic].

P096
HEME SENSOR PROTEINS AS POTENTIAL BIOMARKERS OF OXIDATIVE STRESS CAUSED BY IONIZING RADIATION

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Ionizing radiation is generally cytotoxic. Oxidative stress induced by ionizing radiation is one of the main reasons for this cytotoxicity. It generates free radicals that damage essential biomolecules leading to acute (acute radiation syndrome, death) or chronic (malignant processes, sterility, *etc.*) consequences. Although there has been done significant development in the field of retrospective biodosimetry recently, there is still not any method fast enough for estimating the dose of ionizing radiation to which the organism was exposed. Incidents like the Fukushima nuclear accident, Japan or the explosion near Nyonoksa, Russia prove that, despite the security precautions, the development of such a method is necessary. Our research is focused on the explanation of mechanisms by which eukaryotic heme sensor proteins respond to oxidative stress induced in cells by ionizing radiation, especially on the role of heme in these processes. We are interested in the interaction of heme and its ligands with the model sensors, heme iron state and cysteine thiolate groups oxidation states in these sensors. Three eukaryotic heme sensor proteins were selected as possible biodosimetric markers:

(i) heme-regulated inhibitor (HRI). It is a kinase of eukaryotic initiation factor 2α and its activity depends on heme concentration. This enzyme is active only in the absence of heme. Binding of heme to HRI causes inhibition of its enzymatic activity.

(ii) tumor suppressor p53. The physiological function of the p53 protein is to regulate transcription of many genes. More recently, a preliminary study stating that the p53 protein is able to interact with heme molecule has been published [Shen J. et al 2014. Cell Rep. 7:180-193].

(iii) the leucine zipper transcription factor Bach1 (acronym for BTB domain and CNC homolog 1). Heme regulates Bach1 transcriptional activity (its DNA binding affinity). It acts as repressor of „enhancers" of hemoxygenase 1 gene. In the case of heme excess, the transcription is activated.

The application of heme sensor proteins as biomarkers of oxidative stress induced by ionizing radiation will be discussed. The results will also shed more light on the mechanism of heme sensor proteins signal transduction as well as on their general properties.

Supported by the grant 158120 from the Grant Agency of Charles University.

P097
ARYL HYDROCARBON RECEPTOR (AHR) MODULATES THE INFLAMMATORY RESPONSE IN HUMAN LUNG ALVEOLAR TYPE II CELLS MODEL

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The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor involved in the metabolism and bioactivation of carcinogens. The ligand-activated AhR translocates to the nucleus where it forms a dimer with the AhR nuclear translocator (ARNT), which in turn binds to xenobiotic response elements in the promoter regions of target genes *e.g. CYP1A1*. In addition to its well-known role in detoxification/bioactivation processes, there is evidence that the AhR is involved in

the modulation of inflammation response. At present, there is only a limited information available regarding the responsible mechanisms of this AhR/inflammation crosstalk within lung alveolar type II (AEII) cells. In this study, we used a human model of AEII cells, lung adenocarcinoma A549 cell line. We exposed both wild type (WT) and AhR knockout (AhR KO) A549 variants, to pro-inflammatory cytokines (such as IL-1β and TNFα) as inflammation inducers, in order to study the role of AhR in the modulation of inflammatory responses (production of cytokines, chemokines and prostaglandins) and to examine the possible mechanisms underlying this role of the AhR. We found that COX2, one of the main enzymes in the conversion of arachidonic acid (AA) to prostaglandins, is markedly upregulated in A549 AhR KO in comparison with WT cells, when treated with IL-1β. Likewise, we found that the concentrations of prostaglandins and proinflammatory cytokines released into the culture medium, such as PGE_2, IL-8 and IL-6, was higher in A549 AhR KO cells. Accordingly, the induction of *PTGS2*, *TNF*, *PTGES*, *CXCL8* and *IL6* genes, was higher in the AhR KO cells in comparison with WT cells. One of the main pathways involved in the inflammatory response is NF-κB. Therefore, we assessed the active state of the principal regulators of NF-κB activity by using phosphospecific antibodies. Here, we found that the both IKKα/β and their target, IKBα, are more efficiently phosphorylated in AhR KO cells. In conclusion, these results suggest that AhR may act as a negative regulator of the inflammatory process in human AEII cells, with NF-κB pathway being the major mechanism responsible for this modulation of inflammatory responses.

Supported by grant no. 18-00145S from the Czech Science Foundation and The National Council of Science and Technology (CONACYT), MEXICO.

P098
ENDOCRINE DISRUPTIVE POTENTIAL OF INDOOR DUST SAMPLES FROM DIFFERENT ENVIRONMENTS

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Population in industrial areas tends to spend the major part of life indoors *i.e.* in homes, schools, workplaces, vehicles, shops, restaurants, *etc.* This behaviour puts us in close and constant contact with chemicals that are released by abrasion or volatilization from several sources *e.g.* electronics, furniture, heating systems *etc.* These compounds once released into these microenvironments can be absorbed and exert adverse effects on human health. The current work has intended to elucidate the endocrine disruption (ED) potential associated with the exposure to the complex mixture of pollutants present in indoor dust. To address the variety of indoor microenvironments, the dust samples were collected from different types of indoor spaces including old and new houses, cars, offices, schools, kindergartens, and public spaces in the Czech Republic. Afterwards, the samples were extracted with methanol or hexane: acetone to obtain two fractions of chemicals differing in their polarity. The ED potential of the extracts was assessed using a set of *in vitro* bioassays addressing activity connected with estrogen, aryl hydrocarbon and thyroid hormone receptors (ER, AhR, and TR, respectively). The assays were based on human cell lines transfected with luciferase reporter gene under transcription control of respective receptor. The data confirmed that there are significant ED potentials in the analyzed samples with different effects in every environment and method of extraction. The data from the biological assays will be interlinked with extensive chemical analyses to identify the effect-drivers in the pollutant mixtures. The responses assessed in the current study have shown that compounds present in indoor dust may contribute to ED in humans. Elucidating the effects and characterizing the toxic compounds in the complex mixture may represent an important step in the risk assessment of the human exposome.

Supported by the grant 19-20479S from Grant Agency of the Czech Republic and EU H2020 research and innovation programme project ERGO (grant agreement No.825753).

P099
EFFECTS OF MATERNAL DEPRESSION AND/OR TREATMENT WITH ANTIDEPRESSANT MIRTAZAPINE DURING PREGNANCY AND LACTATION ON THE HEALTH OF MOTHER'S AND NEUROBEHAVIORAL DEVELOPMENT OF RAT OFFSPRING

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Depression is one of the most prevalent forms of mental illness and stress-related disorders, such anxiety and depression, are the most common complications during pregnancy. Suffering from depression during pregnancy often represent a key point of ceasing anti-depressant treatment, that could end up in serious health problems for both the mother and the offspring. Antidepressants cross the placenta and are excreted into the breast milk, so treatment with antidepressants during this critical period can interfere with brain neurodevelopment of the fetus and/or neonate, leading in turn to long-term neurobehavioral changes. The debate resides in treating or not treating maternal depression, as both may result in serious consequences for the mother and child. Several clinical and experimental studies showed that SSRIs drugs, considered the antidepressants of choice during pregnancy, can have adverse effects on fetal and neonatal development. Nowadays research is focused on new generation of antidepressants, such mirtazapine, a

noradrenaline and selective serotonin reuptake antidepressant (NaSSA). Mirtazapine acts at central α2 inhibitory autoreceptors and heteroreceptors, enhancing the serotonergic and noradrenergic activity. It has a unique pharmacological profile, with a suggested faster onset of action and greater efficacy compared to other antidepressants. However, knowledge on the effects of this compound on fetal and postnatal development is limited. Our aim was to study the effects of maternal depression and/or antidepressant mirtazapine treatment on health of pregnant and lactating rat and neurobehavioral development of their offspring. Basic reproductive variables and results from behavioral investigations (anxiety-, depression-like behavior and cognitive abilities) will be presented.

This study was supported by the grant VEGA 2/0124/19.

P100
ENVIRONMENTAL STRESSORS AND LIPID DROPLETS ACCUMULATION: A SYSTEMATIC REVIEW

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A systematic review (SR) is a 21st-century tool in the era of evidence-based toxicology to critically asses, collect and report the data from individual studies. In this study, we performed a review following a variety of steps which are (a) problem formulation, (b) protocol development, (c) identification and selection of studies(d) critical appraisal of the selected studies (quality, and quantity assessment), and (e) data synthesis and reporting including gaps identification. Lipid droplets are the understudied cytosolic organelles involved in many pathological conditions such as Non-alcoholic fatty liver diseases (NAFLD). Exposure to many environmental stressors can result in the accumulation/disruption of the lipid droplets leading to a variety of adverse outcomes. In this study, we focused on lipid droplets as a target of the effects of environmental stressors. Firstly, we looked up in four databases (PubMed, Web of Science, SCOPUS, Ovoid) using a pre-defined systematic search strategy followed by uploading the articles on the online tool Rayyan QCRI to accelerate the screening of articles. The included studies assessed the effects of environmental stressors in humans, *in vitro* or *in vivo* mammalian models leading to the accumulation/disruption of the lipid droplets. The included studies were individually appraised for their risk of bias/ quality, *in vitro*, and *in vivo* studies using SciRAP (Science in Risk Assessment and Policy) and epidemiological studies using OHAT-based risk of bias rating tools. All the steps in this review were individually carried out by two reviewers to avoid any bias. In this review, we came across many environmental stressors including microcystin-LR (MC-LR), a toxin produced by cyanobacteria. MC-LR will serve as an example of an environmental stressor causing adverse outcomes by interactions with lipid droplets. MC-LR is known to be a potent hepatotoxin inducing acute and chronic liver toxicity *in vivo* and *in vitro*. Recently, MC-LR was associated with chronic liver diseases, possibly through lipid accumulation in the liver. Thus, exposure to the environmental stressors could lead to the accumulation of lipid droplets, which could further result in chronic diseases like non-alcohol fatty liver diseases.

Supported by the Czech Science Foundation project No. GA19-19143S.

P101
SENSITIVITY OF ZEBRAFISH (*DANIO RERIO*) EMBRYOS TO HOSPITAL EFFLUENT COMPARED TO *DAPHNIA MAGNA* AND *ALIIVIBRIO FISCHERI*

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The Fish Embryo Acute Toxicity (FET) Test was adopted by the Organisation for Economic Co-operation and Development as OECD TG 236 in 2013. The test has been designed to determine acute toxicity of chemicals on embryonic stages of fish and proposed as an alternative method to the Fish Acute Toxicity Test (OECD TG 203). In recent years fish embryos were used not only in the assessment of toxicity of chemicals but also for environmental and wastewater samples. In our study we investigated the acute toxicity of treated wastewater from seven hospitals in the Czech Republic. Our main purpose was to compare the suitability and sensitivity of *Danio rerio* embryos with the sensitivity of two other aquatic organisms commonly used for wastewater testing – *Daphnia magna* and *Aliivibrio fischeri*. For the aim of this study, in addition to the lethal endpoints of FET test, sublethal effects such as delayed heartbeat, lack of blood circulation, pericardial and yolk sac edema, spinal curvature and pigmentation failures were evaluated. The comparison of three species demonstrated certain differences in reaction to the acute toxicity of the samples. The inclusion of sublethal endpoints improved the sensitivity of the FET test. Based on our results, the FET test, especially with the addition of sublethal effects evaluation, can be considered as a sufficiently sensitive and useful additional tool for ecotoxicity testing of the acute toxicity potential of hospital effluents.

Supported from ERDF/ESF project „International competitiveness of NIPH in research, development and education in alternative toxicological methods" (No. CZ.02.1.01/0.0/0.0/16_019/0000860).

P102
EFFECT OF COMBINATED PARP-INHIBITOR AND CHEMOTHERAPY TREATMENT IN BRCA WILD-TYPE AND BRCA2 DEFICIENT HUMAN PANCREATIC CANCER CELLS

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Clinical data have shown that patients with ovarian and breast cancer with *BRCA* deficiency have better and durable response to PARP-inhibitors and influence the chemosensivity to chemotherapy. The aim of this *in vitro* study was to evaluate the chemosensitivity of human PDAC cell lines to PARP-inhibitors, and its combination with various cytostatic agents with regard to their *BRCA* status. Human adherent pancreatic *BRCA* wild type PDAC cell lines BxPC-3, MiaPaCa-2, PaCa-44 and *BRCA2* deficient Capan-1 were treated with gemcitabine, cisplatin, paclitaxel, doxorubicin, cyclophosphamide, 5-fluorouracil and olaparib. Combinations of those cytostatic agents with olaparib were also tested. IC50 values were determined and cross comparison of sensitivity/resistance to tested cytostatic agents and combinations was made. Significant differences in chemosensitivity were observed concerning tested cell lines. Unexpectedly, *BRCA2* deficient cell line Capan-1 was not the most sensitive to cisplatin. All lines were not sensitive to olaparib as a single agent. PaCa-44 was resistant to olaparib in combination with all used cytostatic agents, BxPC-3 was sensitive in combination with cyclophosphamide and MiaPaCa-2 with gemcitabine. *BRCA2* deficient line Capan-1, shown sensitivity across the whole spectrum of used cytostatic agents in combination with olaparib. Our study pointed out the interesting differences in sensitivity to cytostatic agents and olaparib in different cancer cell populations. *BRCA2* deficient pancreatic cancer cell line Capan-1 has better response to olaparib when compared to *BRCA*-wild type lines but only when was administered in combination with cytostatic agents. Some *BRCA* wild type cancer cell populations are sensitive to olaparib administered in combination with gemcitabine and cyclophosphamide, what may be caused by another defect in homologous recombination and must be verified by further studies.

This work was supported by the Ministry of Health of the Czech Republic grant no. NV-19-03-00097 and student grant IGA_LF_2020_005 from Palacky University.

EP1
STILBENE COMPOUND TRANS-3,4,5,4´-TETRAMETHOXYSTILBENE REGULATES CONSTITUTIVE ANDROSTANE RECEPTOR (CAR) TARGET GENES, BUT DOES NOT POSSES PROLIFERATIVE ACTIVITY IN MOUSE LIVER

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The constitutive androstane receptor (CAR) is a primary regulator of drug detoxification. CAR activation is connected with mitogenic effects leading to liver hyperplasia and tumorigenesis in rodents. Therefore currently known mouse CAR activators, including phenobarbital and the potent agonist TCPOBOP (1,4-bis[2-(3,5-dichloropyridyloxy)]benzene), are considered rodent non-genotoxic carcinogens. Recently, the stilbenoids resveratrol and *trans*-3,4,5,4´-tetramethoxystilbene (TMS) have been shown to alleviate *N*-nitrosodiethylamine/phenobarbital-induced liver carcinogenesis. Thus in the present work, we examined if TMS may be an inverse agonist of mouse CAR, which suppreses or stimulate hepatocyte proliferation and liver hyperplasia.

We used luciferase reporter assays, *in silico* docking, primary human hepatocytes or we performed animal experiments with C57BL/6 mice.

Unexpectedly, we identified TMS as a novel moderate murine CAR agonist. This was consistently observed in *in vitro* analyses comprising reporter gene experiments, *in silico* docking experiments, studies in mouse hepatocytes and AML12 hepatic cells, as well as in C57BL/6 mice *in vivo*. TMS significantly up-regulated *Cyp2b10, Cyp2c29* and *Cyp2c55* mRNAs, typical murine CAR target genes, but down-regulated expression of genes involved in gluconeogenesis and lipogenesis such as *Pck1*, *G6pc*, *Scd1, Acaca* and *Fasn* to a similar degree as TCPOBOP, a prototype mouse CAR ligand. Importantly, TMS did not promote EdU incorporation in AML12 cells, did not increase liver weigh and had no statistically significant effect on Ki67 and Pcna labeling indices in mouse liver *in vivo*. In line with that, TMS did not induce genes involved in liver proliferation or apoptosis such as *Mki67, Foxm1, Myc, Mcl1, Pcna, Bcl2, Bax* or *Mdm2* in mice, but slightly up-regulated *Gadd45β* mRNA.

We conclude that TMS is a novel mouse CAR ligand with limited effects on hepatocyte proliferation, while at the same time controlling CAR target genes involved in xenobiotic and endobiotic metabolism.

Funded by GAČR 19-14497S to P.P. and S.M and by OPVVV project InoMed (CZ.02.1.01/0.0/0.0/18_069/0010046) to J.D. and P.P.

EP2
IN VITRO ASSESSMENT OF RENAL TOXICITY IN BRAF INHIBITORS

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One of the most serious malignant skin diseases is currently melanoma. The treatment of advance melanoma has changed in recent years due to introduction of several new drugs including BRAF inhibitors. Targeted therapy based on the inhibition of oncogenic mutations of BRAF by their specific inhibitors, dabrafenib, vemurafenib or encorafenib presents an important component of the progress. However, available clinical data for BRAF-inhibitors have suggested an adverse effect on the kidney. But Detailed information on mechanism/s of BRAF inhibitor nephrotoxicity is very limited. The available data have suggested that the toxic effect can be directed primarily at podocytes in the glomerular membrane. The aim of the study is to investigate whether the toxic effect of BRAF inhibitors in the kidney is specifically targeted at podocytes or whether it is more universal and includes other types of renal cells. The in vitro experiments were performed using four human cell lines representing different types of kidney cells (HEK-293, PODO/TERT256 and HK-2) and liver cells (HepG2) as a comparator. The cells were incubated with the tested compounds at different incubation concentrations for 24 h and 48 h. The experiments also included a comparison of BRAF inhibitor toxicity with a drug exerting clear nephrotoxic effect (amphotericin B – positive control) or a drug without known nephrotoxicity (paracetamol – negative control). A colorimetric method based on measurement of cell metabolic activity was employed. The IC50 values were determined by analysis of inhibition curves. The found results showed significant toxic effects of BRAF inhibitors on all employed renal cells in vitro because the effect on human podocytes was similar in comparison with the other model renal cells. Vemurafenib and encorafenib were significantly more toxic than dabrafenib in the renal cells with toxicity even higher than in amphotericin B. Hepatotoxic effect of BRAF inhibitors in vitro was also significant, in some cases was found at similar concentrations as the nephrotoxic effect. In conclusion, the damage of the kidney by individual BRAF inhibitors may be quantitatively different and probably involves multiple types of renal cells, including renal tubular cells.

Supported by the grants of Charles University Progres Q42 and SVV 260414.

EP3
CHANGES IN BIOCHEMICAL PARAMETERS OF RATS DEPENDING ON THE FRUCTOSE CONTENT IN THE DIET

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Natural sugar fructose is used in the food industry as a common sweetener and is widely consumed, especially in Western diet. High fructose intake can lead to the development of metabolic syndrome. Metabolic syndrome is a disease that is characterized by a damaged lipid profile, obesity, hypertension, oxidative stress and insulin resistance. Our work is focused on determining the effect of high-fructose diet on the parameters of oxidative stress and on the lipid profile in Wistar rats. The diet was enriched with 20% fructose or 60% fructose. We found that rats consumed much more food in the first week of the diet if they had a 60% fructose content in pellets. 60% fructose in the diet caused an increase in triacylglycerols (TAG), an increase in lipid peroxidation in the liver. On the other hand, we found that this fructose content in the diet caused a decrease in oxidative damage of lysosomes in the cortex and kidneys and kept the lipid peroxidation rate in serum at the same level as the control group. The 20% fructose content caused increased lipid peroxidation in the kidneys and greater lysosomal oxidative damage in the liver and a decrease in total cholesterol (TC) in the blood serum of rats, however TC levels did not differ among groups at the end of experiment. Our results are interesting in terms of elevated TAG levels and increased lipid peroxidation in the liver, but contradictory in terms of oxidative stress results. Biphasic regulation of lysosomal activity by oxidative stress is considered. So it is very important to continue testing and investigating the mechanism of action of fructose on oxidative stress parameters and lipid profile.

This work was supported by the project VEGA 2/0120/19.

EP4
IMPACT OF EARLY DEVELOPMENTAL PHTHALATE MIXTURE ON MOTOR ACTIVITY AND STEREOTYPIC BEHAVIOUR OF LABORATORY RAT PUPS

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Phthalates (Pht) are chemicals belonging to the group of endocrine disruptors. They interfere with the endocrine system of animals and when administered during early developmental stages, they are capable of disrupting normal brain development and causing behavioural changes.

There have even been concerns that these chemicals may play a role in the emergence of neurodevelopmental diseases such as ADHD. Our goal was to investigate the effect of prenatal and early postnatal exposure to Pht mixture on motor activity and stereotypic behaviour in weaning, puberty and adulthood. Pregnant Wistar rats were divided into two groups: control (Ctrl) and exposed to the mixture of Pht (Ft) – Di(2-ethylhexyl) Pht (DEHP), Diisononyl Pht (DiNP), and Di-n-butyl Pht (DBP) in dose of 4,5 mg/kg/day each. The mixture was diluted in peanut oil as a vehicle, and delivered to an animal orally from gestational day 15 to postnatal day 4. Open field test was performed in weaning, puberty (Ctrl: ♂ n=12, ♀ n=12; Ft ♂ n=12, ♀ n=12) and adulthood (Ctrl: ♂ n=12, ♀ n=10; Ft ♂ n=10, ♀ n=12) to assess motor activity and stereotypic behaviour through ontogenesis. We did not find any significant changes in motor activity between the groups in weaning, puberty or in adulthood. Stereotypic behaviour was seen only in puberty in a small number of animals and could not be statistically evaluated. These results suggest that prenatal and early postnatal exposure to the mixture of phthalates did not have an effect on the motor activity and stereotypic behaviour of Wistar rat pups.

Supported by the grants VEGA 2/0166/16; VEGA 2/0154/20

EP5
IMPACT OF LATE GESTATIONAL HYPOXIA ON THE DEVELOPMENT AND BEHAVIOR OF THE RAT OFFSPRING

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An unmutilated course of pregnancy and delivery is inevitable for healthy development of the fetus. Prenatal hypoxia (restricted supply of oxygen) remains between the greatest threats to the fetus during development. The aim of our study was to assess the impact of hypoxia on postnatal development and behavioral changes of the rats, whose mothers where exposed to hypoxic conditions (10,5% O2) during critical period of brain development an maturation on gestational day 20 for 12 h. This prenatal insult resulted in a significant delay of sensorimotor development of hypoxic pups compared to the control group. Air-righting (p<0.01) and startle reflex (p<0.05) test were affected by hypoxia and a survival of male pups was also decreased in the hypoxic group compared to normoxic pups from the postnatal day (PD) 8 (p<0.05), which was probably caused by a delayed brain injury seen in other hypoxic models. Hypoxic pups also showed hypoactivity and lower anxiety-like behavior in the open field on PD 25 that was normalized in adulthood (PD 85). Moreover the sociability of hypoxic offspring was significantly decreased in adulthood. Although we did not see differences between control and hypoxic groups in grimace scale (reflecting the degree of lived negative emotions) in the home cage, hypoxic animals (but only male sex) showed lower levels of RGS in immobilization (restrain) chamber compared to control group. In conclusion, our study shows a deleterious impact of late maternal hypoxia on the early development of offspring. The fact that in the most cases changes in observed parameters are manifested in male offspring confirms that male sex is more sensitive to prenatal effects.

Supported by the grant VEGA 2/0154/20.

EP6
EFFECT OF COMMERCIAL PESTICIDES ON CELL VIABILITY, OXIDATIVE STRESS INDUCTION AND DNA DAMAGE IN *CANDIDA SPP.*

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Candidiasis represents a common disease found in humans and animals after microbiota imbalance. The effect of three common pesticides, Tango® fungicide (6, 12 and 25 μg.ml^{-1}) and two insecticides Mospilan® (25, 50 and 100 μg.ml^{-1}) and Calypso® (60, 120 and 250 μg.ml[1]) respectively, was studied in *C. albicans* and three non-albicans species *C. pulcherrima*, *C. glabrata* and *C. tropicalis* to assess cell viability, oxidative stress induction and genetic changes after their exposure. Assessment of cell viability after pesticide treatment showed that the Tango® fungicide concentrations tested decreased the cell survival rate for *C. albicans*, *C. pulcherrima* and *C. glabrata*. A slight increase in the fraction of dead cells was observed in *C. tropicalis*. The largest fraction of *C. pulcherrima* dead cells was observed after applying Mospilan® at a concentration of 25 μg.ml^{-1}. In the case of Calypso® insecticide, the highest mortality rate was observed among *C. pulcherrima* (60 μg.ml^{-1}; p<0.01) and *C. glabrata* (250 μg.ml^{-1}). Next, oxidative stress damage was evaluated using the mitochondrial superoxide level. Significantly elevated levels were observed after Tango® treatment in *C. tropicalis* cells (6 and 12 μg.ml^{-1}; p <0.001), and in *C. pulcherrima* and *C. glabrata* cells (12 μg.ml^{-1}; p<0.05). Both insecticides did not cause any significant changes in peroxide levels. Finally, we assessed the occurrence of DNA damage using the alkaline comet assay. In all *Candida* species, Tango® was able to induce significant levels of DNA breaks at concentration 6 μg.ml^{-1} (p<0.01, p<0.05). Mospilan® caused a significant increase in DNA damage in *C. pulcherrima* and *C. tropicalis* at concentration 100 μg.ml^{-1} (p<0.05). Calypso® (250 μg.ml^{-1}) caused increased levels of DNA damage in all tested strains but without statistical significance. Our results have

shown that even insecticides widely used in agriculture against different kinds of pests can lead to accumulation of ROS in cells, causing oxidative damage of DNA and consequently may promote *Candida spp.* intercellular variability and indirectly influence their pathobiology, just like fungicides.

Supported by the grant 1/0242/19 from the Slovak Scientific Agency VEGA and IGA 01/2019: Assessment of genotoxic and cytotoxic effect of acetamiprid insecticide in cell cultures.

EP7
OPTIMIZATION OF FILAMENT MODIFICATION BY SILVER NANOPARTICLES FOR 3D PRINTING OF ANTIBACTERIAL MATERIAL

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In spite of modern medical methods, bacterial infections are a considerable health problem. As a result of infections, caused by resistant bacterial strains, 700 000 patients die worldwide each year. Infections can be prevented by using a suitable antibacterial agent as various types of metal nanoparticles (NPs), especially silver nanoparticles (AgNPs). The aim of the present work was to incorporate AgNPs into the plastic material used for 3D printing and subsequently to study its toxicological properties. AgNPs were prepared by green synthesis. By replacing chemical reducing agents in the synthesis of NPs with an extract from *Salvia officinalis*, their efficiency can be increased by modifying the surface with substances contained in the plant. The used filament was ABS (acrylonitrile-butadiene-styrene, ø1.7 mm, white). The extruder was heated to 230 °C and the plate temperature was 80 °C, the jet ø0.5 mm. The standardised JIS Z 2801 method was used to evaluate the antibacterial activity (*S. aureus*) of the material. Variable AgNPs concentrations/printed material were used (1 mg/g, 10 mg/g, 20 mg/g, 40 mg/g). The best antibacterial activity was demonstrated in the material at a concentration of 40 mg AgNPs/g ABS. High antibiofilm (antibacterial) activity and efficacy were confirmed by the standard JIS Z 2801 method. When culturing *S. aureus* ($c=1\times10^6$ CFU/mL), an average of 3 colonies of S. aureus were observed compared to the negative control, in which separate colonies could be recognized only when the bacterial suspension was diluted ($c=1\times10^3$ CFU/mL). At a bacterial concentration of 1×10^5 CFU/mL cultured on the material, no colony was observed compared to the positive control, *i.e.* a 100% inhibitory effect of the material on bacterial culture growth was found compared to unmodified ABS. Using AgNPs as an antibacterial agent, the growth of microorganisms resistant to commonly used antibiotics can be inhibited. When using 3D printing, many different devices can be prepared – handles, keyboards, tables, various stands, and other potentially contaminated objects.

Supported by the grant COST Action CA15114, CA LTC18002 and FVHE/Pikula/ITA2020

EP8
THE 3'-UNTRANSLATED REGION (3´-UTR) CONTRIBUTES TO THE PREGNANE X RECEPTOR (PXR) EXPRESSION DOWNREGULATION BY PXR LIGANDS AND UPREGULATION BY GLUCOCORTICOIDS

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The pregnane X receptor (PXR) is a key ligand-regulated transcription factor governing expression of drug-metabolizing enzymes. Over the past years, PXR has been thoroughly examined regarding its function. However, less attention has been paid to a regulation of PXR expression *per se*.

In our study, we observed that PXR is under a negative feed-back regulation upon its activation by prototype PXR ligand rifampicin in a set of primary human hepatocytes. In the same line, hepatic PXR expression was downregulated by a rodent PXR ligand PCN in C57/BL6 mice.

MiRNAs are small, noncoding RNAs, which predominantly suppress mRNA expression via binding to their responsive elements within the 3´-UTR. Specifically, we demonstrated that full-length 3´-UTR of PXR inserted into luciferase reporter or expression plasmid contributes to decreased PXR expression. Among tested miRNAs, miR-18a-5p reduced not only PXR expression but also induction of PXR target gene CYP3A4. Next, we found an increased in miR-18a-5p expression 6 h after rifampicin treatment, which may indicate a potential mechanism behind PXR feed-back regulation.

Moreover, glucocorticoids enhanced PXR expression through the PXR promoter region but also via 3´-UTR. The latter may be associated with a glucocorticoid-induced decrease in miR-18a-5p expression.

In conclusion, miR-18a-5p seems to mediate a dual role in PXR regulation such as a PXR downregulation upon treatment with PXR ligands and PXR upregulation by glucocorticoids.

Supported by the grant EFSA-CDN (No. CZ.02.1.01/0.0/0.0/16_019/0000841, Czech Republic) co-funded by ERDF.

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ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

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Instructions for Authors **2020**

Interdisciplinary Toxicology is the official electronic and printed journal of the Slovak Toxicology Society SETOX and Institute of Experimental Pharmacology, Slovak Academy of Sciences (www.setox.eu). The international, peer-reviewed journal is covering the fields of Toxicology, Pharmacology, Physiology and related areas such as reproductive medicine, embryology, teratology, molecular biology, biophysics, behavioral pharmacology, biochemistry, neurochemistry, receptor studies, animal breeding, and others.

The Journal publishes Original Papers, Review Articles and Clinical Reports on research related to toxicity of chemicals at molecular, cellular, tissue, target organ and whole body level *in vivo* (by all routes of exposure) and *in vitro*/*ex vivo* and a broad range of disciplines related to toxicology. Papers from both basic research and clinical research will be considered for fast publication.

Ethical/Legal Considerations

Originality and Scientific Rigor of Published Work

It is expected that manuscripts submitted to the journal be original works not previously published or under consideration elsewhere (except in abstract form). Manuscripts published in the journal must not be subsequently published elsewhere in similar form, in any language, without the consent of the Slovak Toxicology Society SETOX. Each person listed as an author is expected to have participated in the study to a significant extent, as guided by the International Committee of Medical Journal Editors, "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication" accessible at http://www.icjme.org/. Although all manuscripts, except selected editorial features, are subject to peer review, the responsibility for the accuracy and legitimacy of the scientific content of the manuscripts rests with the authors. It is also the responsibility of the authors to notify the editor in writing of all potentially perceived conflicts of interest, financial or otherwise.

Protection of Human Subjects

It is the authors' responsibility to ensure that all research manuscripts, including investigative case series, have been properly reviewed for adequate protection of human subjects as required by the authors' institution(s). Such protection should be described in the Methods of the manuscript.

Copyright

After a manuscript is accepted, all authors must sign the journal's applicable "Copyright Transfer Form." Authors planning to reproduce previously copyrighted materials, such as figures, tables, or direct quotations, must solicit and obtain written permission from the copyright owner(s) prior to publication. It is the author's responsibility to secure such permission.

Protection of Animal in Research

When reporting experiments on animals, authors should be asked to indicate that they followed institutional and/or national guides for the care and use of laboratory animals. Such statement should be included in the Methods section.

Disclosure

Interdisciplinary Toxicology expects all authors to disclose all financial relationships with all companies regarding products mentioned in a manuscript. If any author has any perceived conflicts, the author is expected to disclose them.

Manuscript Submission

All manuscripts should be submitted through e-mail in electronic form in plain English by email to the Editor-in-Chief: Michal Dubovický, PhD. (intertox@setox.eu) according to the Instructions for Authors.

The files should be named with the following convention: first author's initials, date of submission (day-month-year), underline, and the word "title", "text", or "Figure 1,2,3..." as appropriate. For example, "MM27-05-2008_Figure1."

Interdisciplinary Toxicology encourages submission of original work in the following journal categories: teratogenesis, (developmental) reproductive toxicology, carcinogenesis, mutagenesis, pharmacokinetics, toxicogenomics and proteomics, pharmacotoxicological and metabolic mechanisms, risk assessment, environmental toxicology and environmental health as applied to humans (including epidemiological studies), forensic toxicology, environmental chemistry, pesticides, dioxins, regulatory toxicology, occupational toxicology and food toxicology.

The Journal's "Letters to the Editor." Suitable for brief novel reports, commentary on previously published manuscripts within the journal, or other correspondence with relevance to medical toxicology.

General

All manuscripts should be submitted in Microsoft Word compatible formats. They should be in 12-pt font and be double-spaced throughout with 2.5 cm margins on all sides. Pages should be numbered, and a descriptive header (blinded, without authors' names) should appear on each page.

Title page

The title page must be submitted as a separate file (the manuscript will be forwarded to reviewers in blinded fashion without the title page). Information required on the title page:

- Complete manuscript title
- Authors' full names, academic degrees, and affiliations
- Name and address for correspondence (include telephone number and email address)
- Sources of funding for project
- Three to five "key words" acceptable for National Library of Medicine indexing

Abstract

The abstract should concisely present the hypothesis being tested, general methods, results, and conclusions. Abstracts of more than 250 words will not be accepted. A word is one or more characters bounded by white space. The abstract must be a single paragraph. IMPORTANT: If your manuscript is accepted, the abstract entered into the online metadata form will appear online EXACTLY the way you enter it during the submission process.

Introduction

This section, which has no heading, must contain a clear statement of the aims of the work or of the hypotheses being tested. A brief account of the relevant background that supports the rationale of the study should also be given. The length of the Introduction should not exceed 750 words.

Methods

This section should contain explicit, concise descriptions of all new methods or procedures employed. Whereas modifications of previously published methods must be described, commonly used procedures require only a citation of the original source. Descriptions of methods must be sufficient to enable the reader to judge the accuracy, reproducibility, and reliability of the experiment(s). The name and location (city and state or country) of commercial suppliers of chemicals, reagents, and equipment must be given. Sources of compounds, reagents, and equipment not available commercially should be identified by name and affiliation here or in the Acknowledgments section.

Results

Contained in this section are the experimental data, with no discussion of their significance. Results are typically presented in figures or tables, with no duplication of information in the text. If a table or figure includes less than four values, the data should be presented in the text rather than as a separate table or figure. Magnitudes of variables reported should be expressed in numerals. Generally, units are abbreviated without punctuation and with no distinction between singular and plural forms (e.g., 1 mg, 25 mg). Sufficient data should be presented to allow for judgment of the variability and reliability of the results. Statistical probability (p) in tables, figures, and figure legends should be expressed as $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$. For second comparisons, one, two, or three daggers may be used. For multiple comparisons within a table, footnotes in lower case, superscript letters are used and defined in the table legend.

Discussion

Conclusions drawn from the results presented are included in this section. Whereas speculative discussion is allowed, it must be identified as such and be based on the data presented. The Discussion must be as concise as possible and should not exceed 1,500 words.

Acknowledgments

The Acknowledgments section is placed at the end of the text. Personal assistance is noted here. Financial support is acknowledged as an unnumbered footnote to the title.

References

References are cited in the text by giving the first author's name (or the first and second if they are the only authors) and the year of publication (e.g., Ruth and Gehrig, 1929; McCarthy, 1952; or Kennedy *et al.*, 1960). In the reference list, the references should be arranged alphabetically by author and numbered. The names of all authors should be given in the reference list. If reference is made to more than one publication by the same author(s) in the same year, suffixes (a, b, c, etc.) should be added to the year in the text citation and in the references list. Journal titles should be abbreviated as given in the Medline abbreviation list linked to the online Instructions to Authors.

References to personal communications, unpublished observations, and papers submitted for publication are given in parentheses at the appropriate location in the text, not in the list of references. Only papers that have been officially accepted for publication may be cited as "in press" in the reference list. The authors are responsible for the accuracy of the references. The format for journal article, chapter, and book references is as follows:

1. Griffiths RR, Bigelow GE, Liebson IA. (1986). Human coffee drinking: Reinforcing and physical dependence producing effects of caffeine. *J Pharmacol Exp Ther* **239**: 416–425.
2. Chernow B, O'Brian JT. (1984). Overview of catecholamines in selected endocrine systems, in *Norepinephrine* (Ziegler MG and Lake CR eds) pp. 439–449, Williams & Wilkins, Baltimore.
3. Tallarida RJ, Murray RB. (1987). *Manual of Pharmacologic Calculations with Computer Programs*. Springer-Verlag, New York.
4. National Institute on Drug Abuse [webpage on the Internet]. Club drugs. Washington, DC: National Institute on Drug Abuse [updated 2005 Apr 14; cited 2005 Aug 15]. Available from: http://www.nida.nih.gov/DrugPages/Clubdrugs.html

Tables

Each table must be double-spaced and begin on a separate page, each page numbered continuous with the rest of the manuscript. Tables are numbered consecutively with Arabic numerals. A brief descriptive title is provided at the top of each table. General statements about the table follow the title in paragraph form. Footnotes to tables are referenced by lower case, superscript letters and defined beneath the table.

Acceptable formats for tables are Word and WordPerfect. Do not embed tables within the body of the manuscript. Tables should be self-explanatory and should supplement, rather than duplicate, the material in the text.

Figures

Each figure must be uploaded as a separate file in a 600+ dots per inch .tif, .eps, or .jpg format and scaled to fit an A4 page. Authors are advised to avoid submitting .ppt files; they do not reproduce as clearly as other formats. Label the front of every figure with the figure number. Lettering on figures should be large enough to be legible after reduction to single-column width of 8 cm. Type sizes after reduction should be 6–8 points. Do not use varying letter type sizes within a single figure; use the same size or similar sizes throughout the drawing. Figures should be ready, in all respects, for direct reproduction. All panels of a multipart figure should be provided in the same file. If symbols are not explained on the face of the figure, only standard print characters may be used. Include figure titles in the legend and not on the figure itself.

Photomicrographs and electron micrographs must be labeled with a magnification calibration in micrometers or Angstrom units. A statement concerning the magnification must appear in the figure legend.

The cost of publishing color figures will be billed to authors at a rate of EUR 50 per figure. If the corresponding author is a SETOX member in good standing when the paper is published and the referees and editor agree that color is necessary to convey the desired information, then the rate is EUR 20 per figure. Multiple-part figures submitted as separate illustrations are charged as separate figures. A color authorization form will be provided and must be completed prior to publication.

Schemes should be placed after tables, but before figures. Appendices should be placed after tables and figures.

Reagents

As a condition of publication the authors agree, whenever available quantities allow, to distribute freely to academic researchers for their own use any reagents (e.g. novel chemicals, DNA, antibodies) developed for the published study that are not available from commercial suppliers. Nucleic acid and protein sequences, as well as X-ray crystallographic coordinates, must be deposited in the appropriate databases with a release date no later than the publication date. Sequence accession numbers must be provided in the text.

Drugs

Generic drug names are used in text, tables, and figures. Trade names may be given in parentheses following the first text reference, but should not appear in titles, figures, or tables. Whereas trade names are capitalized, generic or chemical names are not. The chemical structure of new compounds (or a citation to the published structure) must be given. The form used in calculating concentrations (e.g. base or salt) must be indicated.

Receptor nomenclature

The nomenclature used to identify receptors and ion channels should conform to guidelines of the Committee on Receptor Nomenclature and Drug Classification of the International Union of Pharmacology. These are published periodically in Pharmacological Reviews and are freely accessible online at www.pharmrev.org.

Revised Manuscripts

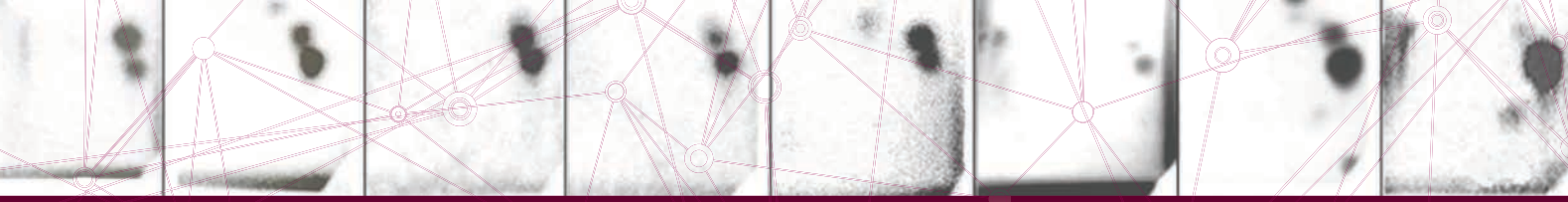
Revised manuscripts must be submitted within the designated time and must contain an itemized list of all changes made, or a rebuttal, in response to each of the reviewers' suggestions. Do not neglect to retrieve the Revision Checklist to correct any publishing format deficiencies pertaining to your manuscript.

Make sure source files are included, figures conform to specifications (see Figures). Publication of accepted papers will be delayed pending correction of any outstanding deficiencies.

After Acceptance

Authors will receive an e-mail with their page proofs. Proofs should be printed out, corrected, and mailed to the compositor. Complete instructions will be included with the page proofs. An order form for reprints will be included with the page proofs.

Interdisciplinary Toxicology
ISSN: 1337-6853 (print version) 1337-9569 (electronic version)



ISSN 1337-6853 (print version)
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