10th Congress of Toxicology in Developing Countries (CTDC10)
12th Congress of the Serbian Society of Toxicology (12th SCT)

Book of Abstracts

Publisher
Serbian Society of Toxicology
Vojvode Stepe 450, 11000 Beograd
E-mail: vevodi@pharmacy.bg.ac.rs

Editor
Vesna Matović

Editorial Assistant
Stefan Mandić-Rajčević

Design and Prepress
Atelje, Beograd
www.atelje.rs

Printed by
Dosije studio, Beograd
www.dosije.rs

Publishing Year
2018.

Circulation
350

ISBN 978-86-917867-1-7
Table of Contents

LECTURES AND INVITED ORAL PRESENTATIONS ................................................................. 5
  Opening Lecture .................................................................................................................. 7
  Plenary Lecture .................................................................................................................. 7

SYMPOSIA .......................................................................................................................... 11
  ANTIBIOTIC RESISTANCE IN HEALTHCARE SETTINGS: EFFICIENCY VERSUS SAFETY ................................................................. 13
  EXPOSURE AND RISK ASSESSMENT OF FOOD CONTAMINATION ........................................................................................................... 16
  APPROACHES, TOOLS, AND ADVANCES ......................................................................... 19
  ADVANCES IN MOLECULAR METAL TOXICOLOGY .............................................................. 21
  EMERGING AND KNOWN NATURAL TOXINS: ENVIRONMENTAL FATE AND HUMAN RISK ................................................................. 24
  MODIFIED MYCOTOXINS – AN EMERGING RISK IN FOOD SAFETY ................................................................. 26
  APPLICABILITY AND LIMITATION OF ALTERNATIVE ANIMAL TESTING IN SAFETY ASSESSMENT ................................................................. 28
  ENVIRONMENTAL POLLUTION AND TOXIC OUTCOMES: DOSES, MOLECULAR BIOMARKERS, AND ASSOCIATIONS ................................................................. 28
  EVALUATION OF SAFETY PROFILE OF HERBAL PRODUCTS ......................................................................................................................... 31
  FROM ASSESSMENT OF INTERNAL EXPOSURE TO CHEMICALS TO ACTION TO PREVENT ADVERSE HEALTH IMPACTS: THE ROLE OF HUMAN BIOMONITORING ................................................................. 33
  THE SIGNIFICANCE OF DRUG/XENOBIOTIC METABOLIZING ENZYME POLYMORPHISMS IN CANCER/DISEASES ................................................................. 36
  SUBSTANCES OF ABUSE: GLOBAL TRENDS, PREVENTION AND MANAGEMENT ......................................................................................................................... 38
  BIOMARKERS IN CHRONIC DEGENERATIVE DISEASES AND RISK ASSESSMENT ......................................................................................................................... 41
  INFLUENCE OF ENDOCRINE-DISRUPTING CHEMICALS (EDCS) ON DEVELOPMENT AND REPRODUCTION ................................................................. 43
  DEVELOPMENT IN METHODOLOGIES TO ADDRESS MIXTURE RISK ASSESSMENT ......................................................................................................................... 46
  TOXICITY OF RESPIRABLE PARTICULATE MATTER IN AMBIENT AIR ......................................................................................................................... 49
  INCORPORATING INFORMATION ON CHEMICAL MIXTURES INTO CHEMICAL RISK ASSESSMENTS ......................................................................................................................... 52

WORKSHOPS .......................................................................................................................... 55
  Plenary Workshop .................................................................................................................. 57
    TOXICOLOGY DATA AND ONLINE TOOLS: AVAILABILITY, SEARCH STRATEGIES, OPEN DATA, AND REPRODUCIBILITY ................................................................. 57
  Workshops ............................................................................................................................ 60
    ARACHNIDS: FALLACIES, CLINICAL MANIFESTATIONS, DIFFERENTIAL DIAGNOSIS AND MANAGEMENT OF SPIDER BITE AND SCORPION STING ......................................................................................................................... 60
    PRODUCT STewardship AND Regulatory TOXICOLOGY IN THE OIL AND GAS INDUSTRY ......................................................................................................................... 62

ROUND TABLES ....................................................................................................................... 67

CONTINUING EDUCATION COURSES .................................................................................. 71

SHORT COMMUNICATIONS ..................................................................................................... 75

POSTERS .................................................................................................................................. 87
  Air Pollution .......................................................................................................................... 89
  Alternative Animal Models .................................................................................................... 90
  Analytics in Toxicology ........................................................................................................ 92
  Biomonitoring and Biomarkers .......................................................................................... 96
  Carcinogenesis ...................................................................................................................... 100
  Clinical Toxicology ............................................................................................................. 102
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Computational Toxicology</td>
<td>103</td>
</tr>
<tr>
<td>Endocrine Disrupting Chemicals</td>
<td>105</td>
</tr>
<tr>
<td>Food Toxicology</td>
<td>109</td>
</tr>
<tr>
<td>General Toxicology</td>
<td>114</td>
</tr>
<tr>
<td>Herbal Products</td>
<td>116</td>
</tr>
<tr>
<td>History of Toxicology</td>
<td>119</td>
</tr>
<tr>
<td>Human and Environmental Risk Assessment</td>
<td>129</td>
</tr>
<tr>
<td>Immunotoxicology</td>
<td>133</td>
</tr>
<tr>
<td>Mechanisms of Toxicity</td>
<td>134</td>
</tr>
<tr>
<td>Metals</td>
<td>139</td>
</tr>
<tr>
<td>Nanomaterials</td>
<td>144</td>
</tr>
<tr>
<td>Natural Toxins</td>
<td>147</td>
</tr>
<tr>
<td>Non Animal Testing</td>
<td>150</td>
</tr>
<tr>
<td>Pesticides</td>
<td>152</td>
</tr>
<tr>
<td>Psychoactive Substances and Substances of Abuse</td>
<td>157</td>
</tr>
<tr>
<td>Regulatory Toxicology</td>
<td>160</td>
</tr>
<tr>
<td>Target Organ Toxicity</td>
<td>161</td>
</tr>
<tr>
<td>Toxicology of Drugs</td>
<td>163</td>
</tr>
<tr>
<td>Toxicology of Mixtures/Mixture Risk Assessment</td>
<td>167</td>
</tr>
<tr>
<td>Other Topics</td>
<td>169</td>
</tr>
</tbody>
</table>

**INDEX OF AUTHORS**                                                                 | 175  |
LECTURES AND INVITED ORAL PRESENTATIONS
**Opening Lecture**

**State of Science and Profession of Toxicology on the African Continent: Lessons Learned from Challenges, Advancements, and Future Developments**

M. Gulumian¹,²

¹National Institute for Occupational Health (NIOH), P O Box 4788, Johannesburg 2000, South Africa
²Haematology and Molecular Medicine Department, School of Pathology, University of the Witwatersrand

Progress in social, political and economic developments experienced by countries within the African continent have also produced challenges in addressing adverse impacts on human health and the environment. The unambiguous role played by toxicologists in developed countries in addressing similar challenges is a foregone conclusion. As yet, reliance on toxicologists in addressing these very challenges may not be the norm in different African countries. The reasons being multifactorial: toxicology is a neglected profession where it is simply not part of the education curriculum, misconception on the recognition of toxicology as a separate scientific discipline, and finally lack of understanding of the role and functions of a toxicologist where the stress should be more on prediction and prevention through risk assessment rather than on a mere confirmation of toxicity.

The establishment of number of toxicological societies in different African countries has helped in addressing some of these challenges. In developed countries risk assessment has grown to a sufficient stature to assess hazard, estimate risk as well as offer rational safe management of these risks. The toxicological societies within these African countries have taken this approach seriously and have embarked in number of training opportunities as well as post graduate courses on the topic. They have also enabled toxicology to be recognised as a profession but most importantly, have succeeded in changing the perception on the role and functions of toxicologists in addressing human health and the environment.

Challenges do however exist in providing opportunities for toxicologists in these countries to contribute in setting of more relevant standards in occupational and environmental surrounding as well as their involvement in regulatory aspects in registration of pesticides pertaining to this sub-discipline of toxicology. Future developments on the continent are therefore aimed at directing efforts to fulfill this important role of toxicologists on the African continent through providing training to the toxicologists and also communication with governmental agencies.

**Keywords:** perception of toxicology, toxicology neglected profession, training needs, challenges faced by toxicologists

**Correspondence:** mary.gulumian@nioh.nhls.ac.za

---

**Plenary Lectures**

**Introduction to the Concept of “Signal Toxicity”**

Jun Kanno¹,²

¹Japan Bioassay Research Center, Japan Organization of Occupational Health and Safety
²Division of Cellular and Molecular Toxicology, National Institute of Health Sciences.

The “Silent Spring” by Rachel Carson (1962) had established the basis for, and the “Our Stolen Future” by Theo Colbone, Dianne Dumanoski and John Peterson Myers (1996) had coined the concept of “Endocrine Disrupting Chemicals” with its mechanistic plausibility to all living organisms. And it took some time to realize that the plausibility is backed by the paradigm of receptor-mediated toxicity or in other words “signal toxicity”.

In classical toxicology, a toxic substance reaches the target molecule and induces malfunction. Such targets are enzymes, lipid membranes, DNA, and other components in the cell. In case of signal toxicity, a chemical binds to a receptor. After that, the chemical itself is not important. The signal from the receptor initiates a cascade of molecular events that leads to various changes in the cells and organs. When the signal is abnormal in terms of quality, intensity and timing, the signal will induce adverse
effects to cells and organs. The target would be not only endocrine system including reproductive, but also immune, and neuronal systems. The dose-response characteristics and the dose-range will depend on the signaling system of concern. If the signaling system is used for organogenesis and its functional maturation, there would be a critical period in developmental phase that the disturbance of such signals may leave irreversible changes to the organ.

Here, experiments to illustrate the “early exposure – late effect” at a so-called “low dose range” and related project on transcriptomics will be presented for further discussion on this matter.

**Keywords:** signal toxicity, receptor mediated toxicity, endocrine disrupting chemicals, perinatal exposure, Percellome Project

**Correspondence:** jun-kanno@jbrc.johas.go.jp

---

### The Search for Safe Replacements for Endocrine Disrupting Chemicals

**Barbara F. Hales**¹, Bernard Robaire¹,²

¹Department of Pharmacology & Therapeutics, ²Department of Obstetrics & Gynecology, McGill University, Montreal, QC, Canada H3G 1Y6

Some of the diverse synthetic chemicals associated with our modern lifestyle have unintended adverse influences on human health, affecting hormone production or action, and thus acting as endocrine disrupting chemicals (EDCs). Polybrominated diphenyl ether (PBDE) flame retardants, phthalate plasticizers, and bisphenol A (BPA), an epoxy resin ingredient, are EDCs. Studies with animals and cells have provided evidence for associations between PBDE exposures and adverse outcomes. Human studies demonstrate that PBDE exposures are associated with adverse effects on neurobehavior and an increase in cryptorchidism. Phthalates have well documented anti-androgenic effects while BPA exposure is associated with estrogenic activity. Many governments have restricted the uses of these chemicals due to their adverse effects, creating a “market gap”. We are now discovering that chemicals introduced as replacements may not be safer than the “legacy” substances. BPA analogues, with similar chemistry, may have similar or greater toxicity than BPA. Organophosphates are “emerging” as the “new” flame retardants; using cell and limb bud cultures we have shown that some of these chemicals have effects similar to or greater than PBDEs. In collaboration with colleagues in Chemical Engineering we have identified novel “green” alternative plasticizers with a promising profile. Improvements in our assessment of alternatives are needed to ensure that strategies to identify replacements that are safer and have a reduced environmental impact will be embedded in society in the future.

Supported by the CIHR Institutes of Human Development, Child and Youth Health and of Population and Public Health. BFH and BR are James McGill Professors.

**Keywords:** flame retardants, phthalates, bisphenols, reproductive toxicity, developmental toxicity

**Correspondence:** barbara.hales@mcgill.ca

---

### From Pre-marketing Studies and Authorization Dossiers to New Prospects for Pesticide Risk Assessment in Rural Enterprises

**Claudio Colosio**, Stefan Mandic-Rajcevic, Federico Maria Rubino

Department of Health Sciences of the University of Milan and International Centre for Rural Health of the S. Paolo Hospital, Italy

The role of pesticides in the modern society has been strengthened by the need for higher yield in food production and the ongoing battle against vector borne diseases in public health. Nevertheless, the toxicity of these chemicals is not fully specific to target organisms, thus posing a potential health threat to humans. In this frame, risk assessment and management are fundamental. In the occupational settings, variability of meteorological conditions, use of different concentrations of variable mixtures, and significant variations in the application times and modalities make this task very complicated, making necessary proposing novel approaches for conducting “in field” preventive activities. The amount of information collected during the process of authorization of a new active ingredient is unique, with a size similar of the one available for human drugs. Therefore, a possible way forward for risk assessment is represented by a better exploitation in the post-marketing phase of the data used for the registration process, combined with the data collected in real-life field studies usable for refining and validate the risk hypothesis generated through modelling. In particular, parameters such as Acceptable Operator Exposure Level (AOEL), acute reference dose (ArD) as well data regarding skin absorption, metabolism and relevant metabolites in animals can find use in
the conduction of risk assessment activities in agricultural enterprises, through the creation of new tools for exposure and risk assessment. Such tool are usable even without conducting complicated and expensive measures, and therefore are adequate for the needs of small and medium sized agricultural enterprises.

**Keywords:** exposure assessment, biological monitoring, modeling, field studies, small and medium-sized enterprise

**Correspondence:** claudio.colosio@unimi.it

---

**Communications in the Area of Toxicology: a Challenging Task?**

Lucia de Luca  
*European Food Safety Authority*

In her talk Lucia will look at the characteristics of communicating risks and in particular those in the area of toxicology. The talk will look at why should experts engage in communication and what are the challenges they should pay attention to? Specific focus will be devoted to todays’ societal background against which toxicological concepts and scientific work needs to be communicated and what role can scientists and toxicologists play in ensuring effective risk communication.

**Keywords:** risk, communication, information, engagement, trust

**Correspondence:** Lucia.DELECA@efsaeuropa.eu

---
SYMPOSIA
ANTIMICROBIAL COATINGS IN HEALTHCARE SETTINGS: EFFICIENCY VERSUS SAFETY

Chair: Anne Kahru¹²  
Co-Chair: Angela Ivask¹

¹National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, 12618 Tallinn, Estonia,  
²Estonian Academy of Sciences, Kohtu 6, 10130 Tallinn, Estonia

Infections and infectious diseases are considered a major challenge to human health in healthcare units worldwide. As antimicrobial materials (such as silver and copper) are inherently toxic, the application of (nano-enabled) antimicrobial surface coatings (AMCs) in healthcare settings may cause harm in addition to benefits. Indeed, in parallel to cutting down the spread of potentially infectious microbes, AMCs may induce (eco) toxicological hazard and antimicrobial resistance. This session will cover briefly all these aspects: potential of AMCs in the development and spread of antimicrobial resistance (A. Ivask), introduction of the adverse environmental effects (A. Kahru), the need for balancing the efficiency and safety of AMCs by ‘safe-by-design’ (I. Vinković Vrček), aspects of industrial up-scaling of safe AMCs (P. Mantecca) and importance of internationally coordinated research and networks (F. Crijns). The session was suggested by Estonian Society of Toxicology and the speakers cooperate in the COST Action AMiCI (AntiMicrobial Coating Innovations, CA15114), supported by the European Cooperation in Science and Technology (COST). More information on this COST network is available in the joint paper of the authors: http://www.mdpi.com/1660-4601/14/4/366.

Keywords: nanomaterials, safety, healthcare associated infections, antimicrobial resistance, risk-benefit analysis

Correspondence: anne.kahru@kbfi.ee  
angela.ivask@kbfi.ee

Antimicrobial Coatings in Healthcare: Possible Benefits and Need for Internationally Coordinated Research

Francy Crijns¹, Rinaldo van Meel¹, Olaf Brouwers¹, Jim Odekerken¹, Patrick van de Poel², Minna Keinänen-Toivola³, Gabrielle Tuïjthof⁴

¹Zuyd University of Applied Sciences, Faculty of Bèta Sciences and Technology, P.O. Box 550, 6400 AN Heerlen, The Netherlands;  
²Zuyderland Hospital, P.O. Box 5500, 6130 MB Sittard-Geleen, The Netherlands,  
³Faculty of Technology, Satakunta University of Applied Sciences, P.O. Box 211, FI-26101 Rauma, Finland

Infections are a continuous threat to human health. According to the European Centre for Disease prevention and Control (ECDC), each year over 4 million people are estimated to acquire a HealthCare Associated Infection (HCAI). A potential and promising weapon against bacterial growth and possibly the development of multi-drug resistant bacteria has been found in AntiMicrobial (nano)-Coatings (AMC). In coatings fortified with an active ingredient, the ingredient is responsible for the elimination of the microorganisms.

Nowadays, a lot of these coatings are commercially available and establish a high reduction of bacterial loads on surfaces, when studied in standardized laboratory conditions. Field studies to test the efficacy of AMC in healthcare settings are scarce. This is one of the main reasons for so far limited use of AMCs in hospitals. Zuyd University is currently performing field studies in Zuyderland Hospital, to assess the efficacy of 2 different antimicrobial TiO₂ coatings in patient rooms. Both coatings have proven efficacy in controlled lab conditions using the intimate contact test ISO-19622. The aim of the current study is to explore the efficacy of these coatings in a living lab situation by assessing bacterial load and diversity. Moreover, experience built up in these living lab studies is valuable to extend field studies through the AMiCI network.

The COST Action AMiCI (AntiMicrobial Coating Innovations, CA15114), supported by the European Cooperation in Science and Technology (COST), brings together universities, research institutes and companies to evaluate the impact of (introducing) antimicrobial coatings in healthcare on spreading of infections.

Keywords: healthcare associated infections, nano-coatings, COST Action AMiCI, living lab, performance assessment

Correspondence: francy.crijns@zuyd.nl
Design and Evaluation of Efficient and Safe Antimicrobial Coatings: Connections with the Industry

Paride Mantecca1, Kaja Kasemets1,2, Ehud Banin3, Ilana Perelsthein4, Aharon Gedanken4
1Department of Earth and Environmental Sciences, Research Centre POLARIS, University of Milano-Bicocca, 1 Piazza della Scienza, Milan, Italy; 2Laboratory of Environmental Toxicology, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia; 3The Mina and Everard Goodman Faculty of Life Sciences, Institute of Nanotechnology and Advanced Materials, Bar-Ilan University, Ramat-Gan, Israel; 4Department of Chemistry and Nanomaterials, Bar-Ilan University Center for Advanced Materials and Nanotechnology, Ramat-Gan, Israel

The increase of infectious diseases is a global scale concern with extremely high social and economic costs. Special concern is spreading of the nosocomial infections typically derived from increased exposure to pathogenic bacterial in hospitals or healthcare units. The development and use of antimicrobial coatings (AMCs) is a promising industrially up-scalable research field to mitigate spreading of infectious diseases and antimicrobial resistance (AMR). The primary goal is to obtain more effective AMCs and currently the devices/materials impregnated with antibiotics or silver are most widely used. As the scientific community has questioned the human and environmental safety of nanosilver, the identification of safer AMCs is urgently needed applying safe-by-design approach. Textiles are among the materials with the highest demand for antimicrobial functionality, due to their ubiquitous use in healthcare settings (e.g. bandages, pajamas, bed-sheets). The growing need for antibacterial textiles has resulted in revolutionary progress in the textile industry, leading to new technologies and products able to improve the antibacterial efficacy and to concomitantly reduce the environmental and health hazard, which finally has the potential to open new market and business opportunities for the companies.

The EU project PROTECT has developed novel nano-enabled AMCs (CuO, ZnO and Zn-doped CuO) to coat textiles to achieve enhanced antibacterial activity (also against resistant bacterial strains), in concomitance with reduced health hazards. Pilot-scaled industrial plants were developed and the refinement of the technologies is ongoing to match the market demand.

EU-H2020 project PROTECT (n. 720851) and EU COST Action network CA15114 AMiCI are acknowledged.

Keywords: COST Action CA15114 AMiCI, antibacterial textiles, nano-coatings, metal oxides, safety
Correspondence: paride.mantecca@unimib.it

Can Antimicrobial Coatings Promote Development of Resistant Microorganisms?

Kaja Kasemets1, Angela Ivask1, Siiri Kõljalg2
1Laboratory of Environmental Toxicology, National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, Tallinn 12618, Estonia; 2Institute of Biomedicine and Translational Medicine, Faculty of Medicine, University of Tartu, Ravila 19, Tartu 50411, Tartu, Estonia

Microbe-caused infectious diseases and development of antimicrobial resistance (AMR) are increasing global concerns associated with significant medical costs. Antimicrobial coatings (AMCs) have been suggested as a promising solution against the spread of microbes and related infections in public and healthcare sectors. Although certain AMCs affect microbial cells via topology or anti-fouling properties, most often AMCs are based on antimicrobial active substances - traditional antibiotics, antimicrobial peptides, enzymes, quaternary ammonium compounds (QACs), or nanoparticles e.g., of silver, copper, zinc - that kill microbes either after release from the surface or by direct contact. Due to the release of these chemicals from AMCs and rapid genetic exchange and mutations in microbes there are concerns that increasing use of AMCs may induce the appearance of new or spread of already existing AMR microbes. The fact that traditional antibiotics, even if released from AMCs only at low concentrations may cause the spreading of AMR microbes via horizontal gene transfer, is well-recognised. However, microbes may develop resistance also against antimicrobial peptides and QACs e.g., via mutations for modified membrane composition, expression of stress-response, repair system, or efflux pump genes. Also various genes granting microbial resistance to metals have been discovered and there is growing evidence of co-resistance between e.g., QACs or metal-ions and traditional antibiotics. Thus, by aiming to control microbial infections by AMCs one has to take care not to open the door for resistant microbes instead. Financial support from COST Action CA15114 AMiCI and Estonian Research Council grants PUT748 and IUT23-5 are acknowledged.

Keywords: antimicrobial surfaces, co-resistance, horizontal gene transfer, mutations, membrane composition, COST Action CA15114 AMiCI
Correspondence: angela.ivask@kbfi.ee
Can Antimicrobial Coatings Pose Risk to the Environment?

Anne Kahru¹ ², Kaja Kasemets¹, Merja Ahonen³
¹Laboratory of Environmental Toxicology, National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, Tallinn 12618, Estonia;
²Estonian Academy of Sciences, Kohtu 6, Tallinn 10130, Estonia;
³Faculty of Technology, Satakunta University of Applied Sciences, P.O. Box 211, FI-26101 Rauma, Finland

Bacteria-caused (infectious) diseases are currently among the most burdensome to patients and society in terms of distress, therapy needs and financial burden to healthcare budgets. The problem expands as microbial resistance to antibiotics increases and can be alleviated by application of antimicrobial coatings (AMCs) in healthcare settings. To this end, nanoparticles (NPs), e.g., silver, copper, and zinc NPs are increasingly used in AMCs (bandages, face masks, privacy curtains, bed-sheets) due to their antimicrobial efficiency. Unfortunately, most AMCs are also toxic to humans, animals and to the environment at large. For example, Ag⁺, Cu²⁺, Zn²⁺-ions that are the drivers of the antimicrobial effects of respective AMCs are highly toxic to algae and crustaceans – important members of the aquatic food-webs, although at low concentrations copper and zinc are essential micronutrients. Therefore, AMCs can be a double-edge sword: in addition to inhibition/killing of noxious microbes adverse effects to environmental organisms can occur via various waste-streams originating e.g., from the production, application, wear and tear, cleaning and/or disposal of AMCs. Thus, the risk assessment of AMCs over the entire life cycle into different environmental compartments is needed for the sustainable application of AMCs. Currently there are some data available for hazard evaluation of AgNPs – the antimicrobial nanomaterial that has been most efficiently studied - but not yet enough to conduct a detailed risk-benefit assessment. The data gaps are even more severe for other (nano)antimicrobials that have remarkably less available information.

Estonian Research Council project IUT23-5 and EU COST Action CA15114 AMiCI are acknowledged.

Keywords: silver, copper, aquatic food-web, risk-benefit assessment, COST Action CA15114 AMiCI
Correspondence: anne.kahru@kbfi.ee

Safe-by-design Approach for Development and Use of Antimicrobial Coatings

Ivana Vinković Vrček¹, Angela Ivask²
¹Institute for Medical Research and Occupational Health, Ksavarska cesta 2, Zagreb, Croatia;
²Laboratory of Environmental Toxicology, National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, Tallinn 12618, Estonia

A state-of-the-art innovation to combat Healthcare Associated Infections (HCAIs) is the creation of self-disinfecting surfaces through the application of antimicrobial coatings (AMCs). Nanotechnology, one of the key enabling technologies, allows for significant improvements in the AMC development providing better healthcare but also enabling the design and clinical use of innovative solutions for HCAIs. However, nano-specific behavior should be taken into account during manufacturing, application and final disposal of nano-enabled AMCs at the Safe-by-Design level. This is especially relevant for exposure, absorption, distribution, accumulation, and toxic effects. The Safe-by-Design is well accepted approach to ensure safety for three different, but interrelated communities – the workplace, consumers and environment.

There are following key issues: identification and characterisation of nano-based biocidal agent; transformation of nanomaterials; dose metrics; fate and kinetics. It is crucial to identify difficulties related to experience and knowledge about safety assessment of AMCs. Opinions are needed to provide response, challenges, needs and recommendation for Safe-by-Design approach in addressing the application of AMCs in healthcare setting.

Financial support from EU COST Action CA15114 AMiCI is acknowledged.

Keywords: nanotechnology, healthcare associated infections, safety, COST Action CA15114 AMiCI
Correspondence: ivinkovic@imi.hr
EXPOSURE AND RISK ASSESSMENT OF PESTICIDE USE IN AGRICULTURE: APPROACHES, TOOLS, AND ADVANCES

Chair: Claudio Colosio¹
Co-Chair: Aristidis Tsatsakis²
¹Department of Health Sciences of the University of Milan and International Centre for Rural Health of the S. Paolo Hospital, Italy,
²Medical School, Division Morphology, University of Crete, Greece

Pesticide exposure remains an outstanding problem for public health, and the attention of the scientific community on the topic is growing. Plant Protection Products are used worldwide, their presence is ubiquitous in the living and working environments and doubts persist on the real health risks they pose, especially for continuous, long term, low-dose environmental and occupational exposures. In assessing pesticide risk, the main challenge is quantifying and qualifying exposure levels. This activity often leads to biases due to under and overestimation of exposure, impairing risk assessment and management. Researchers face the problem of choosing adequate analytical methods and approaches for their study, including both pre-analytical and analytical phases, and, in any case, any analytical approach is hampered by the difficulties created by the instability of the climatic conditions and the variation of the exposure timing and patterns. In the meanwhile, the continuous development of new technologies and computational approaches creates the possibility to perform risk assessment without doing measures, using models and simulations. This Symposium is aimed at offering data regarding the sources of difficulty in doing biological and environmental monitoring, and at over-viewing the different methods available for pesticide exposure and risk assessment, from the regulatory field to real-life exposure studies. A particular attention will be addressed at the new tools which may be developed using modern technologies. Each Speaker will first offer a short theoretical background on each method presented, followed by the practical approach and experience. Each presentation will discuss also prospects for development of these activities.

Keywords: pesticides, exposure assessment, risk assessment, biological monitoring, modeling

Correspondence: claudio.colosio@unimi.it
aris@med.uoc.gr

From Regulation to Risk Assessment: Outlining the Process of Regulatory Exposure and Risk Assessment and the Practical Use of Limit Values

Martin F. Wilks
Swiss Centre for Applied Human Toxicology, University of Basel, Switzerland

The process for human health risk assessment of pesticides involves 4 steps: identification of toxicological hazards; characterization of dose-response relationships; assessment of human exposure; and comparison of exposure estimates to a limit value that is defined as the dose which will not harm humans. Limit values are normally set using threshold levels at which no adverse effects are seen in animal studies and applying a number of uncertainty or modifying factors to account e.g. for interspecies and interindividual differences, or sensitive subpopulations. Different limit values are set for different regulatory purposes: the Reference Dose (RfD) is the amount of a chemical that a person can be exposed to on a daily basis that is not anticipated to cause adverse health effects over the entire lifetime. For pesticide handlers, the Acceptable Operator Exposure Level (AOEL) is the maximum amount of active substance to which the operator may be exposed without adverse health effects. Both RfD and AOEL can also be calculated for acute exposures (typically 24 h or less). An alternative approach to the setting of limit values involves calculating the margin between an observed (adverse) effect and the measured or estimated exposure (MoE). Although current risk assessment methodologies have been successfully used for many years, they need to be developed to address existing and future challenges, e.g. how to integrate novel data (in vitro, omics, biomarkers) into risk assessments; how to deal with chemicals for which no threshold can be established; or how to account for exposure to chemical mixtures.

Keywords: hazard, threshold, reference dose, acceptable operator exposure level, margin of exposure

Correspondence: martin.wilks@unibas.ch

Introduction to Pesticide Exposure Monitoring, Practical Guidance, and Perspectives

Claudio Colosio, Stefan Mandić-Rajčević, Federico Maria Rubino
Department of Health Sciences of the University of Milan and International Centre for Rural Health of the S. Paolo Hospital, Italy
The use of pesticides has continued to grow since their introduction to modern agriculture, and more than 2 billion kilograms of these substances are used per year worldwide. Their main characteristic, that they impact living organisms, makes them a chemical hazard, and their use results in potential health risk, especially in agricultural workers. In agriculture, there is a notable instability of working conditions, disregard of good agricultural practices, and misuse of personal protective devices. Estimating the exposure and absorbed dose in this scenario is, therefore, extremely challenging. Pesticide field studies still represent the main way to collect real-life exposure data, to perform absorbed dose and risk assessment, and to verify the presence or absence of health effects from pesticide use. Here we present the main methods for pesticide exposure monitoring in real-life field conditions, with their advantages, disadvantages, and ways to improve them. Since most of the exposure in open field conditions comes from dermal exposure, OECD “patch” and “whole-body” methodologies are the two most widely used methods for exposure assessment. The main advantages of the “patch” methodology is that it preserves the real-life working conditions and allows the combined use of personal exposure and biological monitoring. The use of biological monitoring is limited in because of the lack of health-based occupational biological exposure limits. Ideally, a method to produce biological exposure limits for pesticide use in agriculture, similar to the AGIHE BEIs, could be developed taking into account skin as the main route of exposure in this setting.

Keywords: methodology, patch, whole-body, biomonitoring, biological exposure limit
Correspondence: claudio.colosio@unimi.it

Duration of Skin Exposure: a Neglected Variable in Absorbed Dose Assessment

Stefan Mandić-Rajčević, Federico Maria Rubino, Claudio Colosio
Department of Health Sciences of the University of Milan and International Centre for Rural Health of the S. Paolo Hospital, Italy

The use of pesticides has become unavoidable in agriculture as it ensures the massive production of food crops and their global trade, as well as solves public health problems by eradicating vectors of human diseases such as malaria. Besides risk assessment done in the pre-marketing phase, field studies allow for the re-evaluation of exposure and risk in real-life working conditions, opening new possibilities for risk assessment and modeling. In agriculture, special attention must be given to the skin as the main route of exposure, but the fixed fractional approach to dermal absorption might not represent the perfect solution to absorbed dose assessment. Here we present a practical method for integrating the information on the duration of exposure into the absorbed dose assessment, using a group of mancozeb applicators as a case study. Assumption of an 8-hour exposure resulted in a gross overestimation of absorbed...
dose from hands’ exposure. Absorbed dose from body exposure was overestimated in those workers working less than 8 hours, but somewhat underestimated in those working more than 8 hours, which is common in agriculture. In total, an 80% reduction of the absorbed dose estimate resulted from the introduction of the duration of exposure as a factor. This reduction did not influence risk assessment significantly for substances with low toxicity such as mancozeb, but implications for modeling might be much more important.

**Keywords:** methodology, patch, biological monitoring, absorbed dose assessment, modeling

**Correspondence:** stefan.mandic-rajcevic@unimi.it

**Integrating Epidemiology Along with other Lines of Scientific Evidence into Pesticide Risk Assessment**

Antonio F. Hernández
Department of Legal Medicine and Toxicology, University of Granada, School of Medicine, Avenida de la Investigación, 11, 18016-Granada, Spain.

Regulatory agencies currently conduct a formal human risk assessment for pesticide active substances based on mandated regulatory toxicology studies. Although this process is mainly based on experimental studies according to specific study protocols, human observational epidemiological studies could add relevant information to the risk assessment process. However, a better use of epidemiology data is needed to improve the understanding and characterization of risks from pesticide exposures. To this end, individual studies addressing the association between pesticide exposure and human health should be subject to a quality assessment of methodology and reporting to meet quality standards. Then, studies should be combined and summarized using systematic reviews and meta-analysis. The impact of evidence synthesis on risk assessment will be particularly useful for problem formulation and hazard identification, though if quantitative data are available dose-response modelling of pooled data could be used for hazard characterization. Therefore, the new paradigm of pesticide risk assessment should be based on all available lines of evidence, from animal regulatory studies to independent peer-reviewed studies, including human data. In addition, novel tools for identifying biological pathways and mechanisms of toxicity (in vitro/mechanistic studies) could provide biological support to the findings observed in animal or human studies. All these lines of evidence can form part of the overall weight of evidence using modified Bradford Hill criteria as an organizational tool to increase the likelihood of underlying causal relationships. This integrative process would allow for a more realistic human risk assessment of pesticides, though a harmonised framework will be required.

**Keywords:** epidemiology, risk assessment, evidence synthesis, weight of evidence, integration of evidence

**Correspondence:** ajerez@ugr.es

**Oxime Efficacy in Acute Organophosphate Poisoning: Challenges and Perspectives**

Biljana Antonijević, Evica Antonijević
University of Belgrade-Faculty of Pharmacy, Department of Toxicology “Akademik Danilo Soldatovic”, Serbia

Organophosphorus (OP) compounds are used as pesticides causing every year thousands of fatalities especially in developing countries, mainly resulting from suicidal or accidental poisonings. Recent homicidal use of chemical warfare nerve agent sarin confirmed that OPs still represent permanent threat on the global scale. High toxicity of OPs is based on inhibition of acetylcholinesterase (AChE), which leads to excessive accumulation of acetylcholine in the synaptic cleft and subsequent overstimulation of cholinergic receptors. Current standard treatment of acute OP poisoning includes a combined administration of causal antidotes (oximes), antimuscarinic drug (atropine) and anticonvulsive drug (diazepam).

The principal mechanism of action of oximes is reactivation of OP-inhibited AChE. Since the synthesis of the first pyridinium oxime in 1955, a number of oximes have been invented and tested in order to identify structures sufficiently potent to restore inhibited AChE and to enhance therapeutic effectiveness. Despite numerous in vitro and in vivo studies none of the known oximes fulfills the crucial requirement, i.e. ability to recover AChE inhibited by a broad range of structurally different OPs. Aimed to overcome present drawbacks and limitations of the oximes, recent research efforts are focused on the development of broad spectrum, less toxic oximes, the synthesis of mono-charged or uncharged reactivators able to penetrate blood-brain barrier, to restore “aged” AChE, and to reactivate OP-inhibited bioscavengers. Designing an oxime with universal antidotal properties is of utmost interest but potentially a never-ending goal. Therefore, alternatively improvement of oximes therapeutic value could be achieved by combination of oximes with complementary spectrum towards critical OPs.

**Keywords:** therapeutic effectiveness, reactivation, drawbacks and limitations, novel strategies

**Correspondence:** abiljana@pharmacy.bg.ac.rs
**ADVANCES IN MOLECULAR METAL TOXICOLOGY**

Chair: Yoshito Kumagai  
*Faculty of Medicine, University of Tsukuba, Japan*

Dr. Naranmandula will especially focus on the anticancer effects of arsenic trioxide and its active metabolites on PML-RARA fusion protein degradation in acute promyelocytic leukemia (APL). He will introduce that the actual arsenic metabolic pathway would be beneficial in elucidating the probable role of different arsenic species in relation to toxicities along with the uses of arsenic as a therapeutic drug. Dr. Hara will discuss recent advances in understanding the change in lipid metabolism in methylmercury (MeHg) and other metals-induced toxicity, focusing on the LC-MS/MS-based lipidomics approach. He will also show the role of some lipid metabolizing enzymes in detoxification of metal-toxicity by using these enzymes gene-engineered mice. Dr. Aschner will discuss recent studies that tested whether the redox sensitive cofactor nicotinamide adenine dinucleotide (NAD(+) is affected by MeHg and whether supplementation of NAD(+) prevents MeHg-induced toxicities. He will show studies in worms treated with MeHg where depletion in cellular NAD(+) levels was prevented by NAD(+) supplementation. Dr. Kumagai will introduce that electrophilic metals such as MeHg and cadmium are able to activate cellular redox signal transduction pathways (e.g., Keap1/Nrf2 pathway, HSP90/HSF1 signaling and PTEN/Akt signaling) involved in adaptive response, quality control of cellular proteins and cell survival at lower concentrations. In addition, he will also introduce that persulfide/polysulfides capture the environmental metals, leading to formation of their sulfur adducts, thereby regulating the activation of redox signaling and cytotoxicity.

**Keywords:** arsenicals, methylmercury, cadmium, molecular toxicology  
**Correspondence:** yk-em-tu@md.tsukuba.ac.jp

---

**Metabolism, Toxicity and Anticancer Activities of Arsenicals**

Hua Narenmandura  
*School of Medicine and Public Health, Zhejiang University, China*

A variety of studies indicated that inorganic arsenic and its methylated metabolites have paradoxical effects, namely, carcinogenic and anticancer effects. Especially, arsenic trioxide (As$_2$O$_3$) is successfully used in the treatment of refractory or relapsed acute promyelocytic leukemia (APL), but its exact antileukemic mechanism in APL is still under investigation. The probable explanation for As$_2$O$_3$-induced cell differentiation is the direct targeting of PML-RARα oncoprotein by As$_2$O$_3$, which results in initiation of PML-RARRa degradation. However, after injection, As$_2$O$_3$ is able to methylated in body to different intermediate metabolites such as trivalent monomethylarsonous acid (MMA$_{III}$) and dimethylarsinous acid (DMA$_{III}$), therefore, it remains unknown that which arsenic specie is actually responsible for the therapeutic effects against APL. Here we have shown the role of iAs$_{III}$ and its intermediate metabolites (i.e., MMA$_{III}$/DMA$_{III}$) in NB4 cells. Inorganic iAs$_{III}$ predominantly showed induction of cell differentiation, while MMA$_{III}$ and DMA$_{III}$ specifically showed to induce apoptosis. Additionally, our results also found that the binding of arsenicals to PML proteins is not associated with the degradation of PML-RARRa fusion protein.

**Keywords:** arsenic trioxide, acute promyelocytic leukemia, PML-RARRa, cell differentiation  
**Correspondence:** narenman@zju.edu.cn

---

**Metal Toxicity and Toxicolipidomics**

Shuntaro Hara  
*Division of Health Chemistry, Department of Healthcare and Regulatory Sciences, School of Pharmacy, Showa University, Tokyo, Japan*

Some metals such as methylmercury cause their toxicity by the induction of oxidative stress in the target organ. Oxidative stress is more likely to enhance lipid peroxidation of membrane phospholipids and formation of bioactive lipid mediators leading to the tissue injury. In order to clarify novel mechanisms of metal toxicity, we focused on lipid molecules as targets of metal toxicity and performed toxicolipidomics, in which we comprehensively investigated...
alterations in lipid profiles including membrane phospholipid composition and production of bioactive lipids. As the results, we found that mouse cerebellum tissue and cerebellar granule cells, both of which are sensitive to methylmercury, contain high levels of polyunsaturated fatty acids in their membrane phospholipids. Methylmercury treatment affected their membrane phospholipid composition but did not enhance the production of eicosanoids. It was also found that some inhibitors of calcium-independent phospholipase A$_2$ (iPLA$_2$s), which have an ability to eliminate lipid peroxides from membrane phospholipids, enhanced methylmercury-induced cell death of cerebellar granule cells. These results suggested that in cerebellum tissues, polyunsaturated fatty acids in membrane phospholipids might be targets of metal toxicity and that iPLA$_2$ might function as detoxifying enzymes. Furthermore, we have established several lipid-metabolizing enzymes gene-engineered mice and found that iPLA$_2$$\gamma$ (one of iPLA$_2$s) or ACSL4 (one of long-chain acyl-CoA synthase isoforms) gene knockout changed membrane phospholipid compositions in mouse tissues/cells. The effects of these gene knockouts on metal toxicity will be also discussed.

**Keywords:** methylmercury, cerebellum, lipidomics, polyunsaturated fatty acids, phospholipase A$_2$

**Correspondence:** haras@pharm.showa-u.ac.jp

---

**NAD$^+$ Supplementation Attenuates Methylmercury Dopaminergic and Mitochondrial Toxicity in Caenorhabditis elegans**

Michael Aschner

*Albert Einstein College of Medicine, Bronx, NY 10461*

We have previously shown that MeHg causes both morphological and behavioral changes in the Caenorhabditis elegans dopaminergic (DAergic) neurons that are associated with oxidative stress. We were therefore interested in whether the redox sensitive cofactor nicotinamide adenine dinucleotide (NAD$^+$) may be affected by MeHg and whether supplementation of NAD$^+$ may prevent MeHg-induced toxicities. Worms treated with MeHg showed depletion in cellular NAD$^+$ levels, which was prevented by NAD$^+$ supplementation prior to MeHg treatment. NAD$^+$ supplementation also prevented DAergic neurodegeneration and deficits in DAergic-dependent behavior upon MeHg exposure. In a mutant worm line that cannot synthesize NAD$^+$ from nicotinamide, MeHg lethality and DAergic behavioral deficits were more sensitive to MeHg than wildtype worms, demonstrating the importance of NAD$^+$ in MeHg toxicity. In wildtype worms, NAD$^+$ supplementation provided protection from MeHg-induced oxidative stress and mitochondrial dysfunction. These data show the importance of NAD$^+$ levels in the response to MeHg exposure. NAD$^+$ supplementation may be beneficial for MeHg-induced toxicities and preventing cellular damage involved in Parkinson’s disease.

**Keywords:** methylmercury, dopamine, nicotinamide adenine dinucleotide (NAD). C. elegans

**Correspondence:** michael.aschner@einstein.yu.edu

---

**Activation of Redox Signal Transduction Pathways Mediated by Electrophilic Metals**

Yoshito Kumagai

*Environmental Biology laboratory, Faculty of Medicine, University of Tsukuba, Japan.*

Methylmercury (MeHg) accumulated in fish such as tuna and cadmium (Cd) contaminated in rice are electrophilic metals that covalently modify protein thiols, resulting in formation of protein adducts. While it is believed that such protein modifications are associated with cell damage and tissue injury due to nonselective and excess modification of cellular protein thiols at higher concentrations, accumulated studies indicate that MeHg and Cd modulate cellular signaling as well. From these observations, we hypothesized that MeHg and Cd would activate redox signaling consisting of sensor proteins and effector molecules through covalent modification of the sensor proteins at lower concentrations because sensor proteins with reactive thiols showing low pKa values should be selectively modified by these metals.

We found that 1) MeHg and Cd modify sensor protein Keap1 and thus repress its activity to hold transcription factor Nrf2, thereby activating Nrf2 and up-regulating proteins responsible for detoxification and excretion of these metals. 2) MeHg causes S-mercuration of phosphatase PTEN, leading to inhibition of its enzyme activity, thereby activating kinase Akt and its downstream transcription factor CREB; as a result, anti-apoptotic protein Bcl-2 involved in cell survival was up-regulated. However, MeHg at higher concentrations disrupted PTEN/Akt/CREB signaling through S-mercuration of CREB. 3) Cd modifies sensor protein HSP90, leading
to blockage of its activity to interact with HSF1, thereby substantially activating HSF1 and up-regulating HSP70 associated with quality control of cellular proteins. 4) Persulfides and polysulfides capture MeHg and Cd, resulting in formation of their sulfur adducts with little toxicity.

**Keywords:** methylmercury, cadmium, covalent modification of proteins, adaptive response, reactive sulfur species

**Correspondence:** yk-em-tu@md.tsukuba.ac.jp

---

**EMERGING AND KNOWN NATURAL TOXINS: ENVIRONMENTAL FATE AND HUMAN RISK**

**Chair:** Emanuela Testai  
*Istituto Superiore di Sanità - Environment and Health Department, Rome, Italy*

Natural toxins are secondary metabolites produced by bacteria, cyanobacteria, fungi, algae, plants and animals. Usually, they are produced in proximity of essential resources for humans and animals, such as food, feed and drinking water. However, their entrance in the food web or their effects after different exposure routes does not represent the only sanitary problem since their impact on environment may affect human welfare.

Although some toxins represent a challenge in toxicological studies and in their management due to the limited availability and not always commercially available, several studies allowed a correct risk assessment and management for some toxins. However, there are no analytical methods for many toxins, and no monitoring data and/or regulations notwithstanding their well-documented toxicities. Examples are represented by “emerging natural toxins”, which, in view of climate changes, may open new scenarios from a toxicological point of view. However, the concept “emerging toxins” is quite subjective, being used for new toxins, known toxins appearing in new geographical areas and non-regulated known toxins requiring additional toxicological evidence before establishing regulations. Hence, an update of the state-of-art of emerging natural toxins and their impacts on the environment and humans is necessary to understand and profile the future goals and challenges to be addressed by the scientific community.

This session will focus on multiple aspects of natural toxins, from the novel findings on the biochemical, molecular and clinical effects, to the new methods for their detection, with special emphasis on the emerging species representing the urgency in the near future.

**Keywords:** human risk, environmental toxicology, natural toxins, human toxicity, monitoring methods

**Correspondence:** emanuela.testai@iss.it

---
The Booming Field of Botulinum Neurotoxins

Marco Pirazzini, Ornella Rossetto, Cesare Montecucco
Department of Biomedical Sciences, University of Padova,
Via Ugo Bassi 58/B, Padova, Italy

Botulinum neurotoxins (BoNTs) are the etiologic agents of botulism, a reversible, yet potentially lethal, neuroparalytic syndrome specifically affecting vertebrates. They are produced by anaerobic bacteria of the Clostridium genus and are the most poisonous toxins known with a MLD_{50} as low as 10 picogram/Kg. Such a potency is due to the specific capability of BoNTs to block peripheral neurotransmission, an essential task for survival, by inactivating the molecular machinery responsible for neurotransmitter release at peripheral nerve terminals. Extreme potency and exquisite neurospecificity make BoNTs a dichotomic reality: from one side they are considered potential bioweapons; on the other, BoNTs have been successfully used since the nineties as remarkable therapeutics and more recently as popular cosmetics.

Traditionally, seven different BoNTs were known, classified according to their immunological properties, yet many novel toxins are now being discovered thanks to the use of NGS, bioinformatics and metagenomics studies. Presently, more than 40 BoNT variants have been isolated from clostridia, and, interestingly, BoNT-like genes and toxins have been found in non-clostridial species. These novelies are now reviving the interest for BoNT basic and applied research. Large efforts are being made worldwide to discover novel antitoxins and for engineering BoNTs both to improve their clinical utilization and to expand the landscape of their therapeutic application. This booming field will be discussed in relation to the recent advances.

Keywords: neuromuscular junction, neurotoxins, peripheral neuroparalysis, metalloprotease

Correspondence: marcopiraz@gmail.com

Cyclic Imines Phycotoxins: Pharmacological Characterization, Biodistribution, Musculoskeletal Effect and Detection of these Emergent Families of Neurotoxic Agents

Denis Servent1, Sophie Creuzet2, Carole Malgorn1, Vincent Dive1, Armen Zakarian1, Romulo Aràoz1,2, Jordi Molgó1,2
1Service d’Ingénierie Moléculaire des Protéines (SIMOPRO), CEA, Université Paris-Saclay, F-91191 Gif sur Yvette, France,
2Institut des Neurosciences Paris-Saclay, UMR 9197, CNRS/Université Paris-Sud, 91191 Gif sur Yvette, France,
3Department of Chemistry and Biochemistry, University of California, Santa Barbara, California 93106, USA

Cyclic imines produced by various species of marine dinoflagellate microorganisms, constitute a widely distributed group of phycotoxins with increasing prevalence in oceanic environment due to recent climate change. During active dinoflagellate blooms, phycotoxins may accumulate in shellfish tissues and can be transferred into fish, marine mammals and ultimately to humans. Among these phycotoxins, pinnatoxins (PnTx-A to H), produced by the dinoflagellate Vulcanodinium rugosum, were recently identified and shown to exhibit the highest oral acute mouse toxicity among cyclic imine toxins. We will describe binding and electrophysiological experiments highlighting the exceptional ability of these toxins to interact with various subtypes of nicotinic receptors (nAChRs), especially the muscle and neuronal (α7) subtypes. Biodistribution analyses by digital radioimaging in rats, after oral or i.v administration of tritiated PnTx-G, revealed the presence of the toxin in various peripheral organs, as well as in the central nervous system, highlighting its property to cross both the intestinal and the blood-brain barrier. Moreover, using the chick embryo as a first model, we show that during embryonic development, PnTx-A exposure reduces embryo motility by decreasing the embryo spontaneous movements, which affected the maturation of the musculoskeletal system. Finally, in order to be able to detect cyclic imine phycotoxin families in the environment, original receptor-based assays were developed in microplate or lateral flow test formats.

Keywords: pinnatoxins, nicotinic receptors, cyclic imines, V. rugosum

Correspondence: denis.servent@cea.fr

Toxic Effects of Co-exposure to Mycotoxins

Maja Peraica
Institute for Medical Research and Occupational Health, 10000 Zagreb, Ksaverska c.2, Croatia

Humans and animals are constantly exposed to mycotoxins mostly through the ingestion of contaminated food and feed, although other ways of exposure are possible. Food is usually contaminated with several mycotoxins due to co-contamination by various molds but also because some mold strains may produce various mycotoxins under modified conditions.
of growth. There are about 400 known mycotoxins that, using very sensitive methods, can be found in food and feed, but only some have been found to have a toxic effect in mammals. Unfortunately, even fewer have known toxicokinetics and mechanisms of toxicity. The mechanisms of toxicity of mycotoxins are rarely studied on experimental animals, while the results of *in vitro* studies on cell cultures are often contradictory. In the available literature on the effect of co-exposure to mycotoxins either in experimental animals or in *in vitro* studies, many different endpoints were measured which creates difficulties in results interpretation. In animals these were changes in body weight, parameters of kidney, liver and immune system lesions, diuresis, activity of plasma, organs and urine enzymes, teratogenicity and mortality rate. In various cell cultures, cell viability, reactive oxygen species production, apoptosis and parameters of oxidative stress were measured. It was found that the same combinations of mycotoxins may have an antagonistic, additive or synergistic effect, depending on the dose of mycotoxins. Although some mycotoxins in combination with others have always had a clear antagonistic effect at any tested dose, the best protection against them is the prevention of their overall appearance.

**Keywords:** additive effect, antagonism, mycotoxins, synergism, toxicity

**Correspondence:** mperaica@imi.hr

---

**Human Health Risks Associated to Cyanotoxins Exposure**

**Emanuela Testai**  
*Istituto Superiore di Sanità - Environment and Health*  
Department, Rome, Italy

Since 3.5 billion years ago, when cyanobacteria were already present on the earth, they have colonized almost all terrestrial and aquatic ecosystems, mainly but not exclusively freshwaters, where they can grow up to very high densities, forming blooms and scums. Cyanobacteria produce a high number of bioactive molecules, among which cyanotoxins, such as microcystins (the most studied group), nodularins, cylindrospermopsin and neurotoxins. Increasing occurrence of blooms, in terms of extension and frequency, associated with excess of nutrients due to anthropogenic activities and climate changes, has given rise to some concern for human health and animal life exposed to cyanotoxins. Numerous cases of lethal poisonings have been associated with cyanotoxins ingestion in wild animal and livestock. In humans few episodes of lethal or severe human poisonings have been recorded after acute or short-term exposure, but the repeated/chronic exposure to low cyanotoxin levels remains a critical issue. Indeed, the cyanotoxins known and studied so far have a large spectrum of toxic effects from hepato- and nephro-toxicity, to neurotoxic effects and tumor promotion, depending on the toxin involved and the exposure scenarios. Despite this, data on the kinetic behavior, toxicological profile and exposure levels are still scant and often limited to few variants within each cyanotoxin group. Nevertheless the WHO organization has derived health based values and considerations on the risks for human health can be drawn to protect the potentially exposed populations.

**Keywords:** cyanobacteria, cyanotoxins, toxicological profile, exposure, risk assessment

**Correspondence:** emanuela.testai@iss.it
MODIFIED MYCOTOXINS – AN EMERGING RISK IN FOOD SAFETY

Chair: Angela Mally
Department of Toxicology, University of Würzburg, Germany

Modified (or “masked”) mycotoxins are metabolites of the parent mycotoxin formed in the fungus, in plants or in animals. Modified forms of certain mycotoxins, which are not captured by routine analysis, may therefore be present in food and contribute to the overall mycotoxin exposure. Preliminary exposure estimates for modified Fusarium mycotoxins indicate that human exposure to certain masked mycotoxins may be as high as exposure to the parent mycotoxin. This suggests that current risk assessment and legal limits, which are based on parent compounds only, may underestimate human health risk resulting from dietary intake of mycotoxins. The European Food Safety Authority (EFSA) therefore identified modified mycotoxins as an emerging risk in food safety. Focusing on fusarium mycotoxins and their modified forms as exemplary mycotoxins of key concern, the session will summarize the current knowledge and highlight data gaps regarding the occurrence, exposure to and toxicity of modified mycotoxins. Recent approaches to risk assessment of modified forms of mycotoxins in food and feed will be presented.

Keywords: modified mycotoxins, mycotoxin exposure, food safety, risk assessment

Correspondence: mally@toxi.uni-wuerzburg.de

Application of Human Biomonitoring to Assess Human Exposure to Mycotoxins and their Modified Forms

Michele Solfrizzo, Lucia Gambacorta
Institute of Sciences of Food Production (ISPA), National Research Council of Italy (CNR), Via Amendola 122/0, 70126 Bari, Italy

Total mycotoxins exposure in humans could be estimated by the combined measures of urinary free mycotoxins, phase I metabolites and their glucuronides and/or sulphates derivatives. The predigestion of urine with β-glucuronidase/sulfatase enzymes deconjugate the glucuronides and sulphates derivatives to form free mycotoxins and phase I metabolites, thus reducing the number of analytes (mycotoxin biomarkers) to be measured. It has been demonstrated that foods can be frequently contaminated by mixtures of mycotoxins and their modified forms. These last can be hydrolysed in the gut to form the parent mycotoxins. Therefore the analysis of urine for multi-biomarker is a powerful approach to identify the type and the number of mycotoxins ingested by each individual. Moreover, the urinary concentrations of mycotoxin biomarkers can be used to estimate the probable daily intake of those mycotoxins for which human excretion rate is available. We have developed an highly sensitive multi-biomarker LC-MS/MS method based on predigestion of urine with β-glucuronidase/sulfatase enzymes and SPE/immunoaffinity concentration and cleanup for the determination of urinary biomarkers of deoxynivalenol (DON), zearalenone (ZEA), aflatoxin B1 (AFB1), fumonisin B1 (FB1), and ochratoxin A (OTA). These are the main mycotoxins frequently co-occurring in food/beverage worldwide and are mainly produced by Fusarium graminearum (DON, ZEA), Aspergillus flavus and A. parasiticus (AFB1), F. verticillioides and F. proliferatum (FB1), Penicillium verrucosum and A. carbonarius (OTA). Our method was used to analyse 356 human urines collected in Italy, South Africa and Sweden and allowed the identification of mycotoxins mostly ingested in each country as well as the probable daily intake of each mycotoxin.

Keywords: mycotoxin, food, urine, biomarker, assessment

Correspondence: michele.solfrizzo@ispa.cnr.it
In Vitro and in Vivo Toxicity of Modified Fusarium Mycotoxins: Current Status and Knowledge Gaps

Angela Mally
Department of Toxicology, University of Würzburg, Germany

Based on current exposure estimates, it appears that application of the threshold of toxicological concern (TTC) concept, which may be used to assess known structures of unknown toxicity present at very low levels in the diet, may not be applicable to modified mycotoxins. Therefore, in order to reliably assess the contribution of individual modified mycotoxins to health risks related to the presence of modified mycotoxins in food, it is essential to understand both their toxic potential and the extent and form in which modified mycotoxins become bioavailable. Due to the lack of commercially available reference standards, however, only limited data on modified forms of fusarium mycotoxins are available so far. The examples of conjugated forms of zearalenone and deoxynivalenol, and of fumonisin bound to components of the matrix demonstrate that conjugated and matrix-associated mycotoxins may be released in gastrointestinal tract and significantly add to the systemic exposure to the free mycotoxin. While in vitro data indicate that conjugates frequently show lower toxic potential compared to the free mycotoxin, the few data available on in vivo toxicity of selected conjugated forms suggest that conjugates may exhibit comparable in vivo toxicity to that of the parent compound, consistent with their cleavage in the gastrointestinal tract. Importantly, a reductive metabolite of zearalenone, (α-zearalenol), was shown to be 60-times more estrogenic than its parent mycotoxin, indicating a potentially large contribution of some modified forms to the overall health risk.

Keywords: modified mycotoxins, fusarium mycotoxins, toxicity, food safety

Correspondence: mally@toxi.uni-wuerzburg.de

Current Approaches to Health Risk Assessment of Modified Mycotoxins in Food and Feed

Nicole Lorenz1, Sven Dänicke2, Lutz Edler2, Christoph Gottschalk3, Eva Lassek4, Doris Marko5, Michael Rychlik6, Angela Mally8
1Federal Institute for Risk Assessment (BfR), Max-Dohrn-Str. 8-10, D-10589 Berlin, Germany
2Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Bundesallee 50, D-38116 Braunschweig, Germany
3German Cancer Research Center, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany
4Chair of Food Safety, Ludwig-Maximilians-Universität München, Schönleutnerstr. 8, D85764 Oberschleißheim, Germany
5Bavarian Health and Food Safety Authority, Luitpoldstr. 1, D-97082 Würzburg, Germany
6Institute of Food Chemistry and Toxicology, University of Vienna, Währingerstr. 38, A-1090 Vienna, Austria
7Chair of Analytical Food Chemistry, Technische Universität München, Alte Akademie 10, D85354 Freising, Germany
8Department of Toxicology, University of Würzburg, Versbacher Strasse 9, D-97078 Würzburg, Germany

A systematic definition consisting of four hierarchical levels introducing the term “modified mycotoxins” for all mycotoxin compounds that are altered in their chemical structure compared to the corresponding parent compound was proposed in order to encompass all possible forms in which mycotoxins and their modifications can occur. Potential exposure to modified mycotoxins due to their presence in food and feed has raised concern that modified mycotoxins may pose a substantial additional risk to human and animal health.

Considering the range of modified forms of zearalenone (ZEN) detected in food and feed, the endocrine activity of several of these metabolites and the fact that estimated dietary exposure to free ZEN alone is already close to the tolerable daily intake, ZEN is used as an exemplary mycotoxin of key concern. Recently published scientific opinions of the European Food Safety Authority (EFSA) are taken as starting point for the evaluation of the current situation and identification of data gaps for a comprehensive health risk assessment of ZEN and its modified forms.

The development of sufficiently sensitive analytical methods for the detection of modified mycotoxins, the application of these methods to generate reliable occurrence data, the reduction of uncertainties in the toxicology of modified mycotoxins, the derivation of appropriate health-based guidance values, the utilization of biomonitoring data to reduce uncertainties in the exposure assessment as well as the development of strategies for health risk assessment of chemical mixtures were identified as key challenges which will be presented along with recommendations for further research.

Keywords: zearalenone, oestrogenicity, dietary exposure, biomonitoring, chemical mixtures

Correspondence: Nicole.Lorenz@bfr.bund.de
APPLICABILITY AND LIMITATION OF NON ANIMAL TESTING IN SAFETY ASSESSMENT

Chair: Emanuela Corsini¹
Co-Chair: Emanuela Testai²
¹ Università degli Studi di Milano, Milan, Italy
² Istituto Superiore di Sanità, Rome, Italy

A fundamental change occurring in toxicity testing to improve efficiency and human relevance in chemical safety assessments is the use of testing strategies, integrating human cell-based in vitro assays, including advanced models such as the microfluidic systems, and new predictive computational tools. This allows to move away from animal-based risk assessment strategies and default factors used for extrapolating to humans and to meet many pieces of regulation in Europe, asking for reduction in animal testing.

The session will present consolidated and emerging methodologies and how industry, regulatory agencies and academy are facing the use of non animal testing methods, highlighting the successful cases in which their applicability has been already accepted, the limitation(s) of their use in other cases and the challenges for the future regarding the increase in their predictivity of in vivo effects in response to chemical exposures.

Keywords: safety assessment, in vitro methods, in silico methods, regulatory acceptance

Correspondence: emanuela.corsini@unimi.it
emanuela.testai@iss.it

Think-exposure-first! Industry Perspective on Predictive Tools for Exposure-based Safety Assessment

Marco Corvaro
Dow Agrosciences, UK

In the global agrochemical regulatory testing programme, risk assessment is driven by “external doses” administered to 4 animal species used (humans clinical testing is not currently possible). Despite not strictly required for registration, translational evaluation of internal exposure alongside toxicodynamic characterisation has been supported by numerous publications and regulations. Introductory traditional examples from animal programs will be given for species sensitivity/human relevance characterisation and dose selection, using more relevant kinetically-derived maximum doses (not MTDs).

A toolbox of non-animal ADME models has been developed for decades in the pharmaceuticals regulatory packages and is further developing with the changes in the cosmetic industry regulation. These tools provide unique opportunity to characterise internal exposure and inter-species toxicokinetic differences. In addition, some of the more complex predictive tools are also being characterised by regulatory agencies for predictivity of target-organ toxicody-namic, with a variety of complex endpoints such as high content or toxicogenomic techniques. The usefulness of these tools for the characterisation of the human relevance of toxicology findings and factors influencing the regulatory uptake will be discussed.

Current and future applications include the use to design more sustainable “green chemistry” in the R&D agrochemical process and the potential for a shift towards (internal) exposure-based safety assessment, already in place in other sectors of regulated industries.

Keywords: non-animal ADME models, toxicogenomic techniques, regulatory acceptance

Correspondence: MCorvaro@dow.com

Regulatory Use of Non Animal Models: the Present and the Future Challenges

Emanuela Testai
Istituto Superiore di Sanità, Rome, Italy

The currently hazard-driven approach in toxico-logical risk assessment has strong reliance on animal testing. However, as a consequence of advances in scientific knowledge, modelling and measuring
techniques, the emphasis of the scientific community is moving towards integrating in vitro human cell-based assays, imaging and high throughput methodologies (including ‘-omics’ and microfluidic organ-on-a-chip) with computational modeling, with a shift to an exposure-driven, more mechanism-based paradigm, with identification of AOP.

Since regulators demand validated and internationally accepted non-animal methods, during the last two decades, a number of in vitro methods were submitted to international validation bodies. This exercise showed that most of the available in vitro and in silico methods still require considerable improvements in design, robustness and reliability to generate data sets useful to support regulatory decisions, the major issues being the establishment of in vitro preparations preserving in vivo original properties for prolonged periods of time, the biokinetics measurement and the establishment of in vitro biomarkers of adversity. EURL ECVAM is currently coordinating the issuing of a guidance on Good In Vitro Method Practices (GIVIMP). ECHA is working on a ‘Report on the regulatory applicability of alternative and non-animal approaches’.

Some animal-free tests have been already adopted as Guidelines and accepted by regulators in different areas (e.g. some non-testing methods such as TTC or read across, and some genotoxicity tests, dermal absorption, skin sensitization testing) and others are included in the data requirements by regulatory authorities (e.g. the in vitro comparative metabolic profile of pesticide active substance asked by EFSA).

Keywords: non animal testing, alternative methods, regulatory acceptance
Correspondence: emanuela.testai@iss.it

Applicability of Non-animal Based Tools for Food and Feed Safety Assessment

Jean-Lou Dorne
EFSA, Italy

Application of non-animal based tools in food and safety is of critical importance both to reduce animal testing and to apply predictive tools dealing with data poor chemicals. In 2017, EFSA published OpenFoodTox: its open source database which provides summary hazard data for more than 5000 chemicals in the human, animal and ecological risk assessment (HRA, ARA, ERA). From openfoodtox and other relevant databases (US-EPA terrestrial database, Fraunhofer RepDose), a number of predictive QSAR models have been developed. For HRA, these include continuous QSAR models predicting NOAEL values in rats for general sub-chronic toxicity and sub-chronic liver toxicity. For ARA and ERA, models include a continuous one for acute contact toxicity in trout and a classification model predicting acute toxicity in honey bees were.

Finally, generic toxicokinetic (TK) models were developed for HRA, ARA and ERA using physiological, metabolism data and their use has been illustrated for a number of single and multiple chemicals. These TK tools provide a means to further integrate exposure, TK processes and toxicity through quantitative modelling of inter-species differences and inter-individual differences.

The future of open source mechanistic tools as alternatives to animal testing is discussed including in silico and in vitro tools and tiered weight of evidence approaches tailored to support risk assessment. International cooperation between national and international scientific advisory bodies and academic institutions concludes as the corner stone for the translation of 21st century toxicological research into harmonised methodologies and tools and for the training of the next generation of risk assessors.

Keywords: food, feed, in silico methods, harmonised methodologies
Correspondence: Jean-Lou.DORNE@efsa.europa.eu

History of a Success: Contact Hypersensitivity

Emanuela Corsini
ESP, Università degli Studi di Milano, Italy
Correspondence: emanuela.corsini@unimi.it

Over the last two decades, an incredible progress has been made in the field of in vitro immunotoxicology, in particular, in the area of contact hypersensitivity. Several in vitro methods to support the discrimination between skin sensitizers (i.e. UN GHS Category 1) and non-sensitizers in combination with other complementary information (i.e. in the context of an Integrated Approach for Testing and Assessment, OECD guidance document No. 256) have been developed. These methods have been formally validated and OECD guidelines are available. In addition to the already validated tests, several other methods will be soon validated for skin sensitization. Currently validated methods are useful for hazard identification, classification and labeling. With no doubt, contact hypersensitivity represents a real success in the field.
of alternative methods to the use of the animals: from the description of the first Adverse Outcome Pathway to the validation of several in vitro methods, this field of research is a history of a success. However, it is important to remember that to achieve a complete replacement of animals in skin sensitization assessment, dose-response information and evaluation of relative skin sensitizing potency to support effective risk assessment are necessary. In this presentation, the state-of-the-art in the field of in vitro assessment of skin sensitization and the in vitro identification of the no induction sensitization level for contact sensitizers will be discussed.

**Keywords**: skin sensitization, integrated testing strategy, effective risk assessment

---

**Future of Non Animal models: 3D Models and Human on a Chip**

**Thomas Hartung**  
*CAAT, Baltimore, USA*

The increasing doubt into the value of animal models given their ethical and pecuniary costs and the concomitant emergence of predictive non-animal models have brought us to a tipping point, where new approaches are more and more dominating life science research. Objective assessments of the reproducibility of animal studies even under quality-assurance by Good Laboratory Practice are showing tremendous problems contributing to the reproducibility crisis perceived in science. In recent years, for example, pharmaceutical companies have dramatically reduced animal usage while shifting to novel tools. Four trends contributing to this paradigm change will be discussed: (1) the favoring of mechanistic assays, (2) the generation and mining of big data, (3) the creation of more relevant organotypic cell cultures (microphysiological systems) and (4) the integration of different information sources in Integrated Testing Strategies, Systems Biology approaches and Systematic Reviews (evidence-based approaches).

**Keywords**: organ on-a-chip, adverse outcome pathway, big data, systematic review

**Correspondence**: Thartun1@jhu.edu

---

**ENVIRONMENTAL POLLUTION AND TOXIC OUTCOMES: DOSES, MOLECULAR BIOMARKERS, AND ASSOCIATIONS**

**Chair**: Hilmi Orhan
**Co-chair**: Ali Esat Karakaya

1*Ege University, Faculty of Pharmacy, Izmir, Turkey,*  
2*Retaired, Gazi University, Faculty of Pharmacy, Ankara, Turkey*

Environmental pollution is a growing problem all over the world. Various chemicals at different emission rates enter one or more environmental compartments by industrial, agricultural and/or household usage, as well as by road traffic. These chemicals directly and/or indirectly affect wildlife, and eventually reach humans by atmosphere, water, and food chain. There have been epidemiological, as well as experimental findings that they may trigger several pathophysiological mechanisms and cause various diseases. The possible link between persistent organic pollutants, metals, therapeutic agents and diseases and proposed hypotheses will be thoroughly discussed and recent data on this field will be presented in this symposium.

**Key words**: Environmental pollutants, exposure, disease, human, biomarker

**Correspondence**: horhan@gmail.com  
aekarakaya@gmail.com
Kidney, Breast and Stomach Cancers in Relation to Internal Concentrations of Persistent Organic Pollutants, DNA Damage Markers and Related Polymorphisms

Hilmi Orhan1, Sinan Süzen2, Rasih Kocaçoğz1, Ilgen Onat1, Merve Demirbüüen2, Burak Turna3, Koray Atilla4, Levent Yeniay5, Banu Sarsık1, Osman Zekioğlu3, Murat Özdemir3
1Ege University, Faculty of Pharmacy, 2Ankara University, Faculty of Pharmacy, 3Ege University, Faculty of Medicine, 4Dokuz Eylül University Faculty of Medicine, İzmir, TURKEY

Environmental pollution has been linking to various diseases including cancers since soon after the industrial revolution. Among the numerous synthetic pollutants, organochlorine pesticides, polychlorinated biphenyls and polybrominated diphenyl ethers are the most concerned ones because of their chemical stability and lipophilicity, and called "persistent organic pollutants; POPs". In majority of the human studies on the link between POPs and cancers, exposure data have been based on self-declaration questionnaire, which potentially diminish the accuracy and reliability of quantitative assessment. In order to accurately assess whether environmental exposure to POPs increase risk of kidney, breast and stomach cancers, tumour tissues as well as related specimen were collected from surgically operated patients. Cellular DNA and protein oxidative damage markers (8-OHdG and dityrosine, respectively), have also been analysed in the patients and healthy control group, and assessed whether there are changes in these parameters because of the disease. The preliminary data suggest that tissue and blood POP concentrations were, although weakly, associated with cancers in patients. DNA and protein damage was found to be higher in patients compared to healthy volunteers, although inter-individual variability and sample size analysed so far prevented statistical significances. Current findings confirmed that glutathione S-transferase theta null-polymorphism is a risk factor for kidney tumours. Data suggest that environmental exposure to POPs may induce tumour initiation and/or progression in humans.

The study was supported by The Scientific and Technical Research Council of Turkey (TUBITAK, grant SBAG-114S310).

Key words: Persistent organic pollutants, cancer, human, DNA damage, biomarker

Correspondence: horhan@gmail.com

Drugs and their Metabolites as Environmental Pollutants

Momir Mikov1,3, Svetlana Golocorbin-Kon2
1School of Pharmacy, Faculty of Medicine, University of Banja Luka, Bosnia and Herzegovina, 2Faculty of Medicine, Department of Pharmacy, University of Novi Sad, Serbia
3Faculty of Medicine, Department of Pharmacology and Toxicology, University of Novi Sad, Serbia

Drugs enter into the environment through various routes. The general perception is that drugs in the environment are the result of inappropriate disposal of expired and/or unused drugs. The reality is that the most drugs detected in the environment are the result of their therapeutic use in humans and animals. When drugs finish their therapeutic role they take another role as environmental pollutants. Ingested or injected drugs are excreted unmetabolized via the urine and feces or as active or inactive metabolites. Domestic sewage is a dominant and hospital sewage is a secondary source of environmental pollution with drugs and their metabolites. Drugs and their metabolites can escape degradation in standard sewage treatment facilities. The metabolites, especially their conjugates can be transformed to the parent drug. Many questions regarding environmental pollution are unanswered like: what is the impact of the short term and long term of low levels of drugs, what is the impact of the exposure to the mixtures of drugs, their metabolites and chemicals, what populations are most vulnerable like elderly, young, pregnant women, disease, what is the significance for the animals and plants which are used as food?

Treatment of patients (human and animal) with drugs is beneficial to them, but its role as environmental pollutant contributor is underestimated. The evaluation of the risk of drugs and their metabolites should be a part of their drug evaluation process before drug registration. The implementation of the system of ecopharmacovigilance is crucial for the monitoring and intervention regarding their impact on the environment.

Keywords: drugs as contaminants, ecopharmacovigilance

Correspondence: momir.mikov@mf.uns.ac.rs
Neurodegenerative and Neurodevelopment Disorders Linked to Chemicals: What are the Underlying Mechanisms?

Antonio F. Hernández¹, Fernando Gil¹, Beatriz González-Alzaga²,³, Clemente Aguilar-Garduño¹, Marina Lacasaña²,³,⁴
¹University of Granada School of Medicine, ²Andalusian School of Public Health, ³ibs.GRANADA, Spain ⁴CIBERESP, Spain;

The developing brain is much more susceptible to toxic injury than the adult brain. The developmental origin of health and disease hypothesizes that the environment during fetal and childhood development affects the risk for many chronic diseases in later stages of life, including neurodevelopmental disorders. Expose to environmental factors, such as neurotoxicants, mental stress and malnutrition during fetal and neonatal periods can induce epigenetic alterations of the offspring. Alternatively, these exposures can disrupt the normal function of neurotransmitter receptors during developmental periods, affecting the mechanisms driving neural progenitor cell proliferation, migration, differentiation, neurite outgrowth, dendritic maturation, synaptogenesis, and apoptosis, eventually leading to neurodevelopmental disorders.

On the other hand, epidemiological studies have identified exposure to neurotoxic substances as a risk factor for various neurodegenerative diseases, particularly in genetically vulnerable people as a result of gene–environment interactions. The underlying pathogenic mechanisms include oxidative stress, mitochondrial dysfunction, epigenetic modifications, impairment of the ubiquitin proteasome system (UPS) and deregulated autophagy. Many environmental factors can produce highly reactive molecules in nerve tissue or affect antioxidant defense mechanisms, leading to oxidative protein modifications, oxidative modification in the mitochondrial DNA, mitochondrial dysfunction and neuronal death. Insufficient cellular repair mechanisms may contribute to premature aging and neuronal apoptosis. Moreover, epigenetic modifications play a pivotal role in the epigenetic regulation of gene expression and many other cellular events, including growth, differentiation, development, learning and memory, and apoptosis. Defects in the UPS and autophagy lead to the accumulation and aggregation of toxic proteins, which may eventually result in neurodegeneration.

Keywords: neurodegeneration, neurodevelopment, mode of action, mechanisms of action, toxicodynamic
Correspondence: ajerez@ugr.es

Cadmium Modulation of Immune Defense and Susceptibility to Inflammatory Diseases: Insight from Animal Models

Milena Kataranovski¹,²
¹Department of Physiology and Biochemistry, Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia
²Department of Ecology, Institute for Biological Research “Sinisa Stankovic”, University of Belgrade, Despota Stefana Boulevard 142, 10000 Belgrade, Serbia

Cadmium (Cd) is important food and water contaminant, which allocates gastrointestinal tract as target for its toxicity. The effect of 30-day oral intake of 5 ppm and 50 ppm of Cd to gut immune reactivity in DA and AO rats (which accumulate similar levels of Cd in the intestine) was assessed. More pronounced intestine damage, inflammation, along with reduction of commensal bacteria of Lactobacillus strain, were noted in DA rats only. Intestinal damage and inflammation was seen in cadmium-exposed AO rats, but immune priming of major gut-associated (mesenteric) lymph nodes (MLN) was absent. Immune priming of MLN [(increase of cell proliferation, inflammatory cytokine (IFN-γ and IL-17) production] in DA rats, is devoted to protect vulnerable intestine from bacterial overgrowth. However, activity in this otherwise tolerogenic lymphoid microenvironment might be an introduction to perturbation of immune-mediated homeostasis in the gut, making MLN a player in the disease pathogenesis. Systemic immune-modulatory effects of oral cadmium intake in rats were also seen in the skin, barrier tissue responsible both for immune defense and immune tolerance to commensal microorganisms. Neutrophil infiltration, mast cells hyper-granulation and change in inflammatory (IL-6, TNF and IL-1) epidermal cell cytokine production and their responsiveness to commensal Staphylococcus epidermidis were observed in the skin of DA rats which consumed cadmium. This depicted skin not only as a place of cadmium accumulation, but also indicate impact on tissue immune homeostasis. Both local and systemic immune-toxic effects should be taken into account when assessing dietary cadmium as health risk factor.
Environmental Lead Exposure in Children: a Problem of Developing Countries?

Petar Bulat1,2, Stefan Mandić-Rajčević3, Zorica Bulat4, Vesna Matović4

1University of Belgrade, Faculty of Medicine, Belgrade, Serbia, 2Serbian Institute for Occupational Health “Dr Dragomir Karajović” Department of Toxicology, Belgrade, Serbia, 3Department of Health Sciences of the University of Milan and International Centre for Rural Health of the San Paolo Hospital, Via San Vigilio 43, 20142 Milan, Italy, 4Department of Toxicology “Akademik Danilo Soldatović,” University of Belgrade - Faculty of Pharmacy, Serbia

Environmental exposure to lead, although not an important cause of mortality, represents one of the main causes of morbidity among children and adolescents. In general, rural communities are expected to have significantly lower blood lead levels (BLLs) than urban communities. However, this is not the case in populations living in the vicinity of lead mines and smelting facilities, where higher BLLs may occur, particularly among young children. Around 50% of Global lead production can be traced back to car battery recycling.

The aim of this study was to quantify blood lead levels (BLLs) of children living near a car battery smelting facility in Serbia, compare the levels with developed and developing countries’ standards, and identify the main determinants of lead exposure in this population. BLLs were quantified in 75 children from Zajača, a village where a car battery smelting factory is located, and 52 children from Paskovac, village 5 kilometers away from Zajača. The median BLL for both groups were 12 μg/dl, 7.60 μg/dl in children from Paskovac, and 17.5 μg/dl in children from Zajača. Even 87% of children from Zajača had the BLL above 10 μg/dl, which is comparable to urban school-children in South Africa and Bangladesh. Although a European country, a candidate country for the European Union, BLLs of Serbian children were comparable to children living near lead smelting facilities in developed countries.

Keywords: combined exposure, blood lead levels, mixed-effects model

Correspondence: petar.bulat@med.bg.ac.rs

EVALUATION OF SAFETY PROFILE OF HERBAL PRODUCTS

Chair: Ahmet Aydin¹
Co-Chair: Nan Mei²
¹Department of Toxicology, Faculty of Pharmacy, Yeditepe University, Turkey; ²Division of Genetic and Molecular Toxicology, National Center for Toxicological Research, U.S. Food and Drug Administration (FDA), United States.

Herbal supplements and plants have a long history in many cultures for use in health improvement and treatment of various illness and ailments. Their long term use is a guaranty of their safety according to the international regulatory documents, although there are some concerns on their safety profile. The complex chemical nature of herbal products makes it difficult to evaluate their efficacy and safety. Published international regulatory issues about herbal products were discussed from safety concern. The toxicological data on some herbal products have been reviewed. Interaction is one of the important topics about herbal use. Possible interactions and endpoints have been investigated during co-administration of herbal product and other therapeutic drugs. Among the toxicological endpoints, genotoxicity attracts great attention because of direct relationship between genotoxicity and carcinogenic development. Evaluation of mutagenic and genotoxic effects of herbal formulations in multiple test systems will be discussed in this session. Threshold for toxicological concern (TTC) is an efficient regulatory tool for risk assessment. This TTC approach, along with innovative applications, will be discussed in order to conduct risk assessment of the genotoxic compounds found in herbal drugs. As an example for genotoxicity evaluation of herbal products, goldenseal, which is available in a wide array of herbal products on the international market and has been used for the treatment of a wide variety of ailments, has been studied for their carcinogenicity potential and mechanism of action. Recent studies suggest that topoisomerase II inhibitory effect might contribute to its carcinogenic development.

Keywords: regulatory issues, threshold for toxicological concern, genotoxicity, interaction, goldenseal

Correspondence: ahmet.aydin@yeditepe.edu.tr nan.mei@fda.hhs.gov
Safety Evaluation of Herbal Products from the View Point of Regulations

Ahmet Aydın
Department of Toxicology, Faculty of Pharmacy, Yeditepe University, Turkey

Herbal products have been used for years in the world for their different health benefits. However, their long-term use is no guarantee of their safety according to the regulatory documents. There are some missing information on their safety profile. Many of the responsible authorities indicate that in case of enough data on animal use for the same type of toxicity test, it should be restricted. According to the European Medicine Agency (EMA), if a herbal product has been used in the world for 30 years and at least 15 years in Europe, there is no need to carry out general toxicity tests except for developmental and reproductive toxicity, genotoxicity, and carcinogenicity. For many of the herbal products due to their-use for a long time, there is generally a gap at these types of data. The published regulatory documents, mainly EMA documents, were reviewed, and the present situation was discussed. Missing information on herbal products should be completed for the need for further evaluation of herbal products.

Keywords: regulatory issues, long term use, genotoxicity

Correspondence: ahmet.aydin@yeditepe.edu.tr

Toxicological Endpoints of Herbal Product-Drug Interactions

Nurşen Başaran¹, Merve Bacanlı¹, A. Ahmet Başaran²
¹Hacettepe University Faculty of Pharmacy Department of Pharmaceutical Toxicology 06100, Ankara, Turkey, ²Hacettepe University Faculty of Pharmacy Department of Pharmacognosy 06100, Ankara, Turkey

In recent years, because of the belief that herbal medicines are safe in the treatment or prevention of diseases, and the protection of the overall health status, their usage has increased. However, as the contents of herbal medicines have many bioactive components, the lack of sufficient study on their efficacy and toxicity, inadequate controls of their availability reduce their safety. Many medicinal herbs and pharmaceutical drugs can be therapeutic at one dose and toxic at another. Interactions between herbs and drugs may increase or decrease the pharmacological or toxicological effects of the active component. In addition, patients with chronic diseases who also use herbal medicines must consider the adverse effects and interactions of these substances. The herbal medicines contain substances which can induce or inhibit enzymes that can take part in drug metabolism. Particularly, drugs with a narrow therapeutic index (warfarin, digoxin, etc.), and many plants that affect drug metabolism (Hypericum perforatum, Panax ginseng, Ginkgo biloba, etc.) when they are used together, may lead to undesirable results. The concurrent use of these drugs with some medicinal plants can cause serious adverse effects and also the decrease of the efficacy of the therapy. In order to prevent unwanted medicine-herbal drug interactions, it is important to have more information about this area and patients using herbal medicines must consult with their physician and pharmacist.

Keywords: herb, drug, interaction, toxicity

Correspondence: nbasaran@hacettepe.edu.tr

Risk Assessment of Genotoxic Compounds in Herbal Drugs from TTC to Innovative Approaches

Heidi Foth
Martin Luther University Halle Saale, Germany

Naturally occurring genotoxic compounds or carcinogens such as estragole, pulegone, or menthofuran cause alerts during evaluation of herbal medicinal products for efficacy and safety. Contaminating plants carry pyrrolizidine alkaloids into the final product. Another example for contamination are polycyclic aromatic hydrocarbons from air pollution or technical sources during processing of the herbs.

In principle, the rules from scientific assessment of risks from herbal products are the same as already established for other sectors such as occupational world or food safety. The progress in toxicological research on genotoxic compounds has generated doubts whether broadly distributed herbs and traditionally consumption patterns are proven to be safe. But observational studies on human health are also vague or missing and it is unclear whether these naturally occurring carcinogens are a real danger.

It may be debatable whether the insight is strong enough to reason the need to lower the use of herbal plants in general. A scientific discussion is needed on signal values and margin of exposure approaches that will give the right impulses for risk management measures that are effective to reduce consumers health
risk on the background of diverse sources of exposure. Many plants used in pharmaceuticals and in medicinal teas have an overlapping exposure scenario with traditional cooking with herbs or with herbal tea consumption.

Keywords: herbal medicine, margin of exposure, natural carcinogens, genotoxicity

Correspondence: heidi.foth@uk-halle.de

Mechanistic Study of Goldenseal-Associated Genotoxicity

Nan Mei¹, Si Chen², Lei Guo²

¹Division of Genetic and Molecular Toxicology, ²Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, AR 72079, United States.

Correspondence: nan.mei@fda.hhs.gov

Goldenseal (Hydrastis canadensis) is available in a wide array of herbal products on the international market, and it has been used for the treatment of a wide variety of ailments. The five major alkaloid constituents in goldenseal are berberine, palmatine, hydrastine, hydrastinine, and canadine. When goldenseal was evaluated by the National Toxicology Program (NTP) in the standard 2-year bioassay, goldenseal induced an increase in liver tumors in rats and mice. It is of great interest to investigate the potential mechanisms that may contribute to its carcinogenicity. In this study, the toxicity of the five goldenseal alkaloid constituents was characterized, and their toxic potencies were compared. As measured by the Comet assay and the expression of γ-H2A.X, berberine, followed by palmatine, appeared to be the most potent DNA damage inducer in human hepatoma HepG2 cells. Berberine and palmatine suppressed the activities of both topoisomerase (Topo) I and II. In berberine-treated cells, DNA damage was shown to be directly associated with the inhibitory effect of Topo II, but not Topo I by silencing gene of Topo I or Topo II. In addition, DNA damage was also observed when cells were treated with commercially available goldenseal extracts and the extent of DNA damage was positively correlated to the berberine content. Our findings suggest that the Topo II inhibitory effect may contribute to berberine- and goldenseal-induced genotoxicity and tumorigenicity. The use of goldenseal or berberine products may be not without risk due to this “off-target” toxicity.

Keywords: goldenseal, berberine, DNA damage, Comet assay, γ-H2A.X
Harmonization of Approaches to Human Biomonitoring: Benefits and Challenges

Irina Zastenskaya, Dorota Jarosinska, Elizabet Paunovic
WHO Regional Office for Europe, WHO European Centre for Environment and Health

Human biomonitoring (HBM) is a reliable tool for assessment of human exposure to hazardous chemicals. HBM provides information on cumulative exposure to chemicals from different sources, opens opportunities for investigating links between exposure and health effects, allows assessing geographical and temporal trends of exposure, and provides the basis for cost-effectiveness and social-economic impact analysis. But HBM isn’t applicable for all chemicals of interest and for monitoring of sources of exposure. Ideally, HBM and environmental monitoring should complement each other. Harmonization of approaches to HBM allows getting comparable data, which enable monitoring of geographical distribution of exposure, collecting knowledge on sources of chemicals to humans, prioritizing chemicals of public health concern, and identifying the effective risk reduction measures. In addition, the WHO recommendations on HBM create opportunities for setting national biomonitoring programs, especially in developing countries.

The first experience of applying a harmonized approach to HBM comes from a WHO/UNEP survey of POPs in breast milk. Conducted in the framework of the Stockholm Convention, the survey provides valuable data on distribution of POPs globally, effectiveness of preventing measures, and allows identifying hotspots, also unexpected.

Another successful experience is the development of a harmonized approach to assessment of prenatal exposure to mercury. Lessons learned from both initiatives show challenges in harmonizing all components of HBM and the need to adapt the WHO recommendations to specificities and capacities in countries. In certain areas, such as methods for analysis of chemicals in biological matrices, harmonization is challenging due to differences in analytical capacities.

Keywords: human biomonitoring, exposure, hazardous chemicals, harmonization, mercury

Correspondence: zastenskayai@who.int

Towards Expanding the HBM in Europe – the European Human Biomonitoring Initiative HBM4EU

Argelia Castaño1, on behalf of the HBM4EU Initiative2

Humans are exposed to a wide range of chemicals in their daily life at the workplace. Exposure to some of those chemicals can seriously damage human health. In order to effectively tackle chemical risks, policies must be grounded in a robust scientific understanding of the chemical exposure and the potential impacts on human health.

HBM measures chemicals or their metabolites in the human body. It provides an integrated measure of the level of exposure to chemicals independently of their source and considering individual susceptibility. As such, HBM is an important tool for assessing exposures of the human population to chemicals, and estimating potential health risks linked to the exposure. Analysed over time, HBM data allow evaluation of trends in exposure and can be used to assess the efficiency of implemented policies.

HBM4EU is a joint effort of 28 countries, the European Environment Agency and the European Commission, co-funded under Horizon 2020. The main aim of the initiative is to coordinate and advance human biomonitoring in Europe. HBM4EU will provide better evidence of the actual exposure of citizens to chemicals and the possible health effects to support policy making. HBM4EU will run for five years, from 2017 to 2021, building on previous activities undertaken at EU and national levels. The HBM4EU initiative represents a novel collaboration between scientists and chemical risk assessors and risk managers, including several Commission services, EU agencies and national representatives. The project will build bridges between the research and policy worlds and deliver benefits to society in terms of enhanced chemical safety.

Keywords: chemical exposure, human biomonitoring, project, Europe

Correspondence: castano@isciii.es

Application of a Harmonized Approach to HBM: Insights from a Multi-country Project on Mercury

Irina Ilchenko1, Tatiana Boyarskaya1, Kamila Timoshenko1, Sergey Lyapunov2, Olga Okina2

1I.M. Sechenov Moscow State Medical University (Sechenov University), 2Institute of Geology Russian Academy of Sciences
Mounting evidence confirms that mercury affects the developing brain at early life. This human biomonitoring (HBM) survey in Russia, aiming at assessment of prenatal exposure to mercury in Republic of Karelia (RK) and its determinants, was conducted using the WHO methodology including the survey protocol and standard operating procedures for sampling of biological matrices and mercury analysis. Some amendments were required to apply the WHO harmonized procedures in the country.

In cross-sectional survey, 252 women from RK were examined. Epidemiological information was obtained using the WHO questionnaire. Mercury level in hair and cord blood was assessed using AAS with cold vapor. One-way ANOVA was used to compare mercury levels with SES trajectories in the region. Determinants of exposure, social gradients in exposure were examined with multiple and logistic regression.

The geometric mean for mercury was 0.534 µg/g in maternal hair, 2.29 µg/L in cord blood. Among the most important determinants of exposure to mercury were municipality and urban vs rural residency as well as maternal education level. There was a significant effect of the education level on concentrations of mercury in hair of women (F=4.30; p<0.01), on mercury in cord blood (F=4.74; p<0.01), urban/rural residence for mercury in hair (F=6.68; p<0.01).

The survey demonstrated applicability of the WHO harmonized approach. The findings provide comparable exposure data, which might be useful for long-term mercury HBM. Results also illustrate the magnitude and distribution of inequality in mercury exposure within Karelia and help to identify target groups and prioritize areas for intersectoral action.

Keywords: mercury, human biomonitoring, prenatal exposure, Republic of Karelia

Correspondence: Irinailchenko9@gmail.com

**Role of HBM in Managing Contaminated Sites: Exposure to Lead Close to Antimony and Lead Mining and Metal-processing Complex in Serbia**

Branislava Matic1, Dragana Jovanović1, Igor Dragičević2

1Institute of Public Health of Serbia, Belgrade, Serbia
2Institute of Public Health of Šabac, Šabac, Serbia

Objective of this cross-sectional study was assessing exposure to lead and its dynamics through examining blood lead levels (BLL) in children, living in the vicinity of the Zajača antimony and lead mining and metal-processing complex, as compared to partially exposed (Paskovac), and non-exposed (Gornja Borina) population.

Two cross-sectional studies were conducted in 2012 and 2013. Atomic absorption spectrometry was used to determine lead concentration in blood. To assess the statistical significance of mentioned comparisons we used univariate methods and the non-parametric tests for the attributable variables: Hi-square test, proportion test. To test significance of the difference between non-parametric variables, we used: Kolmogorov-Smirnov Z test for testing distribution's normality, ANOVA for the normal distribution, and Kruskal Wallis test in cases without normal distribution.

142 participants were tested on lead in blood: exposed (Zajača, 58 children, 18 adults), partially exposed (Paskovac, 38 children), and non-exposed (Gornja Borina, 30 children). Mean BLLs in children living less than 1 km from the smelter was 18.98 µg/dl (SD 6.06), at the first test series (year 2012), and 12.21 µg/dl (SD 6.41) in the second round (year 2013). For those living further than 3 km mean BLLs at first series was 8.30 µg/dl (SD 1.51), and at the second series it was 5.85 µg/dl (4.39).

Blood lead levels in children from Zajača are high and risk reduction measures are necessary to minimize the exposure. Due to the complex pattern of multi-source exposure to lead, cadmium and arsenic, abatement and further monitoring are needed at the site to assess the effectiveness of risk reduction measures, primarily, continuous HBM.

**Key words:** human biomonitoring; blood lead levels; exposure; children, lead smelter

**Correspondence:** brankicam@batut.org.rs
THE SIGNIFICANCE OF DRUG/XENOBIOTIC METABOLIZING ENZYME POLYMORPHISMS IN CANCER/DISEASES

Chairs: Mumtaz Iscan1, Ann K. Daly2
1Department of Toxicology, Faculty of Pharmacy, Ankara University, Tandogan, Ankara, Turkey,
2Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK

Cancer is the worldwide leading cause of mortality. In addition, neurodegenerative diseases, like Parkinson’s disease (PD), are also known to be very important health problem. Besides inter-individual differences that are important in susceptibility to these diseases, great inter-individual differences in response to therapy treatment also occur among these patients. The biotransformation enzyme systems inactivate environmental carcinogens and certain drugs used in therapy depending on their genetic polymorphisms. Therefore, it is important to identify individual genetic factors that modify such diseases prognosis in order to develop preventive and therapeutic strategies.

T. Simic will focus on whether common GST polymorphisms confer the risk and the progression of oxidative stress associated malignant (clear renal cell carcinoma, ccRCC) and non-malignant (end stage renal disease, ESRD) renal diseases.

A.O. Ada will present their recent findings with respect to the association between non-small cell lung cancer (NSCLC) risk and polymorphisms of CYP2E1 and GST genes encoding these xenobiotic metabolizing enzymes and TP53.

A.K. Daly will report the associations of CYP polymorphisms with risk of various cancers including lung, liver, colon and nasopharyngeal based on recent results of both meta analyses and genome-wide association studies.

I.Novakovic will present their recent data on the association between polymorphisms in genes encoding for brain derived neurotrophic factor (BDNF), apolipoprotein E (APOE) and catechol-O-methyltransferase (COMT) and response to therapy and adverse outcomes in PD patients.

M.Iscan will provide their recent findings with respect to the role of CYP and GST gene polymorphisms in response to chemotherapy and survival in NSCLC patients.

Keywords: risk, prognosis, polymorphisms, CYPs and GSTs, COMT
Correspondence: iscan@pharmacy.ankara.edu.tr

Role of GST Polymorphisms in Malignant and Non-malignant Diseases of the Kidney

Tatjana Simic
Institute of Medical and Clinical Biochemistry, Faculty of Medicine, University of Belgrade, Belgrade, Serbia

Almost all members of the mammalian cytosolic glutathione transferases (GSTs) exhibit genetic polymorphism, resulting in inter-individual differences in GST isoenzyme profile that might affect both biotransformation and antioxidant capacity. Therefore, we aimed to discern whether common GST polymorphisms confer the risk and the progression of oxidative stress associated malignant (clear renal cell carcinoma, ccRCC) and non-malignant (end stage renal disease, ESRD) renal diseases. GST genotypes were determined by various PCR methods whereas oxidative stress by-products and soluble adhesion molecules were measured using ELISA method. GSTP1*Val (variant) and GSTM1-null genotypes showed a significant individual association with ccRCC risk. On the other hand, GSTM1-null genotype was associated with better survival in ccRCC. Regarding ESRD patients, the results of this study showed that variant forms of genes encoding antioxidant enzymes GSTA1, GSTM1, GSTP1, GSTT1 and GSTO2 were more frequent in ESRD patients than in corresponding healthy subjects. Besides, the presence of variant genotypes was associated with the higher levels of oxidative stress byproducts and soluble adhesion molecules in these patients. Moreover, GSTM1-null genotype conferred higher risk and worse prognosis in terms of overall and cardiovascular survival in patients with ESRD. Taken together, the results presented in this study suggest that GST genotypes might serve as a valuable indicator in both risk assessment and prognosis stratification.

Keywords: GST polymorphism, risk, prognosis, RCC, ESRD
Correspondence: tatjana.simic@med.bg.ac.rs

Relevance of CYP Polymorphisms to Cancer Susceptibility

Ann K. Daly
Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK

Associations of cytochrome P450 polymorphisms with risk of cancer have been reported widely. Studies on lung cancer susceptibility have been particularly common. There are a large number of published
studies on polymorphisms in CYP1A1, CYP2D6 and CYP2A6 as lung cancer risk factors. However, recently both meta analyses and genome-wide association studies suggest that only the CYP2A6 association, where genotypes associated with low activity decrease susceptibility, appears significant. This may be due to the role of CYP2A6 in nicotine metabolism. Associations with lung cancer susceptibility have also been reported for CYP1A2, CYP1B1 and CYP2E1 polymorphisms but these, though biologically plausible, have not been well replicated. For cancers where exposure to non-tobacco related xenobiotics affects risk, cytochrome P450 polymorphisms may also be relevant. Examples include CYP1A2 for colon cancer associated with heterocyclic arylamine exposure, CYP3A for hepatocellular carcinoma due to aflatoxin exposure and CYP2E1 for nitrosamine-related nasopharyngeal cancer. Data supporting each of these associations will be considered. In general, cytochrome P450 polymorphisms are relevant to risk for some cancers but the importance of this risk may have been overstated in the past.

Keywords: lung cancer, tobacco smoke, nicotine, cytochrome P450, polymorphism

Correspondence: a.k.daly@ncl.ac.uk

Pharmacogenetics of Drug Response in Parkinson’s Disease

Ivana Novakovic, Eleonora Dzoljic, Milena Jankovic, Ana Marjanovic, Marija Brankovic, Natasa Dragasevic, Vladimir Kostic
Clinic of Neurology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia

Parkinson’s disease (PD) is the most common movement disorder affecting 1% of population at the age of 60 years. Individual therapy optimization is one of the important issues for medical doctors, pharmacists and health insurance in whole. There are recent evidences that variable efficacy of treatment and risk of motor and mental complications could have genetic basis. Pharmacogenetic and pharmacogenomic studies of PD plays leading role in that research. Variability in genes coding for drug-metabolizing enzymes, drug receptors and proteins involved in drug pathway signaling is an important factor determining inter-individual variability in drug responses. In this study we have analyzed polymorphisms in genes encoding for brain derived neurotrophic factor (BDNF), apolipoprotein E (APOE) and catechol-O-methyltransferase (COMT) in a group of PD patients on standard antiparkinsonian drug treatment. Molecular genetic analysis was performed using real time PCR method and pre-designed TaqMan genotyping assays. Interpersonal differences in drug responses have been clearly documented although individualized treatment of PD was not widely applied. Treatment with antiparkinsonian drugs was associated with the development of complications, such as L-DOPA-induced dyskinesia (LID), hallucinations and excessive daytime sleepiness. Carriers of specific genetic polymorphisms showed particular susceptibility to development of some of these drug adverse effects. Our results confirm that many genetic variations and polymorphisms in different proteins can influence individual response to anti-PD drugs.

Keywords: antiparkinsonian drugs, individual response, gene polymorphisms

Correspondence: novivana@eunet.rs

Association of Drug/Xenobiotic Metabolizing Enzyme Polymorphisms with Treatment Outcome of Advanced Non-small Cell Lung Cancer Patients with Platinum-based Chemotherapy

Mumtaz Iscan
Department of Toxicology, Faculty of Pharmacy, Ankara University, Tandogan, Ankara, Turkey

Lung cancer is an increasing worldwide public health problem. Most of the lung cancer patients have non-small cell lung cancer (NSCLC). These patients are mainly treated with standard platinum based chemotherapy. The poor response and great inter-individual variety in response to this chemotherapy occur among these patients. There are accumulating evidences to support the hypothesis that genetic polymorphisms alter the drug response and survival. The cytochrome P450 (CYP) and glutathione S-transferase (GST) enzymes metabolize xenobiotics including antineoplastic drugs and involve in drug resistance. The polymorphic CYPs and GSTs have altered enzyme activities and thus they may have influences on the response to chemotherapy and survival in lung cancer patients. Herein, our recent findings with respect to the role of CYP and GST gene polymorphisms in response to chemotherapy and survival in NSCLC patients will be evaluated which could be useful for the clinicians in the prognosis of these patients treated mainly with platinum based chemotherapy.

Keywords: CYPs, GSTs, polymorphisms, response to chemotherapy, survival

Correspondence: iscan@pharmacy.ankara.edu.tr
SUBSTANCES OF ABUSE: GLOBAL TRENDS, PREVENTION AND MANAGEMENT

Chairs: Slavica Vučinić¹, Hans Maurer
¹National Poison Control Centre MMA, Medical faculty University of Defense, Belgrade, Serbia, ²University of Saarland, Homburg, Germany, advisor to the U.S. Federal Bureau of Investigation

Over the past 20 years, the spectrum of substances available on the global drug market has widened considerably, parallell with the significant increase in the number of drug users. In 2015, about 250 million people used drugs at least once, of these, around 29.5 million suffered from drug use disorders. Opioids were the most harmful drug type in health terms, with almost 12 million years of „healthy“ life (disability-adjusted life years – DALYs) lost worldwide. Overall, hepatitis C has caused greatest harm among drug users. Albeit the opioid market is becoming more diversified and the cocaine market is expanding, marijuana is top drug in every country by the proportion of users. The World Drug Report 2017 of UNODC reinforced the importance of united action to address drug challenges and the need for science and rights-based drug use prevention and treatment.

The world is witnessing an alarming problem of an unprecedented increase in the number, type and availability of new psychoactive substances (NPS) in Europe with over 620 being monitored by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). The EMCDDA’s main responsibilities in this field are to operate the EU Early Warning System (EWS), with its partner Europol and to undertake risk assessments of NPS when necessary. The EU EWS works by collecting information on the appearance of NPS from the 28 EU Member States, Turkey and Norway, allowing the EU to respond rapidly to emerging threats. Serbia has promoted the National EWS in 2016, with the National Poison Control Centre as one of key subjects.

The strong EWS can play a critical role in ensuring a timely response in order to protect public health.

Keywords: designer drugs, new psychoactive substances, synthetic cannabinoids, early warning system, liquid chromatography

Correspondence: nckt@vma.mod.gov.rs hans.maurer@uks.eu

Analytical Strategy for Effective Clinical Toxicology Services

Hans H. Maurer
Department of Experimental and Clinical Toxicology, Saarland University, D-66421 Homburg (Saar), Germany

Various analytical tools allow today a broad range of analysis in clinical toxicology, particularly in big centers. Of course, long distances may limit the usefulness. Current strategies for efficient analytical diagnostics in clinical toxicology are presented. The tasks for such diagnostics, different analytical strategies and various methods are reviewed. They cover procedures mainly using low and high-resolution mass spectrometry coupled to gas or liquid chromatography (GC-MS or LC-(HR)MS) for target or comprehensive screening for drugs (of abuse), poisons, and their metabolites as well as for quantification in blood. Quality control aspects are discussed as well as strategies for competent interpretation of the analytical result in correlation with the clinical signs presented by the patient. However, the service must be available around the clock and reliable results must be provided in a short time frame relevant for treatment and at reasonable costs.

Keywords: clinical toxicology, analytical strategy, mass spectrometry, toxicological interpretation, quality control

Correspondence: hans.maurer@uks.eu

The Role of the National Poison Control Centre in the Early Warning System on New Psychoactive Substances

Slavica Vučinić
National Poison Control Centre, MMA, Medical Faculty, University of Defense, Crtomiravska 17, Belgrade, Serbia

The drug market in Serbia is characterized with the availability of all types of drugs, including new psychoactive substances (NPS), the steadily rising number of patients treated for overdose, and great difficulties that come with NPS analysis and determination of substance-specific effects. From January 2013 to December 2016, 58 patients, aged 14 to 25, with the effects of synthetic cannabinoids, were treated in the NPCC. Products “Biljni tamjan”, “Beli slez”, “Rainbow Special” etc, were obtained from tobacco shops or online as natural herbal products and air fresheners, and consumed by smoking or inhalation of smoke. Liquid chromatography-electrospray
ionization-mass spectrometry with Xterra column, liquid chromatography with PDA detector and GC-MS (ion trap detector) were used for analysis. The clinical picture included: tachycardia in 54 (93.1), mydriasis in 31 (53.4%), somnolence, nausea, vomiting, agitation in 16 (27.6%), dizziness in 10 (17.2%), disorientation in 9 (15.5%), chest pain, dispnea in 5 (8.6%), loss of consciousness, pallor, paresthesia, muscle twitches and short-term memory impairment in 2 (3.4%) patients. After receiving treatment, all patients had fully recovered within up to 8 hours in the emergency ward and were discharged shortly afterwards. AB-PINACA AB-FUBINACA, JWH 18, JWH-122, JWH-210, 5F-AKB48, MDMB-CHMICA and AB-CHMINACA were detected in urine samples and/or herbal products.

The efficient and systematic collection of the relevant data on synthetic cannabinoids and other NPS, adverse effects, changes of purity and composition of controlled PS and NPS-based products, along with raising the public awareness on the NPS will pave the way to the national EWS.

Keywords: synthetic cannabinoids, clinical picture, analytics, poison control centre

Correspondence: nckt@vma.mod.gov.rs

The EU Early Warning System – 20 Years of Monitoring New Psychoactive Substances in Europe

Rita Jorge, Ana Gallegos
EMCDDA, Lisbon, Portugal

Over the past 20 years, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) has played a central role in Europe’s response to new psychoactive substances (NPS). Its main responsibilities in this field are to operate the EU Early Warning System (EWS), with its partner Europol and to undertake risk assessments of new substances when necessary. The EU EWS works by collecting information on the appearance of new substances from the 28 EU Member States, Turkey and Norway, and then monitoring them for signals of harm, allowing the EU to respond rapidly to emerging threats.

Over the last two decades there has been an unprecedented increase in the number, type and availability of NPS in Europe. The EMCDDA is currently monitoring in excess of 620 new psychoactive substances, which are not covered by international drug controls. The rapid increase in growth in this market is evidenced by the fact that approximately 70% of these new substances were detected in the EU in the past 5 years alone.

The growth in the market is also responsible for the increase in serious harms reported to the EMCDDA in recent years. Most of these concern non-fatal intoxications and deaths, but also include broader social harms. During 2016, serious harms requiring urgent attention led the EMCDDA to undertake investigations into 3 new substances – the synthetic cannabinoid receptor agonist MDMB-CHMICA, and two fentanyl derivatives: acryloylfentanyl and furanylfeitnaly.

It is likely that the growth of the market in NPS will continue to pose a range of challenges for public health and drug policy over the next few years i.e. the speed at which they appear, their open sale, and the little or no information provided on their effects and harms. It is here that strong early warning systems can play a critical role in ensuring a timely response in order to protect public health.

Keywords: EMCDDA, international drug controls, synthetic cannabinoids, fentanyl derivatives

Correspondence: Ana.Gallegos@emcdda.europa.eu

Alternative Biological Samples for Determination of Psychoactive Substances

Snežana Djordjević
National Poison Control Center, Military Medical Academy, Medical Faculty, University of Defence Belgrade, Serbia

In recent years unconventional biological samples such as saliva and hair are used for determination of psychoactive substances (PAS), although the drug levels are often lower than the corresponding ones in urine or blood.

The aim of this study was to describe the possibility of determination of PAS in saliva and hair samples. Saliva samples were collected from patients after abuse of alcohol, benzodiazepines and heroin, and hair samples were taken from patients who abused PAS.

Alcohol in saliva was determined by gas chromatography with flame ionization detection, and presence of benzodiazepines and heroin metabolites was confirmed after alkaline chloroform extraction, by liquid chromatography with mass spectrometry. After decontamination, pulverization and solubilization, hair samples were prepared by alkaline liquid-liquid extraction with chloroform for benzodiazepines and heroin and acidic extraction with n-hexane:ethylacetate (9:1) for THC-COOH. The chromatographic
separation was performed on XTerra®RP18 column, using a gradient of acetonitrile/formic acid 1% and formic buffer pH 3.5 as the mobile phase.

Our result showed good correlation of alcohol and benzodiazepines concentration between blood and saliva, as well as heroin metabolites in urine and saliva. We also proved presence of benzodiazepines, heroin and THC metabolites in hair samples of chronic abusers.

Saliva and hair samples could be used as an alternative to blood or urine. The detection of PAS in saliva is a sign of recent drug use and it may be used for their detection. Hair analysis may provide useful information about abusing PAS and the history of usage after their intake.

**Keywords:** saliva, hair, liquid chromatography with mass spectrometry

**Correspondence:** ivezicnela@gmail.com

---

**Drugs of Abuse: Trends in Croatia**

**Irena Brčić Karačonji, Andreja Jurič, Nataša Brajenović**

*Analytical Toxicology and Mineral Metabolism Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia*

According to data provided by the European Monitoring Centre for Drugs and Drug Addiction, approximately one quarter of Europeans have used an illicit drug at some point in their lives. A study on illicit drug use in Croatia carried out in 2015 on 4,992 respondents showed that 20.3% of the population aged 15-64 years used illicit drug at least once during their lifetime. Cannabis was the most frequently used illicit drug in Croatia with a lifetime prevalence of 19.4%, followed by amphetamines (3.5%), ecstasy (3%), cocaine (2.7%), LSD (1.7%), and heroin (0.3%). The largest concern is the high lifetime prevalence (6.9%) of taking new psychoactive substances, mainly synthetic cannabinoids, in the 15-24 age group.

Advances in the sensitivity of analytical techniques have enabled analyses of substances of abuse in alternative biological matrices such as hair. Since 1999, over 1700 hair samples were analysed by gas chromatography-mass spectrometry at the Institute for Medical Research and Occupational Health, Croatia for the presence of methadone, cocaine, heroin, codeine, morphine, and amphetamine-type stimulants. A total of 22% of the tested samples were positive for one or more drugs of abuse. Ecstasy was the most frequently detected substance (in 10% of samples).

Croatia’s National Strategy on Combating Drug Abuse was adopted in 2012, with an aim to reduce both the demand and the supply of drugs of abuse. Its implementation should significantly contribute to protecting the health of individuals and the general community through an integrated approach to the problem of drug abuse.

**Keywords:** substances of abuse, new psychoactive substances, hair analysis, statistical data

**Correspondence:** ibrcic@imi.hr
BIOMARKERS IN CHRONIC DEGENERATIVE DISEASES AND RISK ASSESSMENT

Chair: Patrizia Hrelia
Co-chair: Sabrina Angelini
*Department of Pharmacy and Biotechnology, University of Bologna, Italy*

The identification of biomarkers is an important stage in the development of strategies for prevention, prognosis and treatment of any disease. Indeed, understanding the relationship between measurable biological process and disease development is vital to expand our arsenal of biomarkers risk assessment and prevention, and of treatments for all disease. Besides these, biomarkers might play a critical role in deepening our understanding of normal, healthy physiology.

**Keywords:** biological process, disease development, prevention, treatment

**Correspondence:** patrizia.hrelia@unibo.it  
s.angelini@unibo.it

---

COPD Risk Evaluation Through the Exposome, Genetic Traits and Enzymatic Activities

**Sabrina Angelini**¹, Francesca Maffei², Silvana Hrelia², Patrizia Hrelia¹

¹Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy; ²Department for Life Quality Studies, University of Bologna, Rimini, Italy

Chronic obstructive pulmonary disease (COPD) is a multifactorial disease with cigarette smoke the main risk factor. Only a small proportion of smokers develop symptomatic disease, suggesting the existence of other risk factors. We perform a multidisciplinary project, *Breath Bologna*, with the aim to uncover socio-economic features, biomarkers of genetic damage and susceptibility, and enzymatic activities as risk factors in COPD. 229 subjects were recruited among outpatients undertaking respiratory function tests at the Pneumology Unit of the Sant'Orsola-Malpighi Hospital, Bologna. Comprehensive socio-demographic, lifestyle and clinical data, revealed that fragility is associated with COPD stage and that comorbidities and the low body mass index are predictors of mortality or hospitalization. Regarding biomarkers of genetic damage, we evaluated micronuclei frequency in peripheral blood lymphocytes. Data showed no significantly different micronuclei frequencies associated with the disease stage. Interestingly, comparing micronuclei frequency in COPD patients and healthy subjects, we observed a trend for a higher micronuclei frequency in patients. With regard to biomarkers of susceptibility, we investigate whether polymorphisms of mEH and other oxidative stress genes (CAT, GR, GPX, SOD) influenced individual susceptibility to COPD onset and severity. Currently statistical analysis of association with the analyzed polymorphisms is ongoing. We also aimed to define biochemical biomarkers of oxidative stress, predicting the risk of COPD, determining by spectrophotometric test the activity of GR, CAT, GPX and SOD. At present, we are evaluating the association of these enzymatic activities with COPD risk and severity. Furthermore, we are assessing the influence of polymorphisms in the observed enzymatic activities.

**Keywords:** COPD, biomarkers, micronuclei, polymorphisms, antioxidant activity

**Correspondence:** s.angelini@unibo.it
hCOMET Network: Results of the First Statistical Analysis and New Findings in the Buccal Micronucleus Assay, as Biomarkers in Human Biomonitoring and Early Disease Detection

Mirta Milić1, Stefano Bonassi2-3, Emilio Rojas4, Claudia Bolognesi5, hCOMET Consortium5
1Institute for Medical Research and Occupational Health, Mutagenesis Unit, Croatia; 2Scientific Institute for Research, Hospitalization and Health Care San Raffaele Pisana; 3IRCCS San Raffaele Pisana, Italy; 4National Autonomous University of Mexico, Mexico; 5Environmental Carcinogenesis Unit, Ospedale Policlinico San Martino, Genova, Italy

The alkaline comet assay is a test for measuring single strand DNA breaks in eukaryotic cells. Method exists for more than 30 years and is used in genotoxic research. Since 2014 (revised 2016) it has become the official method for assessing genomic damage \textit{in vivo} after exposure to both physical and/or chemical agents (Organization for Economic Co-operation and Development- OECD Test No. 489). With its additional variants it is also used in the biomonitoring of exposed populations, but also as an auxiliary method for disease diagnosis and in therapies for patients’ health improvement, but for such an official application, the method should be improved and validated. That task has been addressed by the newly formed group hCOMET that has gathered results on human biomonitoring studies from 41 laboratories, including 90 methods, 108 studies and more than 19 000 individuals. We will discuss the efforts and the results of the group, and will also talk about a relatively new method of micronucleus buccal test (buccal mucosa) that has a potential to replace or to be added value to the classical \textit{in vitro} micronucleus method on mammalian cells. Much shorter assay than the classical micronucleus method \textit{in vitro}, the assay has already shown possible application in the study of exposure to asbestos and the occurrence of Alzheimer’s disease. We will discuss the efforts already done in this area and further steps.

Keywords: comet assay, human biomonitoring, buccal micronucleus assay, human biomarkers, early disease detection

Correspondence: mmilic@imi.hr

---

Genotoxicity Biomarkers in the Clinical and Environmental Molecular Epidemiology Studies for Children
Gonca Çakmak
Gazi University, Faculty of Pharmacy, Department of Toxicology, Ankara, Turkey

Children are among the most susceptible group of the population regarding environmental exposures, diseases and medical treatments. There is an increasing attempt to determine the sensitivity of children to environmental agents and clinical outcomes, especially to predict likely cancer development. Genotoxicity, as the intermediate step in cancer can be evaluated by use of early effect biomarkers. In order to protect children’s health there is crucial need for genotoxicity data derived directly from children studies. The information gained from those studies could be crucial in the hazard identification step of risk assessments to drive regulatory actions and in developing efficient and safe treatment strategies. Additionally, to maintain conscious future generations, increasing the awareness of children on indoor and outdoor chemicals could be complementary. Herewith the focus point is to overview the molecular epidemiology studies on children for environmental exposures (air pollution) and for clinical outcomes (children with thalassemia minor, chronic kidney disease, etc.), in which genotoxicity was the main endpoint.

Keywords: genotoxicity, children, clinical biomonitoring, environmental biomonitoring, awareness

Correspondence: gcakmak@gazi.edu.tr

---

Role of Oxidants and Antioxidants in Degenerative Diseases Development: \textit{in vitro} Models

Ksenija Durgo, Ana Huđek, Ana Belsčak-Cvitanović, Arijana Bušić, Draženka Komes, Višnja Bačun-Družina
Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia

Cells of our organism are permanently exposed to different endogenous and exogenous oxidants, originated from basal metabolism, immune reactions, xenobiotics, detoxifying processes and intermediates formed during biotransformations. At cellular level, oxidants cause DNA damage, oxidation of the proteins and destruction of lipids by lipid peroxidation chain reactions. On the other hand, numerous chemicals (of exogenous or endogenous origin) easily interacts with reactive oxidative species, preventing
macromolecules destruction and consequently, damages of the cells and tissues. Significant role in this oxidative/antioxidative balance play bioactive compounds from plants, fruits or vegetables. Under certain circumstances, these compounds can decrease level of oxidative damage, but at the same time, under some other circumstances they can enhance pro-oxidative response. There are different assays for measurement of the antioxidant status and oxidative damage. The simplest ones are chemical in vitro reactions and tests in cell cultures. They can yield useful information about mechanisms of action, but extrapolation to effects of dietary antioxidants in vivo is not simple and often it may be incorrect, because uptake from the gastrointestinal tract, influence of microflora and metabolism are not considered. In this presentation, some of in vitro models for detection of pro-oxidative/antioxidative effects will be presented, including the most common biomarkers that are measured in order to determine changes of cellular macromolecules that are the first step in the development of degenerative diseases.

**Keywords:** oxidative damage, antioxidants, degenerative diseases, biomarkers, phytochemicals

**Correspondence:** ksenija974@gmail.com

---

**INFLUENCE OF ENDOCRINE-DISRUPTING CHEMICALS (EDCs) ON DEVELOPMENT AND REPRODUCTION**

Chair: Djuro Macut

Co-chair: George Mastorakos

1Clinic of Endocrinology, Diabetes and Metabolic Diseases, Faculty of Medicine, University of Belgrade, Belgrade, Serbia. 2Endocrine Unit, ARETAIEION hospital, Athens Faculty of Medicine, National and Kapodistrian University of Athens, Athens, Greece

Endocrine-disrupting chemicals (EDCs) are ubiquitous chemicals that affect all endocrine systems. Male and female reproduction is a supreme physiological process that is under the control of signals from central nervous system and gonadal steroid. Reproduction could be under the influence of various disruptors among which chemicals are playing still unresolved role. EDCs may influence gonadal system on the level of hypothalamus, pituitary, testicles, ovary, prostate and uterus, and resulting in subfertility, infertility, improper hormone production, estrous and menstrual cycle abnormalities, anovulation, and early reproductive senescence. These substance could interfere with developmental processes and mediate carcinogenesis as well. First lecture of the symposium will provide an introduction to the complex issue of EDCs. Second lecture of the symposium will demonstrate how the xenograft system could be used for the demonstration important species differences in the effects of exposure to chemicals such as di-n-butyl phthalate, diethylstilboestrol and that paracetamol could interfere with testosterone production and germ cell development in the human fetal testis. Third lecture will emphasize the effects of EDCs on reproductive system in males, namely semen quality, infertility, urogenital tract abnormalities, and testicular germ cell cancer. Fourth lecture will provide clinical data on the effects of phthalates and bisphenol A in polycystic ovary syndrome as common female reproductive disorder. Fifth lecture will provide preclinical data on the endocrine disrupting potential of herbal dietary supplements on estrogen receptor function, and estrogenic activity of mixtures of persistent organic pollutants in breast cancer patients.

**Keywords:** endocrine-disrupting chemicals, testosterone, infertility, polycystic ovary syndrome, estrogen receptor

**Correspondence:** djmacut@gmail.com mastorakg@gmail.com
EDCs: an Introduction

Djuro Macut
Clinic of Endocrinology, Diabetes and Metabolic Diseases, Faculty of Medicine, University of Belgrade, Belgrade, Serbia

Endocrine disrupting chemicals (EDCs) are substances present in the environment, food, and consumer products that could interfere with hormone biosynthesis, metabolism, or action. A variety of synthetic chemicals could affect human development, as well as fertility at different periods of humans life, endocrine-related cancers as breast and prostate ones, neuroendocrine pathways, thyroid function, obesity and metabolism. Among EDCs we are aware on the influence of pesticides (organochlorines, organophosphates, carbamates, pyrethroids, triazines), heavy metals (arsenic, lead, mercury), plasticizers, different pharmaceutical compounds and others. The mechanisms of EDCs involve different receptors and enzymes including estrogenic, androgenic, thyroid, peroxisome proliferator-activated receptors, retinoid, other nuclear receptors, steroidogenic enzymes, neurotransmitters. Moreover, we are concerned on the non-monotonous effects of even small concentrations of the specific chemical or their mixtures, latency to the occurrence of clinical manifestation of the derangement or disease as well as their transgenerational effect. Results obtained from studies on animal models, in vitro analyses and epidemiological data on humans led us to the conclusion for the existence of EDCs on human development and general health, and epidemiological studies converge to implicate EDCs as a significant concern to public health.

Keywords: endocrine disrupting chemicals, hormones, receptors, neurotransmitters, reproduction

Correspondence: djmacut@gmail.com

EDCs: Assessing the Risks of Exposure to Environmental Chemicals and Pharmaceuticals

Rod Mitchell1,2
1MRC Centre for Reproductive Health, Queens Medical Research Institute,
2Department of Diabetes and Endocrinology, Royal Hospital for Sick Children, Edinburgh, UK

Many industrial chemicals and pharmaceutical products have been proposed to result in endocrine disruption in humans. This includes potential effects on reproductive development in males and females. Chemicals that have been proposed to impact on male reproductive development include plasticizers, synthetic oestrogens and analgesics. Much of the data on the effect of exposure to these agents are based on studies conducted in rodent models; however, confirmation of such findings in human model systems at human-relevant exposure levels are lacking.

We have developed model systems to determine the effects of exposure to a variety of proposed ‘EDCs’ on human fetal testis development and function. Using a xenograft system designed to reproduce normal fetal testis development and in-utero hormonal environment, our results demonstrate important species differences in the effects of exposure to chemicals such as di-n-butyl phthalate (DBP) and diethylstilboestrol (DES) in terms of testosterone production. We have also demonstrated that exposure to analgesics, such as paracetamol, result in a significant reduction in testosterone production and also impact on germ cell development in the human fetal testis. Importantly, these effects are apparent at therapeutic levels of exposure using a standard therapeutic regimen.

Our work, in addition to that of several other groups, highlight the importance of choosing an appropriate model species, experimental system and relevant exposure regimen in order to determine the potential impact of EDC exposure in humans. Findings from rodent studies should, where possible, be confirmed using human tissue models in order to determine the relevance to human health.

Keywords: testis development, testosterone, phthalate, paracetamol, diethylstilboestrol

Correspondence: Rod.Mitchell@ed.ac.uk

Endocrine Disruption and Male Gonadal Function

George Mastorakos
Endocrine Unit, ARETAIEION hospital, Athens Faculty of Medicine, National and Kapodistrian University of Athens, Athens, Greece

Humans are exposed to dozens of endocrine-disrupting substances (EDCs). Some act as antiandrogens while androgenic EDCs have been identified. The latency between exposure to EDCs and occurrence of clinical disorders can be long or transgenerational. Three male reproductive health endpoints are affected by EDCs: disrupted reproductive function (semen quality, infertility); urogenital tract abnormalities, (hypospadias, cryptorchidism); and testicular germ cell cancer (TGCC). These endpoints have
been connected to EDCs and genetic factors. Cryptorchidism, hypospadias, oligospermia, and testicular cancer have been associated with the so-called testicular dysgenesis syndrome (TDS). TDS was reproduced in rodents with phthalates and PCBs. Reduced anogenital distance was observed in the rat and in epidemiological studies in human male newborns. Inverse relationship between maternal phthalate levels and human offspring’s anogenital index has been described. Prostate hyperplasia has been described after exposure to BPA. Regarding PCBs, pesticides, and phthalates, limited evidence supports a relationship between adult exposure and reduced semen quality; timing of exposure to dioxins has been associated to semen quality. The dramatic recent upward trend in the incidence rate of TGCC indicates that apart genetic factors, environmental and lifestyle factors might be involved. Blood organochlorine levels measured in mothers, decades after their sons’ birth, were predictive of increased TGCC risk in the latter. Because the current scientific evidence on associations of EDCs with male reproductive health endpoints remains limited there is need for further research.

**Keywords:** endocrine-disrupting chemicals, male reproduction, infertility, prostate, testicular cancer

**Correspondence:** mastorakg@gmail.com

---

**Influence of Phthalates and Bisphenol A on Fertility in Woman**

Milica Medic Stojanoska

University of Novi Sad, Faculty of Medicine, Clinical Center of Vojvodina, Novi Sad, Serbia

Endocrine disrupting chemicals (EDCs) as bisphenol A (BPA) diphenylmethane derivate and phthalates, often called plasticizers (diestars of 1,2-benzendicarboxonic acid), are the most produced chemicals worldwide. Numerous studies indicate that they can adversely affect ovaries, uterus, anterior pituitary and/or steroid hormone production, gonadotropins, GnRH pulsatility and signaling. This can lead to reproductive disorders, such as early puberty, infertility, premature ovarian failure, endometriosis and adverse pregnancy outcomes. It is known that fetuses, infants and / or young adolescents are more susceptible groups. Exposure to them may have trans-generation effects on female fertility. Recently published studies suggest association between BPA and some phthalates (diethyl-hexyl phthalates – DEHP and others) with polycystic ovary syndrome (PCOS). Our previously performed examination confirmed association of BPA and DEHP with obesity and other parameters of metabolic syndrome, which can exacerbate PCOS phenotype and infertility. Now we found increased level metabolites of DEHP and diethyl phthalate (DEP) and BPA in some groups of patients with PCOS. Little information is available on the effects of phthalates and BPA on the other causes of infertility in humans. Mechanisms by which BPA and phthalates disrupt folliculogenesis and steroidogenesis are not studied enough and they include estrogenic, anti-estrogenic, oxidative stress response or PPAR activation. The evaluation in vitro of acute BPA exposition in the panel of mammalian cell lines was confirmed to be toxic, which may also suggest its toxic model of action. Conclusion: BPA and some kinds of phthalates have a proven negative influence on the female fertility by insufficiently studied mechanisms.

**Keywords:** EDCs, BPA, phthalates, infertility, PCOS

**Correspondence:** milica.medlic1@gmail.com

---

**Exploring EDCs and the Mechanisms by Which They Adversely Affect Reproduction**

Hande Gurer-Orhan

Department of Toxicology, Faculty of Pharmacy, Ege University, Izmir, Turkey

Exposure to various endocrine disrupting chemicals (EDCs) is suggested to play an important role in hormone-dependent cancers and reproductive disorders. A recent comprehensive meta-regression analysis reported a significant decline in sperm counts among men from developed countries suggesting an urgent need for research on the causes of this continuing decline. OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters suggests a battery of in vitro assays providing data about selected endocrine mechanism(s)/pathways(s) at Level 2. Our group has been evaluated endocrine disrupting potential of active molecules from widely used herbal dietary supplements. Their estrogen receptor (ER) binding affinities, ER agonist/antagonistic effects in MCF-7 proliferation assay and aromatase inhibitory potential have been investigated. Some of our findings were confirmed by molecular modelling (OECD Level 1) and estrogenic activity observed in vitro was further investigated by in vivo uterotrophic assay (OECD Level 3). Recently we are working on investigating possible utilization of total estrogenic activity of adipose tissues from breast cancer patients as biomarker for exposure to mixtures of persistent organic pollutants (POP). For this reason we improved an existing
method to separate POPs in the tissue samples from endogenous hormones by HPLC, and the estrogenic potential of the eluates containing POPs was evaluated in the E-Screen bioassay. The amount and type of POPs in the samples were further analysed by LC-tandem MS. Results of this assay make it possible to evaluate the total estrogenic potential of the mixtures of POPs at various combinations and concentrations.

**Keywords:** endocrine disruptors, screening, mechanism of action, phytoestrogens, persistent organic pollutants

**Correspondence:** hgurer@gmail.com

---

**DEVELOPMENT IN METHODOLOGIES TO ADDRESS MIXTURE RISK ASSESSMENT**

**Chair:** Alan Boobis  
*Imperial College London, UK*

Deriving methodologies for human-relevant risk assessment for mixtures is leading to a number of approaches driven by exposure. These approaches need to be scientifically-based, feasible within and across chemical sectors, consistent globally and provide relevant assessments to protect public health using the most current tools such as read-across, *in silico* or *in vitro* approaches and AOPs. Problem formulation and exposure mapping will aid in first focusing the drivers for risk assessments to address relevant population concerns.

This symposium will describe approaches which are being developed for combined risk assessment of multiple chemicals.

**Keywords:** mixtures, human-relevant risk assessment

**Correspondence:** a.boobis@imperial.ac.uk
Human Health Protection from Combined Exposure to Multiple Chemicals in the Global Context

Alan R. Boobis
Department of Medicine, Imperial College London, London W12 0NN, UK

Exposure to chemicals never occurs in isolation; rather there is co-exposure to multiple chemicals, both natural and synthetic. It is not possible to eliminate all such exposure, and indeed it may not be desirable as some chemicals are beneficial to health. Hence, some means of assessing which combinations are of concern, that is both efficient and effective in protecting human health, is needed. As it is not feasible, or indeed necessary, to test all potential combinations, a tiered approach to determining which chemicals should be considered in combination has been proposed. An assessment should start with problem formulation, which includes explicit statement of the purpose of the assessment and the risk management goals. Given the breadth of chemical space involved, it is essential to consider which chemicals should even be considered within the scope of the assessment. The chemicals are then triaged using tiered exposure and hazard assessments, higher tiers resulting in less uncertainty but involving greater resources. The outcome is identification of a set of chemicals that should be assessed together, a cumulative assessment group. The combined toxicity of the group is estimated using an appropriate model, usually dose addition, but if supported by the data, response addition might be more appropriate. If the margin of exposure is such that there is potential concern for effects on public health, dialogue with the risk manager is necessary to determine whether there is time for further refinement or given the magnitude of potential concern, immediate action is necessary.

Keywords: cumulative assessment groups, dose addition, problem formulation, tiered assessment

Correspondence: a.boobis@imperial.ac.uk

Opportunities for Grouping/Read-across in the Risk Assessment of Combined Exposures to Multiple Chemicals

Andrea-Nicole Richarz, Andrew Worth, Stephanie Bopp
European Commission Joint Research Centre, Directorate for Health, Consumers and Reference Materials, Ispra (VA), Italy

Grouping approaches are considering similar chemicals together, defining similarity based not only on chemical structure, but also biological/toxicological similarity. The definition and justification of the similarity are crucial for the assessment approaches built upon the grouping. One widely discussed use of grouping for mixture assessment is the grouping into assessment groups. It is assumed that based on the similarity of mode of action of the chemicals, concentration addition methods can be used for combined risk assessment. Read-across is inferring unknown properties, such as toxic effects, for the target substance(s) based on available data from the similar (source) substance(s). It is useful for predicting toxicity of large groups of chemicals in absence of actual testing. Again, the crucial step is to find the relevant similar analogues. In view of applying read-across to combined exposure assessment, ideally analogues for the whole mixture should be considered. For mixtures in the sense of the REACH (IUCLID) substance definition, databases such as AMBIT can be searched. For non-defined mixtures and general combined exposure the read-across approach has to be further explored, starting with reading across toxicity for single substances or specific chemical groups within the mixture. In that case, however, possible synergistic effects are not taken into account and have to be considered additionally. The adverse outcome pathway (AOP) framework can support this approach by identifying modes of action and thus chemicals acting towards the same adverse effects, even if relating to different molecular initiating events, or influencing each other through the AOP network.

Keywords: mixtures, safety assessment, mode of action, adverse outcome pathways

Correspondence: andrea.richarz@ec.europa.eu

Chemical Mixtures in Food – What Tools and Test Strategies will the EUROMIX Project Deliver?

Jacob van Klaveren
RIVM National Institute for Public Health and the Environment

The main deliverable of EuroMix is a mechanism-based test strategy, aiming to reduce the use of animals in testing, for refining risk assessment of multiple chemicals from multiple exposure routes. EuroMix developed Adverse Outcome Pathway (AOP) networks and practical tests for measuring the mixture effect at several key-events. The EuroMix
consortium tested QSARs, molecular docking and exposure tools for pesticides, additives and contaminants aiming to set test priorities. EuroMix will provide in vitro tests and standard operating procedures describing how these tests can be used. The in vitro testing will be validated against in vivo testing, which is required in current risk evaluation. The test results will refine uncertainties embedded in current risk assessment of mixtures such as worst-case assumptions on in- or exclusion of chemicals into cumulative assessment groups, the dose addition and the potency of each separate chemical in a mixture. The results will be used in deterministic and probabilistic exposure and hazard modelling approaches. The EuroMix will develop a web-based model and data toolbox available for stakeholders involved in the chemical safety evaluation. Case studies addressing several chemical classes will illustrate how the web-based toolbox can be used overarching regulatory sectors such as pesticides, additive and contaminant legislation. Practical guidance will be written and training will be given on how to use the EuroMix toolbox. Countries outside Europe participating in the Codex Alimentarius will try to reproduce these case studies using consumption data of their countries, which will simulate international implementation and discussions on harmonising approaches.

Keywords: mixtures, AOP, risk, probabilistic, harmonisation

Correspondence: Jacob.van.klavren@rivm.nl

Utility of AOPs for Mixture Safety Assessments

Angelo Moretto
Department of Biomedical and Clinical Sciences, Università degli Studi di Milano, and ICPS at ASST-Fatebenefratelli-Sacco, Milano, Italy

Adverse Outcome pathways (AOPs) consist of a sequence of KE (Key Events) connecting an MIE (Molecular Initiating Event) to an AO (Adverse Outcome). For compounds that share the same MIE (and AOP) (also identified as compounds with similar MOA) the approach is rather straightforward. However, when compounds have different MIEs, that, further down the chain of events, lead to the same KE(s) and AO (dissimilar MOA), the approach becomes more complex. Quantitative and temporal definition of dose-response relationships with KE(s) are key to determine whether the additive model for combined exposures needs to be applied or whether it entails excessive conservatism, particularly at environmentally relevant doses that are expected to cause sub-threshold KEs.

Keywords: outcome pathways, key events, molecular initiating events, adverse outcomes

Correspondence: angelo.moretto@unimi.it

Exposure-Driven Cumulative Risk Assessment in Practice

Tina Mehta
Toxicology and Risk Assessment Leader, Human Health Assessment, Dow AgroSciences, UK

Deriving methodologies for human-relevant risk assessment for mixtures is leading to a number of approaches driven by exposure. The problem formulation applied to cumulative risk assessment of agrochemicals needs to be explored further. An exercise in mapping and integrating scenarios enables the risk assessor to identify and clarify the cumulative exposure for which a risk assessment is needed. The aim of this presentation is to present a case example of how to map such an exposure scenario and then apply the relevant tools to addressing the risk. The types and extent of data that would be required will differ according to the defined protection goals. A primary outcome is to identify the risk drivers and thus focus the assessment to the relevant populations or exposure scenario of concern, and define individual substances leading to that concern. This will then enable appropriate risk mitigation or risk reduction measures to be applied, thus leading to relevant decision-making for achieving the risk management goals.

Keywords: cumulative assessment groups, problem formulation, exposure scenario, exposure data, tiered assessment

Correspondence: jmehta@dow.com
TOXICITY OF RESPIRABLE PARTICULATE MATTER IN AMBIENT AIR

Chair: M. Jovašević-Stojanović¹
Co-chair: P. Mantecca²

¹Vinča Institute of Nuclear Sciences, University of Belgrade, Serbia,
²University of Milano-Bicocca, Department of Earth and Environmental Sciences, Research Center POLARIS, Milan, Italy

Air pollution is the single largest environmental health risk in Europe. Recently, a new WHO air quality model confirmed that 92% of the world’s population lives in places where air quality levels exceed WHO limits. Numerous studies underlined contribution of exposure to toxic spices contained in ambient airborne respirable particulate matter (RPM) for development different cardiopulmonary diseases and lung cancer. In addition, air pollutants and its most toxic past that is RPM associated with increase in incidence of numerous additional diseases. IACR designated outdoor air pollution as a Group 1 carcinogenic substance. In addition RPM mixture, that contain variable amount of organic (e.g. PAHs, OC) and inorganic (e.g. metals and metalloids) species was evaluated separately and also classified in the Group 1. Current research on airborne particle-induced health effects investigates the critical characteristics of RPM that determine their biological effects. It need to be take in account that the size of the airborne particles and their surface area determine the potential to elicit inflammatory injury, oxidative damage, and other biological effects. Associations between chemical compositions and particle toxicity tend to be stronger for the fine and ultrafine particulate matter size fractions. Extractable organic compounds contribute to various mechanisms of cytotoxicity, in addition, the water-soluble faction (mainly transition metals with redox potential) play an important role in the initiation of oxidative DNA damage and membrane lipid peroxidation.

Keywords: respirable particulate matter (RPM), particle size, particle composition, toxicity
Correspondence: mjavst@vin.bg.ac.rs
paride.mantecca@unimib.it

Comparative Toxicity of Airborne Fine and Ultrafine Particles from Different Regions and Emission Sources

P. Mantecca¹, S.K. Hassan², A.A. El-Abssawy³, W.H. Shetaya³, A. El-Mekawy³, E.F. Mohamed³, A.M.F. Mohammed³, R. Bengalli¹, S. Marchetti¹, A. Zerboni¹, E. Longhin¹, M. Camatini¹

¹University of Milano-Bicocca, Department of Earth and Environmental Sciences, Research Center POLARIS, Milan, Italy;
²Air Pollution Department, Environmental Sciences Division, National Research Centre, Dokki, Giza, Egypt

Air pollution, mainly with airborne particulate matter (PM), is one of the leading global risks to human health. The toxicity of airborne PM is linked to particles’ dimension and chemical composition and is greatly affected by regional and seasonal variations, as well as by the local emission sources. Fine (FP) and ultrafine (UFP) particles are nowadays considered the most deleterious fractions, because they penetrate into the profound lung, where can hit the respiratory barrier and translocate to secondary organs.

In this presentation, the cytotoxic and genotoxic effects of PM2.5 collected in Milan (Italy) and Giza (Egypt) on lung cells will be displayed. The variations in PM2.5 chemical composition, especially polycyclic aromatic hydrocarbons (PAHs) and heavy metals, will also be discussed in relation to the geographical and seasonal variations.

Since diesel and biomass combustion-derived ultrafine particles (UFPs) largely contribute to the PM2.5 pollution, additional toxicity studies have been performed by testing combustion particles from different diesel and biomass sources. The results clearly indicate the variability in the cytotoxic effects exerted by UFPs derived from different sources and burning conditions.

The chemical and toxicity data presented will improve our knowledge on the health hazards associated with PM2.5 and UFP exposure and may be considered in the planning of future mitigation strategies.

Acknowledgments: Italian Ministry of Foreign Affairs and International Cooperation (proj. ID PGR00786); Egyptian Science and Technology Development Fund (STDF) (proj. ID 26001); Fondazione Cariplo (proj. ID 2013-1038)

Keywords: PM2.5, ultrafine particles, cytotoxicity, genotoxicity, emission sources
Correspondence: paride.mantecca@unimib.it
The Influence of Metal Components on the Health Effects of Ambient Particulate Matter

Jasmina Jović-Stošić¹, Dragan Alavantić², Milena Jovašević-Stojanović²
¹National Poison Control Centre, Military Medical Academy, Belgrade,
²Vinča Institute of Nuclear Sciences, Belgrade

In order to assess the contribution of metal fraction to particulate matter (PM) toxicity, we made a review of the relevant literature, comparing results of epidemiological studies, experimental research and professional exposure.

Except for heavy metals, well known for their toxicity, epidemiological studies tended to be internally consistent in identifying some transition metals (iron, nickel, vanadium, zinc) more often than others (arsenic, copper, manganese, selenium) as potentially affecting health. The limitations of these studies are primarily related to characterization of exposure and the presence of co-pollutants possibly contributing to the same effects. Occupational studies may not be representative for general population exposure, but provide valuable information on metals toxicity. Metal fume fever as acute illness, and pulmonary fibrosis, nasal and lung tumours after chronic exposure, are examples of metals toxicity. In such cases, concentrations of metals are much higher than those measured in polluted air. These observations are consistent with the experiments demonstrating that metals from ambient PM can produce acute inflammatory responses only in doses which are much greater than in ambient air.

Epidemiologic studies have found statistically significant association between certain metals in ambient PM and health effects (respiratory and cardiovascular morbidity and mortality). However, experimental research and experience from professional exposure indicate that toxic effects occur at doses many orders of magnitude greater than in airborne PM. From the medical point of view, some metals are normal constituents of the body and we may suppose that relatively low ambient air concentration may be controlled by body homeostasis.

**Keywords:** air pollution, PM, metal, toxicity

**Correspondence:** drjasminajovicstosic@gmail.com

---

(Bio)monitoring of Polycyclic Aromatic Hydrocarbons

Simone Morais

Polycyclic aromatic hydrocarbons (PAHs) are a large group of ubiquitous pollutants. Many PAHs present cytotoxic and mutagenic properties being some of them considered carcinogenic or probable/possible carcinogenic to humans. PAHs monitoring is complicated by their partitioning between air particulate and vapour phases. Main exposure routes to these chemicals are inhalation, food ingestion, and dermal contact, which make biomonitoring the most appropriated way to assess total exposure to PAHs. Thus urinary metabolites of PAHs, monohydroxy-PAHs (OH-PAHs), constitute valuable biomarkers.

Children are a sensitive group because their systems (respiratory, central nervous, etc.) are not fully developed. Firefighters are an occupational group that are regularly exposed to a high number of pollutants released during fires, including PAHs. Thus ongoing research aims to attain a comprehensive characterization of preschool children and firefighters exposure to PAHs based on biomonitoring and environmental monitoring.

Airborne PAHs with 2–3 rings were the most abundant compounds, being followed by 4-6 ring PAHs. A similar distribution profile was observed between airborne PAHs and urinary OH-PAHs, with 1-hydroxynaphthalene and 1-hydroxyacenaphthene contributing the most for ΣOH-PAHs, followed by 2-hydroxyfluorene, 1-hydroxypyrene, and 1-hydroxyphenanthrene. Levels of 1-hydroxypyrene were lower than the proposed benchmark level; urinary 3-hydroxybenzo[a]pyrene, the PAH biomarker of carcinogenicity, was not detected. Regardless the group considered, strong to moderate Spearman correlations were observed between the levels of ΣPAHs and ΣOH-PAHs, suggesting air as the one of the major exposure source of PAHs.

This work was supported by EU (FEDER/COMPETE) and National Funds (Fundação para a Ciência e Tecnologia) through project UID/QUI/50006/2013.

**Keywords:** air pollution, polycyclic aromatic hydrocarbons, total exposure, biomarkers of exposure, urine.

**Correspondence:** sbm@isep.ipp.pt
Toxicity of Indoor and Outdoor Respirable Particulate Matter in Schools/Kindergartens

M. Jovašević-Stojanović1, A. Filipović2, M. Živković1, M. Jovanović3, I. Lazović3, M. Davidović3, R. Kovačević3
1Vinča Institute of Nuclear Sciences, University of Belgrade, Serbia
2Public Health Institute of Belgrade, Serbia,
3Mining and Metallurgy Institute Bor, Serbia

Kindergartens and later schools are the microenvironments where adolescents spend significant period of time. Outdoor ambient particulate matter (PM) originate mainly from soil, traffic and industrial sources, while indoor PM in schools represent mixture of both, infiltrated and indoor-generated particle. In many studies there are notified high concentrations of PM in classrooms comparatively with typical home indoor environment. In addition level and content of indoor PM in schools are under influence: air exchange rate; cleaning practice; resuspension; age and physical activity of children in relation to room dimension, due to higher inhalation rates and levels of physical activity children may be more exposed than adults.

PM in ambient air shows differences in size, physical and chemical properties and thus in the toxicological, mutagenic and/or carcinogenic effects. Hazardous pollutants such as Polycyclic Aromatic Hydrocarbons-PAHs and heavy metals are strongly associated with finer particles (< PM2.5). PAHs generated primarily emitted to atmosphere during the incomplete combustion of organic materials such as coal, oil, petrol and wood. Level and characteristics of PAHs in school indoor environment depends of different factors including building location (traffic/residential area), season (heating/nonheating), heating (local/district), etc.... Metal-enrichment in PM is of particular importance for schools located in industrialized regions since anthropogenic metal emissions to the ambient outdoor atmosphere can transport indoors and negatively impact children’s health.

For data collected in last decade risk of exposure to PM, bonded PAHs and metals in selected school in Serbia will be presented and compared with similar studies performed in other countries.


Keywords: indoor air pollution, Respirable particulate matter, schools, toxicity

Correspondence: mjovst@vin.bg.ac.rs

Development of an Assay to Assess Genotoxicity by Particulate Matter Extract

Alexandros Pritits1, Konstantinos Papikinos1, Marina Koukoulani1, Efthalia Kerastioti1, Dimitrios Stagos1, Konstantinos Konstantinopoulos2, Demetrios Spandidos3, Marianthi Kermenidou4, Spyros Karakitsios5, Dimosthenis Sarigiannis6, Aristidis Tsatsakis6, Demetrios Kouretas1
1Department of Biochemistry and Biotechnology, University of Thessaly, Larissa 41500, Greece,
2Coffee Island S.A., Patras 26334, Greece,
3Laboratory of Clinical Virology, University of Crete, Medical School, Heraklion 71409, Greece,
4Aristotle University of Thessaloniki, Department of Chemical Engineering, Environmental Engineering Laboratory, 54124 Thessaloniki, Greece,
5Institute of Advanced Study (IUSS), Environmental Health Engineering, Piazza della Vittoria 15, 27100 Pavia, Italy,
6School of Medicine, University of Crete, Greece

The current study describes a method for assessing the oxidative potential of common environmental stressors (ambient air particulate matter), using a plasmid relaxation assay where the extract caused single strand breaks, easily visualized through electrophoresis. This assay utilizes a tiny amount (11μg) of particulate matter (PM) extract compared to other, cell-based methods (~3000μg). The negative impact of air pollution on human health has been extensively recognised. Among air pollutants, PM holds an eminent role reflected in the broad scientific and regulatory interest. PM toxicity highly depend on its composition (metals and organic compounds), which in turn has been linked to multiple health effects (such as cardiorespiratory diseases and cancer) through multiple toxicity mechanisms; oxidative stress induction is consider one major mechanism among them. In this study the PM levels, the oxidative potential, the cytotoxicity and the genotoxicity of PM in the region of Larissa were examined using the plasmid relaxation assay. Finally, coffee extracts from different varieties, derived from both green and roasted seeds, were examined for their ability to inhibit particulate matter induced DNA damage. These extracts also displayed an inhibitory activity towards xanthine oxidase and catalase, while having no effect against superoxide dismutase. Overall, the study highlighted the importance of assays for assessing the oxidative potential of widespread environmental stressors (PM), as well as the antioxidant capacity of beverages and food items, with the highlight being the development of a plasmid relaxation assay to assess the genotoxicity caused by PM using only a tiny amount.

Keywords: particulate matter, genotoxicity, antioxidant activity, coffee, polyphenols

Correspondence: dkouret@uth.gr
INCORPORATING INFORMATION ON CHEMICAL MIXTURES INTO CHEMICAL RISK ASSESSMENTS

Chair: Richard Brown
Co-Chair: Mykola Prodanchuk
1World Health Organization HQ, Switzerland, 2LI Medved Research Centre of Preventive Toxicology, Ukraine

Combined exposures to multiple chemicals is a concern in many environmental matrices, including chemicals in food, in drinking water and in air. Many tools have been developed for risk assessment of combined exposures of chemicals, including by the World Health Organization (WHO).

This symposium will describe an electronic tool for prioritization of chemicals for risk assessment using monitoring data of chemical mixtures in indoor air, and a published framework for assessing mixtures of chemicals in drinking water using tiered approaches for hazard and exposure assessment. Use of chemical registers to characterize chemical mixtures in various sectors, and development of “in silico” methods to identify chemicals of concern will also be described. The web-based platform set up by the European Commission for searching for and gaining access to chemical monitoring programme data from multiple sources will be demonstrated. A case study of assessing interactions between chemicals in mixtures (cadmium and PCBs) will also be presented.

Keywords: chemical mixtures, combined exposures, tools, cocktail effect, prioritization
Correspondence: brownri@who.int

Tools Available and Developing for the Risk Assessment of Mixtures of Chemicals in Indoor Air

Irina Zastenskaya, Dorota Jarosinska, Elizabet Paunovic
WHO Regional Office for Europe, WHO European Centre for Environment and Health

Indoor air is a source of exposure to a number of chemicals. Around 1000 chemical substances have been identified in indoor air to date. They can interact, and synergistic and cumulative effects of exposure on human health are of particular concern.

To assess health risks due to exposure to multiple chemicals, WHO, supported by leading experts, has developed a framework for a tiered exposure and hazards assessment, which allows also assessing risks having limited data on exposure. The framework can be applied for assessment of health risks of chemicals in indoor air.

A number of tools for cumulative risk assessment (CRA) exist to enforce the EU legislation on chemicals. They were applied to assess risks of indoor pollution in several projects. Risks of irritative and respiratory health effects were assessed in relation to acute and long-term exposure to indoor air pollutants emitted during household use of selected consumer products.

To assist countries in implementing CRA of exposure to indoor chemicals, WHO is working on developing an electronic tool to facilitate assessment of risks using existing monitoring data. Given the complexity of the task, a pragmatic approach is taken for the development of a tool for assessment of risk of chemical mixtures in indoor air.

Development of the tool will contribute to the implementation of the Ostrava Declaration on Environment and Health in priority areas such as improvement of quality of the indoor environment and assessment of risks of chemical mixtures.

WHO Europe acknowledges the government of Germany for financial support of the project.

Keywords: chemical mixtures, indoor air, exposure, risk assessment
Correspondence: zastenskayai@who.int

Assessment and Management of Chemical Mixtures in Source and Drinking Water

M.E. (Bette) Meek
McLaughlin Centre for Risk Science, University of Ottawa, Canada
A recently released WHO Publication on Chemical Mixtures in Source and Drinking Water provides an overview of available tools for assessment of combined exposures. It also includes practical recommendations to support the prioritization of mixtures in drinking and source water for risk assessment and management. Illustrative case studies address pharmaceuticals, non-steroidal anti-inflammatory drugs, statins, microcystins, carbamate insecticides and natural and synthetic oestrogens. Prioritization of pesticides which co-occur in drinking water on the basis of risk ranking and detection frequency is also addressed in an additional case study.

The pragmatic recommendations in the publication are based on experience in the drinking water sector in application of the World Health Organization (WHO) International Programme on Chemical Safety (IPCS) Framework on combined exposures to multiple chemicals. This framework, which was developed drawing from assessments internationally, includes formal problem formulation followed by step-wise consideration of both exposure and hazard in several tiers of increasingly data-informed analyses.

Implications of this additional experience are considered. This includes the critical role and impact of the early consideration of exposure in setting priorities and additional aspects related to treatment and analysis. Priorities for further work to facilitate more systematic consideration of combined exposures in drinking and source water including the potential application of bioanalytical tools are also identified.

**Keywords:** drinking-water, problem formulation, prioritization, risk assessment

**Correspondence:** bmeek@uottawa.ca

---

**Sharing Data on Chemicals in Different Environmental Matrices and Humans – How Data from Chemical Monitoring Programmes Can Be Easily Discovered, Accessed and Retrieved (the European Commission’s IPCHEM Platform)**

**Stylianos Kephalopoulos, Silvia Dalla Costa**

**European Commission, DG Joint Research Centre**

Directly following-up to the European Commission’s Communication “Combined Effects of Chemicals - Chemical mixtures” (EC, 2012), DG JRC has developed the EC’s reference Information Platform on Chemical Monitoring data (IPCHEM).

IPCHEM supports a more coordinated approach for searching, accessing, retrieving, assessing and sharing data related to the occurrence of chemicals and chemical mixtures across various media (e.g. environment, humans, food & feed, indoor air and consumer products) (https://ipchem.jrc.ec.europa.eu/).

IPCHEM was designed and implemented as a distributed infrastructure, providing where feasible, remote access to existing relevant information systems and data providers.

European Commission services (DGs ENV, SANTE, JRC, RTD), European Agencies (EFSA, EEA, ECHA) and EU MS are actively involved in IPCHEM’s development, promoting its use and engaging new data providers.

IPCHEM’s primary objectives are focused on: (1) Assisting policy makers and scientists to discover and access chemical monitoring data on existing, new, emerging and less-investigated chemicals covering a range of matrices and media; (2) Hosting data currently not readily accessible (e.g. outcomes of research projects, off-line stored monitoring data, etc.) that will be searchable and accessible through the platform; (3) Providing chemical monitoring data and information of defined quality in terms of spatial, temporal, methodological and metrological traceability; (4) Facilitating exposure and risk assessment practices in support of EU policies to adequately address the risks from exposure to multiple chemicals from different sources and pathways.

IPCHEM contains already a wealth of data thanks to the contributions of several EU bodies, government agencies and research consortia: more than 33 million of chemical concentration measurements data are accessible, retrievable and downloadable today via IPCHEM.

**Keywords:** information platform, chemical monitoring data, chemical mixtures

**Correspondence:** stylianos.kephalopoulos@ec.europa.eu

---

**Development of National Chemical Registers and Using Information on Chemical Mixtures to Prioritize Chemicals for Risk Assessment. Use of In Silico and Testing Methods**

**Mykola Prodanchuk, Serhii Kolesnyk, Oleksandr Kravchuk, Petro Zhminko, Olena Ryabuha**

**L.I. Medved’s Research Center of Preventive Toxicology, Food and Chemical Safety, Ministry of Health of Ukraine**

There are several chemical registers in Ukraine: registers of Hazardous Factors, Food Additives, Flavorings, Pesticides and Agrochemicals, Disinfectants.
According to the legislation, chemical and biological substances are permitted for use only being certified through the state registration procedure. Most information required for classification, labelling and for safety datasheets is included in the certificate: product identification; physico-chemical properties; use and storage; risk of ignition and burning; environmental and human toxicity and biological effects; safety standards; hazard class; analytical methods; first aid for acute poisoning; ecological safety. Thus, existing registers include some information necessary for risks assessment of mixtures (RAM).

To pull out data necessary for RAM quickly and effectively, the existent chemical registers should be digitalized and unified. Changes in regulations are also needed to advance work on RAM.

Given high attention to endocrine disrupting properties of pesticides and biocides, algorithms for using “in silico” methods for identification of substances with high affinity for the same nuclear receptors are being developed in our center for further identification of groups for toxicity assessment and RAM.

We are conducting a research of effects of combined exposure to mixtures of pesticides in the pesticide formulations. Acute toxicity studies were performed and showed that combined effects of two or more active substances contained in the same product often lead to potentiation of toxicity. Further studies of repeated dose toxicity are planned. In addition, a study of combined action of pesticides that are a part of single-component plant protection products but used in one agrotechnology is also interesting.

**Keywords:** chemical mixtures; pesticides, in-silico methods, toxicity, risk assessment

**Correspondence:** skolesnick@gmail.com

---

**Assessing Possible Interactions in Chemical Mixtures: Case Study on Cadmium and Polychlorinated Biphenyls**

**Aleksandra Buha, Vesna Matovic**

*Department of Toxicology “Akademik Danilo Sodatović”, University of Belgrade-Faculty of Pharmacy, Serbia*

Humans are concurrently exposed to a great number of chemicals from different sources, thus toxicological evaluation should be focused on their “cocktail effects”. Up to date, several types of models for assessing the mixture toxicity have been proposed; all having certain limitations and/or being difficult to interpret.

The aim of the study was to assess possible interactions between chemicals in mixtures using the concept that analyzes the differences in the slope of dose–response curves for single chemicals and their mixtures. A mixture of cadmium (Cd) and polychlorinated biphenyls (PCBs) was chosen as a representative one.

The experimental design consisted of twenty-three groups of rats orally treated during 28 days with different doses of Cd (six groups), different doses of PCBs (six groups), different dose combinations of Cd and PCBs (nine groups), and vehicles (two controls). Investigated end points were effects on blood, liver, kidney, and thyroid function.

The study revealed certain important limitations of the applied method. Namely, for some investigated parameters, data on the assessed interactions between chemicals in mixtures were dependent on the chemical chosen as a comparative one. Furthermore, if the effect was not dose dependent it was not possible to assess whether chemicals acted independently or produced joint actions.

It can be concluded that defining an adequate model for the mixture toxicity assessment i.e. the model that will consider interactions between chemicals in mixtures, is of paramount importance in predicting the entire range of mixture toxicity.

**Keywords:** cocktail effects, slopes, dose-response curves, limitations

**Correspondence:** aleksandra@pharmacy.bg.ac.rs
WORKSHOPS
PLENARY WORKSHOP

TOXICOLOGY DATA AND ONLINE TOOLS: AVAILABILITY, SEARCH STRATEGIES, OPEN DATA, AND REPRODUCIBILITY

Chair: Philip Wexler
National Library of Medicine

Toxicology information in the form of online databases and related data resources is widespread. The challenge is knowing what is available, how to fit the right resource to the need, and to recognize what may be missing. This session will address the issue by offering an overview of two extensive portals to toxicological data, followed by a practical look at how to access information to answer particular questions, and conclude with ways to enhance data repositories in the interests of transparency and reproducibility. The session will begin by highlighting two major toxicology data portals, the U.S. National Library of Medicine’s TOXNET system and the OECD’s eChemPortal, together covering a vast array of both references to toxicological literature and data, and free to the public. Among TOXNET’s many databases are the Hazardous Substances Data Bank (HSDB), containing toxicologically oriented data on some 6,000 chemicals, and TOXLINE, a bibliographic file of over 4 million references. eChemPortal draws from a multiplicity of sources offering data on chemical use, hazard, exposure, and risk information. Another presentation looks at the particular question of identifying reliable information on workplace chemical exposures and offers guidance. The two concluding papers take different but related approaches to look at the broader issues of research and publication and how a greater transparency can enhance the credibility and reliability of results. One of these focuses on the importance of reproducibility and data sharing while the other highlights the increasing move to open data, both within the context of toxicological research.

Keywords: TOXNET, eChemPortal, occupational health data, reproducibility, open data

Correspondence: wexlerp@mail.nlm.nih.gov

The US National Library of Medicine’s TOXNET System and Other Toxicology Information Resources

Philip Wexler
National Library of Medicine

The US National Library of Medicine’s (NLM) Toxicology Information Program, established in 1967, evolved into today’s Toxicology and Environmental Health Information Program (TEHIP). TEHIP offers a comprehensive and globally accessible online portal to free information on toxicology and allied disciplines. These include databases, bibliographies, tutorials and other scientific and consumer-oriented resources. Among its most widely consulted databases, offered via the TOXNET system, are HSDB (Hazardous Substances Data Bank), a peer-reviewed file covering some 6,000 chemicals with information on their human health effects, emergency medical treatment, animal toxicity studies, environmental fate/exposure, chemical/physical properties, and much more and TOXLINE (Toxicology Literature Online) containing over 4 million references to technical literature on the biochemical, pharmacological, physiological, and toxicological effects of drugs and other chemicals and ChemIDplus, a chemical dictionary of over 400,000 chemical, including names, synonyms, structures, toxicity data, and links to additional information such as regulatory lists. Specialized databases cover topics such as drugs and lactation (LactMed), developmental and reproductive toxicology (DART), toxic releases (TRI), household products (Household Products Database), occupational exposures (HazMap), and risk assessment (IRIS and TRI). Radiation Emergency Medical Management (REMM) and Chemical Hazards Emergency Medical Management (CHEMM) and two tools offered by TEHIP for the emergency response community. Resources such as ToxTown and ToxMystery are designed specifically for students and educators. TEHIP also provides links to information on a diverse range of subjects in environmental health and toxicology, disasters, chemicals, drugs, and special populations. This presentation will also touch upon other global toxicology databases.

Keywords: TOXNET, databases, informatics, NLM, online searching

Correspondence: wexlerp@mail.nlm.nih.gov
eChemPortal - The Global Portal to Information on Chemical Substances

Sally de Marcellus, Bob Diderich
Organisation for Economic Co-operation and Development (OECD)

In a continuous effort to build chemicals management capacity to protect human health and the environment, the Organisation for Economic Co-operation and Development (OECD), in cooperation with the European Chemicals Agency and with contributions from governments, international organisations, non-governmental organisations and the chemical industry, developed an Internet portal which provides access world-wide to regulatory relevant chemical information.

eChemPortal is an efficient instrument for finding and accessing hazard and risk assessments, chemical property data sets, classification results, and exposure and use information on a chemical substance across existing and new industrial chemicals, pesticides, and biocides.

Searches by substance identity, property and effects (physical-chemical properties, environmental fate and behaviour, ecotoxicity, and toxicity), or classifications lead to the websites of multiple data sources, targeting the location of the specific information searched. Searches by classifications according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) also display a table view for a specific chemical of classifications which have been reviewed and made available by a regulatory body or intergovernmental organisation.

The OECD encourages use of the eChemPortal and encourages regulatory authorities to make chemical information freely available on the Internet and via eChemPortal.

Disclaimer: The opinions expressed and arguments employed herein are those of the authors and do not necessarily reflect the official views of the OECD or of the governments of its member countries.

Keywords: chemical, hazards, environment, health, classification

Correspondence: sally.demarcellus@oecd.org; bob.diderich@oecd.org

How to Find Reliable Online Information Regarding Chemical Exposure at Work?

Gert van der Laan1,2,3, Pieter van Broekhuizen4, Frank van Dijk1,3,4,5
1Free University of Amsterdam Medical Centre (VUMC), The Netherlands. 2International Centre for Rural Health, University of Milano, Italy. 3Foundation for Learning and Developing Occupational Health (LDOH) 4University of Amsterdam, Amsterdam, The Netherlands. 5Arbeits- und Umweltsepidemiologie & Net Teaching, Ludwig-Maximilians-Universität, München, Germany

Health professionals all over the world dealing with toxicological and occupational hygiene problems often have just basic knowledge of toxicology. They face two main categories of toxicological questions to solve. Substance-oriented questions concerning characterisation of the substance, human exposure and health effects, methods of measuring and monitoring, treatment of adverse health effects or occupational illnesses, prevention and risk-communication. And questions concerning a disease: is this caused by exposure to specific substances and how should this be assessed?

For identification of toxicological and occupational hygiene data a guidance is developed with an overview of relevant, open access databases. The databases are distinguished under different headings:

- From trade name into generic name to chemical name/ CAS number
- Searches and search engines for MSDS
- Chemical data overview for regulatory purposes
- Chemical profiles of industrial processes
- Risks assessments documents of chemicals
- Data on specific chemical classes, such as pesticides, solvents
- Databases starting from medical endpoints/ diseases
- Bibliographic databases, such as PubMed

The guidance will be integrated in OSH-online and in the update of the book OSH online. How to find reliable information (Van Dijk, Caraballo-Arias).

Several practical issues may be difficult to manage. An example is the practice that workers are often exposed to complex workplace-generated mixtures such as welding fumes, diesel exhaust fumes, dirt and other undefined fumes generated by heating, combustion or machining of materials. Another example is assessing industrial chemicals with limited hazard information. Finally there might be practical problems of dealing with poor Internet connections and language issues.

Keywords: occupational toxicology, free data-bases, search strategy

Correspondence: g.vanderlaan@occmed.eu
The Importance of Open Data in Toxicological Research and Publishing

Petar Bulat1,2, Zorica Bulat3, Stefan Mandić-Rajčević4
1University of Belgrade-Faculty of Medicine, Belgrade Serbia, 2Serbian Institute of Occupational Health, Belgrade Serbia, 3University of Belgrade-Faculty of Pharmacy Department of Toxicology “Akademik Danilo Soldatović” Belgrade, Serbia, 4Department of Health Sciences, University of Milan, Milan, Italy

Every year thousands of toxicological studies are performed around the World. These studies are most commonly funded by Governmental, Non-Governmental agencies, Universities, Faculties, and in the last 50 years even more so by funds provided by research funding schemes such as the Horizon 2020 and the Framework Programmes in the European Union, or the National Institutes of Health in the United States. As part of these studies, thousands of experiments and field studies are done, collecting millions of tables of data ranging from genotypes and cell-culture reactions to chemical agents, to epidemiological data on populations from different towns and countries. In addition, governmental agencies and private companies measure a vast amount of parameters regarding the environment. Recently, a very specific risk has come to attention of research funders: the results of a specific research project are published in a peer review journal, thus satisfying the basic requirements of the project call, and the raw data which was collected remains buried (or lost) in the hard-disk of participating researchers. To resolve this situation, many institutions have adopted the „Open Data” policy, which should allow the data collected by these institutions or projects funded by them to be freely available to use and re-use by others. This philosophy was also adopted by many journals which now allow the authors of published articles to store even the raw data in their online repositories. This new trend, which might soon become a rule in the scientific publishing world, considering there are journals specifically designed to store datasets and study protocols, increases the use of already collected data, facilitates data re-use and new discoveries, but also helps authors achieve a higher impact and recognition than by just publishing their work.

Keywords: research data, raw data sharing, open data policy

Correspondence: petar.bulat@med.bg.ac.rs

Improving the Quality of Toxicological Research Findings Using Modern Principles of Reproducible Research

Stefan Mandić-Rajčević, Federico Maria Rubino, Claudio Colosio
Department of Health Sciences of the University of Milan and International Centre for Rural Health of the San Paolo Hospital, Via San Vigilio 43, 20142 Milan, Italy

Reproducibility represents the foundation of scientific work and publications, and the materials and methods section in each published article should allow any researcher to repeat the experiment in question and get the same or similar results. Nevertheless, in most scientific papers the data analysis procedure is rarely described well, and it often contains just the basic information on statistical procedures performed. We present all of the basic steps in doing reproducible data analysis, with all the advantages and disadvantages over the non-reproducible methods, on a case study of pesticide exposure and risk assessment. Data is imported from multiple sources (text, excel, access database), and basic description of acquired data, visual and numerical comparison between groups, and modelling of data acquired in real-life studies of pesticide exposure in agriculture are presented. The final products of the data analysis process, tables and figures which are ready for the revision process, are compiled using the R Language and Environment for Statistical Computing and additional packages. Considering the more strict requirements for funding and the increased competition, as well as the slow (but certain) move towards open access, open review and data exchange, doing data analysis the reproducible way will become inevitable in toxicology, as well as other scientific fields. Popularization and training on using free statistical and reproducible research tools should be a priority for young researchers entering this field, as this will result in the improvement of the quality of toxicological research, leading to easier publishing.

Keywords: R language, statistics, modeling, pesticide studies

Correspondence: stefan.mandic-rajcevic@unimi.it
Scorpion sting is an important cause of envenomation in Africa, e.g. Morocco, South Africa and Zimbabwe. Important toxicoepidemiological characteristics of scorpion sting include the clinical presentation of envenomation, characteristics of venomous scorpions and clinical management. In Morocco large numbers of scorpion sting occur, therefore a special surveillance program was implemented together with education and communication to health workers and the public. These measures have shown a decreased in the morbidity and mortality of patients with scorpion sting. Scorpion antivenom is often available in countries where large numbers of scorpion stings occur. Timeous administration of antivenom may play an important role however, supportive treatments appear mandatory to efficiently improve patient outcomes. Evidence for additional antivenom use is still questionable and its cost-effectiveness remains limited. The evidence in favor or against the clinical usefulness of antivenom will be debated.

The medically important spiders of southern Africa and Europe include the neurotoxic spiders belonging to the genus *Latrodectus* (widow spiders) and the cytotoxic spiders: the genera *Cheiracanthium* (sac spiders) and *Loxosceles* (violin spiders). The *Latrodectus* species cause a serious neurotoxic syndrome known as latrotoxicism. Cytotoxic spider bite usually manifests as local pain and erythema. This may develop into a necrotic ulcer. Necrotic arachnidism is often a convenient diagnosis for unexplained local tissue injury/dermal necrosis. The spectrum of spider bites in southern Africa are not well documented therefore a retrospective analysis of spider bite using two different data-recording systems of a PIC was conducted in an attempt to improve documentation, identification, diagnosis and management.

**Keywords:** scorpion sting, antivenom, necrotic arachnidism

**Correspondence:** bruno.megarbane@aphp.fr
caw@sun.ac.za

---

**Antivenom to Treat Scorpion Sting: Is it Useful?**

**Bruno Mégarbane**

*Department Medical & Toxicological Critical Care, Paris-Diderot University, Paris, France*

Scorpion sting represent a major public health issue in almost all continents including resource-limited countries. Scorpion venoms contain low molecular-weight peptides that rapidly distribute and induce catecholaminergic storm, accounting for the clinical consequences. Life-threatening but reversible cardiomyopathy represents the major cause of death, mainly from Old World scorpions. Neutralizing and redistributing the venom from its target may be life-saving if rapidly administered. Although well-done experimental studies have evidenced antivenom contribution to improve survival and reduce toxicity following envenomation by scorpions, its usefulness to treat scorpion-stung patients is still controversial. Time from sting to admission generally limits the utility of antivenom action to prevent mediator release and consequently reduce lethality. Additionally, highly species-specific antivenom is needed but still lacking for *Androctonus mauritanicus* and *australis*, the most fatal species in Morocco and Tunisia, respectively; whereas poorly refined antivenom may induce severe side-effects. While supportive treatments appear mandatory to efficiently improve the patient, evidence for the additional contribution of antivenom is still missing. Moreover, its cost-effectiveness remains limited. Since authorities have to rationalize expenses, efforts to decrease scorpion-related lethality should be focused on standardizing supportive care, training professionals, launching information campaigns and implementing clinical audit. Before recommending its use, antivenom efficacy should be established by randomized controlled studies. This lecture will review the evidence in favor or against the clinical usefulness of...
antivenom going from basic science to bedside indications and use.

**Keywords**: scorpion, envenomation, antivenom, severity assessment, cost-effectiveness

**Correspondence**: bruno.megarbane@lrb.aphp.fr

---

**Spider Bite in Southern Africa**

**Cherylynn Wium, Gert Muller**

Division of Clinical Pharmacology, Stellenbosch University, Cape Town, South Africa

The medically important spiders of southern Africa can be divided into neurotoxic and cytotoxic groups. The neurotoxic spiders belong to the genus *Latrodectus* (widow spiders) of which six species occur in southern Africa. The cytotoxic spiders include the genera *Cheiracanthium* known as sac spiders (nine species) and *Loxosceles*, known as violin spiders (six species). The *Latrodectus* species cause a serious neurotoxic syndrome known as latrodectism. Symptoms and signs reflect peripheral nervous system stimulation e.g. generalised muscular pain and cramps, a feeling of tightness in the chest, anxiety and profuse sweating with board-like rigidity of the abdomen. This syndrome is well known to mimic other disease states. The differential diagnosis includes scorpionism, snake bite, acute abdomen, myocardial infarction, alcohol withdrawal syndrome and organophosphate poisoning. *Latrodectus* antivenom is available in South Africa and is very effective. Spiders in southern Africa suspected of causing the most cases of necrotic arachnidism include the sac spiders (*Cheiracanthium*) and the violin spiders (*Loxosceles*). Although both are widely distributed, the sac spider is more abundant. Symptoms and signs are similar and usually manifests as local pain and erythema. This may develop into a necrotic ulcer, often slow to heal. Treatment is symptomatic and supportive, which may include antibiotics. No antivenom is available. Necrotic arachnidism is an over-diagnosed clinical entity and is often a convenient diagnosis for unexplained local tissue injury/dermal necrosis. The clinical picture, differential diagnosis and management of latrodectism and necrotic arachnidism will be discussed and debated.

**Keywords**: latrodectism, arachnidism, differential diagnosis, antivenom

**Correspondence**: caw@sun.ac.za

---

**Scorpion Sting in Southern Africa: The Zimbabwean Experience**

**Dexter Tagwireyi, Louis Gadaga**

Drug and Toxicology Information Service, University of Zimbabwe, Harare, Zimbabwe

Scorpion sting continues to be an important cause of envenomation in some parts of Africa, including Zimbabwe. Whilst many stings have not been reported as being fatal in Zimbabwe, there are a number of documented deaths in the southern parts of the country. In this paper, important toxicoepidemiological characteristics of scorpion sting in Southern Africa (as exemplified by Zimbabwe) will be presented.
These include the clinical presentation of scorpion envenomation, characteristics of venomous scorpion to be aware of as well as clinical management of scorpion stung individuals.

**Keywords:** Scorpionism, toxicoepidemiology, envenomation

**Correspondence:** dextagwireyi@gmail.com

---

### Scorpion Stings in Morocco

**Naima Rhalem, Rhizlane El Oufir, Ilham Semlali, Rachida Soulaymani-Bencheikh**

*Poison Control and Pharmacovigilance Centre of Morocco, Rabat, Morocco*

Objective: In Morocco, A national strategy for stings and scorpion envenomations was setup in 2001, focused on training of health professionals, education and communication to the public and improving management of cases. Indicators of morbidity and mortality were set up to evaluate the strategy. We examine the relevance of the strategy by monitoring the chosen indicators. Methods: We collect records to study regional and national demographics, economics and evolving features of Scorpion Stings (SS) between first January 2001 and 31 December 2016. Results: Totally, 404 500 cases were reported. Patients were in class I (Simple sting without envenomation) in 91.4%. Cases were admitted to a healthcare facility reflecting envenomation (class II for 7.3% and class III for 1.3% of cases). Lethality rate in patients with SS varied according to year (0.4% in 2005 to 0.2% in 2016). Envenomation lethality rate in patients with class II and class III was at 5.5 in 2001 and at 2.4% in 2016; the lethality rate by SS in children less than 15 years decreased from 2.1% in 2001 to 0.8% in 2016. Conclusion: Continued improvement in the surveillance of morbidity and mortality from scorpion stings is still necessary to reach zero deaths.

**Keywords:** scorpion stings, Poison control centre, Morocco

**Correspondence:** nrhalemcap@gmail.com

---

### PRODUCT STEWARDSHIP AND REGULATORY TOXICOLOGY IN THE OIL AND GAS INDUSTRY

**Chair:** Salmaan Inayat-Hussain

*Petroleum Nasional Berhad (PETRONAS) Malaysia*

As part of the commitment to SAICM, product stewardship plays a significant role in promoting chemical safety through a sound management of chemicals throughout their lifecycle to ensure that chemicals are produced and used in ways that minimize significant adverse impacts on human health and environment by 2020. This 2020 goal was adopted by the World Summit on Sustainable Development in 2002, as part of the Johannesburg Plan of Implementation. In this respect, developed countries have established chemical regulations and there is now an emerging shift where developing countries are also introducing regulations to ensure governance on the manufacture, import and use of chemicals. This workshop will discuss the current approaches and examples of in the product stewardship and regulatory toxicology especially in the oil and gas industry.

**Keywords:** Product Stewardship, Regulatory Toxicology, Oil and Gas, Toxicity Testing, Reprotoxins Management

**Correspondence:** salmaan.inayat@petronas.com
Product Stewardship and Regulatory Toxicology in the Oil and Gas Industry

Salmaan H Inayat-Hussain
Petroliam Nasional Berhad (PETRONAS), Kuala Lumpur, Malaysia

As part of the commitment to SAICM, product stewardship plays a significant role in promoting chemical safety through a sound management of chemicals throughout their lifecycle to ensure that chemicals are produced and used in ways that minimize significant adverse impacts on human health and environment by 2020. This 2020 goal was adopted by the World Summit on Sustainable Development in 2002, as part of the Johannesburg Plan of Implementation. In this respect, developed countries have established chemical regulations and there is now an emerging shift where developing countries are also introducing regulations to ensure governance on the manufacture, import and use of chemicals. The oil industry needs global coordination of GHS activities to promote the development and adoption of technically correct and consistent guidelines for petroleum substances classification. IPIECA developed the GHS guide in 2010 (currently being updated) on the classification and labeling of petroleum substances. This workshop will discuss the current approaches and examples of in the product stewardship and regulatory toxicology especially in the oil and gas industry.

Keywords: product stewardship, regulatory toxicology, oil and gas

Correspondence: salmaan.inayat@petronas.com

Integrated Approaches for Testing and Assessment of Chemicals for Regulatory Decision-making

Bob Diderich
Organisation for Economic Cooperation and Development

Governments are struggling with three competing requirements: (1) assessing and managing the risks of more chemicals in a shorter time, (2) reducing the number of laboratory animals used for the safety testing of chemicals, and (3) increasing the knowledge-base on which to base the risk assessments and reducing their uncertainty.

While traditionally risk assessments are based on a set of predetermined set of standardised laboratory tests, such as OECD Test Guidelines, many of which use laboratory animals, regulatory risk assessors more and more rely on a wide array of information used in a weight of evidence assessment. These so-called Integrated Approaches for Testing and Assessment (IATA) may include results from (Q)SARs, read-across, in vitro, ex vivo and in vivo methods.
While IATAs indeed have the potential to speed up the risk assessment process and to reduce the reliance on test results involving laboratory animals, the integration of different types of data into hazard characterisation may prove to be complex. The OECD has therefore promoted the concept of Adverse Outcome Pathways (AOPs) as a way of organising and integrating results from different types of methods. An AOP is a description of how the interaction of chemicals at the molecular level in an organism triggers adverse effects at the cellular, organ, whole organism or even population level.

The mechanistic knowledge gathered in AOPs can be used to structure and evaluate the existing data available for a chemical from different methods and help decide whether additional targeted data needs to be generated (integrated testing strategy) before a conclusion can be taken regarding the hazard characterisation of a chemical. The mechanistic knowledge gathered in AOPs can be used (1) for the development of (Q)SARs, (2) for grouping chemicals into chemical categories, (3) for interpreting the results from non-standard test methods and (4) for selecting test methods for further standardisation.

Disclaimer: The opinions expressed and arguments employed herein are those of the author and do not necessarily reflect the official views of the OECD or of the governments of its member countries.

Keywords: OECD test guideline, QSAR, adverse outcome pathway

Correspondence: bob.diderich@oecd.org

A Concawe Perspective on Alternatives to Animal Testing under Regulatory Programmes

Hans Ketelslegers
European Petroleum Refiners Association - Concawe division, Brussels, Belgium

Petroleum substances (PS) are a prototypical example of UVCBs (Unknown or Variable composition, Complex reaction products and Biological materials), which present enormous challenges for science-informed regulatory decision making. Therefore, regulators and industry have a common interest to define a process for (petroleum) UVCBs to ensure that there is no underestimation of hazards and at the same time minimize or eliminate the use of animals in toxicology testing to ensure safe use.

Over the past decades, major advancements have been made in biotechnology that have changed, and are changing, the field of toxicological sciences. Concawe, recognising both the extensive opportunities but also appreciating the current shortcomings of these new technologies, has several ongoing research efforts aiming to progress the risk assessment of petroleum UVCBs - focusing on either directly informing human health hazard assessments (e.g., reprotoxicity and carcinogenicity endpoints) and indirectly by informing and underpinning read-across approaches. Experience with both applications of high content screening tools on PS will be presented, with a particular focus on Cat-App: a multi-year research consortium initiated in 2016 by Concawe, applying high-content screening data to underpin grouping and read-across under regulatory programmes such as REACH.

Overall, an overview will be given of the feasible alternative approaches that are being developed for PS, working towards a more sustainable framework for 21st century human health assessment of PS with a focus on minimizing the reliance on animal testing in regulatory submissions.

Keywords: petroleum substances, UVCB, risk assessment, alternatives to animal testing, in-vitro screening

Correspondence: hans.ketelslegers@concawe.org

Management of Reproductive Toxic Chemicals in the Oil and Gas Industry

Masao Fukumura1, A Muiz Aziz1, Linda Roberts2, Salmaan H. Inayat-Hussain1
1Petroliam National Berhad (PETRONAS) Malaysia, 2Chevron Energy Technology Company (retired)

Oil & gas industry uses hazardous chemicals throughout the value chain beginning from exploration through the final stage of petrochemical manufacturing. Management of hazardous chemicals, including reproductive toxicants, is important to protect workers from adverse health outcomes. This is particularly true with the increase in female workers in our industry, equating to a greater need to manage reproductive hazards for the wellbeing of workers and their offspring.

Management of reproductive health involves an understanding of inherent hazards, exposures, and reproductive risk assessment. Reproductive toxicity, as defined by the Globally Harmonized System (GHS), includes adverse effects on adult reproductive capacity and developmental toxicity in the offspring. Although workplace chemicals may cause reproductive toxicity, a chemical health risk assessment (CHRA), beginning with hazard identification, is an efficient way to protect workers. A CHRA assesses a chemical’s potential reproductive or developmental hazard
and the exposures associated with effects. Management of reproductive hazards ensures that risks are controlled and practices are protective.

To better understand chemicals used in the energy sector, profiling from 20 upstream and downstream operating units identified approximately 100 chemicals as reproductive toxicants from GHS classifications. A major challenge was inconsistent hazard classification by different suppliers for the same chemical resulting in inconsistent CHRA outcomes. Of note is that only a small percentage of these chemicals have occupational exposure limits based on reproductive endpoints.

This presentation describes an approach to managing reproductive toxicants, highlighting challenges and opportunities in the oil and gas industry.

**Keywords:** reproductive toxicity, hazard identification, GHS, chemical health risk assessment

**Correspondence:** Masao.fukumura@petronas.com
ROUND TABLES
WOMEN IN TOXICOLOGY IN DEVELOPING COUNTRIES

Chairs: Tao Wang¹, Nurser Basaran²
Panelists: Nurser Basaran³,
Silvia Berlang de Moraes Barros⁴, Hanan Gchantous⁵,
Mary Gulumian⁶, Anne Kahru⁶, Vesna Matovic⁷
¹Achaogen, Inc, USA;
²Hacettepe University, Turkey;
³University of Witwatersrand, South Africa;
⁴Food and Drug Administration, USA;
⁵University of Sao Paulo;
⁶National Institute of Chemical Physics and Biophysics, Estonia;
⁷University of Belgrade, Serbia

Although the global share of women in research has been increased for the last decades, women are still remain underrepresented in many areas of science including toxicology not only in developing countries but also in many developed regions. It is actually difficult for women to get a good position in research. Gender differences in researchers are even more pronounced in some developed countries such as UK, Japan and Canada compared to some developing countries. According to the report of Elsevier (1) in 12 comparator countries and regions over 20 years, in developed countries, the percentage of women in science and research in the area of Pharmacology & Toxicology and Pharmaceutics and also in the implementation of policies and legislation are still lower than men. But compared to other areas, a relatively high portion of women among researchers in Pharmacology & Toxicology and Pharmaceutics has been observed. The data about the situation of women in toxicology research in developing countries is not clear but it seems that female representation is still lagging behind in scientific bodies. The push for gender equality in developed and in developing countries is not easy and seems to need time. The panel is aimed to describe the situation of women researchers in toxicology in some developing countries and also to increase and foster the awareness of power of women in the areas of toxicology.

Keywords: women, toxicology, developing countries

Correspondence: twang@achaogen.com
anursesnbasaran@gmail.com

PROMOTING UNDERGRADUATE TOXICOLOGY EDUCATION AND CAREER OPPORTUNITIES FOR STUDENTS IN DEVELOPING COUNTRIES

Chairs: Blase Billack⁸, Petar Bulat⁹
Panelists: Nurser Basaran³, Claudio Colosea⁴,
Emanuela Corsini⁴, Elaine Faustman⁵,
Mary Gulumian⁶, Anne Kahru⁶
⁸St. John’s University, New York, USA;
⁹University of Belgrade, Belgrade, Serbia

We invite all conference participants, especially undergraduate students from developing countries who are majoring in science and science advisors of such students, as well as other interested researchers and toxicologists, to actively participate the Round Table. The aims of this discussion are to increase awareness about career choices and opportunities in toxicology and to increase interest and motivate undergraduate students to pursue graduate biomedical education pertinent to toxicology. Attendees will hear from toxicology leaders from both developed and developing nations, who will not only provide personal perspectives but also describe education programs in toxicology in their home countries as well as related educational initiatives to promote the science of toxicology which are being put forth by large toxicology societies such as SOT and EUROTOX. At the conclusion of the formal Round Table presentation, and to foster interactive dialog, the panelists will meet with small groups composed of students and graduate student mentors. The Panel will also describe potential career opportunities after the PhD in toxicology across different employment sectors (academia, government, and industry).

Keywords: toxicology education, career perspectives, career opportunities, undergraduate students, developing countries

Correspondence: billackb@stjohns.edu
bulatp@gmail.com
TOXICOLOGY FOR HEALTH IN THE UNITED NATIONS SUSTAINABLE DEVELOPMENT GOALS

Chairs: Richard Brown¹, Dorota Jarosinska²
Panelists: Sameeh Mansour – National Research Centre, Egypt;
Salmaan H. Inayat-Hussain – Petronas, Malaysia;
Claudio Colosio – International Centre for Rural Health, Italy;
Cherylynn Wium – Tygerberg Poison Information Centre, South Africa;
Arina du Plessis – Tygerberg Poison Information Centre, South Africa;
Bob Diderich – Environment Directorate, Organisation for Economic Co-operation and Development (OECD);
Emanuela Corsini – International Union of Toxicology (IUTOX)
¹World Health Organization HQ, Switzerland,
²WHO Regional Office for Europe, WHO European Centre for Environment and Health, Germany

In 2015, countries in the United Nations adopted a set of goals for the next 15 years, which are the backbone of the 2030 Sustainable Development agenda. These Sustainable Development Goals (SDGs) include several targets, which directly relate to chemicals and health, including targets on reducing health impacts of hazardous chemicals and poisoning (target 3.9), sound management of chemicals through the life-cycle (12.4) and chemicals in water (6.3). In addition to the targets specific for chemicals, toxicology can also contribute towards achieving other SDGs, such as the goals relating to food production, working conditions, innovation (environmentally sound technologies) and waste management in cities.

This Round Table discussion will feature different perspectives on how toxicology can contribute to the wider SDG agenda. The Round Table participants will bring together views of different sectors and stakeholders. They will represent academia, industry (petrochemical sector), occupational health (agricultural sector), a poisons centre, an international organization and a professional society.

The Round Table aims to frame developments in toxicology, such as those presented in the Congress, in the wider context of contributing to the Sustainable Development agenda.

Keywords: sustainable development, health, poisoning, life-cycle

Correspondence: brownri@who.int
jarosinskad@who.int
CONTINUING EDUCATION COURSES
Carcinogenicity Studies: Perspectives on Design and Execution for Successful Product Registration
Organized by the American College of Toxicology

Owen McMaster1, Hanan Ghantous1, Scott Boley2, Thomas Larsen3, John Vahle4
1ACT, 2MPI Research, 3Covance Laboratories, Inc. 4Lilly

The guidelines for carcinogenicity testing of drugs, biologics, and environmental chemicals have undergone recent revisions. Evaluation of the carcinogenic potential of therapeutic agents is now a very complex, multi-step process which is conducted only for chemicals which meet certain criteria. The practical aspects of running these large studies create challenges even for those with experience. This course will begin by exploring the history of carcinogenicity testing followed by an overview of the current international guidelines. The design and execution of carcinogenicity studies will then be discussed in detail, with topics including dose justification, strain differences, routes of administration, diet, the choice of negative controls, positive controls, sentinel animals, biomarkers, and formulations. The collection, evaluation and categorization of histopathology data will be detailed including topics such as background lesions, historical control data, toxicity vs neoplastic findings, toxicity vs exaggerated pharmacology, peer reviews, Pathology Working Groups and various statistical approaches. The procedures for evaluating data from carcinogenicity studies differ in the various regulatory and environmental agencies. The interpretation of carcinogenicity data will be discussed, including considerations of the context of use of the test compound. This will be followed by an examination of the future of the ICH S1 regulatory paradigm and an update to the status of the proposed changes to the S1 Guidelines by ICH’s Expert Working Group. In the final presentation, there will be analysis of the impact of positive carcinogenicity results on ongoing trials, approvals, prescribing information and post marketing events, with 2 or 3 case studies.

List of topics:
- Introduction and overview of guidelines.
- Practical aspects of design and testing.
- Pathology, statistics and presentation of data.
- Evaluating carcinogenicity studies including status update on proposed changes to the ICH S1 Guidelines.
- Carcinogenicity Study Outcomes: Impact on biopharmaceutical safety assessment, product approvals, labeling, and post marketing surveillance.

For whom the course is intended:
This course is intended for those working in academia, pharmacology, toxicology, regulatory affairs and drug development who are interested in obtaining the latest information regarding the carcinogenicity testing of drugs and environmental chemicals.

Carcinogenicity, ICH, Regulatory, Nonclinical, Drug.

Health-Based Limits for Toxicological Risk Assessment: Setting Acceptable Daily Limits for Pharmaceutical and Chemical Safety
Organized by the American College of Toxicology

Patricia Weideman1, Andrew Maier2, Brad Stanard3, Robert Sussman4
1Sakari Consultants LLC, Stratham NH USA, 2University of Cincinnati, Cincinnati OH USA, 3Medimmune, Gaithersburg MD USA, 4SafeBridge Consultants Inc., New York, NY USA

Health-based exposure limits (HBELs) have been used for many years to assure safety or assess risks from potential adverse health-related effects arising from exposures to xenobiotics. Acceptable Daily Exposure (ADE) and Permitted Daily Exposure (PDE) are terms referencing assessments that can be considered as the bases for a variety of health-based assessments associated with the development and manufacture of industrial chemicals and pharmaceuticals. ADEs/PDEs have similar overall intent and definition as other HBELs and have increasing regulatory implications. As an example, the transition to the use of HBELs (i.e. ADEs) to protect product quality of pharmaceuticals has gained industry and regulatory interest. HBELs rely on robust hazard assessments that can be used as the basis for subsequent risk assessments, such as occupational exposure limits (OELs) and the derivation of limits for cleaning validation processes and control of cross-contamination. Default approaches for limit-setting (e.g. 10 ppm) have not been based on current health-based risk assessment methods. In contrast to the default approaches, derivation of ADEs and subsequent limits includes the use of robust datasets. Although the datasets for pharmaceuticals are generally more complete than those for chemical manufacturing, many aspects of the evolving methods in deriving HBELs for either industrial chemicals or pharmaceuticals are the same with application of appropriate adjustment factors to better inform hazard and risk decisions. The use of ADEs is a step toward better informed science- and health risk-based decisions. Methods used to derive ADEs are
complex and are not harmonized among various regulatory constituencies and practitioners.

List of topics:
- Regulatory and Industry Trends in Deriving Health-Based Exposure Limits
- Application of Data-Derived Health Limits Versus Default Limits
- Point of Departure As a Central Aspect of ADE Derivation
- Fine-Tuning Health-Based Assessments: Applying Adjustment Factors and Use of Pharmacokinetic Data

For whom the course is intended:
This session will provide background and tools for toxicologists and regulators to better understand the basis, derivation, and application of ADE/PDE assessments for protection of human safety as an attempt to provide more consistency in approach and outcomes.

Keywords: ADE, PDE, hazard assessment, risk assessment

---

RISK21: A Practical Framework for Risk Assessment in the 21st Century
Organized by the British Society of Toxicology

Michelle Embry1, Alan Boobis2, Angelo Moretto3
1ILSI Health and Environmental Sciences Institute,
2Imperial College London,
3University of Milan

In 2007, a report on Toxicity Testing in the 21st Century ("TT21C") by the United States National Research Council laid out a vision to leverage scientific advances to make toxicity testing faster, less expensive, more relevant to human exposures, and less reliant on animal testing.

To complement the global effort to fulfill this vision, in 2009, the International Life Sciences Institute (ILSI) Health and Environmental Sciences Institute (HESI), launched its multi-partite, Risk Assessment in the 21st Century (RISK21) Project. RISK21 is a tiered evaluation strategy which emphasizes a problem formulation-based, exposure-driven approach to risk assessment. A key component is the RISK21 Matrix, a freely available electronic tool, which enables the visualization of the intersection of exposure and toxicity data, and facilitates risk communication beyond those with technical expertise. The end result is a systematic and transparent approach to risk-based decision making.

The objective of this course is to teach the risk assessment approach developed by the RISK21 to emerging and established scientists. The specific goals are 1) Communicate the overarching principles of the RISK21 approach; 2) Provide an introduction to the roadmap and visualization matrix; and 3) Conduct hands-on case studies using the visualization matrix.

List of topics:
- Problem formulation
- Risk assessment
- Risk communication
- Tiered evaluation strategies
- Cumulative risk

For whom the course is intended: The course is intended for toxicologists, exposure scientists, and risk assessors involved in evaluation of chemicals for prioritization, screening, product development, alternatives assessment, risk assessment, etc., as well as those interested in/engaged in risk communication. The course is applicable to all chemical sectors and exposure scenarios.

Keywords: RISK21, risk assessment, problem formulation, tiered evaluation strategy, risk communication

---

Water Security: Integrating Lessons Learned for Water Quality and Sustainability

Ellaine Faustman
Institute for Risk Analysis and Risk Communication,
Department of Environmental and Occupational Health Sciences, University of Washington

The purpose of the course is to provide a common risk-based framework for issues of water pollution and water quality. It uses WHO documents to present the issues from the global problems of ensuring water quality. Risk management examples are presented by the course faculty and are linked to the basic toxicology and illustrations of how toxicology informs this topic. We will also choose a set of abstracts from those submitted to the CTDC to have regional fellows and junior scientists from around the world share their experiences on water quality. These case studies are discussed by the group during the one-day course workshop. This course framework provides a context for issues of water security but illustrates issues faced by the local and international developing countries and communities. This course will follow the successful approach used in Brazil and Mexico at other IUTOX-ICSU sponsored congresses and will have the recently published book chapter (Cambridge Press series) for context for these discussions.

---
The Use of Selected Marine Organisms in Nanoeotoxicology: Experiments on Mussels, Sea Urchins and Crabs

Petra Burić1, Maja Levak1, Lorena Perić2, Ines Kovačić3, Dijana Pavičić-Hamer1, Daniel Mark Lyons1
1Laboratory for Marine Nanotechnology and Biotechnology, Center for Marine Research, Ruđer Bošković Institute, Rovinj, Croatia, 2Laboratory for Aquaculture and Pathology of Aquatic Organisms, Division for Marine and Environmental Research, Ruđer Bošković Institute, Zagreb, Croatia, 3Juraj Dobrila University of Pula, Pula, Croatia

With the growing use of nanoparticles in a broad range of industrial and consumer applications there is an increasing likelihood that such nanoparticles will enter the aquatic environment and be transported through freshwater systems, eventually reaching marine waters. Due to silver’s known antimicrobial properties and widespread use of silver nanoparticles (AgNP) in consumer products, the latter’s environmental fate and impact is therefore of particular concern. In this context, we have investigated the effect of low, environmentally-relevant, concentrations (from 1 up to 1000 µg L⁻¹) and different sizes of AgNP (10, 20, 40, 60 and 100 nm) in selected marine invertebrates: sea urchin embryos (*Arbacia lixula* and *Paracentrotus lividus*), mussels (*Mytilus galloprovincialis*) and crabs (*Carcinus aestuarii*). The results have showed that 50-1000 µg L⁻¹ AgNP have a negative effect on the sea urchin embryonal development, fertilisation success and have cytogeneotoxic potential toward sea urchin embryos. This findings were dependent on the investigated sea urchin species and the size of nanoparticles used. Mussels were sensitive to 50 µg L⁻¹ AgNP (60 nm) showing a stress response at the entire organism level, destabilisation of hemocite lysosomal membranes, elevation of enzymatic glutathione S-transferase activity and metallothionein content in mussel tissue. However, no effect was observed in the activity of acetylcholinesterase after AgNP treatment. AgNP concentrations from 100-1000 µg L⁻¹ (60 nm) caused mortality in crabs, while up to 500 µg L⁻¹ caused increased total hemocyte numbers. Thus, the selected marine organisms have shown to be useful models for investigating the toxicity of nanoparticles.

**Keywords:** silver nanoparticle, embryo, lysosomal membrane stability, glutathione S-transferase, metallothionein

**Correspondence:** buricpetra@gmail.com

---

The Reference Value for Biomonitoring in Chemicals Risk Area in Thailand

Nalinee Sripaung
Bureau of Occupational and Environmental Diseases, DDC, Ministry of Public Health, Thailand

The workers’ biological standard value (WBSV) is generally used to assess chemicals health risks in community. It may cause the deviate biomonitoring. Therefore, this study was aimed to set the pilot reference value for exposed people to chemicals. The study was divided into two phases. Phase I was proceeded in one industrial zone in Thailand during the year 2012 - 2014 to collect blood and urine samples of 402 working - age people in community accompanied with in-depth interview. The 4 heavy metals (arsenic, cadmium, lead, mercury) in blood and urine were analyzed by AAS - analysis and the metabolites of 4 VOCs (benzene, toluene, styrene, xylenes) in urine were analyzed by HPLC - analysis. The volunteers’ 8 average chemicals concentration (VACC) were calculated. Phase II was proceeded during the year 2015 - 2016 to compare risk group identification between usage of VACC and usage of WBCV. The results were presented in percentage. The results showed approximate 90 percent of VACC were lower than WBSV. The exceptional result was volunteers’ average urinary arsenic concentration. It was clearly higher than WBSV. The comparative results showed the adjusted amount of risk people by VACC was higher than the adjusted amount risk people by WBSV. This study indicated that general people’s average chemicals concentration should be used as the reference value for biomonitoring and active health surveillance. Besides, daily intake of chemical mixtures in food chain should be concerned.

**Keywords:** reference value, biomonitoring, chemicals health risks, exposed people, active health surveillance

**Correspondence:** nsripaung@gmail.com

---

Evaluation of Manganese, Copper, Zinc and Selenium Levels in Patients with Primary Epithelial Ovarian Cancer

Aydan Caglayan1, Doruk Cevdi Katlan2, Zafer Selçuk Tuncer3, Kunter Yüce3
1Hacettepe University, Faculty of Pharmacy, Department of Toxicology, Ankara, Turkey, 2Suleymaniye Research and Education Hospital, Obstetrics and Gynecology, İstanbul, Turkey, 3Hacettepe University, Faculty of Medicine, Department of Obstetrics and Gynecology, Ankara, Turkey
Ovarian cancer is the fifth most frequent cause of cancer deaths in women in European Union countries whereas seventh among all cancers and second among gynecological cancers in Turkey. Epithelial ovarian tumors (EOC) comprises 90% of malignant, 60% of all ovarian tumors, and 75% of EOC are characterized by serous histologic subtype. EOC has been associated with oxidative stress due to epithelial inflammation which makes ovaries more vulnerable to the deleterious effects of reactive oxygen species (ROS). However, antioxidant enzymes (AOEs) [manganese-superoxide dismutase (Mn-SOD), copper/zinc-superoxide dismutase (Cu,Zn-SOD), catalase (CAT) and glutathione peroxidase (GPx1)] protect cells against the biological damage of ROS and support cancer prevention by maintaining normal cell cycle progression, inducing apoptosis, and inhibiting proliferation/tumor invasion/angiogenesis/inflammation.

In the present study, we aimed to measure the trace elements [manganese (Mn), copper (Cu), zinc (Zn) and selenium (Se)] which are structurally/functionally associated with the AOEs by inductively coupled plasma/mass-spectrometry (ICP/MS) in blood samples of patients with EOC (M, n=30) and compare the data with healthy subjects (C, n=40). Serous EOC (M1, n=18) data were also evaluated according to the tumor grading [well or moderately well differentiated (G1-2) vs. poorly differentiated or undifferentiated (G3)] and disease staging [stage I-II (SI-II) vs. stage III (SIII)]. We obtained; i) The Mn and Se levels of M were significantly lower (∼1.4 and ∼1.2 times) than C, ii) only Mn levels were changed [(G3(Mn) < G1-2(Mn), 32%) in M1, iii) significant correlations were observed between [Cu and Zn levels (r=0.701, p=0.036) in G1-2 and (r=0.686, p=0.041) in G3; Cu and Se levels (r=0.960, p=0.000) in G3; Mn levels and Mn-SOD expression (r = 0.551, p=0.006) in M, (r=0.857, p=0.007) in G1-2 and (r= 0.690, p=0.056) in G3; Se levels and erythrocyte GPx1 activity (r=0.660, p=0.053) in G1-2, Se levels and erythrocyte Cu,Zn-SOD activity (r=0.693, p=0.038) in G3]. The study revealed that the trace elements particularly Mn and Se may be of value in the EOC and although Mn level is important in terms of discriminating the tumor grades, mutual correlations between Cu-Zn and Cu- Se levels are remarkable in all grades. Moreover, Se is the major important trace element correlates significantly with the erythrocyte AOEs.

**Keywords:** epithelial ovarian cancer, manganese, copper, zinc, selenium

**Correspondence:** aydanc@hacettepe.edu.tr or aydancaglayan@hotmail.com

---

**Acute Poisoning in Children in Azerbaijan**

**Ismayil Afandiyev**
**Azerbaijan Medical University, Azerbaijan, Baku**

Acute poisoning in children is one of the leading causes of morbidity and mortality in many countries. Data on the epidemiology of pediatric poisoning in Azerbaijan is very scarce. In this observation study, all children poisonings over eight years period (1st January 2009 to 31st December 2016) admitted to Poison Center (PC) in Baku, Azerbaijan were reviewed using electronic and paper medical records. There were a total 2949 pediatric admissions to PC (17.9% of all poisoning cases). Among them only 6.2% were intentional poisonings. One thousand six hundred fifty-two patients (56.2%) were male. Pharmaceuticals (n=1276, 43.3%), organic solvents/oil products (n=562, 19.1%), and corrosive substances (n=380, 12.9%) were the most common agents responsible for poisoning in children. Thirty fatal cases reported during the study period. Mortality in children (1.02%) was significantly lower (p<0.05) than mortality in adult group (2.56%). Adoption of educational prevention program for parents as well as enforcement child-resistant packaging and reducing toxicity of household chemicals can decrease poisoning cases among children.

**Keywords:** children poisoning, epidemiology, Azerbaijan

**Correspondence:** efendiyevi@gmail.com

---

**Toxic Effects of Bisphenol Analogues: Are Alternatives Safe?**

**Sheikh Raisuddin, Shikha Sharma, Jasim Khan**
**Department of Medical Elementology & Toxicology, Jamia Hamdard (Hamdard University), New Delhi 110062, India**

There is enough literature on bisphenol A (BPA), as it is one of the most studied bisphenol analogues and its production is bulk. Due to sustained media coverage, there is public awareness about the hazardous nature of BPA. This has led to search for alternatives (substitutes) to BPA. Over a dozen bisphenol analogues have been in market. Safety data on these substitutes are limited. However, these analogues appear to be active and available literature indicates their propensity to bind with molecular targets. We conducted a comprehensive in silico study on the binding efficiency of selected bisphenol analogues with their likely target peroxisome proliferator-activated...
receptors (PPAR). We selected mouse PPARα. Upon interaction with PPARα BPA and its halogenated analogues showed binding energy scores (Kcal/Mol) in the order: tetrabromobisphenol A (-8.75) > bisphenol Z (-8.09) > BPC = tetrachlorobisphenol A (-7.77) > bisphenol E (-7.24) > bisphenol B (-7.15) > bisphenol F (-7.02) > bisphenol S (-6.02) > BPA (-5.60) > bisphenol AF (-5.39). Lowest binding energy score of positive control GW 7647 was -7.89. Certain BPA analogues showed higher binding efficiency with PPARα than BPA. In silico results suggest that some of the halogenated analogues of BPA have strong binding affinity with PPARα. Taking bisphenol S (BPS) in vivo study in mice showed hepatotoxic effects of BPS which included lipid peroxidation in a dose-dependent manner along with mild histopathological changes. These results reveal that a proper risk assessment profiling is desirable to ensure safety aspects of BPA alternatives.

Keywords: endocrine disrupting chemicals, nuclear receptors, binding, bisphenol A, bisphenol analogues.

Correspondence: sraisuddin@jamiahamdard.ac.in

---

**Endocrine Disrupting Activity in Sewage Sludge: Screening Method and Cost-effective Strategy for Detoxification**

Dânia E.C. Mazzeo1,2, Andrea Misovic3, Maria A. Marín-Morales2, Mary Rosa R. Marchi1, Jörg Oehlmann2

1Department of Analytical Chemistry, Institute of Chemistry, UNESP — Univ Estadual Paulista, Araraquara, SP, Brazil,
2Department Aquatic Ecotoxicology, Goethe University Frankfurt am Main, Germany,
3Department of Biology, Institute of Bioscience, UNESP — Univ Estadual Paulista, Rio Claro, SP, Brazil

Sewage sludge (SS) presents a high agronomic potential due to the presence of organic matter, macro and micronutrients, encouraging its recycling in agriculture as a soil conditioner. However, the presence of toxic substances can preclude this practice. Among these substances, the endocrine disrupting chemicals (EDCs) require special attention since they can potentially interfere with the hormonal system of exposed organisms, resulting in metabolic and reproductive disorders, even when present in low concentrations. To enable a safe disposal of this waste in agriculture, this study verified the estrogenic, androgenic and retinoic-like activities of anaerobic SS samples by means of yeast-based reporter-gene assays. Additionally, aiming to eliminate the EDCs from SS, a detoxification process combining microorganisms and biostimulating agents (soil, sugarcane bagasse, and coffee grounds) was performed for periods of 2, 4 and 6 months. A fractionation procedure of samples, dividing the target sample extract into several fractions according to their polarity, was conducted in order to decrease the matrix complexity. Before the detoxification process, the studied SS induced significant agonistic activity at the human estrogen receptor α (hERα), androgen receptor (hAR) as well as the retinoic acid receptor (RARα) but not at the retinoid X receptor (RXRα), in, at least, one of the tested fractions. However, after the SS detoxification, no significant activities were observed, showing that the technology applied here was efficient to eliminate receptor-mediated toxicity. Moreover, the recombinant yeast assay and the fractionation procedure proved to be valuable methods to easily detect EDCs in SS.

**Financial support:** FAPESP-BEPE Process 2017/10198-3

**Keywords:** endocrine disruptors, receptor-mediated toxicity, in vitro bioassay, chemical fractionation, biostimulation

**Correspondence:** daniamazzeo@gmail.com

---

**Incidence of Enrofloxacin its Primary Metabolite and Chlortetracycline Residues in Eggs and Broiler Meat from Tamilnadu, India**

Ghadevaru Sarathchandra
Pharmacovigilance Laboratory for Animal Feed and Food Safety, Centre For Animal Health Studies, TANUVAS

The administration of fluoroquinolones and Tetracyclines to food animals without an adequate withdrawal time may lead to violative concentrations of residues in foods destined for human consumption. To ensure control over the presence of antibiotic residues in food stuffs of animal origin, European Union and Japan have set maximum residue limits for antibiotic residues in edible animal tissues. Since 2005 the U.S. Food and Drug Administration has banned the use of enrofloxacin in poultry/food animals. In view of this, the prevalence of enrofloxacin and its primary metabolite ciprofloxacin residues in broiler meat and organ samples of field origin was explored in the present study. A total of 180 numbers of broiler chicken edible tissue samples (liver, kidney, breast muscle, thigh muscle and skin each 36 numbers) were randomly purchased from various retail outlets in
Chennai, India. Sampling was carried out as per the guidelines prescribed by Codex Alimentarius. Liver, kidney, muscle, skin and egg samples were extracted and subjected to enrofloxacin and ciprofloxacin quantification by validated High Performance Thin Layer Chromatography (HPTLC) - Fluorescent Densitometry.

Liver (20%), kidney (15%) and skin (10%) samples had enrofloxacin residues higher than the MRLs and found to be violating the regulations of Japan. Indeed, all muscle and egg samples were found to be safe as per EU and Japan MRLs. This study therefore stresses the need for adhering to the withdrawal period as prescribed by various regulatory bodies (FDA, EU and Japan) and the need for stringent regulation for the use of antimicrobial drugs in the poultry industry as well as the inspection of chicken for antimicrobial residues prior to marketing in India.

Keywords: veterinary drug residue, HPTLC, food safety

Correspondence: sarathchandraghadevaru@gmail.com

Antioxidant Potentials of Cedrelopsis grevei Leave Extract against Chlorpyrifos Induced Oxidative Stress in Kidney of Male Rats

Tarek Heikal
Narcotics, Ergogenics and Poisons Dept, Medical Research Division, National research Centre (NRC), Cairo, Egypt

In the present study, the protective effect of Cedrelopsis grevei (C. grevei) leave extracts against renal oxidative damage and nephrotoxicity induced by chlorpyrifos (CPF) in male rats was undertaken. Four groups containing six rats each were selected. Group I served as control. Groups II rats were received 300 mg/kg extracts by oral gavages. Groups III rats were given a single daily oral doses of CPF (13.5 mg kg⁻¹, 1/10 LD₅₀, in corn oil) for 28 consecutive days. Groups V rats were simultaneously given the same doses of extract and CPF as in groups II and III, respectively. In CPF-treated rats, significant reduction in body weight and elevation in kidney weight were observed compared with control. Also, significant perturbations of renal function as evidenced via increase in blood urea nitrogen (BUN) and serum creatinine level and oxidative damage as evidenced via augmentation in kidney lipid peroxidation (LPO) as well as depletion in kidney antioxidant enzymes; catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) in CPF-treated rats in comparing with control. Histopathological analysis of the kidney revealed that supplementation with C. grevei resulted in nil to mild vacuolization, swelling and degeneration in the endothelium of glomerular tuft and the epithelium of lining tubules. In conclusion, the use of C. grevei extract appeared to be beneficial to rats, to a great extent by attenuating and restoring the damage sustained by insecticide exposure.

Keywords: antioxidants, chlorpyrifos, kidney, oxidative stress, renal toxicity

Correspondence: tarekh@yahoo.com

Toxicological Assessment of Aqueous Leaf Extract of Bryophyllum pinnatum on Body and Organ Weights, and Haematologic Parameters in Rodents

Omoniyi Yemitan¹, Akinsuyi Akinsegun², Sunday Olayemi³
¹Department of Pharmacology, Therapeutics & Toxicology, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria,
²Department of Pharmacology, Therapeutics & Toxicology, College of Medicine of the University of Lagos, Idi-Araba, Lagos, Nigeria.

The acute and subchronic toxicities of the aqueous leaf extract of Bryophyllum pinnatum (ALBP) was studied on the body and vital organs and haematologic parameters in rodents, with a view to predicting its safety in humans.

To five groups of albino mice (n = 5), acute oral or i.p. 24h -14 days toxicity was tested for ALBP, up to 5000 mg/kg, then observed for morphologic changes and mortalities. For the 90-day subchronic study, male rats (16/group) received daily 10, 100, 1000 mg/kg extract doses or distilled water (control); body weights were measured weekly; after 90 days, some rats were sacrificed (10/group) for vital organs weights and haematology. Remaining rats (6/group) were retained, untreated, for reversibility study.

Oral ALBP caused sedation, tachycardia and hypercapnia; intraperitoneal doses produced LD₅₀ of 1650 mg/kg. Subchronic test caused significant (P<0.05) weight reduction. At 1000 mg/kg, ALBP significantly caused increased weights of kidneys, liver and spleen, but reduction in weights of lungs and testes. Reversal of weights of the liver and spleen, but not the lungs and testes, was recorded. At 100 mg/kg, increases in PCV % & WBC, but at 1000 mg/kg, significant reduction of RBC, Hb concentration, PCV % and platelets were recorded. For WBC, significant and
irreversible elevation was recorded at 100 & 1000 mg/kg of ALBP. At high doses, ALBP has potential to cause toxic effects on lungs and testes, as well as anaemia, thereby monitoring of during long-term use is required.

**Keywords:** Bryophyllum pinnatum, toxicity, body weight, testicular toxicity, anaemia

**Correspondence:** kayodeyemitan@yahoo.com

---

**Immunomodulatory Properties of Zinc Oxide**

Pasqualla Fagundes dos Santos1, 2, Kathrin Becker1, Harald Schennach2, Dietmar Fuchs1, Johanna M. Gostner3

1Division of Biological Chemistry, Medical University of Innsbruck, Austria,
2Central Institute of Blood Transfusion and Immunology, University Hospital, Innsbruck, Austria,
3Division of Medical Biochemistry, Medical University of Innsbruck, Austria

Zinc oxide (ZnO) nanomaterials are used not only in technical and chemical industry, but also pharmaceutical, food and cosmetic applications are frequent, due to the relatively low toxicity and biodegradability. While the use of ZnO and nanomaterials is considered not to pose a risk of adverse effects after dermal exposure, there are concerns regarding ZnO containing aerosols.

Human peripheral blood mononuclear cells (PBMC) have been applied successfully to investigate immunomodulatory effects of compounds and materials. For such studies, the interferon-gamma-induced metabolic pathways of tryptophan breakdown to kynurenine via indoleamine 2,3-dioxygenase (IDO-1), and neopterin formation via GTP-cyclohydrolase can be used as readout.

ZnO bulk material and nanoparticles were analysed in this *in vitro* setting in a concentration range from 2.3 to 37.5 µg/ml. Nanoparticles and bulk materials showed different toxicity, the nanoparticles reducing cell viability in a concentration-dependent manner up to 70% with the highest concentration, at which the first effects of the bulk material became obvious. The kynurenine to tryptophan ratio, a measure of IDO-1 activity, decreased with all materials dose-dependently in mitogen-stimulated cells. However, nanoparticle treatment of unstimulated cells increased IDO-1 activity, while this effect was less prominent with the bulk treatment. Neopterin formation via GTP-cyclohydrolase can be used as readout.

ZnO bulk material and nanoparticles were analysed in this *in vitro* setting in a concentration range from 2.3 to 37.5 µg/ml. Nanoparticles and bulk materials showed different toxicity, the nanoparticles reducing cell viability in a concentration-dependent manner up to 70% with the highest concentration, at which the first effects of the bulk material became obvious. The kynurenine to tryptophan ratio, a measure of IDO-1 activity, decreased with all materials dose-dependently in mitogen-stimulated cells. However, nanoparticle treatment of unstimulated cells increased IDO-1 activity, while this effect was less prominent with the bulk treatment. Neopterin concentrations showed the same trend.

Data indicate that within a certain concentration range, ZnO nanoparticles may have some activating effect on unstimulated PBMC, while in stimulated
cells and with higher concentrations immunosuppressive effects prevail. A closer elucidation of these effects is clearly warranted.

**Keywords:** ZnO nanoparticles, *in vitro* setting, immunosuppressive effects

**Correspondence:** Johanna.gostner@i-med.ac.at

---

**Combined Effects of BEA and ENB on Jurkat T-cells at the Transcriptomic Level**

**Lara Manyes, Laura Escrivá, María José Ruiz, Manuel Alonso**

Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Spain

Beauvericin (BEA) and enniatins (ENs) are mycotoxins produced by various *Fusarium* species. BEA produces cytotoxicity, intracellular reactive oxygen species and lipid peroxidation and EN B is an ionophore antibiotic. These mycotoxins are normally concomitant in cereal-based products. Even if both able to reach bloodstream, luckily they show poor bioavailability. The study of transcriptional changes provoked by the individual treatment of BEA on Jurkat T-cells (1.5-3-5 µM during 24, 48 and 72 h), showed a large number of differentially expressed genes mainly related to respiratory chain, apoptosis, and caspase cascade activation. When these cells were exposed to EN B at the same time and concentration, pathways affected were electron transport chain and oxidative phosphorylation. The aim of this work is to study the transcriptional changes in Jurkat T-cells after exposure to the combination of BEA and EN B. Firstly, a bioinformatics approach was conducted. The comparison between the differentially expressed genes (DEGs) from individual mycotoxin exposure (BEA= 5719 and EN B= 5750) show coincidences in 2693 DEGs. Moreover, from 96 genes related to the respiratory chain, 32 are DEGs in both individual exposures. Other identical DEGs were found related to caspase activation, apoptosis, and programmed cell death. Interestingly, only 14 DEGs were found for both treatments and for all conditions belonging 11 of them to mitochondrial genome. Secondly, RT-PCR confirmation was carried out. In conclusion, the results point to mitochondrial toxicity of BEA and EN B through electron transport chain inhibition and oxidative phosphorylation disruption.

**Keywords:** mitochondria, mycotoxin, oxidative phosphorylation, respiratory chain, PCR

**Correspondence:** lara.manyes@uv.es

---

**Effects of BPA on Global DNA Methylation and Global Histone 3 Lysine Modifications in SH-SY5Y Cells: An Epigenetic Mechanism Linking the Regulation of Chromatin Modifying Genes**

Mine Senyildiz1, Ecem Fatma Karaman1, Serap Sancar Baş2, Pelin Arda Pirincci2, Sibel Ozden1

1Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Istanbul University, 34116-Beyazıt, Istanbul, Turkey, 2Department of Biology, Faculty of Science, Istanbul University, 34134-Vezneciler, Istanbul, Turkey.

Bisphenol A (BPA), an estrogenic endocrine disruptor, is widely used in the production of polycarbonate plastic and epoxy resins, resulting in high risk on human health. In present study we aimed to investigate the effects of BPA on global and gene specific DNA methylation, global histone modifications and regulation of chromatin modifying enzymes in human neuroblastoma cells (SH-SY5Y). Cells were treated with BPA at 0.1, 1 and 10 µM concentrations for 48 and 96 h. IC50 value of BPA was determined as 183 and 129 µM in SH-SY5Y cells after 24 h by MTT and NRU tests, respectively. We observed significant alterations on the 5-mC% levels (1.3 fold) and 5-hmC% levels (1.67 fold) after 10 µM of BPA for 96 h. Significant decrease was identified in H3K9me3 and H3K9ac after 10 µM of BPA for 96 h while decrease was observed in H3K4me3 at 10 µM of BPA for 48 h. Alterations were observed in chromatin modifying genes including G9a, EZH2, SETD8, SETD1A, HAT1, SIRT1, DNMT1, RIZ1 and SuV39h1 after 96 h of BPA exposure. We suggest that especially dramatic increase of SIRT1 regulation according to the decrease on H3K9ac might be useful target for the BPA inducing toxicity. In addition, SuV39h1 regulation might involve to the mechanisms of the BPA toxicity. Our study might provide a new perspective for epigenetic alterations of BPA in SH-SY5Y cells due to the fact that limited studies are available on the epigenetic effects of BPA toxicity in neuroblastoma cells.

**Keywords:** bisphenol A, DNA methylation, histone modifications, chromatin modifying genes, neuroblastoma cells

**Correspondence:** stopuz@istanbul.edu.tr

---

**Levels of Heavy Metals and Ochratoxin A in Medicinal Plants Commercialized in Turkey**

Hakan Ozden

Division of Botany, Department of Biology, Faculty of Science, Istanbul University, 34134, Suleymaniye, Istanbul, Turkey,
The aim of this study was to determine the levels of lead, cadmium and ochratoxin A (OTA) in frequently used medicinal plants. Totally twenty-one samples of linden, chamomile and sage were obtained during the spring and summer period of the year 2016 from local markets and traditional bazaars in Istanbul, Turkey. Microwave-assisted digestion was applied for the preparation of the samples and inductively coupled plasma technique with optical emission spectrometry (ICP-OES) was used for the determination of lead and cadmium. Determination of OTA was carried out using high performance liquid chromatography with fluorescence detector (HPLC-FLD) after immunoaffinity column clean-up. OTA was detected in only one chamomile sample with a low concentration level of 0.034 µg/kg. According to the results of ICP-OES analysis, lead in the concentration range of 4.125-6.487 mg/kg, 3.123-5.769 mg/kg and 3.229-5.985 mg/kg and cadmium in the concentration range of 0.324-0.524 mg/kg, 0.365-0.51 mg/kg and 0.321-0.474 mg/kg was found in linden, chamomile and sage teas, respectively. We indicated that levels of Pb and OTA were found below the maximum permissible level whereas high levels of Cd were observed in medicinal plants which may not pose health risk for the consumers according to the exposure assessment. However, it is suggested that other mycotoxins and heavy metal content should be carefully considered in medicinal plants.

Keywords: lead, cadmium, ochratoxin A, linden, chamomile, sage

Correspondence: ozdenh@istanbul.edu.tr

OECD Country’s Research Productivity on Nanotoxicity Research: a Bibliometric Analysis

Mahmoud Abudayyak
Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Karadeniz Technical University, Trabzon, Turkey

The superior properties of nanomaterials have dramatically risen the usage of these materials in various applications that include industrial and medical sectors, leading to higher exposure, which in turn increases the concern about their safety. As the toxicity of nanomaterials is still controversial, more high quality research is needed in order to clarify this matter. The aim of this study was to evaluate the OECD country’s research productivity on nanotoxicology. For this, SciVerse Scopus was used to search and analyse the documents with specific key words related to nanotoxicology. Our analyses show that 44659 research articles were published worldwide, more than half in the last five years. About 60% of these papers originate from the OECD countries and among these 26590 articles produced by OECD countries about 72% were original articles and 14% were reviews. About 2% of the research articles were published by the Biomaterials Journal (Impact factor 4.2). The most productive country was USA (n =10812 article) with the highest quality of nanotoxicity research (h-index= 82). The significant relation between the countries’ collaboration and quantity - quality of the nanotoxicity researches was observed. The current study emphasizes the need for collaboration in this field between the OECD countries and other countries, especially China and India, which could affect not only the number of the articles but also the quality of the research.

Keywords: bibliometric, OECD countries, nanotoxicity, research productivity

Correspondence: abudayyak@ktu.edu.tr

The Distinct Properties of Natural and GM Cry Insecticidal Proteins

J.R. Latham1, M. Love2, A. Hilbeck3
1The Bioscience Resource Project, Ithaca, NY, USA; 2Independent scholar; Melbourne, Victoria, Australia; 3Swiss Federal Institute of Technology (ETH), Zurich, Switzerland

The Cry toxins are crystal-forming proteins produced by the bacterium Bacillus thuringiensis. There are many distinct Cry toxins which, in their natural forms, vary widely in toxicity towards a very wide range of organisms, from gastropods and nematodes to insects, including Lepidoptera. Cry toxins, such as Cry1, Cry2, and Cry9 are used worldwide in GMO crops to protect against insect pests. It is widely assumed that Cry toxins naturally active against coleopterans will remain active against coleopterans when produced in GMO plants. Some published data contradicts this assumption, showing that Cry toxins produced in GMO plants can have an altered and broader spectrum of activity than naturally-occurring Cry proteins. Such anomalies caused us to catalogue the differences between natural Cry toxins and commercial GMO Cry toxins in a search for an explanation. Our results, which were obtained primarily from documents submitted to support GMO approvals, show that there are potentially important chemical differences between natural and GMO Cry toxins. Natural Cry proteins are crystals, for example, whereas GMO Cry proteins are soluble. GMO Cry proteins are often
truncated or mutated by developers or altered by the plant itself. Such differences generally predict a broadened toxicological activity spectrum for the Cry proteins in GM crops because they represent a progression towards the processed and activated form of the protein. This enhancement of toxicity is supported by toxicological studies carried out by applicants, suggesting again that in many cases GMO Cry toxins have greater and broader toxicological activity than natural ones.

**Keywords:** Cry toxins, *Bacillus thuringiensis* toxins, risk assessment, GMO, plant

**Correspondence:** jrlatham@bioscienceresource.org

---

**Investigation of the Cytotoxicity of Medical Devices on Cell Cultures of Laboratory Animals**

Maryna Anisovich, Nastassia Ahamava, Svetlana Petrova

*Republican unitary enterprise «Scientific practical centre of hygiene»*

A study of the cytotoxic properties of medical devices was made on cell cultures of various genesis (experiments were carried out on primary cultures of cells of those tissues with which medical devices will interact when used).

The study was conducted in accordance with the recommendations of ISO 10993-5:2011. Extracts of medical devices were studied. As a positive control a solution of ethyl alcohol (1%) was used.

A study of the cytotoxicity of heart implants was conducted on a primary culture of rat cardiomyocyte cells. Suture surgical material was studied on mouse primary dermal fibroblasts. The absence of the cytotoxic effect of the medical devices in vitro was shown.

A scheme for analyzing the cytotoxic effect of medical devices on cell cultures was developed. At the first stage, the cytotoxicity of medical devices was evaluated in rapid tests - in the MTT test (the analysis of metabolic activity) and in the LDH test (the analysis of integrity of cell membranes). At the second stage, if more detailed studies are needed, within 14 days the analysis of the cell population by different methods was carried out. Estimation of growth dynamics of cells, the analysis of cell morphology is conducted microscopically on 1,3,5,7,11 and 14 day of the experiment. As well the cell population was analyzed by cytofluorimetric methods: determination of the number of apoptotic cells, micronuclei, cell ratio at different stages of the cell cycle.

**Keywords:** cytotoxic properties, primary cell cultures, MTT test, LDH test, cytofluorimetric methods

**Correspondence:** erlemarina@gmail.com

---

**Impact of Pesticide Residues in Feed on Animal Health**

Anne Schmitt, Katrin Franke, Lars Niemann

*German Federal Institute of Risk Assessment (BfR), Berlin*

Current legislation on authorization of plant protection and biocidal products requires risk assessment with regard to animal health. At present, no harmonized procedure exists at EU level.

A case study was performed. In cooperation with EFSA (European Food Safety Authority), Germany considered the impact of glyphosate residues in feed on animal health. Relevant information was taken from the Renewal procedure for glyphosate (RAR, 2015). Furthermore, results of a cooperation project (between the BfR and the University of Veterinary Medicine, Hannover) were taken into account. This research project examined the effects of a glyphosate-based herbicidal product on bacterial communities in an *in vitro* system by means of the "Rumen Simulation Technique (RUSITEC)". The relevant toxicological data were compared to the expected exposure of farm animals. For porcine, equine and avian species, no health concerns were found. No final conclusion could be drawn for the assessment of the impact of glyphosate on bacterial communities in the ruminant gastrointestinal tract.

As shown for glyphosate, the database regarding effects on animal health is usually scarce. All possible sources of information have to be considered. Further research is needed, in order to elucidate whether the reference doses established for humans as a general rule can be applied to farm animals or whether different uncertainty factors or critical toxicological effects need to be considered when extrapolating from toxicological studies performed in laboratory animals.

**Keywords:** risk assessment, farm animals, plant protection products

**Correspondence:** Anne.Schmitt@bfr.bund.de

---

**Pesticide Mixtures Induced Hepato-renal Dysfunction and Oxidative Stress in Pregnant Mice and Their Pups: The Role of Antioxidants**

Sameeh Mansour, Marwa Gad
Environmental Toxicology Research Unit (ETRU), Pesticide Chemistry Department, National Research Centre, Dokki, Giza, Cairo, Egypt

In real life, humans are exposed to a cocktail of pesticide residues in food. Women may expose to these pesticide residues during pregnancy. Highly lipophilic compounds, such as organic pesticides, have the ability to cross the placenta and reach the fetus. This study evaluates toxicity of a mixture of three pesticides (atrazine, chlorpyrifos and endosulfan; ACE) added to the rodent diet allowing the mice to ingest the equivalent of the Acceptable Daily Intake (ADI) of each pesticide, in addition to oral administration of vitamin E (α-tocopherol; 100 ul/mouse). During gestation (21d), the mouse dams were received one of the following treatments: (a) diet free of ACE; (b) diet enriched with ACE; (c) diet free of ACE + oral vitamin E; and (d) diet enriched with ACE + oral vitamin E. During lactation, the dams didn’t receive any chemical treatments. After weaning (42d), selected organs and blood samples were collected for analyses. Compared with the control results either in dams or their pups, the ACE mixture induced high elevation in AST, ALT, ALP, urea and MDA and high decline in BuChE, SOD and CAT. The pups were more affected than the dams with respect to alterations in MDA and BuChE activities, while the opposite was achieved with respect to SOD activities. Supplementation of vitamin E in conjunction with the pesticide mixture revealed the powerful effect of this vitamin; based on the estimated “Amelioration Indices; AI”. The findings revealed the ameliorative effect of vitamin E against toxicity of the tested mixture.

Keywords: pesticide mixture, oxidative stress, vitamin E, amelioration, pregnant mice

Use of Benzodiazepines in Methadone Treatment Patients

Daniela Chaparoska, Niko Bekjarovski, Danil Petrovski, Aleksandra Babulovska, Irena Jurukov,Dushan Petkovski,Tanja Petrushevska
University Clinic of Toxicology, Medical Faculty, University ‘Sts Cyril and Methodius’, Skopje, Macedonia

Introduction: The aim of the study is to explore the use/abuse of benzodiazepines by patients on methadone treatment and indicate needs and recommend intervention.

Material and Methods: The research is quantitative analytical study which was implemented from December 2016 to July 2017. A randomized anonymous survey, in terms of the use/abuse of benzodiazepines, was conducted on 458 addicts who are on methadone substitution treatment Results: The prevalence of abuse of BZD in patients on methadone treatment is 86.66%. The average age in the first try of BZD is 21.9+/−6.1 years. For 24.1% BZD were prescribed by a doctor the first time when it was used. BZD without prescription were use by 85.8% of the respondents. At the moment treatment with BZD prescription receive 22.4 %, usually 38.7% use more than the prescribed dose of BZD, use prescribed dose 17.3%, use less than the prescribed 6.8% and never had prescription for BZD 37.2% vs 50%. BZD use before entering the methadone program 70.7% of respondents in the experimental group. BZD has increased started after the methadone program 60.1%. In the life always used only BZD which were prescribed by a doctor 7.8% of respondents in the experimental group. In the total sample of patients of methadone treatment as the most common health problem is selected heroin at 42% followed by hepatitis C in 41.6% and anxiety/stress at 33.5%. Prescribed treatment for psychological/emotional problems (including depression or anxiety), receiving 54.3% of the participants in the experimental group.

Keywords: benzodiazepine use, methadone program, substances abuse, epidemiology

Effects of Subtle Chemical Changes on Drug-induced Liver Toxicity: A Structure-toxicity Relationship Study on Non-steroidal Anti-inflammatory Drugs

Yi Yun Pang, Han Kiat Ho
Department of Pharmacy, National University of Singapore

Diclofenac and lumiracoxib are non-steroidal anti-inflammatory drugs (NSAIDs) carrying the 2-phe- nylaminophenyl acetic acid scaffold. They exemplify an interesting case study of a pair of compounds that exhibit subtle chemical differences, and yet producing highly contrasting hepatotoxicity. To elucidate the role of these substituents to the toxicophore of the compounds, their chemical differences were extrapolated by synthesizing a series of 24 analogues, differing only in the size of their aliphatic and halogen side chains. Their pharmacological activities as well as in vitro toxicity were profiled and analyzed. Among which, the most toxic in the series was found to be the most
lipophilic and brominated compound. A clear trend could be observed between toxicity with metabolic stability, as well as with electrophilic reactivity. Bio-activation by Phase I metabolism is a prerequisite for toxicity whereby the propensity to generate electrophilic intermediate as measured by glutathione depletion correlated well with the extent of toxicity. Further LC-MS/MS analysis of the glutathione-trapped intermediates facilitated the identification of putative structures of such reactive metabolites. Pooling this effort together with COX-1 and COX-2 evaluations of the compounds unravels a divergent role of the same substituents to its pharmacology, thereby providing an approach to optimize the safety and efficacy of NSAIDs.

**Keywords:** hepatotoxicity, structure-toxicity relationship, reactive metabolites, non-steroidal anti-inflammatory drugs, diclofenac.

**Correspondence:** phahohk@nus.edu.sg
POSTERS
Air Pollution in Novi Sad (Serbia) – Long-term Level of Benzene (2011-2017)

Ljilja Torović1,2, Stanka Bobić2, Milan Jovanović2, Maja Ćirković2, Nataša Dragić1,2, Emil Živadinović2, Sanja Bijelović1,2
1Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia, 2Institute of Public Health of Vojvodina, Novi Sad, Serbia

The scope of the study was monitoring of volatile aromatic hydrocarbons included in BTEX group in ambient air of the City of Novi Sad. In the context of human health risk, benzene as group 1 carcinogen is of special interest.

Urban traffic (A) and industrial (B) site were monitored during seven years (2011-2017). Samples were collected on charcoal sorbent tubes (24 hours), with annual time coverage 27-34% (except 2011 - A 16%, 2014 - B 22%). Analytes of interest were determined by GC-MS, as described by reference method EN 14662-2. Temporal distribution in terms of mean monthly concentrations over calendar year, and between years during monitoring period, as well as spatial distribution between monitoring sites was considered.

The highest concentration of benzene recorded on site A ranged from 4.1 in 2011 to 37.1 mg/m³ in 2015, whereas on site B the range was 7.1-27.3 mg/m³ (2012-2016). The month with the highest mean benzene level was December or January, except in 2011 and 2012. On site A, annual mean benzene levels were 0.5, 1.2, 1.2, 3.9, 1.9, 2.1, and 2.5 μg/m³, during consecutive years, with the overall mean at 1.9 μg/m³. Site B was characterized with higher annual mean benzene levels than site A (except in 2012): 1.0, 0.9, 1.7, 5.0, 3.2, 3.0, and 3.2 μg/m³ (overall mean 2.6 μg/m³). The highest annual mean was recorded in 2014, on both monitoring sites.

Importantly, regulatory annual mean benzene limit value of 5 μg/m³ was not exceeded, although it was reached in 2014.

Keywords: ambient air, benzene, GC-MS
Correspondence: ljilja.torovic@mf.uns.ac.rs

Comparison of Methods for Determination of Sulphur Dioxide Concentrations in Ambient Air

Nenad Petrović
Public Health Department in Cuprija, Serbia

Air pollution is one of the major environmental issues and one of the main pollutant of concern in ambient air is sulfur dioxide (SO2). One of the most frequently used method for determining concentration of sulfur dioxide in ambient air is tetrachloromercurate (TCM)/pararosaniline method in which tetrachloromercurate is present in absorption solution. The objective of the present study was to compare tetrachloromercurate/pararosaniline method with methods that don’t contain mercury in the absorption solution and determine if they can be used as suitable replacement for TCM/pararosaniline method. Two methods were compared with TCM/pararosaniline method: spectrophotometric thorin/barium perchlorate method and nepheleometric barium chloride method. Samples were prepared using certified reference gas cylinder of sulfur dioxide and gas calibrator MCZ-CMK 5. All samples were prepared by transferring diluted gas through absorption bottle filled with a 20 ml of absorption solution with constant flow of 0,5 l/min. The three methods in the order tetrachloromercurate/pararosaniline method, spectrophotometric thorin/barium perchlorate method and nepheleometric barium chloride method gave the following results: limit of quantification(LOQ), 3,6 μg/m³, 22,2 μg/m³ and 18,3 μg/m³; measurement range up to 106,7 μg/m³, 222,2 μg/m³ and 275,1 μg/m³; sensitivity 0,72 μg/m³, 1,94 μg/m³ and 1,97 μg/m³; precision expressed as relative standard deviation(RSD%) 3,3 %, 6,2 %, and 5,6 %; accuracy 98,3-103,9 %, 92,6-102,9 % and 94,7-108,4 %. Although it has been shown that tetrachloromercurate/pararosaniline method has more advantages concerning sensitivity, (LOQ), accuracy and precision, conclusion is that both spectrophotometric and nepheleometric method can be used as suitable replacement for tetrachloromercurate/pararosaniline method.

Keywords: tetrachloromercurate, pararosaniline, thorin, nepheleometry, SO₂
Correspondence: amb.vazduh@zzjzcuprija.com
Inflammatory Response Modulation of Human Airway 3D-model Depending on the PM’s Chemical Composition

Khaled Boukerma, Emeline Seurat, Sophie Achard
Université Paris Descartes, Faculté de Pharmacie de Paris, EA 4064 “Impact des pollutions sur la santé”, Paris, France

Many epidemiological studies describe the existence of an association between air pollution and some respiratory disorders like asthma and allergic rhinitis. Within the major pollutants, the particulate matter (PM) is a complex mixture depending on the emitting sources.

To provide elements of biological plausibility to the epidemiological observations, the present study evaluates the impact of fine particles (PM$_{0.3-2.5}$) coming from two different places: Dunkerque (France) for PMD and Cotonou (Benin) for PMC; with similar sizes but different chemical compositions.

An innovative and sensitive in vitro 3D-model of human reconstituted airway epithelium (MucilAir, Epithelix®), cultured in an air-liquid interface was used.

The epithelia were exposed to PM at 30 or 60µg once or twice a week for three consecutive weeks. Forty hours after each exposure, the culture medium on basal side was collected and inflammatory response was assessed. At the end of each week the membrane integrity was evaluated using the TEER (transepithelial electrical resistance) test.

Our results showed that IL-8 production was time-dependent, whatever the PM sample and the quantity tested: a significant increase of the inflammatory response was observed from 6h to 96h after exposure. In addition, the IL-8 production was the highest during the first week of exposure, certainly due to the adaptation capacity of our 3D-model to its environment. Lastly, measured inflammatory response was higher after PMC exposure compared to PMD exposure, probably in relation with its chemical composition.

**Keywords**: airborne particulate matter, human respiratory reconstituted epithelium, repeated air-liquid exposures, inflammatory response

**Correspondence**: sophie.achard@parisdescartes.fr

---

ALTERNATIVE ANIMAL MODELS

Development of an Alternative Method to Evaluate In Vitro Skin Sensitisation Potency of Chemicals

A. Buzzella$^{1,2}$, R. Vicini$^2$, G. Mazzini$^{1,3}$, C. Angelinetta$^2$, O. Pastoris$^1$

$^1$Department of Biology and Biotechnology “L. Spallanzani”, University of Pavia, Pavia (Italy),
$^2$Bio Basic Europe S.r.l., Pavia, (Italy),
$^3$Institute of Molecular Genetics (IGM-CNR), Pavia (Italy)

Allergic contact dermatitis, a common condition for human health, is an inflammatory reaction caused by repeated skin exposure to specific chemicals. In order to comply with the requirements of European legislation, concerning the risk assessment of potential skin sensitizers, considerable progress has been made in developing alternative methods, such as human cell line activation test (h-CLAT). H-CLAT is based on measurement, obtained by cytometric analysis, of fluorescence variations emitted by anti-CD54 and anti-CD86 antibodies in THP-1 cells. Following this method, a range of substances have been analysed; the emitted fluorescence, generally at low intensity, has caused problems concerning the interpretation of results, preventing the discrimination between sensitizers and non-sensitizers.

For this reason, the aim of the study was to find an alternative cytometric parameter for evaluating the sensitising potential of chemicals. Cells have been analyzed with flow cytometry after treatment with sensitising compounds administered at non-cytotoxic concentrations. A set of cells have not been exposed to any product (negative control).

Sensitizers, once in contact with cells, were able to induce alterations in cell morphology to a more "condensed" one allowing the identification of cells under microscope as a “sensitized” subpopulation. These variations cause similar modifications in "scattering" parameters, making thus cells easily monitorable by flow cytometry. No changes have been observed in cells treated with non-sensitizers or in untreated cells.

In conclusion, this method based on the analysis of forward scatter and side scatter parameters, can be used as a valid alternative method for identifying sensitisation potential of chemical compounds.
An Innovative Approach for Evaluating the Safety of Cosmetic Products Through a Combination of in vitro and in vivo Methods

Riccardo Vicini¹, Alice Buzzella¹,₂, Giuliano Mazzini³, Claudio Angelinetta¹, Eliana Regola⁴, Fernando Bianchi⁵, Ornella Pastoris²

¹Bio Basic Europe S.r.l., Milan, Italy; ²Dpt. of Biology and Biotechnologies, University of Pavia, Italy; ³Institute of Molecular Genetics, CNR, Pavia, Italy

European Regulation no. 1223/2009 prohibits animal testing for cosmetic products and it requires their safety for human health. Hence the need to use alternative validated methods based on in vitro techniques on cell cultures, which are easy to manage and rapid, but on the other hand they provide forecasting models not always suitable for in vivo system. Our aim was to develop a multi-step test to evaluate the high tolerability of cosmetic products that involves the sequential execution of in vitro and in vivo tests.

We performed a pro-sensitising test on human monocytes on 193 cosmetic products, evaluating by flow cytofluorimetry the variation of cell morphology and CD86/CD54 expression. The products were then subjected to in vivo patch test on 25 volunteers, and to a subsequent clinical investigation on 20 volunteers for 28 days, to check the long-term skin tolerability.

182 products (94.3%) did not show any in vitro pro-sensitising activity. On these products also patch test (absence of erythema/oedema), and skin tolerability test (absence of undesirable effects) were negative. 11 products (5.7%) showed in vitro pro-sensitizing effect, but were negative to patch test and skin tolerability test.

We can conclude that in vitro tests on cell cultures can predict with good approximation the in vivo safe use of cosmetic products. Nevertheless, the in vitro presence of an undesirable effect seems not to cause the appearance of the same reaction in vivo. In these cases it is therefore appropriate to deepen the analysis with specific in vivo tests.

Keywords: alternative methods, cosmetic products, skin sensitization, integrated test strategy

Correspondence: alice.buzzella01@ateneopv.it

Development of a Full-thickness 3D Autologous Skin Equivalent Model to Determine Immunogenicity of Therapeutics

Asif S. Tulah, Shaheda S. Ahmed, Anne M. Dickinson

Alcyomics Ltd, Newcastle upon Tyne, United Kingdom

There is growing demand for human-based assays to test compounds/therapeutics for adverse immune reactions prior to entering clinical trials. Human skin equivalent models are useful in vitro testing platforms. We describe a unique full-thickness 3D autologous skin equivalent model made from primary human tissue which is representative of normal human skin and an autologous platform for testing therapeutics under development.

Primary human fibroblasts and keratinocytes were grown from healthy volunteer tissue. The full-thickness skin equivalent model was generated by first forming a dermal equivalent by culturing fibroblasts on a scaffold before adding donor-matched keratinocytes and culturing at the air-liquid interface. Histology and immunofluorescence for protein markers (involucrin, cytokeratin 14 and collagen 3) was completed and compared to normal skin. We co-cultured the model with activated peripheral blood mononuclear cells (PBMCs) and observed similar immune damage to that in our skin explant assay. Immunofluorescence staining for heat shock protein 70 (HSP70) as a marker of apoptosis was used to confirm these findings.

Our model was representative of normal human skin, showing similar structure observed by haematoxylin and eosin staining and positive immunofluorescence staining for protein markers of epidermal differentiation and skin structure (involucrin, cytokeratin 14 and collagen 3). Our data also suggest this model could be used to detect immune damage as shown by positive HSP70 staining.

We have generated an autologous 3D skin equivalent model, representative of normal human skin. Preliminary data has shown this to be a useful additional platform for testing drugs under development.

Keywords: 3D skin model, immunogenicity testing, safety testing, non-animal

Correspondence: asif.tulah@alcyomics.com

Ecotoxicity Assessment of Waste Hazard Using Clutches of the Mollusk Lymnaea stagnalis

Boris Olga, Ilyukova Irina, Shevtsova Svetlana

Republican unitary enterprise «Scientific and practical centre of hygiene», Minsk, Belarus
The method for studying embryotoxicity of waste in the test-model *Lymanea stagnalis* for the application to determine the hazard class of industrial waste on hazard characteristic «ecotoxicity». A study of more than 30 production waste identified toxic effects from exposure to waste and set evaluation criteria harmful effects, scientifically substantiated rational setting conditions of the experiment, the methodological aspects as well as the objective criteria for evaluating the ecotoxicity of waste and test validity criteria.

Such estimation criteria as EC50 (the average effective concentration), EC15 (the threshold concentration) and the acute zone have been developed. The use of these indicators will provide further investigations of waste ecotoxicological profile. The threshold concentration is useful for making out waste concentrations that should be considered conditionally safe in case of contact with the environment.

The clutches in the gastrula stage of snails are used in this test-system. The total number of hatchlings as well as the total number of dead embryos are counted per each experimental group. The experiment with *Lymanea stagnalis* test-system is aimed to estimate the inhibition of hatching efficacy.

The development allows reducing the negative impact of waste production on the environment well-being and preventing negative consequences for the human health.

**Keywords:** the waste products, *Lymanea stagnalis*, biotesting, ecotoxicity.

**Correspondence:** olgaboris88@gmail.com

---

**Analytics in Toxicology**

---

**Analytical Method Development of Methylisothiazolinone, a Preservative, in Rat Plasma Using LC-MS/MS**

Hyang Yeon Kim, Yong Jae Lee, Kyu-Bong Kim
College of Pharmacy, Dankook University,
119 Danwae-ro, Dongnam-gu, Cheonan, Chungnam 31116, Republic of Korea

Methylisothiazolinone (MI) is a preservative in personal care products to control bacteria and fungi. It might be one of the toxic sensitizer and irritant on the skin, and initiator of lung diseases, so it is important to understand the characterization and toxic mechanism of MI in the body. In this study, we developed the HPLC-MS/MS analytical method in rat plasma using multiple reaction monitoring (MRM) technique, which is following the fragments of target metabolite. MRM transition of MI was m/z 116 → 101 and lower limit of quantification (LLOQ) was set at 10 ng/mL. Including the concentration of LLOQ, seven-point calibration curve have good linearity (R²= 0.9998) and its intra- and inter-day accuracy and precision values were within 15% of the relative error (RE%) and standard deviation (RSD%) along with FDA guideline. In the pharmacokinetic study using rats, presented analysis method was useful for detecting the profile of MI in the plasma. We determined half-life, clearance and distribution of MI by WinNonlin software and these might be useful information for the study on the toxicokinetics in body.

**Keywords:** methylisothiazolinone, HPLC-MS/MS, MRM, pharmacokinetics

**Correspondence:** kyubong@dankook.ac.kr

---

**Determination of Methadone in Blood by Solid-phase Extraction and Liquid Chromatography-Mass Spectrometry**

Tatjana Ćebović1, Vesna Kilibarda2, Snežana Dordević2
1Faculty of Medicine Novi Sad, Clinical centre of Vojvodina, Novi Sad, Serbia;
2Military Medical Academy, Poison Control Centre, Belgrade, Serbia
Methadone, synthetic opioid analgesic, belongs to a class of compounds referred to as diphenylpropylamine derivatives. In spite of the introduction of new agents such as L-LAAM and buprenorphine, methadone continues to be the drug of choice for the treatment of heroin addiction. Due to the wide variation in half-life among individuals (reported values 13-58 hours), there is a significant overdose risk. Together with the requirement for effective monitoring of replacement therapy, it emphasizes the need for development of fast, precise and reliable analytical method for quantification of methadone in biological samples.

In this research, liquid chromatography-mass spectrometry (LC-MS) method for determination of methadone in blood was developed, using solid-phase (SPE) extraction for the preparation of samples.

Methadone was determined by LC-MS method in a single ion monitoring mode (SIM) at m/z 310. The chromatographic separation was performed on X Terra MS C18 column, using a mixture of formic buffer (pH 3.5) - acetonitrile (60 : 40 V/V) as mobile phase, with the flow of 0.5 mL/min.

Linearity was achieved in the range from 0.05 to 2 mg/L. Retention time of methadone was 5.1 min. Limit of detection and limit of quantification were 1.0 ng/mL and 3.2 ng/mL, respectively. The intra-assay precisions (RSD) were about 0.07-1.01%. The inter-assay precisions (RSD) were 0.18-2.11%. The accuracy varied from 92.0 to 101.1% for intra-assay and 99.7 to 100.9% for inter-assay.

Described LC-MS method for analysis of methadone in blood is precise, accurate, reproducible and reliable, with significantly shortened time of analysis (run time 10 min).

Keywords: methadone, overdose, therapeutic laboratory monitoring, validation

Correspondence: cebovic.tatjana@gmail.com, tatjana.cebovic@mf.uns.ac.rs

Determination of Carbamazepine Concentration in Serum – Comparison of Methods

Milanka Ljubenovic1, Vladan Cosic1, Jasna Lalic1, Svetlana Stojiljkovic1, Maja Vujovic2, Biljana Milosavljevic2, Bojan Ljubenovic2

1Centre of medical biochemistry, Clinical Centre Nis, Serbia, 2Laboratory of toxicology, Institute of Forensic Medicine, Nis, Serbia, 3Faculty of medicine, University of Nis, Serbia

Carbamazepine is antiepileptic widely used for the treatment of epilepsy. Due to low therapeutic index of carbamazepine, there is a need for routine measuring its concentrations in serum. Monitoring of the drug level helps to adjust the dose and achieve optimal therapeutic effects while avoiding subtherapeutic and toxic level. The aim of this study was to determine serum concentration of carbamazepine in patients with epilepsy who are on monotherapy, as well as to show the correlation of different methods on different systems for determination of the drug in serum (spectrophotometric and chromatographic method).

For the study were used sera of the patients with epilepsy, 35 sera total. The samples were analyzed with homogen immuno-enzyme test on the Beckman Coulter Analyzer System and high performance liquid chromatography with diode array detector (HPLC/PDA) on Waters system.

Concentration of carbamazepine in serum, in the interval of reference values had 71.5% of patients, the lower values had 28.5% patients, and there were no increased values. The average value of carbamazepine concentration in serum determined photometric on Beckman Coulter Analyzer System is \( \bar{X} = 5,44\pm2,06 \). The correlation coefficient obtained by comparing the spectrophotometric and chromatographic methods is, \( r = 0,896, p > 0,05 \).

Our results show that the most of patients on carbamazepine therapy have the correct dosage regimen. The statistical difference between different systems and methods is not significant. There is a strong correlation and connection, so the photometric method is reliable and applicable for routine operation.

Keywords: carbamazepine, analytics, immuno-enzyme test, HPLC/PDA

Correspondence: mljubenovic60@gmail.com

The Analysis on Physicochemical Parameters to Evaluate the Water Quality of Wellspring at Mountain Kukavica Exploited for Drinking

Tomislav Tosti1, Bojan Nikolic2, Radoslav Daljevic3, Katarina Karljikovic Rajic2

1University of Belgrade Faculty of Chemistry-Department of Analytical Chemistry, 2University of Belgrade Faculty of Pharmacy-Department of Analytical Chemistry, 3Institute of general and physical chemistry, Belgrade, Republic of Serbia

At mountain Kukavice, in the southeast part of Serbia, several wellsprings are adapted and exploited for drinking for any person walking or working in this
region, but without any notice on safe usage. The objectives of this study were to evaluate water quality of wellspring based on physicochemical parameters: pH, TDS (total dissolved solids), specific conductivity, total water hardness (ppm CaCO₃) determined by classical complexometric titrations and compared with ICP-OES analysis, as well as ICP/MS for trace analysis of micro elements.

The results of volumetric analysis of total water hardness (27.5 ppm CaCO₃) with the ratio Ca²⁺/Mg²⁺=2.57, classified investigated water sample in the type of very soft (<75 ppm CaCO₃) and were in accordance with ICP-OES analysis: 28.0 ppm CaCO₃ with slightly higher ratio Ca²⁺/Mg²⁺=3.06. The satisfactory accuracy of volumetric analysis was confirmed by Ca²⁺ content of 9.74 ppm in commercial drinking water “Rosa” (labeled 9.6 ppm Ca). For filtered sample the obtained TDS revealed 77 ppm (180 °C) correlated with results for unfiltered sample (TDS 126 ppm and TSS 47.8 ppm at 105 °C). The pH value and specific conductivity were 6.91 and 81.5 mS/cm (18.3 °C), respectively. The calculated TDS from specific conductivity was 61.2 ppm. The ion chromatography (IC) analysis for anions provided adjacent chloride and nitrate content 0.87 and 0.82 ppm, respectively and 12.57 ppm of sulphate. The results of micro elements analysis obtained by ICP/MS provided concentrations (As; Cd; Pb) lower than maximum allowable limits, confirming safe water usage of investigated adapted wellspring.

**Keywords:** adapted wellspring, water quality, total water hardness, TDS and TSS, anions’ analysis

**Correspondence:** kkrajic@pharmacy.bg.ac.rs

---

**Tools for Identification of Unknown Compounds After Gas Chromatographic-mass Spectrometric (GC-MS) Analysis of Urinary Volatile Organic Metabolites (VOMs)**

Tanja Živković Semren, Irena Brčić Karačonji, Andrea Jurić, Blanka Tariba Lovaković, Natasa Brajenović, Alica Pizent

*Analytical Toxicology and Mineral Metabolism Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia*

Volatile organic metabolites (VOMs) in human urine can be used in non-targeted metabolomics research to identify specific metabolites that may be useful as new diagnostic, predictive, and prognostic disease biomarkers. Determination of urinary metabolite profile provides novel information on phenotypic characteristics of an organism that cannot be obtained from target measurement. The headspace-solid phase microextraction (HS-SPME) technique coupled with gas chromatography-mass spectrometry (GC-MS) proved to be the most successful in VOMs analysis. After data processing of raw GC-MS data crucial step is to identify compounds of interest. Due to the complexity of the matrix, wide chemical diversity of the metabolites and their wide concentration range, metabolite identification is intrinsically difficult. In our laboratory, we use automated mass spectral deconvolution and identification system (AMDIS) for identification of unknown VOMs. AMDIS first deconvolutes the raw GC-MS data file to find all components, and then compare mass spectral data against a library of target compounds (e.g. National Institute of Standards (NIST) mass spectral library). To reduce possible solutions of identification offered by NIST Kovats retention index (RI) is used. A Kovats retention index system uses a series of standards, homologous series of n-alkanes applied as reference peak. Despite some limitations, presented methods could be very useful in VOMs identification. To confirm identification of unknown VOMs unequivocally, analysis of available analytical standards using the same GC-MS conditions is recommended.

**Keywords:** metabolomics, HS-SPME, AMDIS, Kovats retention index, NIST

**Correspondence:** tzivkovic@imi.hr

---

**Identification of Synthetic Cannabinoid MMB CHMICA in “Spice-like” Herbal Mixture: Update of the Serbian Situation for the October 2017**

Vera Lukić¹, Ružica Micić²*, Tatjana Verbić³, Anja Jokić²

¹Institute of Forensic Medicine, Faculty of Medicine, University of Belgrade, ²Faculty of Science and Mathematics, University of Priština, Kosovska Mitrovica, ³Faculty of Chemistry, University of Belgrade, Belgrade

Synthetic cannabinoids, which were synthesized to improve the therapeutic effects of cannabis, have become a major issue when they are abused. They have different chemical structures from tetrahydrocannabinol (THC) but similar effects on endocannabinoid receptors. “Spice” named products have more serious side effects than cannabis and can even cause death. These mixtures are prepared by spraying chemicals onto small pieces of herbs and are being dishonestly sold as “natural” and “legal” products over the internet. Their popularity is continuously increasing. Although,
various products are labeled with warnings “not for human consumption”, they are intended to mimic psychoactive effects of illicit drugs of abuse. This paper shows the application of accurate-mass quadrupole time-of-flight (Q-TOF) LC/MS method and GC-EI-MS for identification of MMB CHMICA (methyl 2-[(1-(cyclohexylmethyl)-1H-indol-3-yl)formamido]-3-methylbutanoate) synthetic cannabinoid in plant material. By reviewing the UNODC Early Warning Advisory on the New Psychoactive Substances, the first time this substance was reported by Slovenia in 2015. In 2017, the emergence of this cannabinoid was reported by two countries, the USA and Cyprus. The plant material was analyzed in October 2017 upon user’s personal request. According to the information we received, it was purchased in one of the smart shops in Belgrade, Serbia, in the original package “Milf”. It is declared as a freshener with a warning that it is not for human use. A sample of plant material was prepared by extraction with methanol in the ultrasonic bath for 30 min. The analysis was firstly performed by GC-EI-MS. The resulting mass spectrum did not give an appropriate match with the base of the spectrum used at that moment in the laboratory. Sample extract was further analyzed by LC-QTOF MS and we got accurate-mass 370.2256 Da, Diff (ppm) -0.76 for our compound and proposed formula C22H30N2O3. On the basis of the obtained GC-EI-MS spectra, the exact mass and the recommended formula, MMB CHMICA was identified in the plant material.

**Keywords:** MMB CHMICA, LC-QTOF/MS

**Correspondence:** ruzica.micic@pr.ac.rs

---

**Ion Chromatography for the Determination of Chlorites in Drinking Water**

**Vesna Milutinović, Sežana Vukčević**

Institute of Public Health, Belgrade, Bulevar despot Stefan 54a, 11000 Belgrade, Serbia

Chlorine dioxide is used and in water-treatment facilities to make water safe for drinking. Its byproduct, chlorite and chlorate ions may be present at low levels in tap water. Chlorite ion is very reactive chemically. The EPA has set the maximum concentration in the drinking water at 1.0 mg/L for chlorite ion and domestic regulation at 0.2 mg/L chlorite.

Available human and animal data indicate that oral exposure to relatively large amounts of chlorite may result in irritation of the digestive tract and increased levels of methemoglobin in the blood, which reduces the ability of oxygen to bind with hemoglobin.

The samples that were analyzed were collected twice a week from the “Vinca” plant, starting from 2014. Chlorite concentrations were determined by ion chromatography with suppressor, sodium carbonate as eluent, conductometric detector (Metrohm 930 IC flex) and EPA 300.1 method. Approximately 5 mL of the sample was injected with pre-nitrogen degassing and with a sample-loop of 50 μL. The quantification limit is 0.005 mg/L chlorite. Accuracy and precision were determined via recovery of the spiked samples into nine repetitions. The values obtained were in the range of 75-115%. During 2014, 2015 and 2016, concentrations above the limit of quantification were determined in the samples. About 2% of the samples from the total analyzed had values above the maximum allowed concentration according to domestic regulations and ranged to 0.35 mg/L. During 2017, the treatment of water with chloridoxide has been temporarily suspended, but it is scheduled for 2018 and next years.

**Keywords:** byproduct, regulation, exposure, ion chromatography

**Correspondence:** milutinovicvesna74@mts.rs

---

**Identification of Gamma-butyrolactone (GBL) by Liquid Chromatography Mass Spectrometric Method**

Gordana Brajković, Snežana Đorđević1,2, Jasmina Jović-Stošić1,2, Vesna Kilibarda1,2, Zorica Brajković3, Snežana Bojović, Slavica Vučinić1,2

1National Poison Control Centre, Military Medical Academy, Belgrade, Serbia,
2Medical Faculty, Military Medical Academy, Ministry of Defence,
3School of Medicine University of Belgrade, Belgrade, Serbia

Recreational use of gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone (GBL) as its precursor is very popular in recent years. They are clear, odourless, oily liquids. GHB is used as an anesthetic and relaxant, and also has euphoric effects. GBL is its precursor. It has legitimate use as a chemical for cleaning. GBL represents a serious threat to public safety because in the body it converts to GHB which is on the list of forbidden drugs. This is the reason why the identification of unknown sample is very important. The aim of this work is to present analytical method for identification of GBL in liquid sample.

Laboratory received a liquid sample suspected of GHB or GBL. The sample was qualitatively analyzed by using a liquid chromatography-electrospray
ionization-mass spectrometer (LC-ESI-MS), on XTerra MS RP18 column under following conditions: source temperature 125° C, desolvation temperature 430° C, flow gas: desolvatcion 400 L/h, cone 50 L/h. The mobile phase: 5 mM ammonium formate (pH 3.5) : acetonitril with 0,1 % formic acid. MS data were recorded in the full scan mode (m/z 40–150). For qualitative analysis of GBL the protonated molecular peaks ([M+H]⁺) of these compounds were monitored in the full - scan. The monitoring ions for GBL were m/z 87, 44, 42, and peak od GBL was detected at 3.48 min.

Presented LC-MS method is a method of choice for reliable identification of unknown sample that can make a difference between GHB and GBL.

Keywords: gamma-hydroxybutyrate (GHB); gamma-butyrolactone (GBL), LC-MS.
Correspondence: gordanatox@gmail.com

Distribution of Environmental Lead in Brown Bear Body Compartments

Maja Lazarus¹, Tatjana Orct¹, Slaven Reljić², Jasna Jurasović¹, Đuro Huber²
¹Analytical Toxicology and Mineral Metabolism Unit, Institute for Medical Research and Occupational Health, ²Department of Biology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

Lead (Pb) is a very stable inorganic environmental pollutant exchanged between blood, mineralized and soft tissue compartments upon absorption. Comprehensive distribution of Pb in the body of brown bears (Ursus arctos) has not been previously investigated. The need for such a study arose upon earlier reports suggesting that 1-5% of the bear population might be at risk of adverse effects caused by hepatic and renal Pb levels over threshold levels established for terrestrial mammals. Therefore, the aim of this study was to explore the bone as the principal deposition compartment and marker of long-term exposure to Pb, and soft tissues (heart, kidney, liver, lungs, muscle and spleen) that reflect recent exposure, but also endogenous Pb sources. Lead was quantified in tissues of 40 brown bears hunted in 2015 according to the Brown Bear Management Plan in Croatia. The highest Pb levels were found in the kidney cortex (median, 4398 µg/kg dry mass), followed by the femoral bone (2502-3980), liver (1761), lungs (303), spleen (209), heart (22.7) and muscle (11.1). Microdistribution inside the bone revealed higher Pb in the compact (3980 µg/kg) than trabecular bone (2502 µg/kg) at the same location (femoral metaphysis). Also, the Pb level differed depending on the location on the bone (epiphysis vs metaphysis and metaphysis vs diaphysis). Age was a significant factor in prediction of bone Pb, which was higher in adults compared to young animals. Unlike soft tissues, the femoral bone of brown bears was confirmed as a good biomarker of Pb, known for its accumulative nature.

Keywords: Ursus arctos, femoral bone, soft tissues, kidney, liver
Correspondence: mlazarus@imi.hr
Ten Years of Human Biomonitoring of Environmental Chemicals in Canada

Julie Yome, Annie St-Amand
Healthy Environments and Consumer Safety Branch, Health Canada. 269 Laurier Ave. W, Ottawa, Ontario, Canada, K1A 0K9

The Chemicals Management Plan is part of the Government of Canada’s comprehensive environmental agenda to ensure the safe management of chemicals. Human biomonitoring of chemical exposure is a key element of this program and is used along with interpretation tools to quantify exposure and provide information for setting priorities and taking action to protect public health. The biomonitoring component of the Canadian Health Measures Survey (CHMS) is the cornerstone of the national biomonitoring program, collecting nationally-representative data on environmental chemical exposure in Canadians aged 3-79 years in ongoing two-year cycles. Over the past decade over 250 chemicals were measured in over 29,000 Canadians at 81 sites across the country. In the first three cycles, half of the chemicals measured were above the limit of detection in 60% of individual blood and urine samples. Indoor air and tap water samples were also collected in selected cycles. Tools have also been developed to interpret and communicate biomonitoring data in a public health context. Reference values (RV95) are statistical tools that allow the identification of individuals with increased exposure. RV95s derived from the CHMS are proposed for heavy metals, and persistent and non-persistent chemicals. Biomonitoring equivalents (BEs) are risk-based tools to prioritize vulnerable populations potentially at risk. Experience gained over the past decade has made Canada a world leader in human biomonitoring. This effort has established population baseline concentrations that have been used to inform regulatory risk assessment and improve evidence-based decision making in public policies to reduce exposure to toxic chemicals in Canada.

Keywords: biomonitoring, exposure
Correspondence: julie.yome@canada.ca

Mercury in Hares (Lepus europaeus Pallas) Collected in the Vicinity of the Natural Gas Treatment Plant in Northern Croatia During the Last Ten Years

Andreja Prevendar Crnić, Emil Srebočan
Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

In this study hares organs were analyzed for total mercury concentration by ICP-MS method as a part of a comprehensive monitoring of the eco-system in the vicinity of the natural gas production and treatment plant Molve in the north of Croatia. During the last ten years the range of the median mercury concentration values (wt weight) in hares organs were 0.00002-0.00436, 0.00417-0.01942, 0.0234-0.11729 and 0.00007-0.00102 µg/g for muscle, liver, kidney and brain, respectively. The results of mercury measurements in muscle and brain demonstrate mostly uniform values with a small increase in concentration values of mercury in muscle. Median Hg concentrations in liver and kidney differ from year to year but generally show consistent pattern in fluctuations, meaning that increased concentration of Hg in liver is followed by the same increase in kidney. Comparing our results with results published in available data on mercury concentration in hare’s tissue it can be concluded that investigated area near Molve belongs to low mercury contaminated region. Results also point to the fact that closed mercury removal system installed in 1993 is effective in mercury removal from natural gas, and that research and production of natural gas contaminated by mercury doesn’t threaten human health and the environment. Nevertheless, further eco-monitoring and mercury measurements in various hares organs are valuable and necessary and will be continued.

Keywords: hares, total mercury, natural gas, environmental monitoring
Correspondence: apcrnic@vef.hr

Can Measurement of Arsenic Concentration be Comparable in Human Buccal Cells, Hair Samples and Urine Samples and Correlate with DNA Damage Assessment with Micronucleus Buccal Cytome Assay?

Simone Brauer1, Walter Goessler1, Mirta Milić2, Vatroslav Šerić3,4, Marija Malić3,4, Ivan Pavičić5, Ana Marija Marjanović Čermak5, Stefano Bonassi6,7, Višnja Oreščanin8, Ivana Vinković Vrček9
1Institute for Chemistry, University of Graz, Graz, Austria, 2Mutagenesis Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia, 3Department of Clinical Laboratory Diagnostics, Osijek University Hospital, Osijek, Croatia, 4Faculty of Medicine, University of Osijek, Osijek, Croatia, 5Radiation Dosimetry and Radiobiology Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia, 6Unit of Clinical and Molecular Epidemiology, IRCCS San Raffaele Pisana, Rome, Italy,
Although chronic arsenic (As) exposure is associated with toxic effects, both malignant and non-malignant, the dose response for As toxicity is still not clearly determined for human population, and therefore there are still not reliable biomarkers of the exposure and the effect for chronic As exposure that would helpful in assessment of DNA damage and prediction of cancer risk development. Sampling of urine, hair and buccal cells is part of noninvasive methods, but the potential of those three sampling types is still not fully investigated. With no literature data on arsenic measured in buccal cells and its correlation with DNA damage in buccal cells and arsenic concentrations in hair and urine samples, the aim of this study was to evaluate the buccal cells measurement with two other sampling materials in order to give an insight whether these methods can be used together. As and other elements were measured in more than 100 samples of the individuals from the Eastern Croatia chronically exposed to As through ground water, drinking water (As levels above 10 µg) and water used for crops. Liquid chromatography coupled to inductively coupled plasma mass spectrometry-ICPMS was used and the results were compared with the DNA damage assessed in buccal cells. Results demonstrated that As concentrations determined in buccal cells were not in the correlation with those determined in hair and urine samples.

The study was financially supported by the Grant of Ministry of Science and Education of the Republic of Croatia and Austrian Federal Ministry of Science, Research and Economy 05/2016 Possible early noninvasive biomarkers of chronic exposure to arsenic.

**Keywords:** arsenic, urine, buccal cells, hair, DNA damage

**Correspondence:** mmilic@imi.hr

---

**Defining the Pesticide Exposome:**
**Characterizing Longitudinal Seasonal and Occupational Trends of Pesticides in House Dust**

Breana Bennett¹², Tomomi Workman¹², Marissa Smith¹², William C. Griffith¹², Beti Thompson³, Elaine M. Faustman¹²

Agriculture often relies on chemical inputs such as pesticides, many of which have known adverse health effects. Children of farmworkers are especially vulnerable, and early childhood pesticide exposure may have lasting health effects. Because children of farmworkers are exposed to a variety of pesticides, we propose using an exposome framework to explore child pesticide exposure.

Using household dust samples from a children’s agricultural cohort, we examined the pesticide exposome for the 2005 and 2011 agricultural seasons. In particular, we analyzed how the pesticide exposome changes over time, and investigated the differences in the pesticide exposome between farmworker and non-farmworker households.

Dust samples were collected from floor areas where children played and analyzed by liquid chromatography-mass spectrometry (LC-MS). Individual pesticides were grouped into pesticide classes using the US EPA pesticide chemical classifications, and trends in concentrations were analyzed at the class level.

Across the entire cohort, the levels of organophosphates, pyridazinones, and phenols significantly decreased between 2005 and 2011, whereas levels of anilides, 2,6-dinitroanilines, chlorophenols, and guanidines significantly increased. Among farmworkers alone, there were significantly lower levels of N-methyl carbamates and neonicotinoids in 2011. For several common agricultural pesticides, changes in use were reflected in the dust of farmworker households.

We have defined the pesticide exposome in a children’s agricultural cohort and examined how that exposome changed between two agricultural seasons six years apart. This study exemplifies the utility of an exposome approach in evaluating longitudinal data on the changes in the pesticide exposome for pesticides.

Supported: NICHD, NIEHS, USEPA

**Keywords:** biomonitoring, epidemiology, exposure, children

**Correspondence:** faustman@uw.edu

---

**The First National Biomonitoring Study of Environmental Metals**

Anita Cvetkovska¹, Biljana Manevska³, Fljamure Zekiri-Keka³, Irena Bojadzieva⁴, Elisaveta Stikova⁵
Biomonitoring is a key tool used as an indicator and quantitative measure of exposure to chemicals in the environment. Biomonitoring is recognized as a standard for assessing people’s exposure to toxic substances and for responding to serious environmental public health issues. Public Health Institute of the Republic of Macedonia in 2014 and 2015 perform many activities, aiming to develop and establish the national human biomonitoring study with emphasis on heavy metals.

The main purpose of this study was to determine the baseline levels of Pb, Hg, Cd, Mn and Zn in blood in the representative sample of the adult population living in the 5 hot spots in the country and one control non-contaminated area, with intention to compare the differences of the concentration of these metals among different populations groups by age, sex, occupations and lifestyle (nutrition, smoking and living area). In this paper are shown the results of the first two years of the study (2014-15). The concentration of heavy metals was measured with electrothermal atomic absorption spectrophotometry and apparatus Analyst 600-Perkin Elmer.

The results showed that average concentration of the BLL was 14.5µg/l (2.1-19.7µg/l) ; Hg was 2.5µg/l (0.3-3.8µg/l); Cd was 0.7µg/l (0.1-2.0µg/l); Mn was 7.3µg/l (1.4-14.0µg/l) and Zn was 4.1µg/l (2.7-5.1 µg/l). Except the average concentration of the Mn in one and Cd in two hot spots, all other concentration are below the recommended levels by WHO, but with statistical significant differences in accordance with the age and living area i.e. environmental exposition, which are shown in the paper.

Keywords: lead, mercury, cadmium, manganese, zinc, blood levels, Macedonia
Correspondence: anicvetkovska@gmail.com

Urinary Excretion of Aflatoxin M1: A Survey
Raul Ortiz-Martinez1, Ma. Carolina de Luna-Lopez1, Arturo G. Valdivia-Flores2, Teodulo Quezada-Tristán2
1Department of Animal Science. Agricultural Science Center. UAA,
2Department of Veterinary Clinic. Agricultural Science Center, UAA

Aflatoxin M1 (AFM1) is one of the less toxic forms of aflatoxin B1 (AFB1). When the presence of AFM1 is detected in urine, means in an indubitable way, that people consume food contaminated whit AFB1.
Precisely, the determination of the AFM1 concentration in urine correlates with the amount of ingested AFB1 so it is a linear indicator associated with its consumption.

A biomarker is a variation in cellular or biochemical components or processes, structures, or functions that is measurable in a biological system or sample; these changes are induced by xenobiotics. Based in this fact, the presence of AFM1 in organic fluids, has been considered an excellent exposure biomarker of AFB1.

The objective of the study is to explore the exposure to AFB1, through measuring AFM1 concentration in urine of volunteer adults.

A convenient sampling (non-probabilistic sampling), was performed in Aguascalientes, Mexico. In the study both female and male gender was included. Informed consent was signed by the participants in the research.

The samples were analyzed according to directions of an ELISA commercial kit (Detection range: 0-4.0 ng/mL).

At the present, 223 volunteer adults, were sampled in order to determine AFM1 in urine. The study has had 46% male and 54% female volunteers. The average age of the sampled participants was 20 year-old. The 60% of samples had detectable levels of AFM1. The average concentration of AFM1 in urine samples was 0.40 ng/mL.

These results implicate the importance of biomarkers as predictive tools to detect early potential alterations and eventually, improve public health.

**Keywords:** aflatoxin B1, aflatoxin M1, biomarkers, urine

**Correspondence:** raormar2000@gmail.com

---

**CARCINOGENESIS**

**Antigenotoxic Effects of Cinnamic Acid in Diabetic Rats**

Hatice Gül Anlar\(^1\), Merve Bacanlı\(^2\), Tuğbagül Çalış\(^1,3\), Sevtap Aydin\(^2\), Nuray Arı\(^4\), İlkü Ündeğer Bucurgat\(^2\), Arif Ahmet Başaran\(^5\), Ayşe Nurşen Başaran\(^2\)

\(^1\)Bülent Ecevit University Faculty of Pharmacy Department of Pharmaceutical Toxicology 67600 Zonguldak, Turkey

\(^2\)Hacettepe University Faculty of Pharmacy Department of Pharmaceutical Toxicology 06100 Ankara, Turkey

\(^3\)Karadeniz Technical University Faculty of Pharmacy Department of Pharmaceutical Toxicology 61080 Trabzon, Turkey

\(^4\)Ankara University Faculty of Pharmacy Department of Pharmacology 06100 Ankara, Turkey

\(^5\)Hacettepe University Faculty of Pharmacy Department of Pharmacognosy 06100 Ankara, Turkey

Diabetes mellitus (DM) is a major health problem worldwide. Cinnamic acid (CA) and its derivatives are synthesized in the plants and increasing attention has been received in the recent years due to the high number of beneficial health properties attributed to their consumption. The aim of this study was to investigate the antigenotoxic effects of CA on streptozotocin (STZ)-induced diabetes in Wistar albino rats. The rats were divided into four groups: Group 1: Sham group (n=6), group 2: diabetic group (n=6), group 3: CA treated group (n=6), group 4: CA treated diabetic group (n=6). CA dose (50 mg/kg b.w. per oral) was selected according to our unpublished studies. At the end of the experimental period, all animals were decapitated under the anesthesia. Whole blood samples were obtained via the intracardiac method. Liver and kidneys were removed. DNA damage was evaluated in the blood, liver and kidney cells of rats by the alkaline comet assay. 8-hydroxy-2’-deoxyguanosine (8-OHdG) levels, a critical biomarker of oxidative stress, were also evaluated by in the plasma samples by spectrophotometrically using kit following the manufacturer’s instruction at 535 nm. As a result of this study, DM caused genotoxic and oxidative damage. CA treatment ameliorated these effects and it seems that CA might have a role in the prevention of the complication of diabetes.
Keywords: diabetes mellitus, DNA damage, oxidative stress, 8-hydroxy-2'-deoxyguanosine, comet assay
Correspondence: haticegulanlar@gmail.com

Estrogen Receptor Antagonists as a Target for Treatment of Estrogen-Induced Carcinogenesis

Elif Ince¹, Alev Tascioglu¹, Ozlem Oztürk-Ceylan², Sibel Suzen², Hande Gurer-Orhan¹
¹Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Ege University, Izmir, Turkey; ²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, Ankara, Turkey

Breast cancer is the leading cause of death among female cancers and prolonged exposure to elevated levels of estrogens is suggested to play an important role in the process. Melatonin (MLT) is reported to exert oncostatic effect which is partly mediated by its selective ER modulator and aromatase inhibitor activities. Although MLT has low toxicity, its short half life limits its therapeutic use. This drawback can be conquered by synthesizing MLT analogues with longer half life. Our group has already been working on investigating antioxidant and aromatase inhibiting activity of indole-based MLT analogues. The aim of the present study is to evaluate potential antiestrogenic activity of analogues which were found to have antioxidant and aromatase inhibitor activity. Their antiestrogenic effect is investigated in a two-stepped, modified miniaturized E-Screen assay, which is based on estrogen dependent MCF-7 cells to proliferate only in the presence of estrogenic compounds. In the first step various concentrations of the compounds are added to the medium containing fixed dose of estradiol. Some of the compounds were found to inhibit estrogen action in a dose-dependent manner. Those analogues were further tested in step-2, in order to evaluate whether this effect was receptor dependent. Increasing concentrations of estradiol are added onto fixed dose of the compound and fortunately three of the compounds are found to be promising ER antagonists. As a conclusion some of our indole-based MLT analogues seem to be promising candidates for breast cancer therapy via their triple benefits; as antioxidant, aromatase inhibitor and ER antagonist.

This work was supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK) Grant 117S065 and 109S099. Analyses are performed at Ege University Faculty of Pharmacy, Pharmaceutical Sciences Research Center (FABAL).

Keywords: breast cancer, estrogen receptor antagonist, melatonin analogues
Correspondence: erkekp@yahoo.com, erkekp@hacettepe.edu.tr

Oxidative Stress and DNA Damage Caused by Helicobacter Pylori in Human Gastric Adenocarcinoma Cells

Didem Oral¹, Gizem Ozkemahlı¹,², Unzile Sur¹,³, Belma Kocer-Gumusel¹, Pınar Erkekoglu¹
¹Hacettepe University, Faculty of Pharmacy, Department of Toxicology, 06100 Ankara, Turkey, ²Erzincan University, Faculty of Pharmacy, Department of Toxicology, Erzincan, Turkey, ³Atatürk University, Faculty of Pharmacy, Department of Toxicology, Erzurum, Turkey

The association between chronic inflammation and the development of cancer has been asserted for a long time. Understanding the role of chronic bacterial inflammation in oncogenesis has been gaining importance for the past 20 years. Certain bacteria can also promote carcinogenesis due to their direct effects on cell transformation. Helicobacter pylori is helix-shaped gram-negative anaerobic bacterium that leads to gastritis and peptic ulcer, and later to gastric cancer. The aim of this study was to investigate whether oxidative stress is one of the mechanisms underlying the toxicity of Helicobacter pylori. Human gastric adenocarcinoma cells were exposed to different multiplicities of infection (MOIs) with the bacterium. The intracellular reactive oxygen species (ROS) production and oxidative stress parameters were elucidated. After incubation of the adenocarcinoma cells with different MOIs (25, 50, 75, 100, 200 and 400) of Helicobacter pylori, we observed that this bacterium causes MOI-dependent cytotoxicity. The median inhibitory concentration 50 (IC50) was found to be 218 bacterium/ live cell and IC30 was 138 bacterium/ live cell. We observed that at IC30 MOI, this bacterium caused significant increases in ROS and DNA damage. Moreover, there were significant changes in intracellular glutathione and lipid peroxidation after human gastric adenocarcinoma cells were exposed to Helicobacter pylori. We can suggest that one of the mechanisms underlying the toxicity of Helicobacter pylori is oxidative stress. More mechanistic studies are needed to prove the toxicity mechanisms of Helicobacter pylori.

Keywords: chronic inflammation, oncogenesis, adenocarcinoma, Helicobacter pylori
Correspondence: erkekp@yahoo.com, erkekp@hacettepe.edu.tr

Keywords: breast cancer, estrogen receptor antagonist, melatonin analogues
Role of Circadian Regulated Gene Expression in Mutagenesis and Carcinogenesis

Helmut Zarbl, Howard Kipen, Mingzhu Fang
Environmental and Occupational Health Sciences Institute, Rutgers University, Piscataway, New Jersey, U.S.A. 08854

Methylselenocysteine (MSC) reduces chemically-induced mammary carcinogenesis in all susceptible rat strains. MSC mediates its effects by restoring circadian regulated expression of hormone receptor and DNA repair genes disrupted by carcinogen exposure. Expression of core circadian genes is regulated by oscillation in the acetylation of histones on specific E-box enhancer elements in their promoters. Acetylation is increased by the intrinsic histone acetylation activity of the Clock protein, while deacetylation is mediated by Sirtuin 1, a NAD⁺-dependent protein deacetylase enzyme. The cellular clocks in an organism are synchronized by entrainment with environmental cues that include light, temperature and metabolism, and can be disrupted by a wide variety of environmental toxicants and stressors. Mechanistic studies showed that carcinogen exposures ablate circadian rhythm by depleting intracellular levels of NAD+/NADH, which in turn inhibits the activity of the Sirtuin 1 histone deacetylase enzyme. Increased sensitivity to circadian disruption by chemicals or shifting light-dark cycles (chronotype) contributes to the differential genetic susceptibility of rodents the mammary carcinogenesis.

Disruption of circadian gene expression by exposure to light-at-night through shift work or jet lag is also mediated by depletion of intracellular NAD⁺/NADH. Animal studies showed that MSC restores circadian gene expression and reduces carcinogenesis in animals exposed to jetlag protocols. These studies were translated into a placebo-controlled MSC intervention trial in shift workers who experience circadian disruption.

Keywords: circadian gene expression, carcinogenesis, MSC

Correspondence: zarbl@eohsi.rutgers.edu

Severe Benzodiazepine Poisoning in Elderly – A Case Report

Natasa Perkovic Vukcevic¹, Gordana Vukovic Ercegovic¹, Vesna Mijatovic², Olivera Potrebic¹, Snezana R. Jankovic³, Dragan Zivanovic¹, Jasmina Jovic Stosic¹
¹National Poison Control Centre, Military Medical Academy, Belgrade, Serbia,
²Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Novi Sad, Serbia,
³Institute for Scientific Information, Military Medical Academy, Belgrade, Serbia

A case of an elderly woman with a prolonged decreased level of consciousness and a decreased rate of bromazepam elimination in acute-bromazepam-overdose settings is described. Previously healthy 67-year-old woman was found unconscious with Glasgow Coma Score of 3, normotensive, normofrequent and euglycemic. MSCT scan was normal. After the flumazenil application, a positive response was observed. The patient reported the ingestion of 90 mg of bromazepam. She was referred to the intensive care unit in coma, while vital and laboratory parameters were within a normal range. Toxicological analysis of the blood identified bromazepam in concentration of 2.73mg/l. Flumazenil treatment was continued (both bolus and infusion). EEG was consistent with the findings in benzodiazepines overdoses. On the fifth day of hospitalization, the patient became febrile. Chest radiography revealed a pneumonic infiltration. Leukocytosis and elevated CRP were detected and an antibiotic treatment was introduced. Rhabdomyolysis (CPK level up to 7900i.u.) was also developed as a complication. Due to very slow recovery of consciousness, the blood bromazepam concentrations were determined repeatedly. At day 7, the bromazepam blood level was 2.32mg/l. The patient was hypotensive. The 20% Intravenous Fat Emulsion was applied. After its administration, the bromazepam blood level was 1.2mg/l and 0.6mg/l. Flumazenil treatment was continued up to the 14th day of hospitalization when the patient was conscious, respiratory and cardiocirculatory stable, with bromazepam blood concentration of 0.1mg/l.
Altered metabolism and slower elimination of benzodiazepines in elderly could be responsible for a more severe clinical course of acute benzodiazepines poisoning.

Keywords: bromazepam, overdose, flumazenil

Correspondence: natavuk67@gmail.com

Society Transition and Trends of Acute Poisonings in Republic of Macedonia

Niko Bekjarovski, Daniela Chaparoska, Zhanina Perevska, Natasha Simonovska, Irena Jurukov, Aleksandra Babulovska
University clinic for toxicology, University campus “Mother Theresa” Skopje, Macedonia

Last three decades big transition happened in all former socialist countries. New economic system developed new habits and illness among citizens in these countries.

The aim of the study is to present the changes and new trends in acute poisonings in last three decades in Republic of Macedonia. We analyzed patients treated in University Clinic for toxicology in Skopje, Macedonia after acute poisoning in the period from 1987 till 2017. The total number of acute poisonings has a trend of slow increase, especially in the last 5 years. The trend of acute poisonings with pesticides dramatically dropped down after 1992. Poisonings with barbiturates are almost totally replaced with acute poisoning with benzodiazepines. Benzodiazepines are the most frequent drugs of abuse, and have a trend of continues rising. The numbers of acute corrosive poisonings in last thirty years is almost tripled and are the second reason for hospitalization in our Clinic.

Poisonings with benzodiazepines and corrosive substances are the new challenges in our everyday clinical practice. Poisonings with pesticides and barbiturate, typical for the 80’s of last century are very rare in now days practice.

Keywords: intoxications, pesticides, corrosive, drugs

Correspondence: nikobekjarovski@gmail.com

Molecular Dynamics Simulation of Novel, Dual-binding AChE Inhibitors

Ilija N. Cvijetić1, Aleksandra R. Božić2, Aleksandar D. Marinković2, Milica M. Karanac1, Tamara Vujatović1, Maja D. Vitorović-Todorović5
1Innovation Center of the Faculty of Chemistry, University of Belgrade, Serbia; 2Faculty of Technology and Metallurgy, University of Belgrade, Serbia; 3Innovation Center of the Faculty of Technology and Metallurgy, University of Belgrade, Serbia; 4University of Belgrade, Faculty of Chemistry, Serbia; 5Military - Technical Institute, Belgrade, Serbia

Acetylcholinesterase (AChE, EC 3.1.17) is involved in the termination of nerve impulse transmission by rapid hydrolysis of the neurotransmitter acetylcholine. It is well known target for the treatment of Alzheimer’s disease. The reversible inhibition of AChE was also suggested as the pretreatment option of nerve agents’ intoxications.

In this work, three reversible ligands were designed and synthesized based on previously derived 3D-QSAR model, comprising tacrine unit and aroylacrylic acid amid scaffold, linked by eight polymethylene units. All three compounds were nanomolar mixed-type inhibitors. Putative noncovalent interactions as well as hydrogen bonds and salt bridge of the most potent derivative with aminoacid residues in AChE active site gorge were estimated by 20 ns long molecular dynamics (MD), performed in NAMD 2.12. In the bottom of the gorge, stable π-π interaction was observed between Trp 86 residue and tacrine subunit. Proximal linker -NH- group forms two hydrogen bonds with Tyr 337 (average distance 2.31±0.27 Å) and Tyr 341 (average distance 3.11±0.43 Å). At the peripheral anionic site of the enzyme, a π-π edge-to-face interaction was observed between Trp 86 residue and tacrine subunit. Proximal linker -NH- group forms two hydrogen bonds with Tyr 337 (average distance 2.31±0.27 Å) and Tyr 341 (average distance 3.11±0.43 Å). At the peripheral anionic site of the enzyme, a π-π edge-to-face interaction was observed between Trp 286 and aroylphenyl ring. A fairly stable hydrogen bond is formed between distal -NH- group and Tyr 72 (average distance 3.66±0.94 Å). Additionally, a highly stable salt bridge was formed between protonated distal -NH- group and a negatively charged Asp 74 residue.

In conclusion, binding mode of novel, highly potent, reversible AChE inhibitor was characterized
using MD. Several stable interactions of ligand with both anionic and peripheral anionic site were maintained throughout the simulation.

**Keywords:** AChE, tacrine, reversible inhibition, molecular dynamics

**Correspondence:** iliija@chem.bg.ac.rs

---

**A Systems Biology Approach to Discovery the Mechanism of Regulation of Repetitive Prophylaxis of Stable Iodide on Sodium/iodide Symporter (NIS)**

David P.A. Cohen¹, Dalila Lebsri¹, Karine Tack¹, Marc Benderitter², Maâmar Souidi¹

¹Institut de Radioprotection et de Sûreté Nucléaire (IRSN), PSE-SANTÉ/SESANE/LRTOX, 92262 Fontenay-aux-Roses, France,
²Institut de Radioprotection et de Sûreté Nucléaire (IRSN), PSE-Santé/SERAMED, 92262 Fontenay-aux-Roses, France

A single dose of potassium iodide (KI) against prolonged exposure to repeated radioactivity might not be effective enough to protect the thyroid. Our group showed that repetitive dose of KI for eight days offers efficient protection without toxic effects on rats. However, we also have shown that the expression of the genes involved in the Wolff-Chaikoff effect changes during this period. This effect may result in a decrease in thyroid hormones and hypothyroidism. Notably, a decrease in the sodium/iodide symporter (NIS) gene expression has been observed. NIS is responsible for the uptake of KI and plays an important role in the Wolff-Chaikoff effect. The mechanism of a single dose of KI on the toxicity of the thyroid is well known in contrast to repetitive administration of KI for eight days.

In the present study, we try to understand the Wolff-Chaikoff regulation and its molecular constituents during repetitive administration of KI. For this we have constructed a biochemical reaction network that is visualised as a “geographical” map of the thyrocyte depicting iodide and thyroid hormone metabolism. Subsequently, Path analysis of the network has been performed to investigate if a path exists from the node iodide going to the node representing “nis transcription”.

This map reviews the most updated information about iodide and thyroid hormone metabolism. Besides as a source of information, it can help to elucidate the mode of action of KI on gene transcription after repetitive KI administration. We have found two mechanisms that might explain the inhibition of nis.

**Keywords:** network, map, mechanism, Wolff-Chaikoff, thyroid

**Correspondence:** david.cohen@irsn.fr

---

**In Silico Methods to Predict the Toxicity of Mixtures: Current Status and Future Directions**

Mark Cronin, Steve Enoch, James Firman, Judith Madden, Samuel Belfield

School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, England

*In silico* models provide invaluable tools to fill gaps where toxicological (and other) information is missing. However, approaches such as the use of (Quantitative) Structure-Activity Relationships ((Q)SARs) and read-across have usually been targeted towards the effects of single chemical substances. This study has reviewed the use of (Q)SARs and read-across for the prediction of the toxicity of mixtures with a view to providing recommendations for their future implementation. The term “mixture” covers many types of chemistries ranging from commercial formulations and products; unknown or variable composition, complex reaction products or biological materials (UVCBs); botanicals; nanomaterials and other substances such as raw products and surfactants of variable chain length. Development of predictive models for mixtures requires that its composition is fully understood to enable mixture toxicity effects such as concentration addition, synergy and antagonism to be captured. Modelling is often based on the identification of the hazard of individual components with a “worst-case scenario” enacted. This is simplistic and in some cases effective, however, it lacks appreciation of relative exposure which can be increased or decreased by the other components in a mixture. A better mechanistic understanding and knowledge of “complex” Molecular Initiating Events within the Adverse Outcome Pathway paradigm will be the cornerstone of the development of better models – especially if the theory can be supported by relevant mechanistic data. The challenges remain the need to obtain such data, the anchoring to in vivo results and extrapolation to human exposure.

**Keywords:** computational, toxicology, QSAR

**Correspondence:** m.t.cronin@ljmu.ac.uk
Phthalates (diethylhexyl phthalate (DEHP) and dibutyl phthalate (DBP)) and Obesity: a Toxicogenomics Approach

Katarina Baralić1, Dragica Jorgovanović1, Danyel Jennen2, Danijela Đukić-Ćosić1
1Department of Toxicology “Academic Danilo Soldatović”, University of Belgrade – Faculty of Pharmacy, Serbia
2Department of Toxicogenomics, Maastricht University, The Netherlands

The etiology of many chronic diseases, including obesity, involves interactions between environmental contaminants and genes that modulate physiological processes. Diethylstilbestrol, bisphenol A, phytoestrogens, and phthalates are considered the most well-known obesogens. People are most often simultaneously exposed to multiple phthalates as a result of their use as plasticizers and additives in a variety of consumer products.

The aim of this study was to explore the relationship between the two most commonly used phthalates (diethylhexyl-phthalate (DEHP) and dibutyl phthalate (DBP)) and obesity, using the Comparative Toxicogenomics Database (CTD).

A set of genes of interest was constructed, consisting of 850 curated genes affected by both phthalates. Obtain results indicate that DEHP and DBP correlate with the development of obesity - DEHP by interacting with 55 genes involved in 63 molecular pathways, and DBP by interacting with 79 genes involved in 47 molecular pathways. Both phthalates affect the activity of 41 genes associated with the development of obesity, involved in 38 common molecular pathways. Among these genes, PPARA, the gene with the majority of curated interactions for DEHP (519), PPARG and ATP citrate lyase gene (ACLY) stand out as key factors in lipid metabolism. Additionally, both phthalates interact with insulin 1 gene (INS1), which is important having in mind that obesity is linked to an increased risk of developing insulin resistance and type 2 diabetes.

These results provide a basis for further in vitro and in vivo studies in order to better understanding the molecular mechanisms of phthalate obesogenic properties.

Keywords: plasticizers, obesogens, toxicogenomics, CTD, genes

Correspondence: katherinekatabarka@gmail.com

Effect of Low Oral Cadmium Exposure During Pregnancy on Steroid Hormones in Mother Rats and Female Offsprings

Anja Mikolić, Martina Piasek, Tatjana Orct, Antonija Sulimanec Grgec, Ljerka Prester, Jasna Jurasović
Institute for Medical Research and Occupational Health, Zagreb, Croatia

Cadmium is a toxic metal and environmental pollutant with the potential to act as an endocrine disrupting chemical of reproduction and reproductive development. The main cadmium exposure source is food. Its gastrointestinal absorption in laboratory rats is up to 1% and increases 2-3 times during gestation. We evaluated the effects of low cadmium exposure (5 mg Cd/L as CdCl₂ in demineralised water) in Wistar rats exposed from gestation day (GD) 1 to 20 on steroid hormones in the placenta and maternal serum on GD20 and in sera of 14-day-old female pups (at weaning) and at the onset of puberty. On GD20, part of the mother rats were euthanized under anaesthesia, blood was taken from the heart and the liver, kidney, placentas and foetuses were sampled. Blood, liver, kidney and brain were sampled in 14-day-old and pubertal female offspring. Tissue cadmium was analysed by ICP-MS and progesterone in placenta and serum by ELISA. In the exposed rats, cadmium increased in all of the sampled maternal tissues, placenta, foetuses and 14-day-old pups’ liver. Placental progesterone did not change, whereas progesterone levels decreased in maternal serum on GD20 and in serum of 14-day-old female pups. Onset of puberty was between postnatal days 47-54, a week later in female than in male offspring, irrespective of cadmium exposure. The results show that low cadmium exposure during gestation may disrupt serum progesterone levels in both mother rats and female pups before puberty, which may have impact on foetal viability in utero and/or female reproductive development.

Keywords: cadmium, gestation, steroid hormone disruption, reproductive development, rat

Correspondence: akatic@imi.hr
Determination of Phthalates in Toys

Marija Stanković, Ana Stanisavljev, Anka Cvjetković, Nenad Vuković
Institute for Public Health, Bulevar Despota Stefana 54a, Belgrade

Phthalates are used as plasticisers, primarily in PVC, and can be also used in paints and adhesives. Phthalates can disrupt our hormones and may cause fertility problems. Furthermore they are connected with childhood, obesity, asthma, neurological problems obesity, asthma, neurological problems, cardiovascular issues and even cancer. In our laboratory we analyze toys before they reach the market. Six phthalates determined for that purpose are: bis (2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP) di-isonomyl phthalate (DINP) and di-isodecyl phthalate (DIDP) and di-n-octyl phthalate (DNOP). Phthalates are extracted from samples with tetrahydrofuran for 30 minutes on rotating mixer or ultrasonic bath. After that hexane is added to release PVC polymer. Samples are left for 15 minutes in fridge, for better precipitation of PVC polymer. After that supernatant is filtrated and content of phthalates is determined by GC-MS.

In our laboratory, 111 toys were analyzed for the content of phthalates in the last year. Content of phthalates in nine samples was above maximum allowed concentration defined by National Regulation, that is less than 10 percent of all samples.

Keywords: plasticizers, GC-MS

Correspondence: marija.stankovic@zdravlje.org.rs

Do Biflavonoid Constituents of St. John's Wort Have Endocrine Modulating Effects?

Alev Tascioglu, Senem Ozcan Sezer, Duysal Uslu, Elif Ince, Hande Gurer-Orhan
Ege University, Faculty of Pharmacy, Pharmaceutical Toxicology Department, Izmir/Turkey

St. John's Wort (SJW) is used as a dietary supplement in depression mostly in postmenopausal women. In addition, an active constituent of SJW, hyperforin, is shown to modulate endogenous estrogen levels. SJW has more active compounds such as naphthodianthrones, flavonoids (flavonol glycosides and biflavonoids) which aren't evaluated for their potential hormone-related effects. In the present study, SJW extract and two of its biflavonoid constituents, amentoflavone and biapigenin, were investigated for their endocrine modulating effects via two mechanisms: estrogen receptor modulating effect was evaluated by E-Screen assay and estrogen synthesis modulating effect was evaluated by aromatase inhibition assay since aromatase is responsible for local estrogen synthesis. E-screen results showed that, SJW extract has agonistic effects on estrogen receptor alpha (ERα) with 0,01 mg/ml EC50 value while its biflavonoids didn't show any effects on ERα. In aromatase inhibition assay, SJW extract inhibited aromatase as well as amentoflavone which showed aromatase inhibition with a 93,6µM IC50 value. The extract of St. John's Wort is a herbal dietary supplement intended to treat symptoms of depression. Possible endocrine modulating effects of SJW found in the present study suggests that the consumption of SJW extract by vulnerable populations, such as pregnant women and children, might cause adverse effects. Furthermore amentoflavone, one of the biflavonoid constituents of SJW, is found to be involved in aromatase inhibitory effect of the extract.

This work was supported by TUBITAK grants 108S202 & 112S375 and Ege University Research Fund grant 13ECZ008. Analyses were performed at Ege University Pharmacy Faculty Research Laboratories, FABAL.

Keywords: St. John's Wort, amentoflavone, biapigenin, E-Screen, aromatase inhibition

Correspondence: alev.tascioglu@gmail.com

Current Status of Public Awareness About Endocrine Disrupting Chemicals in Slovenia

Lucija Kolar1,2, Igor Muršec2
1Complementarium, Institute for Environmental Technologies and Research of Nature (CMP Lopata), Lopata 60, SI-3000 Celje, Slovenia.
2Environmental Protection College, Trg mladosti 7, SI-3320 Velenje, Slovenia

Our research shows current situation regarding public recognition of the term endocrine disrupting chemical (EDC) and public awareness about pollution with them at three different sites in Slovenia. EDCs may interfere with the body’s endocrine system and produce adverse developmental, reproductive, neurological, and immune effects in humans and wildlife. Chemicals commonly detected include DDT, polychlorinated biphenyls (PCBs), bisphenol A (BPA), polybrominated diphenyl ethers (PBDE’s), and variety of phthalates.

267 respondents answered the questionnaire we used in the research. There were several important
findings carried out. Our focus was public opinion and awareness about which EDCs are most widely recognized and what the most important routes of body entering are.

Respondents mainly include plant protection products, pharmaceutical agents, phytoestrogens and metals in the EDC group. Slovenians know that EDCs are introduced to the body primarily through food, medicines and cosmetics. They are aware that EDCs have an impact on the organism already in small quantities and that the most endangered are children. We also investigated how many Slovenians know whether there are EDC polluted areas (Mežica Valley, the Krupa River, north-eastern part of Slovenia). Most of the respondents knew about the problems of the Mežica valley but not many were aware about the other two areas. Respondents also gave an opinion on whether substances containing EDCs should be properly labeled or even withdrawn, and we received an affirmative majority answer to both questions.

With this research we set the basics to further raise the awareness and spread the knowledge about EDCs.

Keywords: endocrine disrupting chemicals, recognition, public awareness, national opinion

Correspondence: lucija@complementarium.si

Teratogenic and Embryotoxic Effects of Diisononyl Phthalate

Vitali Hrynchak, Sergei Sychik, Irina Il’yukova
Republican unitary enterprise «Scientific practical centre of hygiene», Academic 8, Minsk, Belarus

A toxicological study was conducted to investigate the effect of diisononyl phthalate on the reproductive system of white rats. The embryotrophic and teratogenic effects were registered taking into account the dynamics of development of offspring in the postnatal period. The presence of anomalies in the development of the internal organs of embryos was determined by the sagittal section method according to W. Wilson. It was found that intragastric administration of the studied compound to females during the pregnancy period at doses of 100, 1000 and 10,000 mg/kg initiated external and internal malformations of embryos (absence of interventricular septum, intestinal and/or liver ejection, microphthalmia, anencephaly, hydrocephalus, acronia, encephalocoele, micrognathia and hypoplasia of the lower lobe of the lung). The exposure level of 10000 mg/kg is characterized by an increase in total postimplantation and embryonic mortality, the presence of multiple embryonic developmental defects. Observations of the process of postnatal development of the rats were carried out starting from the day of birth of the rats up to 60 days of age. It was established that postnatal mortality at a level of exposure to diisononyl phthalate of 10000 mg/kg significantly increased by 22.7%. According to the parameters of physical development (weight and body length), the female rats of the experimental groups receiving phthalate did not differ from the control groups. Also, there were no reliable deviations in the estimated coefficients of testes and appendages, functional spermatozoa.

Keywords: diisononyl phthalate, embryotoxicity, teratogenicity, toxicity

Correspondence: grinchakva@gmail.com

In vitro Estimation of Bisphenol S Toxicity

Maja Milanović¹, Dračana Četojević-Simin², Nataša Milošević¹, Milica Medić Stojanoska³, Nataša Milić¹
¹University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Novi Sad, Serbia,
²University of Novi Sad, Faculty of Medicine, Experimental Oncology Department, Oncology Institute of Vojvodina, Sremska Kamenica, Serbia,
³University of Novi Sad, Faculty of Medicine, Clinic for Endocrinology, Diabetes and Metabolic Diseases, Clinical Center of Vojvodina, Novi Sad, Serbia

The association of bisphenol A (BPA) with a range of health disorders such as fertility problems, diabetes, obesity, cardiovascular diseases and increased carcinogenic risk was confirmed in the considerable number of studies. Due to the extensive exposure to BPA through the variety of everyday products, the major issue is to find the safe replacement of this compound. Nowadays, bisphenol S (BPS) is used as BPA alternative without restriction. Toxicological data on BPS are very scarce. However, similar physico-chemical properties could lead to the potent toxicological profile. Therefore, in vitro toxicity after acute BPS exposure was estimated in the panel of mammalian cell lines. Cell growth effects of BPS were evaluated in human cervix carcinoma (HeLa), breast adenocarcinoma (MCF7), colon adenocarcinoma (HT-29) fetal lung (MRC-5) and rat hepatoma (H-4-II-E) cell lines using colorimetric sulforhodamine B assay. EC₅₀ values were calculated from the concentration response curves following 48 h exposure time. BPS significantly impaired the growth of all investigated cell lines, i.e. the EC₅₀ values were reached in the range from 35.94 to 103.15 mg/mL. When
compared to BPA, BPS was from 3 to 15-fold less toxic. Narrow span of EC<sub>50</sub> values that were obtained at low concentrations indicate general toxic mode of action of BPS and raises awareness of the health risks associated with its ubiquitous presence in the environment.

Acknowledgment: This research has been financially supported by the Provincial Secretariat for Science and Technological Development, AP Vojvodina, Republic of Serbia, Grant No 114-451-2216/2016.

Keywords: endocrine disruptors, bisphenol S, toxicity, cell lines

Correspondence: maja.milanovic@mf.uns.ac.rs

---

Levels of Bisphenol-A in Thermal Paper Receipts from Serbia and Greece

Milan Milenković1,2, Tatjana Nedeljković1, Zorica Blagojević1
1Institute of Public Health of Serbia “Dr Milan Jovanović Batut”,
2University of Belgrade-Faculty of Pharmacy

Bisphenol A (BPA) is a chemical substance which is globally produced in great quantities, and widely used as a color developer in thermal paper. Thermal paper is every day present in daily life, in form of cash register receipts. BPA used for this purpose is in unbound, free form, therefore risk of human exposure is present. BPA is one of the most common endocrine disrupting chemicals, and is also linked to certain developmental disorders, cardiovascular disease, and diabetes. Aim of this study was to determine concentration levels of BPA in thermal paper, in two countries. For this study, 20 thermal paper receipts, 10 from each country, were collected from various market places, randomly selected in Serbia and Greece, and analyzed. BPA was extracted and its analysis was performed using liquid chromatography/fluorescence technique. LOQ of method was 0.001% (0.01 mg BPA/g paper). BPA was detected in 19 samples. Samples concentrations were found in range of 0.59 – 1.32% (5.9 and 13.2 mg BPA/g paper), and 0.02 – 1.61% (0.2 and 16.1 mg BPA/g paper), in Serbia and Greece, respectively. Only one sample from Serbia had concentration below LOQ. The BPA concentrations measured in thermal paper receipts were comparable to those reported in similar studies.

Keywords: BPA, thermal paper receipts, endocrine disrupting chemicals, HPLC-FLD

Correspondence: mmmilann_92@yahoo.com

---

Changes in the Oocyte Integrity and Bone Marrow Induced by 3-Methylcholanthrene and Prevented by α-Naphthoflavone

EA Rhon-Calderón1, RA Galarza1,2, S. Zurita1,
AG Faletti1,2
1Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Centros de Estudios Farmacológicos y Botánicos (CEFYBO), Facultad de Medicina, Buenos Aires-Argentina,
2Universidad de Buenos Aires, Facultad de Medicina, Dto. de Toxicología y Farmacología, Buenos Aires-Argentina

3-Methylcholanthrene (3MC) is an environmental pollutant that causes reproductive toxicity and genotoxicity. Previous studies showed that 3MC alters the ovarian function by affecting the follicle integrity, and causes DNA damage in peripheral blood, bone marrow and oocytes. Now, we studied the effect of daily exposure to 3MC on the oocyte integrity, induction of chromosome aberrations in bone marrow and the ability of α-naphthoflavone (αNF) to prevent this action. Immature rats were daily injected with a combination of 3MC (0.1 and 1 mg/kg) and αNF (80 mg/kg) for 20 days. Oocyte integrity, by morphological parameters; oocyte viability, by fluorescent dyes; and chromosome aberrations (CA) in bone marrow were evaluated. Compared with controls (C), both doses of 3MC increased the area (C 12.4±0.2 μm²; 3MC 14.5±0.7 μm²; P<0.001), perimeter (C 39±1 μm; 3MC 48±2 μm; P<0.001), and perivitelline space (C 1.6±0.1 μm; 3MC 2.3±0.1 μm; P<0.001); and decreased the thickness of the zona pellucida (C 1.7±0.1 μm; 3MC 1.1±0.1 μm; P<0.001) and the number of viable oocytes (C 86±3%; 3MC 60±3%; P<0.001). 3MC-treated rats exhibited a higher number of CA (C 7±2%; 3MC 20±4%; P<0.001), especially metaphases with dicentric chromosomes (C 2.4±0.5%; 3MC 8±2%; P<0.001) and fragments of chromosomes (C 1.0±0.5%; 3MC 3.6±0.4%; P<0.001). All these changes were prevented by daily treatment with αNF. In conclusion: i) daily exposure to 3MC alters the oocyte integrity; ii) low doses of 3MC are enough to induce CA in bone marrow; and iii) αNF prevents both the systemic and oocyte toxic effect of 3MC.

Keywords: 3-methylcholanthrene, α-naphthoflavone, oocyte, bone marrow.

Correspondence: eric.rhoncalderon@gmail.com
Resveratrol Inhibits Ovary Cells Proliferation Induced by Low Doses of Polychlorinated Biphenyls

Marina Miletić, Teuta Murati, Sanja Marđetko, Ivana Kmetič
Laboratory for Toxicology, Faculty of Food Technology and Biotechnology, University of Zagreb, Pierotti St 6, 10000 Zagreb, Croatia

Toxic potential of PCBs is linked to various deleterious effects on human health and, in 2016 IARC has upgraded the classification of PCBs to Group 1 “Carcinogenic to humans”. Increased ROS levels by PCBs are able to promote tumor growth and malignant progression. This study was focused on low doses effects of PCB 77 congener at ovarian cellular level with the intention of reducing PCB induced proliferation by plant polyphenol-resveratrol. Resveratrol was selected because of protective effects on reproductive tissues and antioxidative activity. Initially, ovary cells (CHO-K1 cell line) were exposed sole to resveratrol in order to determine non-toxic doses to further experiments. Dose dependent cell growth inhibition after resveratrol exposure (2.5-150 μM) was confirmed with Trypan Blue method with significant effects in concentrations ≥ 5 μM (p<0.025-p<0.001). Neutral Red method, unequal to results obtained with Trypan Blue method, showed statistically significant cell growth inhibition in doses above 50 μM (p<0.001), while lower doses have shown beneficial effects on cell viability. Cytfluorimetric analysis confirmed elevated late apoptotic/death cells fraction for resveratrol ≥50 μM and therefore concentrations lower than 50 μM were further used in experiments. Cells were pre-treated with resveratrol, then treated with 1 μM PCB 77 (most effective dose in stimulation of cell proliferation) and after 24h cell viability was determined.

Presence of resveratrol efficiently suppressed PCB elevated cellular proliferation–statistically significant in doses 15-50 μM (p<0.05-p<0.01) confirmed with Trypan Blue method. These findings suggest that resveratrol alters cellular progression and may be effective in suppression of malignant progression.

Keywords: PCB 77, protection, plant polyphenol, cell viability, cancer
Correspondence: ikmetici@pbf.hr

Biochemical Distrubances in Testes of Albino Rats with Metabolic Syndrome Induced in Prepubertal Age and Metformin Treatment

Oleksandr Tkachenko, Ganna Shayakhmetova, Alla Voronina, Valentina Kovalenko
SI “Institute of Pharmacology & Toxicology of NAMS of Ukraine”, Kyiv

Adolescence is decisive with regard to the formation of reproductive function, so almost in half of cases childhood and adolescence diseases are causes of male infertility. To date no emphasis has been placed on examination of effect of childhood MS on testes parameters in puberty. The aim of present study was an estimation of biochemical indices in rat testes following MS induced in childhood and metformin treatment. Wistar albino male rats of 21 days age were divided into 3 groups (10 animals in each): (1) control, (2) MS, (2) MS+metformin (266 mg/kg, 1 month). MS was induced by full replacement of drinking water by 10% fructose solution. Development of MS in childhood greatly affected testicular pro- and antioxidant systems: we recorded lipid peroxidation processes intensification 2,2 times, increase of catalase activity 22% and decrease of glutathione content 12% as compared with control. In group with metformin administration a tendency for inhibition of lipid peroxidation processes was detected. At the same time metformin forwarded full restoration of testicular reduced glutathione content and catalase activity. Thus, induction of MS in childhood provoked remote disturbances in testicular cells at biochemical level. These and other pathological events in testicular tissues need to be investigated profoundly as processes affecting spermatogenesis and probably underlying male infertility in adults. It should be highlighted that in very high-risk insulin-resistant children, pharmacotherapy with metformin may be indicated and this medicine use could also protect male reproductive parameters, probably due to attenuation of insulin-mediated oxidative stress.

Key words: metformin, male gonads, lipid peroxidation, glutathione, catalase
Correspondence: falkorn027@gmail.com
**Multi-element Profile of Wines from Fruska Gora (Vojvodina)**

Danijela Lukić, Milan Jovanović, Ivana Beara, Ljilja Torović, Milan Jovanović, Ivana Beara, Ljilja Torović, 1Institute of Public Health of Vojvodina, Novi Sad, Serbia, 2Department of Chemistry, Biochemistry and Environmental Protection, University of Novi Sad Faculty of Sciences, Novi Sad, Serbia, 3Department of Pharmacy, University of Novi Sad Faculty of Medicine, Novi Sad, Serbia

The aim of this study was to provide elemental profile of wines from Fruska Gora, a vineyard in Vojvodina vine growing region, by reporting analysis of 23 elements (Be, B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Sn, Sb, Te, Ba, Hg, Ti, Pb) in 91 bottled wines from 24 wineries, originating from 2009-2015. Analysis was carried out using ICP-MS, on diluted samples with internal standard addition. Levels of elements that could pose a health risk (Pb, Cd, Sn, As, Fe, Cu, Zn) in all analysed wines were far below the current regulatory limits. It is of interest to note the content of As, due to the widely known problem of As contaminated ground water in Vojvodina: mean levels in red and white wines were 3.84 and 4.43 mg/kg, respectively, with maximum concentration (8.92 mg/kg) measured in a white wine. Generally, there were no substantial differences in mean levels of analysed elements between red, rosé and white wines, with the exception of Be, Al and Ni. Al concentration varied from 137 to 4671 mg/kg, with mean at 861 and 1536 mg/kg, in red and white wines, respectively.

Elemental composition of wine is influenced by endogenous sources and winemakers interventions. Certain elements can affect sensory properties of wine (Zn, Fe), some present health risk; furthermore, multi-element profile could contribute to differentiation of wines in terms of geographical origin. Results of this study should contribute to the recognition and positioning of wines from Fruska Gora.

**Keywords:** metals, wine, ICP-MS

**Correspondence:** danijela.lukic@izjzv.org.rs

---

Investigation of Toxic Metals, Nitrate and Nitrite in the Commercial Packed Drinking Water in Mashhad, Northeastern Iran

Seyed Reza Mousavi, Mahd Balali Mood, Sam Elmi, Mahmood Sadeghi, Monavar Afzalaghaee, Bamdad Riahi Zanjani, 1Medical Toxicology Research Centre, Mashhad University of Medical Sciences, Mashhad, Iran

Due to the increasing consumption of bottled water in recent years, we planned to determine the concentration of 6 toxic metals, nitrate and nitrite components in the bottled water which commonly sold in Mashhad, Iran.

The 11 best-selling brand of packaged drinking water in Mashhad’s market were identified. Eight bottles from each brand were randomly prepared and stored in a fridge at -4 °C. The concentrations of metals (lead, mercury, arsenic, chromium, aluminum, cadmium) in the samples were measured with atomic absorption spectrometric methods. Nitrate and nitrite were determined by a spectrophotometric method. The results were analyzed by SPSS software and compared with the WHO and Australian guidelines. Also, the discrepancy between measured components and the depicted labels’ values were compared by means of one sample T-test, by assessing the P value of which being less than 0.05 would be assumed as significant difference.

The mean and SD of concentrations of the toxic metals in 11 brands were as below: lead 1.62±0.86 (µg/L), chromium 1.03±0.84 (µg/L), cadmium 0.17±0.07 (µg/L), mercury 3.86±1.57 (µg/L), arsenic 0.89±0.46 (µg/L), aluminum 6.56±4.54 (µg/L). The mean and SD measured quantities of nitrate, nitrite and pH were 9.96±5.95 (mg/L), 0.01±0.03 (mg/L) and 7.92±5.95, respectively. There were significant difference between the labels values and quantified levels of constituent totally (p-values between 0.000 and 0.003).

The toxic metals, nitrate and nitrite concentrations in all samples were within the national and WHO and Australian ranges, except for mercury in 9 samples that exceeded the Australian standard.

**Keywords:** nitrate, nitrite, toxic metals, drinking water, bottled water

**Correspondence:** mousavir@mums.ac.ir

---

Food Additives in Food Intended for Children in the Republic of Srpska Market

Ljubica Bojanić, Miodrag Marjanović, Mirjana Đermanović, Zorica Jusupović, Janja Bojanić, 1Public Health Institute of Republic of Srpska, 2Medical Faculty, University of Banja Luka

Food additives are added to food in order to improve the technological and sensory properties, but may also have adverse health effects. Children,
especially in pre-school age, are particularly vulnerable. This age is characterized by intense growth and development of the organism and intense metabolism, which leads to increased energy and nutrients requirements, and consequently increased food intake per kg of body weight compared to adults.

According to legislation in the Republic of Srpska, it is allowed to use food additives for children older than three years, including preservatives, sweeteners and colours.

The aim of this paper is to present the potential exposure of children to additives by controlling the declarations of products labeled as intended for children.

Declarations of 25 randomly taken samples were examined, of which 11 soft beverages, 9 candies, 3 lollipops and 2 chewing gums. Of these, Na-benzoate and K-sorbate preservatives were declared on 8 beverages, 5 samples contain artificial sweeteners ace-sulfame K and aspartame (3 beverages and 2 chewing gums), while the presence of colors was found in all tested samples, including natural E100, E120, E141, E150, E153, E160a, E160e, E162, E163, E171 and E172, and artificial E102, E110, E122, E124, E131, E132, E133 and E151, depending on the sample.

The obtained results give a general insight into the quality of products intended for children in our market and show that a significant number of these products contain additives, which indicates the need for further and more detailed research on the assessment of the exposure and impact of additives on children's health in the Republic of Srpska.

Keywords: food additives, children, exposure, Republic of Srpska

Correspondence: ljubica.bojanic@gmail.com

Dietary Exposure to Mycotoxins Through Ready-to-eat Food Consumption

Guillermina Font¹, Dionisia Carballo², Emilia Ferrer¹, Houda Berrada¹
¹Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Spain,
²Faculty of Agricultural Science, National University of Asunción, Paraguay

Knowledge of human exposure to mycotoxins through processed diet is an important component of food safety strategies. The present study investigates the evaluation of mycotoxins in ready-to-eat meals as a reliable tool for risk assessment. For this objective, the presence of twenty-seven mycotoxins including AFB₁, AFB₂, AFG₁, AFG₂, EN A, EN A₂, EN B, EN B₁, BEA, FB₁, FB₂, STG, DON, 3-ADON, 15-ADON, NIV, NEO, DAS, FUS-X, ZEA, αZAL, β-ZAL, αZOL, β-zol, T2 and HT-2 toxin, was assessed in 25 ready-to-eat food samples using QuEChERS extraction and determination by chromatographic methods coupled to mass spectrometry in tandem.

Results showed presence of deoxynivalenol at 36% of samples (2.61-21.59 µg/kg), enniatin B at 20% of samples (9.83-86.32 µg/kg), HT-2 toxin at 16% of samples (9.06-34.43 µg/kg) and aflatoxin G₁ at 4% of samples (2.84 µg/kg). Mycotoxins were detected mainly in ready-to-eat food samples prepared with cereals, vegetables and legumes at levels below those often obtained from raw food. The obtained results indicated that mycotoxins are present in different ready-to-eat food samples which point out the need to perform continuous surveys to insure consumer health. The dietary exposure of the adult population was estimated using the deterministic approach, through the evaluation of the consumption and data of foods mycotoxin contamination to assess the estimated daily intake (EDI) and values obtained resulted below the tolerable daily intake for the selected mycotoxins.

This research was supported by the Spanish Ministry of Economy and Competitiveness (AGL2016-77610-R) and Government Scholarship program “Carlos Antonio López – Paraguay.

Keywords: Mycotoxins, ready-to-eat food, mass spectrometry, dietary exposure

Correspondence: guillermina.font@uv.es

Investigation of the Relative Hepatotoxic and Genotoxic Potency of Selected Pyrrolizidine Alkaloids

Lan Gao, Lukas Rutz, Karl-Heinz Merz, Dieter Schrenk
Food Chemistry and Toxicology, University of Kaiserslautern, 67663 Kaiserslautern, Germany

Pyrrolizidine alkaloids (PAs) having a 1,2-unsaturated pyrrolizidine core are known to be cytotoxic, genotoxic and carcinogenic occurring naturally in many plant species. They exert toxic effects through metabolic activation which form the corresponding dehydropyrrolizidine derivatives, primarily in the liver, catalyzed by cytochrome P450 monoxygenases. The presence of PAs as contaminants in food and feed, depending on the dose, has become a relevant safety issue in recent years. Although hints for a structure dependent toxicity exist, no sufficient conclusions on the relative toxicities of individual PAs can be drawn due to the limited and not comparable data.
In order to investigate further the connection between structure and toxicity, a series of PAs congeners were tested at doses ranging from 1 to 300 µM for mutagenicity in the Ames fluctuation assay with Salmonella typhimurium in the strains TA98 and TA100. None of the selected PAs showed mutagenicity despite metabolic activation with S9-mix. Cytotoxicity was assessed by the Alamar blue assay in HepG2 cells and primary rat hepatocytes. In HepG2 cells, possibly due to the lack of CYPs, none of the selected PAs showed cytotoxic effects. On the contrary, the probable structure-dependent cytotoxicity was demonstrated with rat hepatocytes in primary culture. Lasiocarpine, an open-chained di-ester with 7S-structure, proved to be the most cytotoxic followed by the other di-esters echimidine, retrorsine, seneciphylline and senecionine. The mono-esters heliotrine, indicine, europine and lycopsamine were much less cytotoxic.

**Keywords:** food contaminants, Ames fluctuation assay, Alamar blue assay, metabolic activation, structure-dependent toxicity

**Correspondence:** gao@rhrk.uni-kl.de

---

**Human Intervention Trial with Strawberry Tree (Arbutus unedo L.) Honey: Impact on DNA Stability and Haematological Parameters**

Andreja Jurič1, Marin Mladinić2, Davor Želježić1, Marija Pezer3, Mirjana Turkalj4, Karlo Jurica5, Nevenka Kopjar1, Irena Brčić Karačonji1

1Institute for Medical Research and Occupational Health, Zagreb, Croatia
2Xellia Ltd, Zagreb, Croatia
3Genos Ltd, Zagreb, Croatia
4Srebrnjak Children’s Hospital, Zagreb, Croatia
5Ministry of the Interior, Zagreb, Croatia

Due to its high phenol content, strawberry tree honey possesses many beneficial health effects. The aim of this study was to evaluate potential DNA and cytoprotective effects of *A. unedo* honey after short-term supplementation. Participants (six healthy men, aged 26-38) consumed 70 g of honey (from Pelješac peninsula, Croatia) dissolved in 200 mL of water daily for 14 days. Their blood samples were collected before honey supplementation and one day after the last consumption (day 15). We monitored changes in leukocyte DNA integrity and haematological parameters. To establish how the supplementation modified the response of DNA to external stimuli with a genotoxic chemical, we performed a challenge test with hydrogen peroxide (H₂O₂) by measuring the levels of spontaneous and H₂O₂-induced DNA damage using the alkaline comet assay. Results showed significant lowering of comet tail intensity compared to the baseline value (0.21% vs. 0.88% DNA in comet tail). We found significant DNA-protective effects in leukocytes of almost all participants after an *ex vivo* challenge with H₂O₂. Comet tail intensity recorded after the H₂O₂ challenge was significantly lower after honey supplementation (2.42% DNA at day 0 vs. 0.38% DNA at day 15). Additionally, a significant increase in neutrophil count was observed after honey supplementation. Although limited by a small number of participants, the results of this intervention trial point to strawberry tree honey having promising DNA- and cytoprotective effects, which should be further clarified in forthcoming studies using other complementary methods on a much larger population exposed for longer periods.

**Keywords:** alkaline comet assay, challenge test, supplementation

**Correspondence:** ajuric@imi.hr

---

**Monitoring of Metals and Metalloids in Samples of Plastic Food Packaging During 2013-2017**

Ž. Ljubičić1, B. Antonijević2, N. Zec Petković3

1Institute of Public Health Sremska Mitrovica, Sremska Mitrovica, Serbia,
2Department of Toxicology “Akademik Danilo Soldatovic”, Faculty of Pharmacy, University of Belgrade, Serbia

Health safety of packaging materials represents the basic prerequisite of their safe use in food and beverage packaging. In contact with food, chemicals can migrate from the packaging to the food content and contaminate it. Therefore, according to the official rules, food contact materials (FCM) have to be investigated in order to ensure that the substances migrating from the material into the food do not endanger human health or change the food itself.

The aim of this study was to present the results of arsenic, copper, barium, zinc, iron, chromium, cadmium, cobalt, lithium, manganese, lead and mercury content in FCM during the last five-year period.

Measurement of the elements in the migration solutions (3% acetic acid, 50% ethanol) was carried out by atomic absorption spectrophotometry FLAAS, with exception of arsenic which was determined by HGAAS. Sample preparation was carried out according to the requirements of EU Commission Regulation
No 10/2011 for plastic materials and articles intended to come into contact with food and the Role book on the conditions regarding the health safety of consumer products that may be placed on the market, Official Gazette SFRJ No. 26/1983, 61/1984, 56/1986, 50/1989 and 18/1991 and standards EN 1886-1, EN 1886-5, EN 1886-9, EN 1886-13 and EN 1886-14. All measurements were done using accredited methods.

The measurements were carried out during the period 2013-2017 in different samples of FCM obtained from several manufacturers on the territory of Srem, Serbia. A total of 745 samples was analyzed. All the concentrations of potential contaminants in migration solutions were below the maximum allowable levels, established by the regulation in the EU and the national regulation, which indicate adequate health safety of FCM covered by this monitoring.

Keywords: food contact materials, contaminants, migration

Correspondence: zaklinaljubicic@yahoo.com

---

Safety Assessment of the Extracts of Overground Part of Hedychium Coronarium Koenig in SD rats

Ling-Shan Tse1, Po-Lin Liao1, Jiunn-Wang Liao3, Jaw-Jou Kang2, Yu-Wen Cheng1*

1School of Pharmacy, College of Pharmacy, Taipei Medical University, Taipei, Taiwan, R.O.C,
2School of Pharmaceutical Science, National Tang Ming University, Taipei, Taiwan, R.O.C,
3Graduate Institute of Veterinary Pathobiology, National Chung Hsing University, Taichung, Taiwan, R.O.C

Hedychium Coronarium (HC) has a long history using in food and folk medicine from different countries, however, the potential toxicity of HC was remained unknown. In this study, groups of male and female SD rats were orally administered 0, 1.5, 3.0 and 5.0 g/kg/day water extracts of HC leaf for 90 days (N=15), ten rats from each group were randomly scarified at 91 days, other 5 rats from each group were stopped gavaging and maintained observation for the following 14 days resting phase as followed up recovery study. There were no toxicologically relevant findings in female groups. In male groups, after treatment with HC 3.0 and 5.0g/kg/day for 90 days, urine volume was increased, imbalanced electrolyte and aldosterone was decreased, those indicating the side effect of HC. Hence, the no-observed adverse- effect level of water extracted HC leaf is suggested as 1.5g/kg/day for male.

Keywords: subchronic toxicity, folk medicine, herb, aldosterone, diuretics

Correspondence: jjkang@ntu.edu.tw,
ywcheng@tmu.edu.tw
Carbamate Derivatives of Short-acting Bronchodilator Albuterol Inhibits Human Acetylcholinesterase and Butyrylcholinesterase

Anita Bosak1, Anamarija Knežević2, Katarina Zlatić2, Robert Kerep1, Zrinka Kovarik1
1Institute for Medical Research and Occupational Health, Zagreb, 2Ruđer Bošković Institute, Zagreb

Novel synthesised biscarbamate and monocarbamate derivatives of the short-acting bronchodilator albuterol (biscalb) resemble the structure of biscarbamate bambuterol, whose bioconversion to the bronchodilator terbutaline in an organism is enabled through the inhibition of butyrylcholinesterase (BChE). Bambuterol’s high therapeutic index is associated with an extremely selective BChE inhibition compared to acetylcholinesterase (AChE). We determined the inhibition potency of biscalb and monocarb toward human BChE and AChE to evaluate their selectivity. Both carbamates proved to be potent inhibitors of both cholinesterases with $k_i$ constants within $10^{-3}$-$10^{-5}$ M$^{-1}$min$^{-1}$. Biscalb and monocarb inhibited AChE only 10 times slower than they did BChE, meaning that the selectivity of both carbamates was very poor compared to the 20,000 times faster inhibition of BChE determined for bambuterol. Therefore, the novel compounds lack potential for prodrug development in analogy with bambuterol. However, due to their inhibition potency, these novel carbamates could be considered structural and functional lead compounds for the design of new compounds, whose primary action would be inhibition of cholinesterases, like pesticides or neurodegenerative disease drugs.

Supported by CSF Grant no. 4307.

Keywords: cholinesterase, selectivity, monocarbamate, biscarbamate, prodrug

Correspondence: abosak@imi.hr

Exposure to Exhaust Gas and Changes in Liver Functions, Biomarkers of Oxidative Stress and Heavy Metals in Automobile Workers

Augusta Nsonwu-Anyanwu1*, Sunday Offor1, Edmund Egbe1, Chinyere Usoro1
1Department of Medical Laboratory Science, University of Calabar, Cross River State, Nigeria

Biomarkers of oxidative stress (malondialdehyde (MDA), glutathione (GSH), nitric oxide (NO), total antioxidant capacity (TAC), total plasma peroxides (TPP), oxidative stress index (OSI)), liver function enzymes (Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Gamma glutamyl transferase (GGT)) cadmium (Cd) and lead (Pb) were assessed in sera of automobile workers. One hundred and twenty (120) men (18-60 years) comprising of 40 automobile mechanics (AMM), 40 automobile spray painters (ASP) and 40 non-automobile workers were studied. The MDA, GSH, NO, TAC, TPP, ALT, ALP, GGT were estimated colorimetrically, Cd and Pb by AAS. Systolic and diastolic blood pressure (SBP & DBP) was obtained. Data were analyzed using ANOVA and correlation at p<0.05.

Non automobile workers had lower SBP, TPP, OSI, NO, ALP, GGT, Pb and Cd and higher TAC and GSH compared to AMM and ASP; and lower DBP, MDA and ALT compared to ASP only (p<0.05). ASP had higher SBP, MDA, ALT, ALP, GGT, Pb and Cd compared to AMM (p<0.05). Duration of exposure correlated negatively with NO ($r = -0.230$, $p=0.040$) and positively with TPP ($r = 0.830$, $p = 0.000$), OSI ($r = 0.801$, $p = 0.000$), Pb ($r = 0.776$, $p = 0.000$) and Cd ($r = 0.674$, $p = 0.000$), ALT ($r=0.274$, $p=0.014$), ALP ($r = 0.473$, $p=0.00$) and GGT ($r = 0.535$, $p=0.000$) in both ASP and AMM. Chronic exposure to automobile exhaust gas is associated with increased liver enzymes activities, toxic elements, lipid peroxidation and depletion of antioxidants in automobile workers studied.

Keywords: exhaust gas, liver enzymes, antioxidants, lipid peroxidation, toxic metals

Correspondence: austadechic@yahoo.com.

Mutagenicity Study of Generic Insecticides Lambda Cyhalothrin in the Mammalian In Vivo Micronucleus Test

Tetiana Tkachuk, Mykola Prodanchuk, Nadiiya Nedopytanska, Oleksandr Kravchuk, Volodyymr Bubalo, Oleksander Tkachuk, Olena Zubko, Olena Kostik
Experimental mutagenicity data is a mandatory part of toxicological assessment of the justifying their safety usage in environment. One of the methods applied in our Laboratory is studying mutagenicity in the mammalian in vivo micronucleus test. The purpose of the micronucleus test is to identify test-substances that cause cytogenetic damage which results in the formation of micronuclei containing either lagging chromosome fragments or whole chromosomes. The test evaluates micronucleus formation in polychromatophilic erythrocytes (PCE) of the mice bone marrow. Following OECD 474 guidelines we modified test for rapid screening of generic pesticides for mutagenicity. The tests are conducted following the SOP, in compliance with GLP requirements.

Two samples of technical pesticide lambda cyhalothrin obtained from different manufacturers and content different purity percentage were studied. The mutagenic activity was studied on CD1 healthy young mice, male. Acclimatization of the animals took 5 days before dosing. The test substances were administered as an aqueous emulsion, orally.

Each sample was investigated in the following doses: 5.0; 1.0; 0.2 mg/kg/bw and was accompanied with positive and negative control. Exposure time - 24 hours.

As a result of analyzing lambda cyhalothrin samples in high concentrations were observed significant (p≤0.05) increase in the frequency of PCE micronuclei in compared to the negative control and historical control data.

All two samples of generic insecticide lambda cyhalothrin showed mutagenic effect in highest doses (5.0 mg/kg/bw) in the micronucleus test in the mice bone marrow in vivo.

Keywords: mutagenicity, micronucleus test, pesticides, lambda cyhalothrin.
Correspondence: tatiana.tkachuk.mail@gmail.com

Humic acid (HA), which are part of the organic structure of soils, are the resultant compounds of the biomolecules that undergo physical, chemical and microbiological transformation. Chemical and infrared spectroscopic analyses have revealed the presence of aromatic rings, and phenolic hydroxyl, ketonyl, quinone carbonyl, carboxyl, and alkoxyl groups in HA. Therefore, HA extracts are the most powerful antioxidants in the world. Because of the anti-inflammatory, anti-allergic, anti-apoptotic, anti-irritant, heavy metal binding and antiviral properties of HA, it is increasingly being used in industrial, agricultural, environmental and biochemical applications. The goals of this study were twofold. First, it aimed to isolation and characterization of HA from soils. Second, it aimed to investigation of in vitro anti-carcinogenic and anti-apoptotic effects of synthetic and isolated HA on human servical cancer cell line. For this purpose, we isolated HA from soil samples collected from Antalya, Turkey by the California Department of Food and Agriculture (CDFA) method. Then, isolated HA was characterized by HPLC and compared to the standard HA. HeLa cells were grown in petri dishes in a humidified atmosphere at 37°C. Five different concentrations were prepared using standard and isolated HA. The cell proliferation was measured by MTT method. On the other hand, the apoptotic mechanisms induced by HA in cancer cells were investigated by measuring caspase-3, 8 and 9 enzyme activities.

According to our results, HA has an growth inhibitory and apoptotic effects on HeLa cells. However, further studies are required to determine the exact mechanism.

Keywords: cancer, CDFA method, humic acid, isolation, soil
Correspondence: sultanmehtap.buyuker@uskudar.edu.tr

Isolation and Characterization, Anticarcinogenic and Apoptotic Effects of Humic Acid

Ayse Demir Aktas¹, Sultan Mehtap Buyuker, Derya Ozsavci¹, Ozlem Bingol Ozakpinar¹
¹Department of Biochemistry, School of Pharmacy, Marmara University, Istanbul, Turkey, ²Department of Pharmacy Services, Vocational School of Health Service, Uskudar University, Turkey

Keywords: cancer, CDFA method, humic acid, isolation, soil
Correspondence: sultanmehtap.buyuker@uskudar.edu.tr
**Effect of Myrtenal on Social Behavior and Memory of Rats**

Stela Dragomanova\(^1\), Radoslav Klisurov\(^1\), Marieta Georgieva\(^2\), Maria Lazarova\(^1\), Christophor Dishovsky\(^1\), Reni Kalfin\(^1\), Lyubka Tancheva\(^1\)*

\(^1\)Institute of Neurobiology, Bulgarian Academy of Sciences, Acad. Georgi Bonchev Str., Block 23, Sofia 1113, Bulgaria;  
\(^2\)Medical University of Varna, 55 Marin Drinov Str., Varna 9002, Bulgaria;  
\(^3\)Medical University of Sofia, 2 Zdrave str, Sofia 1000, Bulgaria;  
\(^*\)Weston Visiting Professor of Weizmann Institute of Science, Israel

Plant monotherpen Myrtenal (M) presents in many essential oils and has rich specter of pharmacological activities. Our previous studies established significant effects of M on behavior of mice.  

Aim of this study was to investigate effect of M on the cognition and social behavior of rats.  

Male Wistar rats were daily injected Myrtenal (40 mg/kg, i. p.) for 9 days. 24 hours after the last treatment the changes in behavior and cognition were tested via Open field test, Step-through test and Mouse-killing test. Brain levels of some neurotransmitters (dopamine - DA, serotonin - 5-HT, acetylcholine - ACh, adrenaline - Adr and noradrenaline - NA) were also measured. Significant improved cognitive performance in M-treated group was established. Learning and memory in this group was better than in the control group at the end of the experiment. Elevated brain neurotransmitter levels of DA, 5-HT and ACh confirmed behavioral data. Interesting changes in social behavior of M-treated animals were recorded, as well. After 3rd day of M-administration increased incidences of dominant and aggressive behavior were established accompanied by vocalization and fighting in the group. We assume that it is due to interaction of M odor with rats pheromones related to animal hierarchy in the group. Brain levels of Adr and NA in M-group were not significantly elevated. At the same time incidences of inter-species aggression were observed according to Mouse-killing test.  

Present study indicates significant effects of M on CNS accompanied by changes in brain mediation systems and deserve further studies.  

**Keywords:** cognition, aggression, mediators, monoterpenes  

**Correspondence:** stela_dragonova@abv.bg

---

**Gentiana Lutea Radix Extract Exerts In Vitro Dose- and Time-dependent Response in Peripheral Blood Mononuclear Cells**

Ana Valenta Šobot, Jelena Filipović Tričković, Dunja Drakulić  

Vinča Institute of Nuclear Sciences, University of Belgrade, Mike Petrovica Alasa 12-14, 11001 Belgrade, Serbia

Although Gentiana lutea radix extract (GRE) is believed to strengthen organism by stimulating circulation and the activity of many organs, it has certain active components that can cause oxidative and genotoxic stress. The aim of the current study was to estimate which concentration (0.5, 1 and 2 mg/ml) of GRE initiates DNA damage and lipid peroxidation, disrupts oxidative balance following 48h lasting treatment, and whether those parameters modulate survival of human peripheral blood mononuclear cells in vitro after 48 and 72h. DNA damage was assessed using alkaline Comet assay, oxidative status by PAB assay, lipid peroxidation by determining MDA levels and cell count by tripan blue dye exclusion test. Obtained results indicate that the lowest tested concentration increases lipid peroxidation along with DNA damage and has a mild cytotoxic effect. Higher concentrations provoke only significant DNA damage, probably due to the other mechanisms involved in initiation of cell death rather than elevated oxidative stress. Albeit cell death after 48h of treatment at the highest tested concentration is not significant, DNA fragmentation is more than doubled compared to control. The impact of detected fragmentation is seen as reduction of cellular survival observed after 72 hours of treatment. According to presented findings DNA fragmentation could be a predictive tool for the cytotoxic effects estimation of GRE treatment since increased fragmentation is observed 24h prior to significant increase in cell death. Parallel monitoring of oxidative status and DNA damage might be valuable parameter in determining sublethal concentrations of GRE in other experimental setups.

**Keywords:** DNA damage, Comet assay, lipid peroxidation, viability

**Correspondence:** anavalenta@vinca.rs
Effects of Commercially Available Dietary Supplement Based on Soybean Extract (*Glycine max.* L.) on Hepatic and Renal Function and Clinically Relevant Interactions with Conventional Drugs

Jelena Hogervorst¹, Aleksandar Rašković², Milan Ubavić³, Ana Tomas¹, Bojana Gaćeša¹, Vladan Borčić², Nebojša Stilinović²
¹Department of Pharmacy, Faculty of Medicine, University of Novi Sad,
²Department of Pharmacology, toxicology and clinical pharmacology, Faculty of Medicine, University of Novi Sad,
³Faculty of Pharmacy, European University, Novi Sad

Dietary supplements based on soybean extracts (DSU) are widely used by general public, but there is very limited scientific data to support their efficacy, safety and potential interactions with conventional therapy. The aim of this study was to examine the effect of commercially available DSU on liver and renal function, oxidative status and interactions with several conventional drugs in animal models. Standard pharmacodynamics tests were performed on Swiss albino mice treated with DSU in addition to several central nervous system acting drugs. Parameters of liver and renal function, and oxidative status in liver homogenates were determined in healthy and Wistar rats subjected to oxidative stress with CCl₄ after treatment with dietary supplement. DSU administration weakened the analgesic activity of codeine, significantly potentiated diazepam induced motor coordination impairment in 10th and 30th minute after the administration of diazepam and had the opposite effect on alprazolam effect. DSU pretreated group also exhibited significantly shorter pentobarbital sleep induction and sleeping time. These findings go in favor of DSU interference with metabolic pathways of tested drugs and subsequent altered pharmacodynamics. DSUs supplementation did not affect the liver and renal function, and ameliorated the liver injury and oxidative stress caused by CCl₄. Despite exhibiting no negative effects on liver and renal function, and demonstrated antioxidant in vivo potential, the safety of DSU in combination with conventional drugs is questionable. The results of our study implicate the potential of DSU for serious herb-drug interactions.

Keywords: phytoestrogens, soybean extract, interactions, Cytochrome P-450, conventional drugs

Correspondence: aleksandar.raskovic@mf.uns.ac.rs

Protective Effect of Ellagic Acid on 6-Hydroxydopamine Hemistriatal Intoxication

Andrey Popatanasov¹, Lyubka Tacheva¹, Maria Lazarova¹, Stela Dragomanova¹², Albena Aleksandrova¹, Elina Tsvetanova¹, Almira Georgieva¹, Reni Kafin¹
¹Institute of Neurobiology, Bulgarian Academy of Sciences, ²Faculty of Pharmacy, Department of Pharmacology, Toxicology and Pharmacotherapy, Medical University of Varna

Ellagic Acid (EA) is used in human diet as ingredient of many fruits and vegetables as pomegranate, grapes, etc. EA is known to have also chemopreventive action on metabolite disturbances, liver intoxications, cancer, dementia etc.

Scope of the study was to evaluate EA effects on hemistriatal intoxication with the neuronal and mitochondrial toxin 6-hydroxydopamine (6-OHDA) known to induce Parkinson’s disease (PD) symptoms in rodents.

The intoxication was produced via right striatal 6-OHDA (2 µg in 2 µl saline) injection of male Wistar rats. PD-symptoms were verified by apomorphine rotations test on the 2nd and 3rd week after the operation and were compared to sham operated animals. EA preventive effect (400 mg/kg i.p. 5 days) was evaluated via behavioral and biochemical tests-learning and memory (Step-through test), motor coordination (Rot-a-rod-test) and antioxidant status in brain (lipid peroxidation-LPO, glutathione, SOD, CAT, GPx).

Significant improving effect of EA on learning and memory processes and motor coordination in treated animals was established (both on 2nd and 3rd week) in compare to PD-controls. At the same time significant antioxidant effects of EA in brains were established -LPO is 45% lower and CAT 336% higher in EA group and some changes in glutathione and SOD, CAT, GPx were established. Interhemispheric differences were also observed (10 to 30%). EA probably “shields” lipid bilayers and thus reducing lipid peroxidation. As chelator and radical scavenger EA probably reduces free radical levels and in this way minimizes the mitochondrial and neuronal damage in 6-OHDA intoxicated animals.

Keywords: Parkinson’s disease, antioxidant, chemoprotection, polyphenols

Correspondence: and_atanasov@abv.bg
Oral Acute Toxicity of Candlenut Seeds (*Aleurites moluccana*) in Rats

Dennis Olivares¹, Nicolás Cáceres¹, María Fernanda Cavieres¹
¹Facultad de Farmacia, Universidad de Valparaíso, Valparaíso, Chile

Ingestion of candlenut seeds (*Aleurites moluccana*) induces severe abdominal pain, vomiting and diarrhea. However, these seeds are intentionally and extensively used as a “safe” and “effective” option for weight loss. Here we evaluate and describe the acute toxicity of seeds bought by contacting a provider on the internet. The protocol was approved by the Bioethics Committee of the Faculty of Pharmacy, Universidad de Valparaíso. 8 weeks old, female, Sprague-Dawley rats (n=3 per treatment), weighing 230 ± 20 g were dosed with an extract of 0, 1/2 or 1 seed in 2 ml of cold or boiling water. Animals were observed for up to 72 h, after which they were euthanized for necropsy and histopathology study. All animals dosed with candlenut seed extracts showed toxicity symptoms including gastrointestinal (diarrhea, constipation), neurotoxicity (central and autonomic). There were no differences in blood biochemistry or hemogram values, except for an increase in white blood cells. Necropsy showed abdominal distension, neurotoxicity and an increase of lymphoid tissue associated to the intestinal mucosa. Brain histopathology showed an increase of leucocytes while gastrointestinal tissues showed necrosis, edema, congestion and leucocytic infiltration. Effects may have a dose-dependency and did not vary between the cold or the boiling water extracts. We show that candlenut seeds induce in rats a gastroenterocolitic syndrome with necrosis of the first and second third of the microvilli which agrees with acute manifestations observed in humans. We also observed neurotoxicity not reported in humans, which may be due to different sensibility of rodents.

**Keywords:** Candlenut seeds, *Aleurites moluccana*, acute toxicity

**Correspondence:** fernanda.cavieres@uv.cl

---

Does Ursolic Acid Protect The Kidneys of Diabetic Rats from the Oxidative Stress?

Merve Bacanlı¹, Sevtap Aydın¹, Hatrice Gül Anlar¹, Tuğbagül Çağ¹², Nuray Ari³, Ülkü Ündeğer Bucurgat¹, A. Ahmet Başaran³, Nurşen Başaran³
³Hacettepe University Faculty of Pharmacy Department of Pharmaceutical Toxicology 06100 Ankara, Turkey

Diabetes, a heterogenous metabolic and chronic disease, is a growing health problem in most countries. It has claimed that diabetes is associated with the increased formation of free radicals and decreased antioxidant potential. Ursolic acid is a well-known pentacyclic triterpene which is commonly used in traditional Chinese medicine due to its health beneficial effects. The aim of this study was to investigate the effects of ursolic acid in the kidneys of Wistar albino rats with streptozotocin (STZ)-induced diabetes. For this purpose, the oxidative stress parameters such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GPx) enzyme activities and total glutathione (GSH) and malondialdehyde (MDA) levels were evaluated. It is observed that diabetes caused increases in GR enzyme activities and MDA levels, as well as decreases in GSH levels and CAT, SOD, and GPx enzyme activities. Ursolic acid treatment was found to significantly decrease GR enzyme activities and MDA levels and significantly increase GSH levels and CAT, SOD, and GPx enzyme activities in diabetic rats. According to our results, it seems that ursolic acid might be beneficial against diabetes induced renal damage.

**This study was funded by a grant from The Scientific and Technological Research Council of Turkey (Project number: 114S919).**

**Keywords:** ursolic acid, oxidative stress, kidney

**Correspondence:** mervebacanli@gmail.com
PREVIEW OF DEONTOLOGICAL BEGINNINGS OF TOXICOLOGY IN SERBIA

Bojana Petrović1, Vesna Matović2, Predrag Vukomanović3, Veljko Todorović4
1 Medical Sanitary School of Applied Sciences “Visan”, Belgrade, Serbia, 2 Faculty of Pharmacy, University of Belgrade, 3 Military Medical Academy

The health situation in Serbia began to change significantly in 1830 when Serbia was recognized for constitutional status of the principality, political autonomy, approved military, police, judiciary, health, education, institution formation. After the opening of the first pharmacies, the development of Serbian pharmacy and toxicology was on the rise. However, the situation in these areas was unsettled, as general stores, in addition to other goods, made and sold drugs and poisons. Legislation was necessary, and in 1865 Prince Mihailo Obrenovic passed the “Law on Pharmacies and Pharmacists, Holding, Selling Drugs and Poisons”, which contributes to regulation of the pharmaceutical practice. On the basis of this law, the Minister of Internal Affairs issued “Pharmaceutical tax for Citizens, Public and Private Pharmacies” and “Short Serbian Pharmacopoeia”. The law defines the rules and ethical standards for the keeping and distribution of drugs and toxins. In addition pharmacies were obliged to make toxicological and chemical analyzes for the needs of state authorities. The law prescribed a list of allowed substances in pharmacies, among which there were extremely poisons: cantaridine, colchicine, strychnine, compounds of arsenic, lead, mercury. In Pharmacopoeia there were recipes for the preparation of the extractive compositions of distinct toxins and opiates, teriyaki, colchicine wine and tincture, cantharidin tincture and patches, extracts of helleborus, hemlock, aconite, opium. Besides the fact that it has laid the foundation of modern toxicological deontology, it testifies to the level of health culture, and provides a complete picture of Serbia in the second half of the XIX century.

Keywords: poisons, history, XIX century, laws
Correspondence: bojanapetrovich@yahoo.com

THE INFLUENCE OF ALCHEMY ON THE DEVELOPMENT OF TOXICOLOGY

Aleksandar Vidaković5, Bojana Petrović2, Predrag Vukomanović3, Milica Nikolić4, Slavoljub Jović6, Veljko Todorović4
1 Serbian Institute of Occupational Health “Dr Dragomir Karajović”, 2 Medical Sanitary School of Applied Sciences “Visan”, Belgrade, Serbia, 3 Faculty of Veterinary Medicine University of Belgrade, Serbia, 4 Military Medical Academy

Alchemy covers a period of nineteen centuries (300 B.C.-1600 A.D.), from the Greek philosophers to the chemists in the 17th century. It was developed on the territory of ancient Egypt, the Middle East, India, China, ancient Greece and Rome. Decline and stagnation of medieval European medicine and pharmacy, and the strengthening of mysticism and superstition, alchemy begins to flourish. Alchemy had two basic approaches, practical skill of possible transformation of non-precious metals a precious, and spiritualistic, soul-purification and the attainment of immortality. Despite the fact that both approaches to alchemy were unsuccessful, the importance of alchemy in the development of toxicology and science is extremely important. The toxicological contribution is reflected in the identification of many toxic substances, and their application. Thanks to the continuous work of alchemists in the search for the Philosopher’s stone, the elixir of life, gold, they found new chemical substances and compounds, the Royal water (a mixture of nitric and hydrochloric acid), ethanol, nitrate, mercury, iron, copper, antimony, arsenic, bismuth, phosphorus, etc. In the late Middle Ages, alchemists used different poisons for therapeutic purposes, opiates in analgesia, iron in the treatment of anemia, mercury in venereal diseases, arsenic in skin diseases, having regard to the influence of the dose on toxicity. In the treatment of alcoholism, the emetic effect of antimony tartarate was used, obtained in reaction of antimony with wine. It is especially interesting to point out that the foundations of modern toxicology and science are the result of numerous accidental discoveries by alchemists.

Keywords: alchemy, poisons, metals, Philosopher’s stone
Correspondence: bojanapetrovich@yahoo.com
Paracelsus - a Man behind a Myth

Vesna Matović, Bojana Petrović, Predrag Vukomanović
1Faculty of Pharmacy, University of Belgrade, 2Medical Sanitary School of Applied Sciences, “Visan”, Belgrade, Serbia

Philippus Aureolus Theophrastus Bombastus von Hohenheim-Paracelsus (1493–1541) was one of the most famous alchemists, a Swiss physician, chemist, the “Father of toxicology”. The most accurate definition of drugs: “All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy”, by Paracelsus is the basic toxicology principle. In spite of criticism of colleagues and toxicity of metals he advocated their usage, antimony for treatment of digestive problems and mercury for syphilis. However, mercury was tacitly used due to the rapid spread of syphilis epidemic. Paracelsus first connected drinking water minerals with goiter. He studied miners diseases in Carinthia (present-day Austria), caused by inhalation of mercury and arsenic fumes. Despite the undisputed contribution to medicine and toxicology, Paracelsus was a controversial figure, confirmed by the fact that he chose pseudonym Paracelsus (“beyond” Roman physician Celsus) to express contempt for the doctors and scientists of that time. During his tenure as professor of medicine at the University of Basel, he publicly burned the most authoritative medical textbook The Canon of Medicine of Avicenna, and declared Galenus’s teaching obsolete. His hard temper led to conflicts with leading scientist of that time, who was Luther of medicine” because his papers and lectures were in German and not in Latin that was common practice. In spite of turbulent career, he introduced the more rational approach to diagnosis and therapy, and the usage of chemical instead of herbal drugs.

Key words: alchemy, Paracelsus biography, poisons, doses

Correspondence: vevodi@pharmacy.bg.ac.rs

Poisons Information Service of South Africa

Kate Balm, Linda Curling, Catharina du Plessis, Carine Marks, Farahnaz Mohamed, Cindy Stephen, Cherylynn Wium
1TPIC, Division Clinical Pharmacology, Stellenbosch University, Cape Town, South Africa, 2RXHPIC, Department of Paediatrics and Child Health, Red Cross War Memorial Children’s Hospital, Cape Town, South Africa

South Africa initiated a Poisons Information service over 45 years ago, which was strengthened by the establishment of the Tygerberg Poison Information Centre (TPIC) and Red Cross Children’s Hospital Poisons Information Centre (RXHPIC). In 2015, the two centres combined their telephone service to form the Poisons Information Helpline of the Western Cape (PIHWC). The PIHWC provides a 24/7 consultant based telephone service to medical professionals and the public. Consultants, known as specialists in poisons information (SPIs), assist with the prevention, diagnosis and management of poisoning. Seven of the fifteen SPIs are medical doctors, one of whom is always available to provide clinical support to non-medical SPIs. Data from poisoning calls are entered, real-time, onto a server-hosted database, the AfriTox TeleLog. The locally-compiled AfriTox poisons information database underpins call data entry, providing for both substance definitions and treatment advice given. The PIHWC provides a system of toxicovigilance, which contributes epidemiological data on poisoning, describes new hazards, provides information on national trends, and identifies potentially vulnerable populations. Where necessary, the WHO is informed about any potentially serious or unusual public health impacts. The PIHWC is actively involved in education and research. It offers regular toxicology workshops and formal lectures to under- and post-graduate students. Public education and information programmes on prevention and immediate management of acute poisoning are essential activities. Research findings are regularly published in peer-reviewed journals and staff members actively participate in national and international conferences, as well as international WHO workshops.

Keywords: Poison information helpline, poisonings data, toxicovigilance

Correspondence: caw@sun.ac.za

Almost Four Decades of History of Spanish Association of Toxicology (AETOX)

Font Guillermina, Ana María Cameán
1Laboratory of Toxicology and Food Chemistry, Faculty of Pharmacy, University of Valencia, Valencia, Spain, 2Area of Toxicology, Faculty of Pharmacy, University of Seville, Seville Spain

The Spanish Association of Toxicology (AETOX) was founded in 1980. It is an entity with more than 300 researchers, professionals and professors of Toxicology of Spain and other countries covering all the fields of Toxicology.
The main objective of AETOX is to promote the relationship and cooperation among its members and with other international societies of Toxicology to coordinate their efforts, in order to contribute to the development and dissemination of scientific knowledge in the different areas of the Toxicology.

The Society edits the “Revista de Toxicología” since 1983 maintaining homogeneity in its editorial line and the purposes of the publication. It is an open access journal, which is designed to facilitate the dissemination of current research in the field of Toxicology.

AETOX has the Spanish Register of Toxicologists to include all those professionals specialized in applied or basic Toxicology who apply for registration. Recognizing and accrediting the abilities and toxicological activities of those people registered, and disseminate this information who may be interested is the objective.

AETOX regularly organizes congresses every two years. The last one was celebrated in Valencia in 2017 being the XXII Spanish Congress of Toxicology and VI Iberomerican. In 2019, the congress will be celebrated in Seville. In addition, sessions, meetings and workshops, on topics of interest, are organized by the different sections.

AETOX primary goals consists of improving health conditions of living beings and the protection of the environment motivating young researchers in the field of Toxicology.

Keywords: Toxicology, society, Spain

Correspondence: guillermina.font@uv.es

An Overview of the Accomplishments of the German MAK Commission

Kyriakoula Ziegler-Skylakakis
Scientific Secretariat of the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Freising, Weihenstephan, Germany

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, the MAK-Commission, is a Permanent Senate Commission of the German Research Foundation, Deutsche Forschungsgemeinschaft (DFG) and was established in the year 1955. Its task cording to DFG regulations is to provide scientific policy advice. Its innovative work has helped improve occupational safety and health in Germany.

In its 62 years of existence, the MAK Commission has evaluated over 1000 chemicals. It has established MAK-values and BAT-Values, as well as having developed approaches to strengthen scientific knowledge about potential hazardous substances in the work area. Further tasks include establishing and examining analytical methods to detect hazardous chemicals in the air or in biological materials.
In the poster, the milestones of the work accomplished by the MAK Commission during the past six decades will be highlighted. In addition the methods used for the derivation of the MAK-values, and for the designation of chemicals as to their carcinogenic potential, their sensitizing potential, their germ cell mutagenic potential and their contribution to systemic toxicity after percutaneous absorption will be presented. The cooperation taking place with different national and international Institutions will be also pointed out.

**Keywords:** MAK-values, BAT-values, health at the work area

**Correspondence:** kyriakoula.ziegler-skylakakis@lrz.tum.de

---

**About the Toxicological Risk Assessment Center in Serbia**

**Biljana Antonijević, Danijela Đukić-Ćosić, Marijana Ćurčić, Evica Antonijević**

*Department of Toxicology “Academic Danilo Soldatović”, University of Belgrade – Faculty of Pharmacy*

Toxicological Risk Assessment Center (TRAC) at the Faculty of Pharmacy, University of Belgrade was established in 2013 and represents a unique institution of its kind in the Republic of Serbia. In 2017, the Center became a member of the World Health Organization Chemical Risk Assessment Network. Two principal goals of the center are training and research through networking and coordinating activities between academia and other professionals dealing with chemicals safety. The research interests primarily focus on mixture toxicology with particular emphasis on understanding the links between the environment and health risks, the mechanistic aspects of toxicity and how emerging knowledge could be translated into prevention strategies. The mission of the TRAC is to promote public/chemical safety and regulatory initiatives, and to train professionals on human health risk assessment by organizing seminars, workshops and consultancy. Short-term trainings and info days regarding hazard assessment of chemicals, REACH implementation, safety assessment of plant protection products, biocides and cosmetics have been designed to increase the national capacity to manage environmental, toxicological and health issues. Being a member of a global network, TRAC is open for true collaboration across scientific community, and with regulatory and industry players, all united in the vision to improve and promote the science of toxicological risk assessment.

**Keywords:** risk assessment, center, mission, vision

**Correspondence:** biljana.antonijevic@pharmacy.bg.ac.rs

---

**History of Undergraduate Teaching in Toxicology at the University of Belgrade, Faculty of Pharmacy**

**Danijela Đukić-Ćosić, Katarina Baralić, Marijana Ćurčić, Evica Antonijević, Aleksandra Buha Đorđević, Zorica Bulat, Dragana Vujanović, Mirjana Đukić, Biljana Antonijević, Vesna Matović, Mirjana Nedeljković**

*Department of Toxicology “Academic Danilo Soldatović”, University of Belgrade – Faculty of Pharmacy, Serbia*

Undergraduate teaching in Toxicology has been carried out as an independent subject at the University of Belgrade for almost 80 years. It was first introduced under the name Toxicological Chemistry with the establishment of Department of Pharmacy at the Medical Faculty in 1939. The first professor of Toxicological Chemistry and chief of the Institute of Toxicological Chemistry was Dr. Momčilo Mokranjac (a student of Gabriel Bertrand and later a member of L’Académie de Pharmacie de Paris), and in 1964, he was succeeded by Dr. Danilo Soldatović. Apart from Pharmacy study program, another study program, Pharmacy-Medical Biochemistry, was introduced at the University of Belgrade, Faculty of Pharmacy in 1984. In 1987, the second mandatory subject, Clinical Analyses in Toxicology, was added to the curriculum of the new study program. With the start of Bologna Process in 2008, the latest curriculum of the Faculty of Pharmacy defined the mandatory course Toxicology with Analytics for both study programs and Clinical Analyses in Toxicology for Pharmacy-Medical Biochemistry. These courses are carried out through lectures, case studies analysis, and practical training. This enables students to acquire the necessary knowledge in general and special toxicology, as well as the skills relevant for laboratory work, with emphasis given to the sample preparation, detection, and determination of the most important poisons. In addition, numerous elective courses are available for both study programs: Acute Drug Poisoning with Analytics, Human Health Risk Assessment, Substances of Abuse with Analytics, Free Radicals and Antioxidants-laboratory analyses, Ecotoxicology, Toxicology in Practice and Chemical Carcinogens.
Paracelsus - the Founder of Toxicology Science

Danijela Đukić-Ćosić¹, Steven Gilbert²
¹Department of Toxicology "Academic Danilo Soldatović", University of Belgrade-Faculty of Pharmacy,  
²Institute of Neurotoxicology & Neurological Disorders, Washington

Paracelsus (Philippus Theophrastus Aureolus Bombastus von Hohenheim, 1493-1541), a contemporary of Leonardo da Vinci, Martin Luther, and Nicholas Copernicus, was a Swiss-German physician, chemist, botanist, astrologer, alchemist, and general occultist. As headstrong, stubborn and independent young man, he decided to be a physician/chemist like his father. He studied at a number of universities in Europe, receiving his baccalaureate in medicine in 1510 and doctorate in 1516 from the University of Ferrara (Italy). Paracelsus stated that knowledge plus experience makes an expert, and therefore traveled throughout Europe, Africa, and Asia Minor to broaden his knowledge and gain experience. His influence on toxicology was enormous. He very clearly expounded the dose-response concept, suggesting that "the dose makes the poison". He used this saying to defend the use of inorganic substances in medicine because his critics claimed that they were too toxic to be applied as therapeutic agents. Apart from the basic concept in toxicology, Paracelsus was the creator of the idea of target organ toxicity. His belief that diseases locate in a specific organ was extended to include target organ toxicity, which means that a chemical has a specific site within the body where it exerts its greatest effect. Paracelsus also encouraged the use of experimental animals to study the beneficial, as well as toxic effects of chemicals. In addition, he wrote the paper On the Miners' Sickness and Other Diseases of Miners documenting the occupational hazards of metalworking, including treatment and prevention strategies. While clearly a pioneer in toxicology, his concept of "dose makes the poison" needs to be considered in the light of our new knowledge of low dose effects and vulnerable populations.

Keywords: father of toxicology, physician/chemist, alchemist, dose response concept

Correspondence: danijela.djukic.cosic@pharmacy.bg.ac.rs

The Secret of Paracelsus' Sword

Danijela Đukić-Ćosić¹, Philip Wexler²
¹Department of Toxicology "Academic Danilo Soldatović", University of Belgrade-Faculty of Pharmacy,  
²National Library of Medicine, Washington

Philippus Theophrastus Aureolus Bombastus von Hohenheim, better known as Paracelsus (1493-1541), was a German-Swiss physician who introduced the dose-response concept to toxicology. The influence of this famous doctor on other sciences, including chemistry, medicine, physiology, and philosophy was enormous as well. Paracelsus' portrait dating from 1540, depicts him holding his sword, gripping its spherical pommel. Posthumous portraits of Paracelsus and his statues in Beratzhausen, Bavaria, where he prepared Paragranum, his main work on medical philosophy, show him in the same pose, his hand on the sword's pommel. He came in possession of this famous longsword during the wars when he served as an army physician. The word "Azoth" was inscribed on the handle of the sword. According to some broadsheets, Azoth was the elixir vitae (mercury), a secret medicine Paracelsus had discovered and kept hidden in the handle of his sword. Paracelsus theorized that materials which are poisonous in large doses may be curative when used in small doses; he demonstrated this with the examples of magnetism and static electricity, wherein a small magnet can attract much larger metals. He was acquainted with the therapeutic powers of the magnet and used it in the treatment of various diseases. His doctrines in regard to effects of magnetism on humans have been confirmed to a great extent since the time of his death. If his sword was forged from magnetite, an iron oxide and the primary component of naturally occurring iron ore, it might suggest that it was used as magnetic therapy.

Keywords: portraits of Paracelsus, long sword, magnet therapy

Correspondence: danijela.djukic.cosic@pharmacy.bg.ac.rs
Meet prof. Momčilo Mokranjac – the Father of Serbian Toxicology

Danijela Đukić-Ćosić, Vesna Matović
Department of Toxicology "Academic Danilo Soldatović", University of Belgrade – Faculty of Pharmacy, Serbia

Prof. Momčilo Mokranjac (1899-1967) was a respected professor at the University of Belgrade and the founder of education in the field of toxicology in our country. He was born in Belgrade in the family of the most famous Serbian composer, Stevan Mokranjac, and Marija Mokranjac, born Predić, a relative of Uroš Predić (one of the most important Serbian painters). Nevertheless, his education was aimed at the natural sciences, far from the music and art. He graduated from the Faculté des Sciences in Paris in 1920, and two years later defended the doctoral thesis (Recherches sur la présence du zinc, du nickel et du cobalt dans les terres arables) at Sorbonne under the supervision of Gabriel Bertrand, “father of microelements”. Upon returning to Belgrade, Dr. Mokranjac worked as a chemist in several institutions. In 1946, he was elected the first professor of Toxicological Chemistry at the Faculty of Pharmacy, University of Belgrade. He was the vice-dean (1947-1949), and then dean (1949-1952) of the Faculty, and spread the importance of pharmaceutical education in our country. Prof. Mokranjac published a book in Serbian Toxicological Chemistry and over 60 important scientific papers. Therefore, he was elected a correspondent member of the Paris Academy of Pharmacy in 1954, and the following year awarded Lavoisie’s medal of the French National Academy of Pharmacy. Prof. Mokranjac was a member of numerous associations as well as the first chief of the Institute of Chemistry of the Serbian Academy of Sciences. This first professor of toxicology in Serbia was a professional, humanist, and “man of science”.

Keywords: professor, toxicological chemistry, Faculty of Pharmacy, University of Belgrade

Correspondence: danijela.djukic.cosic@pharmacy.bg.ac.rs

Founding of the Society of Toxicology
Society of Toxicology, USA

On Saturday, March 4, 1961, a small group met in Washington, DC, to talk about the need for providing a forum where toxicologists could meet and share their research findings. By the end of their day-long meeting the Founders had concluded that the advantages of forming a society outweighed the disadvantages. They had even suggested a name, “The Society of Toxicology” (SOT) and it was to be an international learned society drawing together persons trained in the various disciplines related to toxicology. The follow-up work from this organizational meeting required preparation of a draft constitution, bylaws, scheduling presentations at upcoming scientific meetings, and notifying key people in the field of their plans. Dr. Lehman accepted the role of Honorary President. Dr. Hodge was elected President; Dr. DuBois, Vice President; Dr. Deichmann, Treasurer; Dr. Hays, Secretary; Drs. Coulston, Drill, Larson and Shaffer, members of the Council. To finance all this, each attendee at the first meeting contributed $5 to the treasury. Accordingly, the Society of Toxicology was launched with assets of $35. In 2018, SOT remains a professional and scholarly organization of scientists from academic institutions, government, and industry representing the great variety of scientists who practice toxicology in the US and abroad. The membership has grown to 8,200+ members with 18 Regional Chapters, 6 Special Interest Groups, and 29 Specialty Sections. The SOT Annual Meeting provides a forum where scientists can share their research findings and learn about cutting-edge science and the impact of toxicology research on human and environmental health. The Society’s mission is to create a safer and healthier world by advancing the science and increasing the impact of toxicology. Recordings of the history of the Society of Toxicology are available via the website [https://www.toxicology.org/about/history/50anniversary.asp].

History of Olimpic Doping Control Laboratory at Faculty of Pharmacy in Sarajevo

Aleksandra Marjanović, Elma Omeragić, Jasmina Dedibegović, Miroslav Šober
Faculty of Pharmacy, University of Sarajevo, Zmaja od Bosne 8, Sarajevo, BiH

The most outstanding period in history and development of Faculty of Pharmacy, University of Sarajevo was during XIV Winter Olympic Games (ZOI’84) when the Doping control laboratory was established. This laboratory was organized within the Department for Quality Control of Drugs and Department for Toxicological Chemistry and originally financed by International Olympic Committee and Organizing Committee of ZOI’84. Laboratory was equipped with state-of-the-art chromatographic and spectroscopic equipment that existed in Bosnia and Herzegovina at that time. Laboratory was organized in six operational
departments: Department for Collecting and distribution of samples; Department for Extraction and derivatization of samples; Department for Thin Layer Chromatography; Department for Gas Chromatography and High Performance Liquid Chromatography; Department for hyphenated technique GC-MS and Department for Radioimmunologic Analysis. The multidisciplinary team was made up of 60 experts from different professions: pharmacists, doctors, chemists and computer scientists.

During January 1984, the Doping Control Laboratory was accredited by the International Olympic Committee and at that point there were eleven such laboratories in the world. At that time Laboratory at Faculty of Pharmacy together with the laboratory at Los Angeles University of California (UCLA), participated in the wider project of the International Olympic Committee, which aimed to establish reliable evidence for the abuse of exogenous testosterone in sports. After the Olympics, the Laboratory was re-accredited for the control of doping at international competitions in 1987 by the International Athletic Amateur Federation (IAAF).

Keywords: ZOI’84, doping control, Faculty of Pharmacy Sarajevo

Correspondence: aca1902@gmail.com

Former Yugoslav and Latter Serbian Society of Toxicology

Vesna Matović1, Dragan Joksović2, Veljko Todorović2, Aleksandar Vidaković3
1Department of Toxicology Akademik Danilo Soldatović*, University of Belgrade-Faculty of Pharmacy,
2Military Medical Academy, 3Serbian Institute of Occupational Health “Dr Dragomir Karajović”

Serbian Society of Toxicology is a national union of toxicologists, dealing with different fields of toxicology: clinical toxicology, environmental toxicology, occupational toxicology, etc. The Society was registered by Bussiness Registry Agency of Republic of Serbia on 4th of March 2010 with the purpose to gather the persons who are actively engaged or interested in research, teaching and application of toxicology. The main goal of the society is to foster the science and education of toxicology, promote the safety of humans, animals and the environment, and protect global health.

However, Yugoslav Society of Toxicology was established back in 1969 and the first Congress of Toxicology was organized in Herceg Novi, Montenegro in October, 1974. This Congress was followed by Congresses in Portorož, Slovenia (October, 1979), in Struga, FYR Macedonia (October, 1982), in Belgrade, Serbia (September, 1985) and in Brioni, Croatia (1990). During nineties, right after the Yugoslavia separation, Society continues to operate through the Section for toxicological chemistry of Pharmaceutical Society of Serbia and newly formed Section for toxicology of Serbian Medical Society. In this period, two congresses were held: VI Congress of Toxicologists on Tara in 1994 and VII Congress of Toxicologists in Igalo (1998) and the journal “Archives of Toxicology, Kinetics and Xenobiotic Metabolism” with international review was published quarterly, VIII and IX Congresses of Toxicology of Yugoslavia were organized on Tara, in 2002 and 2006, respectively.

Serbian Society of Toxicology organized X Congress of Toxicology of Serbia with international participation held in Palić in 2010 while XI Congress of Toxicology accredited as International Congress was held in Sremski Karlovci in 2014. Both congresses gathered eminent international scientists.

Serbian Society of Toxicology has been member of EUROTOX and IUTOX ever since 2010.

The joint10th Congress of Toxicology in Developing Countries and 12th Serbian Congress of Toxicology is going to be held in April 2018, 18-21, Belgrade, Serbia.

Keywords: toxicology, society, Yugoslavia, Serbia, congresses, mission

Correspondence: vevodi@pharmacy.bg.ac.rs

About the Milestones in Toxicology and Toxipedia

Dragica Jorgovanović, Katarina Baralić, Danijela Đukić-Ćosić
Department of Toxicology “Academic Danilo Soldatović”, University of Belgrade – Faculty of Pharmacy

Toxipedia is a free toxicology encyclopedia initiated by Steven G. Gilbert on June 16, 2006. It provides a way to share the knowledge about Toxicology for the benefit of the common good by offering articles and resources about toxic chemicals, health conditions, ethical considerations, history of toxicology, laws and regulation, etc. Toxipedia is a project of the Institute of Neurotoxicology and Neurological Disorders, a nonprofit organization that distributes scientific information about the health and environmental impacts of toxic chemicals, empowering the public
and policymakers to make choices that create healthy communities and environments.

Through the ages, Toxicology shaped civilization and taught us very important life lessons. Significant milestones and discoveries in Toxicology can be seen in the poster presentation, made by Steven G. Gilbert and Antoinette Hayes in 2006, available as an interactive version at www.asmalldoseof.org. This poster presentation has been translated into 16 different languages- Amharic, Arabic, Chinese, French, German, Italian, Japanese, Korean, Persian, Portuguese, Russian, Serbian, Slovenian, Spanish, Turkish and Vietnamese. Beside the beginnings of Toxicology, the examples in this poster address further milestones that occurred from antiquity to the postmodern era. The poster also explains how toxicology, which is now a separate science with multidisciplinary approach, interacted with other disciplines in the past, including religion and politics.

In 2016, 10 years after the founding of Toxipedia, Steven G. Gilbert was recognized by the Society of Toxicology with Public Communications Award for broadening the public’s understanding of toxicology.

**Keywords:** free toxicology encyclopedia, Steve Gilbert, Milestones of Toxicology

**Correspondence:** dragica.jorgovanovic@gmail.com

---

The Slovenian Society of Toxicology

Lucija Perharič, Jernej Kužner, Lucija Peterlin Mašič, Žiga Jakopin, Marjan Vračko
Gerbičeva 60, 1000 Ljubljana, Slovenia

Founded in March 2000, the Slovenian Society of Toxicology (SST), a member of EUROTOX and IU-TOX, is an independent, voluntary, non-profit organization of professionals working in toxicology. SST aims to foster and promote toxicology in Slovenia and to facilitate information exchange amongst various fields of toxicology (http://www.tox.si/). The SST is also a recognized stakeholder within the Slovenian chemical safety network (http://www.euro.who.int/__data/assets/pdf_file/0005/324293/Chemical-safety-protection-human-health-Slovenian-experience.pdf). The SST’s official journal is the Archives of Industrial Hygiene and Toxicology (https://archiv.imi.hr/index.php/archiv), co-edited with the Croatian Society of Toxicology. In January 2018, the SST counted 52 members from various professional backgrounds including biochemistry, biology, chemistry, food science, medicine, microbiology, pharmacy, sanitary engineering and veterinary medicine, working in a range of institutions. Four SST members are European Registered Toxicologists.

Within its mission, the SST organized a number of lectures and public debates, a symposium Toxicology (2001), a workshop Environmental Biomarkers in Bioindicators, supported by IUTOX (2002), and national Congresses with international participation (2004, 2015, 2017). The latest congress was devoted to pharmaceutical pollutants in the environment, risk communication and contradictions concerning glyphosate. To encourage education in toxicology the SST organized teaching courses in 2004 and 2005/06. The EUROTOX Basic Toxicology course held in Ljubljana in 2004, was the first of its kind. In co-operation with the University of Ljubljana, the SST prepared an MSc programme in toxicology, unfortunately not accredited due to financial restraints.

In 2018, the SST plans to organize a specialized EUROTOX course in Regulatory Toxicology and continue with the preparations for hosting the EUROTOX2021.

**Keywords:** Slovenia, Society of toxicology, mission, activities

**Correspondence:** slotox@gmail.com

---

Postgraduate Studies in the Field of Toxicology - Faculty of Pharmacy, University of Belgrade

Vesna Matović, Dragana Javorac, Evica Antonijević, Aleksandra Buha Đorđević, Marijana Curčić, Danijela Đukić-Čosić, Zorica Bulat, Dragana Vujanović, Mirjana Đukić, Biljana Antonijević, Mirjana Nedeljković

University of Belgrade – Faculty of Pharmacy, Department of Toxicology “Akademik Danilo Soldatović”

Since the founding of the Department of toxicology in 1939 postgraduate studies have been established for the students to advance towards the Master of Sciences degree which, from 1966 on, became a precondition for defending a doctoral thesis. In the 1960s, specialist studies began to be organized with the aim of educating experts in the field of toxicology for the needs of healthcare. In addition to these studies, since 2013, academic specialist studies have also been conducted.

Among the first doctoral dissertations at the Faculty of Pharmacy were dissertations in the field of toxicology: Contribution to the question of the application of polarography in toxicological and chemical analyses, by candidate Dušan Jovanović and Contribution to cobalt as a microelement, by
candidate Zagorka Pavković-Filipović, under the supervision of professor Momčilo Mokranjac in 1956. A few years later, in 1963, among the first three master theses at the Faculty of Pharmacy was Matej Makšimović’s thesis in the field of toxicology, also under mentorship of professor Momčilo Mokranjac. In 1970 Knežević Mileva became the first specialist of Toxicological chemistry. Since the founding of the Department of Toxicology until today, 27 doctoral theses, 20 master theses and over 60 specialist papers were defended.

Nowadays, the Department of Toxicology “Akađemik Danilo Soldatović” organizes postgraduate studies: PhD studies in Toxicology, specialist studies in Toxicological chemistry and specialist academic studies in Toxicological risk assessment, with the aim of acquiring and improving knowledge and skills in the field of toxicology.

Keywords: doctoral dissertations, master theses, specialist studies, Momčilo Mokranjac

The Toxicology Laboratory of Institute of Forensic Medicine in Belgrade – from State Chemistry to Reference

V. Lukić, K. Denić, B. Zdrale, D. Stojkov, D. Cvetskovic, V. Zivković, S. Nikolic
Institute of Forensic Medicine, School of Medicine, Belgrade University

In October 1859, Prince Milos Obrenovic signed the decree on the foundation of the chemistry laboratory within the Sanitary Department of the Ministry of Interior, where samples for the State would be analysed. Its first warden was Pavle Ilic. The first building of this, State Chemistry Laboratory was built in 1882 by Ferdinand Sems, the chemist and laboratory warden since 1873, in Kralja Milutina Street – where it still stands. In this laboratory, chemical analyses for forensic, medical and technological purposes were performed for the needs of all Ministries. From 1893 until 1920, laboratory’s third warden was Marko Leko. From 1929 until 1941, this laboratory was part of the Central Hygienic Institute. In 1947, its Toxicology Department became part of the Faculty of Medicine, specifically Toxicology Sector of the Institute of Forensic Medicine. After World War II, laboratory executives were Dusan Gregovic and Bisenija Hristic. The laboratory was still located in 25 Kralja Milutina Street and a small improvised laboratory was established at the Institute of Forensic Medicine for alcohol detection in biological samples. In 1980, this laboratory was officially moved to a new building of the Institute of Forensic Medicine, Faculty of Medicine. Around 150 years after its foundation, this laboratory became the Reference Laboratory for Identification and Determination of Psychoactive Controlled Substances.

Keywords: State Chemistry Laboratory, Toxicology Sector of the Institute of Forensic Medicine

The Toxicology Society of South Africa (TOXSA)

Werner Cordier, Mary Guluman, Carine Marks, Vanessa Steenkamp, Wilna van Rijssen, Robyn van Zyl, Melissa Vetten, Cheryllyn Wium
Toxicology Society of South Africa

TOXSA was established in 2001 with the aim of promoting and advancing the study and application of toxicology in all its aspects in South Africa. Our first national conference was held in 2002 and the Society was admitted to IUTOX in 2004. In 2012, following extensive negotiations through TOXSA, South African Council for Natural Scientific Professions (SACNASP) accepted Toxicological Sciences as a separate, recognized discipline. TOXSA has hosted a number of international and local conferences, including the 7th Congress of Toxicology in Developing Countries (7CDTC) in 2009, the 7th International Symposium on Nanotechnology, Occupational and Environmental Health (NanOEH) in 2015, and participated in the 17th World Congress of Basic & Clinical Pharmacology (WCP2014) in 2014. TOXSA has provided training in the form of risk assessment and hazard identification workshops and, recently, courses on basic, advanced, and clinical toxicology. Later this year, TOXSA is teaming up with various local societies to co-host the First Conference of Biomedical and Natural Sciences and Therapeutics (CoBNeST 2018) to be held in Stellenbosch, South Africa. In addition, TOXSA is proud to present a session at CTDC10 entitled “Arachnids: Fallacies, Clinical Manifestations, Differential Diagnosis and Management of Spider Bite and Scorpion Sting”, and looks forward to fruitful discussions and possible collaborations.

Keywords: TOXSA, South Africa, Africa, societies

The Toxicology Society of South Africa (TOXSA)

Werner Cordier, Mary Guluman, Carine Marks, Vanessa Steenkamp, Wilna van Rijssen, Robyn van Zyl, Melissa Vetten, Cheryllyn Wium
Toxicology Society of South Africa

TOXSA was established in 2001 with the aim of promoting and advancing the study and application of toxicology in all its aspects in South Africa. Our first national conference was held in 2002 and the Society was admitted to IUTOX in 2004. In 2012, following extensive negotiations through TOXSA, South African Council for Natural Scientific Professions (SACNASP) accepted Toxicological Sciences as a separate, recognized discipline. TOXSA has hosted a number of international and local conferences, including the 7th Congress of Toxicology in Developing Countries (7CDTC) in 2009, the 7th International Symposium on Nanotechnology, Occupational and Environmental Health (NanOEH) in 2015, and participated in the 17th World Congress of Basic & Clinical Pharmacology (WCP2014) in 2014. TOXSA has provided training in the form of risk assessment and hazard identification workshops and, recently, courses on basic, advanced, and clinical toxicology. Later this year, TOXSA is teaming up with various local societies to co-host the First Conference of Biomedical and Natural Sciences and Therapeutics (CoBNeST 2018) to be held in Stellenbosch, South Africa. In addition, TOXSA is proud to present a session at CTDC10 entitled “Arachnids: Fallacies, Clinical Manifestations, Differential Diagnosis and Management of Spider Bite and Scorpion Sting”, and looks forward to fruitful discussions and possible collaborations.

Keywords: TOXSA, South Africa, Africa, societies

The Toxicology Society of South Africa (TOXSA)

Werner Cordier, Mary Guluman, Carine Marks, Vanessa Steenkamp, Wilna van Rijssen, Robyn van Zyl, Melissa Vetten, Cheryllyn Wium
Toxicology Society of South Africa

TOXSA was established in 2001 with the aim of promoting and advancing the study and application of toxicology in all its aspects in South Africa. Our first national conference was held in 2002 and the Society was admitted to IUTOX in 2004. In 2012, following extensive negotiations through TOXSA, South African Council for Natural Scientific Professions (SACNASP) accepted Toxicological Sciences as a separate, recognized discipline. TOXSA has hosted a number of international and local conferences, including the 7th Congress of Toxicology in Developing Countries (7CDTC) in 2009, the 7th International Symposium on Nanotechnology, Occupational and Environmental Health (NanOEH) in 2015, and participated in the 17th World Congress of Basic & Clinical Pharmacology (WCP2014) in 2014. TOXSA has provided training in the form of risk assessment and hazard identification workshops and, recently, courses on basic, advanced, and clinical toxicology. Later this year, TOXSA is teaming up with various local societies to co-host the First Conference of Biomedical and Natural Sciences and Therapeutics (CoBNeST 2018) to be held in Stellenbosch, South Africa. In addition, TOXSA is proud to present a session at CTDC10 entitled “Arachnids: Fallacies, Clinical Manifestations, Differential Diagnosis and Management of Spider Bite and Scorpion Sting”, and looks forward to fruitful discussions and possible collaborations.

Keywords: TOXSA, South Africa, Africa, societies

The Toxicology Society of South Africa (TOXSA)

Werner Cordier, Mary Guluman, Carine Marks, Vanessa Steenkamp, Wilna van Rijssen, Robyn van Zyl, Melissa Vetten, Cheryllyn Wium
Toxicology Society of South Africa
A Brief History of the Department of Toxicology “Academic Danilo Soldatović” at the Faculty of Pharmacy, University of Belgrade

Danijela Đukić-Čosić, Evica Antonijević, Aleksandra Buha Đorđević, Marijana Ćurčić, Zorica Bulat, Dragana Vujanović, Mirjana Đukić, Biljana Antonijević, Vesna Matović, Mirjana Nedeljković

The current Department of Toxicology “Academic Danilo Soldatović” was established as the Institute of Toxicological Chemistry at the Faculty of Pharmacy, University of Belgrade in 1946. The first chief of the Institute and professor of Toxicological Chemistry was prof. Momčilo Mokranjac. At the time, the Institute consisted only of a small room at the University Children’s Clinic, where practical work with students and scientific research were performed. After three years, thanks to the efforts of prof. Mokranjac, the Institute developed and expanded. The Institute of Toxicological Chemistry had separate laboratories for students, professors and assistants within the Institute of Pathology building, as well as a physical chemistry laboratory. After 45 years of establishment, it was transferred to the dedicated premises of the new building of the Faculty of Pharmacy, where teaching and a part of the scientific research are carried out today. In 2006, the Institute of Toxicological Chemistry changed the name into the Department of Toxicology “Academic Danilo Soldatović”. Even today, this Department provides the widest education in the field of toxicology, not only in Serbia, but throughout the former Yugoslavia. Apart from education in undergraduate and postgraduate studies, its academic staff participates in numerous national and international projects, and works with the expert group of ministries and agencies in Serbia responsible in the field of chemicals, biocides, plant protection products, genotoxic impurities, psychoactive substances, cosmetics. Also, the Department cooperates with scientific and professional institutions in the country and abroad in order to improve the knowledge about toxic substances.

Keywords: Institute, education of Toxicology, scientific research, Serbia

Correspondence: danijela.djukic.cosic@pharmacy.bg.ac.rs

Danilo Soldatović-Great Toxicologist, Pharmacist and Man

Uroš Čakar, Vesna Matović
Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11000 Belgrade, Serbia

History of toxicology in Serbia does not remember many experts who were known in the world too. Professor Danilo Soldatović is one of them, and he left a significant mark in the world toxicology. He gained university education from the Faculty of Pharmacy, University of Belgrade in 1952, and PhD degree in toxicology on the same institution in 1959 where he continued professional career. He was also exceptional volleyball player and member of National team. During the years he brought to homeland new achievements from the field of toxicology, which he acquired from famous professors in France, Belgium and Switzerland. Besides his activity in academia and science, he left a great mark in the public health, especially regarding the professional poisoning and ecotoxicology. His most significant research was regarding the ability of magnesium as an antagonist of toxic metals. His exceptional scientific findings from toxicology were recognized by French National Academy of Medicine. In 2006, Danilo Soldatović was first pharmacists from Serbia who became a member of it respectful institution. The achievements of professor Soldatović point out that he was important person for the Serbian toxicology, pharmacy and ambassador of our science in the world.

Keywords: toxicology, pharmacy, magnesium

Correspondence: uroslion@gmail.com

The History of the National Poison Control Centre in Serbia

Slavica Vucinic, Jasmina Jović-Stosic, Vesna Kilibarda, Nataša Perkovic-Vukcevic, Snezana Djordjevic, Gordana Babić, Dragana Djordjevic, Olivera Potrebic, Gordana Vukovic-Ercegovic, Tomislav Rezic, Gordana Brajkovic
National Poison Control Centre, Military Medical Academy, Belgrade, Serbia

The National Poison Control Centre (NPCC) is a national referential institution in which the medical services of prevention and therapy of poisoning by chemical agents, detection of chemical substances in biological and other materials, education and scientific research work in the field of toxicology and pharmacology are carried out.
The forerunner of the present Centre is the Department of Toxicology of the Military Medical Academy (MMA), established in 1961, with the main task to treat acute poisonings and study the poisoning that are of particular interest to the medical corps service. The first Head of the Department was col. dr Mirko Kramer. In 1984 the Department became a Clinic for toxicology.

The toxicology we practice today is a complex scientific discipline, developed in accordance with the growing risks for human health and the environment. With this regard, we can speak about the serbian analytical, experimental and clinical toxicology that have functioned separately in the Army for decades. According to the guidelines of the WHO, IPCS and the EAPCCT, the Ministry of Health of the former Federal Republic of Yugoslavia, established the NPCC in 1997. Since its foundation up to now the NPCC has grown into the most prestigious institution of its kind in South-Eastern Europe. The Heads of the NPCC were col.prof. dr Dusan Jovanovic (1998-1999; 2007-2010) and col. prof. dr Dragan Joksovic (1999-2007). Since 2010 to date Prof. dr Slavica Vucinic is the Head of the NPCC.

In terms of medical prevention and rational treatment of human poison exposures in Serbia, the current organization of NPCC has so far proven to be highly effective, which was recognized by the serbian government that has declared the NPCC as the institution of national importance for the defense of the country.

**Keywords:** poison control centre, toxicology, poisonings

**Correspondence:** slavicavunicic406@gmail.com

---

**HUMAN AND ENVIRONMENTAL RISK ASSESSMENT**

**Positive Results From a Four-year Educational Campaign on Proper Disposal of Pharmaceutical Waste**

Predrag Vukomanović1, Bojana Petrović1

1Medical Sanitary School of Applied Sciences, “Visan”, Belgrade, Serbia

Pharmaceutical waste includes medicines, primary packaging, and accessories for the use, which have become unusable due to expiration date, defects in quality, contamination, or unusable for other reasons. Incorrectly disposed pharmaceutical waste can cause toxic, corrosive, teratogenic, infectious, carcinogenic and other harmful effects on living beings and the environment.

The educational campaign was conducted with the aim of raising public awareness about the importance of proper disposal of expired medicines.

It was conducted in the period from 01/01/2014-31/12/2017. at the Health Center “Lekovita”, Mladenovac, which has 12 pharmacies. In each drug store, it was clearly pointed out that the expired drugs can be brought by citizens and other pharmacies. The container was prominently located (a red-black hard plastic waste bucket, with a good breathing lid). Education was carried out in pharmacies via flyers and personal contact with pharmacists. The results of the action were followed by measuring the amount of waste material annually.

During the first three years of the campaign, the amount of waste material was collected on average over 20kg, in 2014, 20.5kg, 2015, maximum quantity 26 kg, and 2016, 24 kg. During 2017, lowest quantity of waste was recorded 16 kg, with a greater response of citizens with small amount of expired drugs which indicates that the citizens reacted positively to the campaign and deposited waste in the previous years, so they only disposed of current. Perennial constant work with patients gave positive results in terms of increasing awareness about the necessity of the proper disposal of drugs.

**Keywords:** management of pharmaceutical waste, environment, compliance, hazardous waste

**Correspondence:** bojanapetrovich@yahoo.com
Exposure Assessment of Female Population to Dibutyl Phthalate (DBP) via Two Cosmetic Products: Application of ConsExpo Tool

Stefan Simeunovic1, Marijana Curcic1, Evica Antonijevic1, Katarina Baralic1, Gonca Cakmak2, Ksenija Durgó3, Biljana Antonijevic1, Danijela Djukic-Cosic1
1University of Belgrade, Faculty of Pharmacy, Department of Toxicology “Akademik Danilo Soldatović”, Serbia, 2Gazi University, Faculty of Pharmacy, Department of Toxicology, Turkey, 3University of Zagreb, Faculty of Food Technology and Biotechnology, Department of Biochemical Engineering, Laboratory for Biology and Microbial Genetics, Hrvatska

Exposure assessment is an important step in determining risks to chemicals in consumer products, including cosmetics, cleaning and pest control products. Exposure models can be used to estimate exposures to chemicals in the absence of biomonitoring data and as tools in chemical risk screening. We have applied a modelling tool ConsExpo (Consumer Exposure) version 5.0 (RIVM, NL) to assess exposure to dibutyl phthalate (DBP), a chemical present in a variety of consumer products, raising health concerns regarding developmental and/or reproductive toxicity.

As a case study, we quantified exposure of female population to dibutyl phthalate (DBP) via two cosmetic products, hair spray and nail polish, containing DBP as plasticizing agent.

Input data on female’s physiology and product usage habits relevant for calculations were selected from ConsExpo default database. The data on DBP weight fraction in products were obtained from available literature. Inhalation and dermal routes were considered relevant for aggregative exposure scenario. Estimated potential chronic dermal exposure (external dose) to DBP was 0.36 mg/kg/day (0.013 and 0.36 mg/kg/day for the hair spray and nail polish, respectively) while the calculated chronic systemic dose (internal dose) was 0.012 mg/kg/day (0.012 and 6.1x10^-6 mg/kg/day for the hair spray and nail polish, respectively). The calculated exposures by inhalation route were negligible. Thus, dermal exposure route was shown to be dominant for both products. Potential dermal exposure was shown to be higher to DBP in nail polish compared to hair spray. However, systemic dose was result of almost only dermal exposure to DBP in hair spray.

Keywords: systemic dose, aggregative, dermal, hair spray, nail polish

Correspondence: danijela.djukic.cosic@pharmacy.bg.ac.rs

National Inventory of Mercury Releases in the Republic of Macedonia

Elisaveta Stikova1, Aleksandar Mickovski2, Marjan Mihajlov3, Trajče Stafilov4
1Institute of Public Health, Medical Faculty, Sts. Cyril and Methodius University, 2POPs Unit, MOEPP Macedonia, 3MANEKO Solutions, 4Institute of Chemistry, Faculty of Science, Sts. Cyril and Methodius University

The intentional use of mercury in products and processes is a large anthropogenic source of Hg to the global environment. The purpose of this paper is to show the sources of the mercury released in the country, to estimate and quantify of those releases and to distribute the same in the corresponding output pathways.

The identification was made by inventory of the known sources with subsequent quantification in main 10 source categories. The distribution in the corresponding output pathways was made by performing analysis of the input factors and output fractions or by using the default factors provided in the UNEP’s toolkit. The inventory was developed in December 2017 and referent base year is 2013.

The results showed that the total mercury release in the country is 3.282,77 kg/Hg/y. The most significant category is waste deposition/landfilling and waste water treatment, contributing with 1,476.8 kg/Hg/y of the total mercury releases. The second most significant source is the waste incineration and burning with total release of 382,90 kg/Hg/y (12%). The leading output of the released mercury is in the air, with total amount of the 1,225.34 kg/Hg/y (37,3%), following by land with 977.79 kg/Hg/y (29.8%). The individual mercury release sub-categories contributing with the highest inputs of new mercury are: production of recycled ferrous metals, coal combustion, thermometers with mercury, dental mercury amalgam and the cement production.

The national inventory on mercury releases shall present a basis for establishing a national inventory of emissions from relevant sources.

Keywords: inventory, mercury release, output pathways

Correspondence: estikova@ukim.edu.mk

Integrated Approaches to Testing and Assessment (IATA) for Risk Assessment: Scoping of Available Guidance with an Emphasis on Uncertainty Assessment

Andrea-Nicole Richarz, Stephanie K Bopp, Raffaella Corvi, Andrew P Worth
European Commission Joint Research Centre, Directorate for Health, Consumers and Reference Materials, Ispra (VA), Italy

Integrated Approaches to Testing and Assessment (IATA) constitute a framework for considering and integrating multiple data sources in a weight of evidence (WoE) to conclude on the hazard/risk of chemicals in view of informing regulatory decision-making. IATA are composed of different, ideally mechanistically derived, building blocks such as data from Test Guideline and non-standard studies, high throughput in vitro assays, omics data, and in silico predictions. They can also be combined in Defined Approaches.

Uncertainty evaluation is recognised as a crucial part of risk assessment. All IATA components are associated with uncertainty at different levels, including overarching issues of data and methodological quality, adding uncertainties of the integration and WoE. It is important to analyse and transparently document all uncertainties, in order to allow for informed regulatory decisions.

A scoping exercise was undertaken to identify guidance related to the IATA components and overarching issues, from official regulatory sources to the peer-reviewed literature. Particular emphasis was placed on identifying practical elements such as user-friendly templates, and guidance on characterisation and reporting of uncertainties. It was concluded that guidance is available at different levels, however more addressing the “technical level” than the integration and weight of information. Many initiatives support uncertainty reduction via standardisation and good practice. However, the guidance is fragmented and sometimes duplicated across sectors, scientific areas, countries and legislations. Some overall integration and harmonisation will be beneficial, allowing to increase confidence in risk assessment results as well as to support the aim of mutual acceptance.

Keywords: weight of evidence, data quality, reliability, relevance, alternative methods

Correspondence: andrea.richarz@ec.europa.eu

Environmental Risk Assessment of Effluent Discharges into the Ogun River from Kara Cow Market, Ogun State, Nigeria

Esther Olaniran¹, Temitope Sogbanmu²
¹,²-Ecotoxicology and Conservation Unit, Department of Zoology, Faculty of Science, University of Lagos, Lagos, Nigeria

Effluent discharges into freshwater ecosystems are sources of pollutants which can have adverse effects on aquatic organisms. The study aim was to assess the environmental risk posed by the discharge of effluents from Kara cow market, Ogun state, Nigeria into the Ogun river. The studies which were conducted following standard methods include: administration of structured questionnaires to market stakeholders, fish species diversity, physico-chemical analysis, acute and sublethal toxicity studies (biochemical and histological indices) of the effluent using *Clarias gariepinus* (African Catfish) over a period of 28 days. The questionnaire survey showed that effluents were not treated and stakeholders were ignorant of the environmental risks. Some effluent physico-chemical parameters were higher than set limits. Total PAHs level in the effluent and sediment was 6.73 mg/L and 8.07 mg/kg respectively. Tetracycline levels in the effluent and surface water were 0.23 µg/mL and 0.85µg/mL respectively. Fish species diversity was lower at the test site (Margalef index - 0.45) compared the control (Margalef index - 0.71). The 96 hLC₅₀ of the effluent was 126 mL/L. Neither histological alterations nor significant biochemical changes were observed in the gills and liver of *C. gariepinus* exposed to sublethal concentrations of the effluent. The results demonstrate the adverse impacts of the effluent constituents on the physico-chemistry of the river and fish diversity though these were not evidenced in the biomarkers assessed. Consequently, it is recommended that more holistic evaluations of the effluent should be conducted in order to provide evidence-based risk communication to the public and stakeholders.

Keywords: abattoir wastewater, biomonitoring, *Clarias gariepinus*, freshwater ecosystems, persistent organic pollutants

Correspondence: tsogbanmu@unilag.edu.ng
assessments (RA). It also coordinates national activities concerning zonal registrations, mutual recognitions, and European Union active substance (a.s.) RA.

In 2003, ARSFSVPP established a multidisciplinary group of professionals with expertise in chemistry, efficacy, toxicology, ecotoxicology, environmental fate and behaviour and residues. The members were initially trained at the British Pesticide Safety Directorate and supported to continuously develop their expertise. The group currently consists of 15 professionals. The National Institute of Public Health (NIPH) was commissioned to carry out work related to human health. Three NIPH professionals assess risk according to the Regulation 1107/2009 and relevant international technical guidelines.

Since 2003, NIPH assessed 464 PPPs, acted as rapporteur or co-rapporteur for five a.s.: Azimsulfuron, Metsulfuron—methyl, Imazosulfuron, Rimsulfuron and Tritosulfuron. We actively participated in the PPP RA group at the European Food Safety Authority and the Expert Advisory Group for the endocrine disruptor (EDs) identification criteria. We surveyed plant protection first aid, decontamination and treatment instructions of PPP labels in order to bring them in line with the current guidelines. We regularly communicate to the professional and lay public, and participate in capacity building on national and international level.

In 2018, we plan to embark on identification of EDs in the context of Regulation 1107/2009, prepare RA monographs for Giberellic Acid and Gibberellins, to continue the routine and ad-hoc RAs and training activities.

Keywords: pesticides, human risk assessment, capacity building

Correspondence: lucija.perharic@nijz.si

One-year Study on Exposure Assessment to Marine Biotoxins via Consumption of Shellfish from the Black Sea, Bulgaria

Stanislava Georgieva1, Zlatina Peteva1, Bernd Krock2, Anelia Gerasimova-Peneva1, Mona Stancheva1
1Medical University - Varna, Department of Chemistry, Marin Drinov 55, 9002 Varna, Bulgaria,
2Alfred Wegener Institute, Helmholtz Zentrum für Polar- und Meeresforschung, Chemische Ökologie, am Handelshafen 12, 27570 Bremerhaven, Germany

Human poisonings after consumption of shellfish contaminated with microalgae toxins (phytoxins) have been recorded in Europe for several decades. In this regard EFSA has set limits of some marine biotoxins present in seafood. Although in Bulgaria no outbreaks have ever been reported, some of the regulated marine toxins - domoic acid (DA), pectenotoxin-2 (PTX-2) and yessotoxin (YTX) have been detected in mussel samples.

The aim of this study was the human acute and chronic exposure to these toxins through shellfish consumption to be evaluated. For this purpose, data of toxins concentrations in samples collected in the period spring - summer 2017 has been linked consumption data obtained from the National Bulgarian Fish and Shellfish Consumption Survey including 4891 adults.

The spring toxin profile represents 61% DA, 17% PTX-2 and 17% YTX as in summer only YTX was detected. It was calculated that the acute exposure to DA, PTX-2, YTX in spring and YTX in summer is respectively 0,62; 0,00055; 0,00054 and 0,003 μg phytoxin /kg bodyweight (bw) that are not exceeding the acute reference doses (ARfDs). Chronic exposure only for DA was calculated as for PTX-2 and YTX no data on their chronic effects in animals were available, so tolerable daily intake (TDI) is not established. With 0,0076 μg DA/kg bw/day the chronic exposure was established to be beneath the tolerable daily intake of 0,075 μg/kg bw per day.

The present study showed that Bulgarian population is posed to no risk through consumption of studied shellfish.

Keywords: shellfish poisoning, domoic acid, pectenotoxin-2, yessotoxin, mussels

Correspondence: stgeorgieva@mu-varna.bg
Predicting Adverse Immune Reactions Using a Novel Human In-Vitro Skin Test

Shaheda Ahmed2, Louis Bibby1, Anne Dickinson1, 2
1Institute of Cellular Medicine, Newcastle University, Framlington Place, Newcastle upon Tyne, United Kingdom NE2 4HH,
2Alcyomics LTD, Bulman House, Regent Centre, Gosforth, Newcastle upon Tyne, United Kingdom, NE3 3LSC

There are currently no preclinical human in vitro tests to determine the potential of hypersensitivity related to adverse drug responses. Here we describe human in-vitro skin tests to assess the safety, efficacy and dose responses to pharmaceuticals from a hypersensitivity perspective. Responses also correlate to T cell proliferation and IFN-γ assays which give a predictive read out for adverse immune reactions. The used tests are human blood and skin biopsies from healthy donors which are co-cultured with the test compound. Skin is assessed for histopathological damage following fixation of skin in formalin for 24 hours and staining for haematoxylin and eosin (H&E). Sections are graded according to the Lerner classification system (grade I negative or grade II-IV positive) which correlates to immune damage. A number of therapeutic biologics and small molecule drugs have been tested in our human in vitro test model. Results show a statistically strong positive correlation with histopathological grading in our skin tests to expected clinical response for biologics (r=0.96, p<0.0001) and small molecules (r=0.72, p<0.01). Additionally, an analogue of TGN (TGN1412) was tested and showed a strong positive response (grade III) in our assay, indicating that this assay could have predicted the serious life-threatening cytokine storm observed in the 2006 Northwick park trial disaster. The novel human in vitro skin tests can be used to determine safety, efficacy and dose responses to biopharmaceuticals including monoclonal antibodies and small molecule drugs as well as providing information on adverse immune reactions from a hypersensitivity perspective.

Keywords: hypersensitivity, in vitro test, biopharmaceuticals
Correspondence: s.s.ahmed@ncl.ac.uk

Safety Assessment of Vaccine Adjuvants: Effect of MPLA and QS-21 on In Vitro Co-culture Model

Carolina Campos Estrada1,2, Benjamin Riquelme1, Maria Fernanda Cavieres1,2
1Escuela de Química y Farmacia, Facultad de Farmacia, Universidad de Valparaíso, Valparaíso, Chile,
2Centro de Investigación Farmacopea Chilena, Universidad de Valparaíso, Valparaíso, Chile

Currently the vaccines are oriented not only to prevention but also the treatment of various pathologies. In this scenario, must be co-administered with an adjuvant to elicit a potent immune response immunological. Thus, there is a major need for developing powerful adjuvants or adjuvant systems for use in these vaccines. Numerous adjuvants are currently subject to studies and many of these formulations include QS21 and MPL. The main drawbacks in the development and approval of new adjuvants are unacceptable side-effects, lack of understanding of their mechanism, unknown safety in special populations, and use of preclinical models with limitations in their prediction of toxic effects. We standardize a co-culture model between HUVECs and PBMCs from healthy donors to evaluate both vaccine adjuvants to obtain physiologically relevant data that deliver genetically-diverse and meaningful pathophysiological evidence for the country. The viability was evaluated by MTT and PI incorporation. The release of 13-Cytokine was determined with multiplex assay. The induction of immune response was evaluated by ECAMs expression, leucocyte adhesion and NFKB activation. We found that both adjuvants were able generate an increase IL-1, IL-6, IFN-β, TNF-α, and TGF β. However, QS-21 but not MPLA was able to increase IL-17 release. Both adjuvants increased ECAMs expression, cell-cell adhesion indicating endothelial activation and leukocyte recruitment. Interestingly, we found an optimum MPLA/QS21 combination, in order to establish the balance between efficacy and safety. Finally, this model could be an alternative to the use of experimental animals, evaluating more precisely the immune response triggered by the adjuvanted vaccines.

Keywords: cytokines, ECAMs, cell-cell adhesion, adjuvants, NFKB
Correspondence: Carolina.campos@uv.cl
MicroRNA Regulation of TNF Signaling; Changing the Balance Between Pro-death and Pro-survival Effects

Samira Ghorbani1,2, Farideh Talebi1, Farshid Noorbakhsh1
1Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran,
2Shefa Neuroscience Research Center, Khatam Al-Anbia Hospital, Tehran, Iran

MicroRNAs are small RNA species which are known to regulate various aspects of cell biology, including cell survival, proliferation and apoptosis. Tumor necrosis factor alpha (TNF), is an innate immune cytokine which is involved in regulating inflammation as well as cell survival. Interestingly, both pro-survival and pro-death effects have been reported for TNF in various physiological and/or pathological settings. Whether binding of TNF to its receptors leads to pro-survival or pro-death effects is mostly attributed to the type of adaptor molecules which are recruited to the cytoplasmic domain of the receptors. Two such adaptor molecules are TRADD (TNF receptor associated death domain) and MADD (MAP kinase activating death domain). In this study, we investigated the potential effects of miRNAs on the expression of MADD and downstream TNF signaling. Initial bioinformatic analyses revealed miR-181a and miR-181b as potential regulators of MADD expression. Molecular analyses using luciferase vectors which contained a luciferase coding sequence upstream of MADD’s 3’UTR sequence confirmed direct interaction between miR-181a and miR-181b and MADD transcripts. Overexpression of miR-181a/b mimicking sequences in L929 fibroblast cells suppressed MADD’s transcript and protein levels, while downregulation of endogenous miR-181a/b levels by antagonirs enhanced MADD’s expression. miR-181b mimic sequences enhanced the occurrence of apoptosis in L929 cells following TNF treatment, a phenomenon that was associated with decreased mitochondrial membrane potential in miR-181b overexpressing cells. Overall, our results point to a potential role for miRNAs in regulating TNF pro-death versus pro-survival signaling.

Keywords: MADD, TNF signaling, microRNA, apoptosis

Correspondence: f-noorbakhsh@sina.tums.ac.ir

Evaluation of Nephrotoxic Effects of Zearalenone in Human Kidney Cells

Ecem Fatma Karaman, Ileyna Arıman, Sibel Özden
Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Istanbul University, 34116-Beyazit, Istanbul, Turkey

Zearalenone (ZEA), produced by various Fusarium species, is a non-steroidal estrogenic mycotoxin. It contaminates cereals such as corn, wheat, oat and soybean resulting in human and animal consumption. Therefore, it has serious health hazards to humans and animals. ZEA causes severe reproductive toxicity in humans on account of its xenoestrogenic activity. However, toxicity mechanism of ZEA has not been elucidated yet. We investigated dose-dependent effects of ZEA (0, 1, 10 and 50 µM for 24 h) in generation of reactive oxygen species (ROS), cell cycle regulation, global DNA methylation and gene regulation related with oxidative damage in human kidney (HK-2) cells. IC50 value of ZEA was determined as 151.27 µM and 60.5 µM in HK2 cells for 24 h by MTT and NRU tests, respectively. ZEA increased ROS levels in dose-dependent manner. BrdU test showed that 50 µM of ZEA treatment altered cell proliferation significantly. ZEA slightly increased levels global DNA methylation and gene expression levels of related DNMT1 and MGMT enzymes. In addition, gene expression levels of IL6, IL8, TNFa, MAPK8, NF-κB1, HO1, α-GST, HSP70, Nrf2, L-FABP genes were investigated using real time PCR in response to ZEA. We observed dramatically increase on gene expression levels of α-GST and HSP70 confirming that protein expression levels of HSP70 also increased by western blot analysis. We suggested that HSP70 may be a key molecular biomarker in the nephrotoxicity of ZEA. Our study might provide a new perspective in toxicity mechanism of ZEA in kidney cells.

Keywords: zearalenone, DNA methylation, oxidative damage, nephrotoxicity, HK-2 cells

Correspondence: stopuz@istanbul.edu.tr
Genotoxic Effects of Metformin in Human Cervix Adenocarcinoma Cells Using Comet Assay

Tuba Saraydın, Tuğba Gül Çalış, Sevtap Aydin, Ülkü Ündeğer Bucurgat, Nurşen Başaran
Department of Pharmaceutical Toxicology, Faculty of Pharmacy, University of Hacettepe, 06100, Ankara, Turkey

Metformin is commonly used in the therapy of type 2 diabetes. Antioxidant effect of metformin has been suggested to be responsible for protecting oxidative stress-related DNA damage. However there are contradictory results in the literature.

This study was focused on the cytotoxic and genotoxic effects of metformin in human cervix adenocarcinoma (HeLa) cells. The cytotoxicity of metformin (0.5-64 μM) was determined by Thiazolyl Blue Tetrazolium Blue (MTT) assay and its genotoxicity (5-1000 μM) was determined by alkaline comet assay in HeLa cells treated with/without H2O2. DNA damage was expressed as DNA tail intensity.

The cell viability significantly decreased at the doses of 32 μM and 64 μM. The IC50 dose of metformin was 76.97 μM for 24h in HeLa cells. Metformin alone did not induce DNA damage at all studied concentrations (5-1000 μM) for 24h. However, at the concentration ranges between 5 μM and 1000 μM, it significantly increased H2O2-induced DNA strand breakage damage (p < 0.05).

In conclusion, our results suggest that metformin has a potential role in increasing the oxidative stress-related genotoxicity. In order to get more pronounced results, more mechanistic studies should be performed.

Keywords: metformin, cytotoxicity, genotoxicity, comet assay, human cervix adenocarcinoma cells

Correspondence: nbasaran@hacettepe.edu.tr

Interaction of Pycnogenol with Cisplatin on V79 Cell Viability

Sevtap Aydin¹, Merve Becit¹, Arif Ahmet Basaran², Nurşen Başaran¹
¹Department of Pharmaceutical Toxicology, Faculty of Pharmacy, University of Hacettepe, 06100, Ankara, Turkey, ²Department of Pharmacognosy, Faculty of Pharmacy, University of Hacettepe, 06100, Ankara, Turkey

Cisplatin is commonly used in the therapy of many solid tumors. In the treatment of cancer, it has been intended to increase anticancer effect and decrease cytotoxicity using various plant-derived phenolic compounds with chemotherapeutic drugs. Pycnogenol (PYC) obtained from Pinus pinaster is commonly consumed as a dietary food supplement because of its strong antioxidant effect and has been the subject of many studies. The mechanisms of the interactions of PYC with cisplatin needs to be clarified, therefore we aimed to determine the effects of PYC on cisplatin cytotoxicity in Chinese lung fibroblast cell lines (V79). The cell viability was determined by MTT assay for 24h and 48h. The IC50 values of PYC were 670 μM and 119 μM for 24h and 48h, respectively. PYC significantly increased the cytotoxicity of cisplatin at concentrations of 500 μM and 1000 μM (1.75 fold and 4.04, respectively, vs. IC50 doses of cisplatin) for 24h and 250-500 μM (2.92 fold, 6.96 fold and 12.43 fold, respectively, vs. IC50 doses of cisplatin) for 48h in a dose dependent manner. In conclusion, our findings show that PYC may interact with cisplatin and play a important role in the chemotherapy; however, further studies are required to confirm their interactions with cisplatin.

Keywords: Pycnogenol, cisplatin, cytotoxicity, V79 cells

Correspondence: nbasaran@hacettepe.edu.tr

Interaction of Zebrafish (Danio rerio) Organic Anion Transporter 2d (Oat2d) with Xeno- and Endobiotics

Jelena Dragojević, Ivan Mihaljević, Tvrtko Smital*
Laboratory for Molecular Ecotoxicology, Division for Marine and Environmental Research, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, Croatia

Organic anion transporters (OATs/Oats) are polypeptide membrane uptake transporters that mediate entrance of compounds into the cell. Consequently, they are key determinants of toxicological response to various xenobiotics. Despite their physiological importance and role in cellular detoxification, the knowledge about uptake transporters in non-mammalian species is scarce. Zebrafish (Danio rerio) has seven OAT orthologs: Oat1, Oat2a-e and Oat3. Mammalian OAT2 was characterized in human, rat and mouse, and was shown to play an important role in uptake and distribution of physiological compounds, as well as anionic toxins and drugs. As OAT2 orthologs have been poorly studied in non-mammalian species, the goal of our study was to determine phylogenetic relationships, tissue distribution and substrate specificity of zebrafish Oat2d. Phylogenetic analysis of OAT/Oat genes confirmed similarities among mammalian and
zebrafish Oat transporters. Zebrafish Oat2d highly expressed in intestine and moderately expressed in testes and brain. Western blot analysis revealed protein band of 60 kDa, and immunocytochemistry showed its correct localization in the plasma membrane. Functional studies using HEK293T cells overexpressing zebrafish Oat2d revealed two model fluorescent substrates of Oat2d: lucifer yellow (LY, Km = 56.36 µM) and 6-carboxyfluorescein (Km = 210.1 µM). The initial screening with various endo- and xenobiotics showed significant inhibition of Oat2d mediated uptake of LY by endogenous compounds (α-ketoglutarate, fumarate, bilirubin and deoxycholic acid) and exogenous compounds (p-aminohippurate, perfluorooctanesulfonic acid, perfluorooctanoic acid, fursemide, chlorpyrifos, tetracycline and diclofenac). Selected potent inhibitors, fumarate and indomethacin, showed dose dependent inhibition of Oat2d transport (IC50 = 68.24 µM and 20.41 µM, respectively).

Keywords: membrane proteins, phylogeny, tissue expression, cell localization, functional analysis

Correspondence: jdragoj@irb.hr, smital@irb.hr

---

Biological Responses to Hybrid Fe-Si Nanoparticles in Caco2 Cells

Mihaela Balas1, Florian Dumitrache2, Madalina Andreea Badea1, Andreea Luminita Radulescu1, Claudiu Fleaca2, Claudiu Locovei2, Eugenia Vasile2,3, Anca Dinischiotu1

1Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Bucharest, 91-95 Splaiul Independenței, 050095 Bucharest 5, Romania
2National Institute for Lasers, Plasma and Radiation Physics (NILPRP), Atomistilor 409, 077125 Magurele, Romania
3Politehnica University of Bucharest, Faculty of Applied Chemistry and Materials Science, Department of Oxide Materials and Nanomaterials, Gh. Polizu 1-7, Bucharest, Romania

Our study aimed to assess the biological responses of hybrid Fe-Si nanoparticles in cancer cells. Hybrid Fe-Si nanoparticles synthetized by laser pyrolysis using Fe(CO)5 vapors and SiH4 as Fe and Si precursors were coated with L-3,4-dihydroxyphenylalanine (L-DOPA) and sodium carboxymethyl cellulose (CMC-Na). Doses ranging from 0 - 200 µg/mL nanoparticles were exposed to colorectal adenocarcinoma cells (Caco2 cell line) for 24 and 72 hours. Cancer cell viability and integrity were evaluated by the level of mitochondrial enzymes (MTT assay) and lactate dehydrogenase (LDH) activities. Biological responses to nanoparticles were evaluated by analyzing reactive oxygen species (ROS) production, reduced glutathione (GSH) content and Nrf-2 protein expression. Cellular morphology and internalization of nanoparticles were also assessed.

The results showed no changes of viability and integrity of Caco2 cells exposed to a dose below 100 µg/mL L-DOPA-coated Fe-Si nanoparticles but over a dose of 50 µg/mL CMC-Na-coated nanoparticles cell viability significantly decreased in a time-dependent manner. Cellular morphology and organization of F-actin filaments were not altered after exposure to 25 and 50 µg/mL from both L-DOPA and CMC-Na-coated Fe-Si nanoparticles. After 72 hours, internalization of both types of nanoparticles were observed in cancer cells. However, the ROS production increased starting with 24 hours exposure to 25 and 50 µg/mL of both types Fe-Si nanoparticles inducing the increase of GSH intracellular level and activation of Nrf-2.

Our findings suggest that Caco2 cells managed to survive after exposure to both L-DOPA and CMC-Na-coated Fe-Si nanoparticles by triggering antioxidant defense mechanisms in response to oxidative stress.

Keywords: Fe-Si nanoparticles, laser pyrolysis, colorectal adenocarcinoma cells, cytotoxicity, oxidative stress

Correspondence: radu_mihaiella@yahoo.com, ancadinischiotu@yahoo.com

---

Saccharomyces cerevisiae – a Simple Unicellular Eukaryotic Model Organism for In Vitro Toxicity Screening of Nanoparticles

Kaja Kasemets, Sandra Käosaar, Anne Kahr
Laboratory of Environmental Toxicology, National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, Tallinn 12618, Estonia

The yeast Saccharomyces cerevisiae is a promising unicellular eukaryotic model organism for the NPs toxicity screening as its cellular structure and function has many similarities (e.g., oxidative stress response) with the higher-level organisms. Moreover, yeast has a short generation time and can be easily cultivated. In addition, the genome of S. cerevisiae has been sequenced and there are lots of mutated strains available for mechanistic studies. Yeast cells have also endocytosis, being a simple particle-uptake model. Differently from the higher eukaryotic cells yeast has rigid cell wall consisting of chitin and glucan – main specific antifungal molecular targets.
In this study we profiled the toxicity mechanism of copper oxide (CuO) and silver (Ag) NPs using S. cerevisiae BY4741 wild-type (wt) and its single gene-deletion mutants (EUROSCARF) defective in oxidative stress response (OS), cell wall/membrane integrity and endocytosis. The growth inhibition assay in organic-rich growth medium (YPD) and cell-viability assay in deionized water (DI) was applied.

Results showed that the studied CuO and AgNPs were ~30–100 more toxic in DI than in YPD. Comparison of the toxicity pattern of wt and mutant strains revealed that the toxicity of CuO and Ag NPs was not caused by the OS or cell wall/membrane disturbance. Confocal microscopy showed that wt but not the endocytosis-deficient mutant internalized AgNPs. The role of dissolved ions in the studied NPs toxicity will be discussed.

This work was supported by the IUT 23-5.

Keywords: CuO and Ag NPs, dissolution, oxidative stress, cell wall/membrane integrity, endocytosis

Correspondence: kaja.kasemets@kbfi.ee

Investigation into Metabolic Profiles Following Diethylstilbestrol on p53+/- Heterozygous Mice Using 1H-NMR Spectroscopy

Mohd Nazil Salleh1, Henkie Isahwan Ahmad Mulyadi Lai1, Taufiq Yap Yun Hin2
1Department of Health Sciences, Faculty of Engineering and Life Sciences, Universiti Selangor, Shah Alam City Campus, 40000 Shah Alam Selangor Malaysia.
2Catalysis Science and Technology Research Centre, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Malaysia

Metabonomic approach based on 1H-NMR Spectroscopy and chemometric analysis has been applied to investigate endogenous metabolite profiles of biochemical effects of clastogenic carcinogen, diethylstilbestrol (DES) in the urine of female wild-type mice after severed changes of genes profiles compared to p53+/- heterozygous mice. Mice (n=80) were housed individually in metabolism cages in a well-ventilated room, at a temperature of 21 ± °C and a relative humidity of 50 ± 10%, with a 12-h light/darkness cycle. Each animal received a single dose (i.p) injection of 500 umole/kg b.w of DES, once daily for four days and animals were sacrificed. Urine samples were collected at various time-points: pre-dose (control), 8, 24, 32, 48, 56, 72, 80 and 96 hours. Chemometric analysis of 1H-NMR data have shown that there are endogenous metabolite changes between vehicle and DES-treated mice between wild-type and p53+/- heterozygous mice. Major biochemical changes included elevated urinary levels of creatine, lactate, taurine, hippurate and reduced tricarboxylic acid cycle (TCA) especially 2-oxoglutarate and trimethylamine-n-oxidase levels in DES-treated compared to vehicle mice. The elevated taurine and creatine levels in the urine of treated mice in response to hepatocyte necrosis has been shown in the histological evaluation. The reduction of 2-oxoglutarate indicates a general increase in energy metabolism. Therefore, the application of 1H-NMR spectroscopy, coupled with chemometric methods analysis, could be of importance for generating new diagnostic tools in the early detection of toxicity.

Keywords: metabonomic, diethylstilbestrol, p53+/- heterozygous, 1H-NMR Spectroscopy

Correspondence: drnazil@unisel.edu.my
available in environmental science for addressing complex environmental toxicity issues, able to determine the identity of the toxic compounds, mechanisms of toxicity and their effects. Accordingly, the main goal of this study was to identify molecular mechanisms of cyanobacterial toxicity in the extract of aquatic cyanobacterial strain Oscillatoria K3 found in toxic cyanobacterial blooms in Vojvodina region (Serbia) using EDA approach. Ecotoxicological end-points that were determined included interaction with toxicologically relevant zebrafish uptake membrane transporters DrOatp1d1 and DrOct1 (phase 0 of cellular detoxification), and CYP1A1 enzymes (phase I). Extracts of Oscillatoria K3 obtained by non-selective and non-target preparation techniques demonstrated the strongest interaction with DrOatp1d1 transporter as well as CYP1A1 enzyme in the first, second and third tier of our EDA study. In conclusion, our data showed that toxic secondary metabolites from Oscillatoria K3 include polar cationic and anionic DrOatp1d1/DrOct1 inhibitors and lipophilic CYP1A1 inducers of the cellular detoxification mechanism.

Keywords: cyanobacteria, secondary metabolites, uptake membrane transporters, CYP1A1 enzyme, effects-directed analyses (EDA)

Correspondence: pmaric@irb.hr

Oxidative Stress and Antioxidative Defense Parameters in Female Workers Exposed to Volatile Organic Compounds

Nina Umicevic1, Jelena Kotur-Stevuljevic2, Evica Antonijevic3, Danijela Djuči–Cosic3, Biljana Antonijevic3

1University of Banja Luka, Faculty of Medicine, Department of Toxicology,
2University of Belgrade, Faculty of Pharmacy, Department of Medical Biochemistry,
3University of Belgrade, Faculty of Pharmacy, Department of Toxicology “Akademik Danilo Soldatović”

Workers in the shoe manufactures are constantly exposed to commonly used volatile organic compounds (VOCs), including xylene, propane, hexane, cyclohexane, butene, toluene, acetaldehyde, ethanol, acetone, dimethyl sulfide, dimethyl disulfide etc. The aim of this study was to investigate relationship between inhalation exposure to volatile organic compounds (VOCs) and VOCs’ capacity to induce oxidative stress in women employed in shoe production facility. The study included 55 female workers and 25 healthy unexposed female controls. The superoxide anion (O2–), advanced oxidation protein products (AOPP), total oxidative status (TOS), prooxidative-antioxidative balance (PAB), oxidative stress index (OSI) and antioxidative defence parameters (superoxide dismutase (SOD) enzyme activities, values of SH groups, paraoxonase-1 (PON1) activities and total antioxidant status (TAS) were determined in plasma in all subjects. The obtained results have shown that all investigated oxidative stress parameters (O2–, AOPP, PAB, TOS and OSI) as well as antioxidative defence parameters were significantly higher (p<0.001) in exposed workers compared to controls. Moreover, paraoxonase-1 (PON1) activities (p<0.001) and total antioxidant status (TAS) (p<0.001) significantly decreased in the workers exposed to VOCs. These results suggest that occupational exposure to even permissible levels of VOCs in the shoe manufactures may induce oxidative stress in the plasma of workers proving that oxidative stress is among the mechanisms, which positively contribute to the development of adverse effects caused by VOCs.

Keywords: volatile organic compounds, occupational exposure, oxidative stress, antioxidative defense

Correspondence: nina.umicevic@med.unibl.org

Influence of Hypothyroidism on Testicular Mitochondrial Oxidative Stress by Activating the p38MAPK and JNK Signaling Pathways in Rats

X-R Chang1, Y-L Yao1, D Wang1, H-T Ma2, P-H Gou2, C-Y Li1, J-L Wang1

1Department of Toxicology, School of Public Health, Lanzhou University, Lanzhou, Gansu Province 730000, China,
2INSERM UMR-S 1131, Institut Universitaire d’Hématologie, Université Paris Diderot, Paris 75475, France

Thyroid hormone (THs) deficiency can impair testicular function. However, knowledge of the effects of MAPK pathways on testicular mitochondrial oxidative damage induced by hypothyroidism is still rudimentary. This study aims to explore the possible mechanisms of testicular mitochondrial oxidative damage in hypothyroidism rats. Wistar male rats were randomly divided into control (C), low- (L) and high-hypothyroidism (H) groups [0, 0.1 and 10 mg propylthiouracil (PTU)/kg respectively] by intragastric gavage for 60 days. Blood samples were collected to measure the levels of serum triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) in all groups. The results showed that serum T3 and T4 levels were significantly decreased in the H group compared to the C group, while TSH levels were significantly increased in the H group. In conclusion, hypothyroidism induced oxidative stress in testicular mitochondria by activating the p38MAPK and JNK signaling pathways.
hormone (TSH). Testicular mitochondrial homogenates were to measure the activities of superoxide dismutase (SOD), catalase (CAT) and Ca\(^{2+}\)-ATPase as well as protein and mRNA expression of androgen receptor (AR), p38 mitogen-activated protein kinase (p38MAPK) and c-Jun NH\(_2\) -terminal kinase (JNK). The results showed that the body weight, testis weight, and levels of T\(_3\) and T\(_4\) were all significantly decreased and the relative testes weights and level of TSH were significantly increased in the H group. There were significant decreases in SOD activity in the H group as well as decreases in CAT and Ca\(^{2+}\)-ATPase activities in the L and H groups. Additionally, protein expression of AR decreased significantly and protein expression of phosphorylated p38MAPK and JNK increased significantly in the H group. Therefore, the study suggests that hypothyroidism could affect male reproductive function by disturbing expression of AR, inducing oxidative stress and changing the activity of Ca\(^{2+}\)-ATPase, leading to activation of p38MAPK and JNK signaling in the testicular mitochondria.

**Keywords:** hypothyroidism, testicular mitochondria, oxidative stress, p38MAPK, JNK

**Correspondence:** wangjl@lzu.edu.cn

---

**METALS**

**Influence of Chelation Therapy on Oxidative Stress Parameters in Occupationally Lead Exposed Workers**

Asli Karacan Dincer, Tugce Cetin, Suna Sabuncuoglu, Gozde Girgin, Turkan Nadir Ozis

*Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey*

Lead (Pb), which has many toxic effects in human organ systems, is a well-known environmental and occupational toxic heavy metal. The mechanism of Pb induced oxidative stress involves structural and functional changes in tissues and cellular components leading to inactivation of enzymes, lipid peroxidation and DNA damage. The present study was designed to investigate the possible influence of chelation therapy on oxidative stress induced by occupational Pb exposure. For this purpose, heparinized blood samples were collected from occupationally Pb exposed workers (aged 25 to 48, n=39) before and after chelation therapy. 8-hydroxy-deoxyguanosine (8-OHDG), malondialdehyde (MDA), protein carbonyl (CO), glutathione peroxidase (GPx) and aminolevulinate delta dehydratase (ALAD) enzyme, which is an important target of Pb, levels were measured in plasma by using ELISA kits. Besides that, to evaluate possible cholesterol oxidation caused by Pb exposure, 7-ketocholesterol (7-KC) and cholestan-3β,5a,6β-triol (triol) levels were also measured using HPLC-MS/MS. The levels of 8-OHDG, MDA, 7-KC and triol levels were also measured using HPLC-MS/MS. The levels of 8-OHDG, MDA, 7-KC and triol significantly decreased when compared its values before chelation therapy (p<0.05). ALAD enzyme activity significantly increased following chelation therapy. Nevertheless, CO and GPx levels were not significantly affected. Our results showed that chelation therapy decreases Pb induced oxidative stress in occupationally Pb exposed workers. Furthermore, cholesterol oxidation is also significantly increased by Pb exposure and decreased after chelation therapy. The data indicate that chelation therapy, which is mainly used to decrease blood Pb level, is beneficial against Pb induced oxidative stress. Further studies are needed to clarify the role of chelation therapy on Pb exposure induced oxidative stress.
Mercury Levels in the Food and Its Biological Derivatives in Turkey
A Meta-Analysis Study

Elif Aslıhan Cayır¹, Sinan Karacabey¹,², Yasser Batsh¹, Ahmet Aydin¹
¹Yeditepe University, Faculty of Pharmacy, Department of Toxicology, Istanbul, Turkey,
²Marmara University, School of Medicine, Department of Emergency, Istanbul, Turkey

Mercury is a toxic heavy metal and the accumulation of it inside food and its biological derivatives is a common and serious issue. Food and Drug Administration (FDA) estimates that most people are exposed, on average, to about 50 ng of mercury per kilogram of body weight per day (50 ng/kg/day) in the food they eat. A large part of this mercury comes from eating fish. However, in addition to fish, many kinds of contaminated food and its derivatives may be a source of toxicity with mercury. In this study, a meta-analysis was done to many kinds of food and biological derivatives which were consumed in Turkey assessing published articles dealt with the level of mercury in these products. PubMed and Medline databases were searched with mercury, food, biological derivatives, and Turkey keywords. We found about 60 articles. The analyzed material in these articles were both food products and biological samples. Most of the articles have been made on various fish species. The lead levels in these studies were in concentrations that would not pose a threat to human health (0.405±0.025 µg/g), but some suggestions were made that some fish species would not be suitable for human consumption. As a conclusion, many factors affected the level of mercury, like the distance from the highways, which has had high effect to the results, in addition to the time of study, and the method of analysis used to determine it.

Keywords: heavy metals, toxic metal, fish, food contamination
Correspondence: aslihancayir@gmail.com

Lead Levels in the Food and its Biological Derivatives in Turkey
A Meta-Analysis Study

Ezgi Talo¹, Neslihan Şahin¹, Sinan Karacabey¹,², Ahmet Aydin¹
¹Yeditepe University, Faculty of Pharmacy, Department of Toxicology, Istanbul, Turkey,
²Marmara University, School of Medicine, Department of Emergency, Istanbul, Turkey

The lead is a bluish gray metal with the symbol Pb and a toxic substance present in our environment in small amounts. People may be exposed to lead from drinking water or food. In 1993, the FDA established a maximum daily intake level (6 µg/day), based on 10 micrograms of lead per deciliter of blood (µg/dL). Large part of lead comes from by contaminated food. In this study, a meta-analysis was done to many kinds of food and biological derivatives which were consumed in Turkey assessing published articles dealt with the level of lead in these products. PubMed and Medline databases were searched with lead, food contamination, heavy metal, and Turkey keywords. We found about 163 different articles. The analysed material in these articles were both food products and biological samples. Most of the articles have been made on various fish species. The lead levels in these studies were in concentrations that would not pose a threat to human health (0.405±0.025 µg/g), but some suggestions were made that some fish species would not be suitable for human consumption. As a conclusion, many factors affected the level of lead, like the distance from the highways, which has had high effect to the results, in addition to the time of study, and the method of analysis used to determine it.

Keywords: heavy metals, lead, food contamination
Correspondence: ezgii.talo@outlook.com mailto:aslihancayir@gmail.com

Selected Effects of in ovo Aluminium Exposure on Developing Nile Crocodiles

J. Christoff Truter¹, Johannes H van Wyk², Natalia Garcia-Reyero Vinas³, Jan G Myburgh⁴, Anna-Maria Botha¹
¹Department of Genetics, Stellenbosch University, South Africa; ²Department of Botany and Zoology, Stellenbosch University, South Africa; ³Environmental Laboratory, US Army Engineer Research and Development Center, US Army Corps of Engineers, USA; ⁴Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, South Africa
High concentrations of aluminium (Al) have been detected in the surface water and sediments of water-bodies impacted by mining and other anthropogenic activities. Nile crocodiles, *Crocodylus niloticus* nest in damp sediment, and developing young may be at risk of Al exposure. Our objectives were to determine whether Al can (1) enter *C. niloticus* eggs and be absorbed by embryos during development, (2) affect hatching success and the general health of hatchlings, (3) result in abnormalities in gonad development and liver morphology, and (4) alter global DNA methylation. *C. niloticus* eggs obtained from a commercial farm were exposed *in ovo* to 3.3 g/L Al (pH 4), a pH control (pH 4) and negative control (pH 7). The animals were sacrificed three months after hatching. Al concentrations were higher in the exposed egg shells than previously shown for *C. niloticus* eggs collected from the wild. A significantly lower proportion of Al exposed hatchlings were able to hatch without assistance relative to the controls, suggesting that Al exposure may compromise survival in the wild. Corresponding growth rates were observed among treatments after three months, indicating that the Al exposed animals did recover. Although Al concentrations in liver, blood and kidney tissues did not vary significantly among treatments, the femurs of Al treated animals contained significantly lower Al levels than the controls, indicating effective detoxification. No apparent abnormalities were observed in ovary and liver tissues. Global 5mC-methylation was significantly higher in the livers of Al exposed individuals, suggesting epigenetic modification in response to Al treatment.

**Keywords**: metal pollution, reptile ecotoxicology, bioaccumulation, DNA methylation.

**Correspondence**: jctruter@sun.ac.za

---

**Evidence of Immunomodulatory Properties of Cadmium; is Oxidative Stress Involved**

Milena Anđelković1,2, Dragana Javorac3, Katarina Baralić2, Evica Antonijević2, Aleksandra Buha Đorđević2, Vesna Matović2, Zorica Bulat2

1Health Center Kosovska Mitrovica, 2University of Belgrade – Faculty of Pharmacy, Department of Toxicology “Akademik Danilo Soldatović”

Cadmium (Cd) is industrial and environmental pollutant with major concern on human health. Among many mechanisms of toxicity, oxidative stress is tightly connected with Cd exposure disease risk. The aim of the study was to find relation between oxidative stress and potential modulation of immune responses after exposure to single doses of Cd.

The study was carried out on 20 male Wistar rats, randomly divided in experimental groups: control, Group 1-received 15 mg Cd/kg body weight (bw), and Group 2-received 30 mg Cd/kg bw, acute by oral gavage. After rats were sacrificed, collected blood was used for total antioxidative status (TAS) assessment, measurement of white blood cells count (WBC) with leukocyte differential count and Cd levels monitoring, while thymus was used for Cd estimation. For statistical analyses parametric and nonparametric ANOVA followed by appropriate post hoc test was used.

TAS were significantly lower in treated groups compared to controls (p<0.05). Also, inside treated groups significantly differences were noticed (p<0.05). Significantly lower relative lymphocytes counts were observed in both treated groups if compared with control (p<0.01) while WBC significantly decreased only in Group 2, for almost 40% (p<0.01), compared with control. Blood Cd levels were significantly higher in both treated groups (p<0.05) as well as in the thymus of Group 2 (p<0.05) compared to control.

These changes in relative lymphocytes counts are in agreement with increasing Cd levels in blood and thymus and decreasing TAS in treated group. Correlation analysis showed strong negative correlations for Cd levels in blood and TAS as well as relative lymphocyte count which is undoubtedly evidence of the involvement of oxidative stress in Cd-induced immune response modulation.

**Keywords**: total antioxidative status, leukocyte differential count, blood, thymus

**Correspondence**: javoracdragana@gmail.com

---

**Heavy Metals Blood Level in Tobacco Smokers**

Aleksandra Repić1,2, Zorica Bulat2, Vesna Matović2

1Serbian Institute for Occupational Health “Dr Dragomir Karajović”, Belgrade, Serbia, 2University of Belgrade – Faculty of Pharmacy, Department of Toxicology “Akademik Danilo Soldatović”

According to World Health Organisation, in 2015, over 1.1 billion people smoked tobacco. Serbian population rank among the highest in Europe: 43.6 % males and 39.7% females aged 15 and older smoke on regular basis. Tobacco is a plant known to accumulate metals, especially cadmium and to a less extent lead, chromium and nickel. Smoking of 20 cigarettes a day could result in the inhalation of 2–4 μg of Cd and 1–5 μg of Pb.
We determined cadmium (Cd-B) and lead (Pb-B) concentration in the blood of 81 smokers and 30 non-smokers using graphite furnace atomic absorption spectrometry. Smokers were divided into groups according to the number of daily smoked cigarettes and cigarette type. We used ANOVA for statistical analysis. Cd-B was 3.5 times and Pb-B 1.5 times higher (21.4 nmol/L and 0.163 µmol/L, respectively) in smokers compared with nonsmokers (6.0 nmol/L; 0.108 µmol/L, respectively). Smokers who smoked less than 10 cigarettes per day had significantly lower Cd-B (11.4 ± 3.9 nmol/L) and Pb-B (0.124 ± 0.024 µmol/L) than participants who smoked 11-20 cigarettes (23.7 ± 5.0 nmol/L and 0.168 ± 0.026 µmol/L respectively) and more than 21 cigarettes (30.5 ± 15.8 nmol/L and 0.237 ± 0.102 µmol/L, respectively). Smoking of manually prepared cigarettes made of tobacco bought at public market, caused significantly higher Cd-B (30.6 ± 9.9) and Pb-B (0.229 ± 0.070) levels than legal cigarettes (19.5 ± 4.4 nmol/L and 0.151 ± 0.022 µmol/L, respectively).

Keywords: lead, cadmium, cigarettes

Correspondence: aleksandrapepic@gmail.com

Reduction of PARK2 Expression among Smelting Workers Exposed to Manganese (Mn)

Wei Zheng¹, Ximin Fan², Wendy Jiang¹, Qiyuan Fan²,³
¹School of Health Sciences, Purdue University, West Lafayette, IN, United States;
²School of Public Health, Zunyi Medical College, Zunyi, Guizhou, China;
³Department of Health Management, Zunyi Medical and Pharmaceutical College, Zunyi, Guizhou, China

Modern industry demands the high quantity of ferroalloys. Smelting workers engaged in production of Mn-containing ferroalloys are frequently exposed to airborne Mn in workplace. Occupational exposure to Mn is known to cause clinical syndromes similar, but not identical to, Parkinson’s disease. Loss-of-function mutations in PARK2 have been associated with certain forms of PD. In this human cohort study, we tested the hypothesis that Mn-exposed smelters had an altered PARK2 expression, leading to Mn-induced neurotoxicity. Mn-exposed smelters (n=26) and non-Mn-exposed control smelters (n=20) were recruited from a Mn-iron (Fe) alloy smelting factory and an Fe smelting factory, respectively, from Zunyi City in China. Subjects were matched with socioeconomic status and background for environmental factors. Analyses by atomic absorption spectrophotometry (AAS) of Mn concentrations indicated that Mn concentrations in plasma, red blood cell (RBC) and saliva, and the cumulative Mn-exposure were about 2.2, 2.0, 1.7 and 3.0 fold higher, respectively, in Mn-exposed smelters than those in control subjects (p<0.01). Total RNA from the blood samples was isolated and analyzed by RT-PCR to quantify PARK2. The data showed that the expression of PARK2 in Mn-exposed smelters was significantly decreased by 42% as compared to controls (p<0.01). Linear regression analysis further established that the expression of PARK2 mRNA was inversely correlated with Mn levels in plasma, RBC and saliva, as well as the cumulative Mn exposure (p<0.01). Taken together, our data suggest that Mn exposure among smelters may lead to a reduced expression of PARK2, which may partly explain the Mn-induced Parkinsonian disorder.

Keywords: manganese, PARK2, worker, smelter, biomarker

Correspondence: wzheng@purdue.edu

Measurement of Arsenic, Arsenic Species and Other Elements in Urine, Drinking Water and Hair Samples- Screening of the Situation in Eastern Croatia

Walter Goessler¹, Simone Brauer¹, Mirta Milič², Vatroslav Šerić³, ivan Pavičić³, Ana Marija Marjanović Ćermak², Stefano Bonassi⁶,⁷, Višnja Oreščanin⁸, Ivana Vinković Vrček⁹
¹Institute for Chemistry, University of Graz, Graz, Austria, 2Mutagenesis Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia, 3Department of Clinical Laboratory Diagnostics, Osijek University Hospital, Osijek, Croatia, 4Faculty of Medicine, University of Osijek, Osijek, Croatia, 5Radiation Dosimetry and Radiobiology Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia, 6Unit of Clinical and Molecular Epidemiology, IRCCS San Raffaele Pisana, Rome, Italy, 7Department of Human Sciences and Quality of Life Promotion, San Raffaele University, Rome, Italy, 8ORESCANIN Ltd., Zagreb, Croatia, 9Analytical Toxicology and Mineral Metabolism Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia

Chronic exposure to arsenic (As) both in organic but also in inorganic (more toxic) forms, is still a major public health problem worldwide. It is estimated
that hundreds of millions of persons are affected with As problem exposure not limited only to developing countries, but developed countries as well, primarily from natural geological sources (ground water, water used for food preparation or crop irrigation and drinking water) or from anthropogenic sources (e.g. industrial activities). In Eastern part of Croatia, naturally occurring inorganic As in ground water can be present in concentrations less than 1 µg/L up to 610 µg/L. Although public actions were taken to provide affected population with safe drinking water (≤10 µg As/L under the EU Drinking Water Directive), progress is gradual and variable and the concentrations are still above the limit. The aim of this study was make a screening of the situation in Eastern Croatia. We have examined As concentrations in drinking water samples and urine As-concentrations and As-species (organic: dimethylarsinic acid-DMA, monomethylarsonic acid-MA; inorganic; and the sum of cationic arsenic species) in more than 100 exposed individuals from Eastern Croatia using liquid chromatography coupled to inductively coupled plasma mass spectrometry-ICPMS and correlated the results of As-concentrations and other elements measured in hair samples in order to get a better insight in the amount of arsenic that could stay in the human body from the chronic exposure to arsenic and to make assessment of the toxic effect of such exposure.

The study was financially supported by the Grant of Ministry of Science and Education of the Republic of Croatia 05/2016 Possible early noninvasive biomarkers of chronic exposure to arsenic.

Keywords: ICPMS, arsenic, urine, human health

Correspondence: mmilic@imi.hr

The Influence of pH on the Removal of As$^{3+}$ from Aqueous Solutions with Acid-activated Clay Modified with Sodium Carboxymethyl Cellulose

Vojkan Miljković1, Milan Jokanović2, Maja Vujović2, Maja Stanković2, Aleksandra Pavlović2

1Faculty of Medicine, Department of Pharmacy, University of Niš, Serbia, 2Faculty of Science and Mathematics, Department of Chemistry, University of Niš, Serbia

In this study, the suitability of the use of acid-activated clay modified with NaCMC to remove As$^{3+}$ from aqueous solutions was investigated.

Since ancient times, arsenic has been known as a toxic element and as such has often been the cause of many deliberate or accidental poisoning. The origin of arsenic in the human environment is mainly industrial. Most arsenic compounds are rapidly absorbed from the digestive tract (about 80% of the amount ingested), and well absorbed through the skin. Chemical compounds in which arsenic is present as As$^{3+}$ are more toxic than compounds with As$^{5+}$, while inorganic compounds are considered more toxic than organic ones. According to the IARC classification, arsenic belongs to the group 1 of human carcinogens. According to the WHO experts, long-term exposure to arsenic through drinking water containing 0.2 mg arsenic/l can cause skin cancer in 5% of the population.

It has been found that adsorption is variable with a pH change. Asorption of As$^{3+}$ is very successful at pH=1, or when adsorption is carried out in a highly acidic medium. Adsorption is also very successful in the base environment, at pH=9, for all three samples of modified clay (GI, GII and GIII).

This study was financed by the Serbian Ministry of Education, Science and Technological Development through the Grant No. TR 34012.

Keywords: clay, NaCMC, SEM-EDS, FTIR, As$^{3+}$

Correspondence: vojkanmm_serbia@yahoo.com

Manganese-induced Brain Mitochondrial Dysfunction is Associated with Impaired Motor Functions in Rats: Protection with Nutrient Metal Mixture Supplementation

Chand Basha Davuljigari1, Katari Sudheer2, Umayaheswari Amineni2, Sreenivasulu Reddy Motireddy1, Rajarami Reddy Gottipolu1

1Department of Zoology, Sri Venkateswara University, Tirupati, India-517 502.
2Bioinformatics Centre, Department of Bioinformatics, Sri Venkateswara Institute of Medical Science University, Tirupati, India-517 507.

Exposure to excessive levels of manganese (Mn) results in a movement disorder which resembles Parkinson’s disease. The pathogenic mechanisms underlying its action are not elucidated. To determine the role of mitochondrial energy metabolism and oxidative stress in Mn-induced neurobehavioral dysfunctions, male pups were lactationally exposed to Mn
(6 mg/Kg body weight) through intraperitoneal injection for a period of two weeks (5 days/week) from PND 15 to PND28. To study the protective effect of nutrient metal mixture, pups were administered calcium (Ca), iron (Fe) and zinc (Zn) in combination as 0.02% by a single gavage together with Mn injection. The results showed mitochondrial succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), and isocitrate dehydrogenase (ICDH) activities decreased in cortex and cerebellum at PND 28, PND 60 and 3 months age group rats following exposure to Mn. Most notably, Mn exposure decreased the activities of thoredoxin reductase (TrxR), aconitase (Acon), superoxide dismutase (SOD), and catalase (CAT) while the MDA levels increased in the cortex, and cerebellum of selected age groups of rats. In silico findings revealed that aconitate hydratase in complex with modified control cluster (S4F3Mn) influences the Acon activity in the presence of the substrate. Mn-exposed rats exhibited deficits in total locomotor activity and grip strength in rats. However, supplementation of the nutrient metal mixture containing Ca, Fe and Zn reversed the effects of Mn on energy metabolism, oxidative damage of mitochondria and motor behaviour of the rats. In conclusion, our findings demonstrate that exposure to Mn during the development of brain greatly increased the mitochondrial dysfunction, subsequently, associated with motor coordination deficits in rats. Furthermore our results suggest that application of nutrient metal mixture may potentially be beneficial in treating Mn- neurotoxicity.

Supported by Science and Engineering Research Board (DST, Govt. of India), File No. YSS/2015/000289.

Keywords: oxidative damage, in silico studies, aconitase, manganese, motor functions

Correspondence: drchandbasha2012@gmail.com

The Removal of Ni^{2+} and Cd^{2+} -ions onto Synthetic Mineral Based Composite Functionalized by Polyethylenimine

Nina N. Obradović1, Jelena D. Rusmiović2, Darko A. Kosanović1, Maja B. Dolić1, Ana L. Popović1, Vladimir B. Pavlović1, Aleksandar D. Marinković1

1Institute of Technical Sciences of SASA, 11000 Belgrade, Serbia;
2Innovation center, Faculty of Technology and Metallurgy, University of Belgrade, 11120 Belgrade, Serbia;
3Vinča Institute Of Nuclear Sciences, University of Belgrade, Serbia;
4Faculty of Technology and Metallurgy, University of Belgrade, 11120 Belgrade, Serbia;
5Faculty of Agriculture, University of Belgrade, 11000 Belgrade, Serbia

This study presents the synthesis of porous cordierite-based ceramics and its surface activation by polyethylenimine/nano-CeO_2 for the heavy metal removal. The synthesis was carried out by the addition of following powders: MgO, Al_2O_3, and SiO_2 in 2:2:5 molar ratios, respectively. The oxide(s) mixture was further processed in two sequential stages: i) ball milled in the ethanol for 40 minutes and palletized under the pressure of 3 t/cm², as a pre-sintering process, and ii) the pallets were further sintered for 2h in the air atmosphere at 1350 °C, under a heating rate of 20 °C/min. The sintered mineral composite was crashed and sieved, and mixed with 20 wt % of nanocellulose, as a pore forming agent. Nanocellulose mixture was pressed into pallets under 5 t/cm² and sintered at 700 °C, under a heating rate of 5 °C/min. The obtained synthetic cordierite was further tested as the adsorbent activated by polyethylenimine/nano-CeO_2 for the removal of Ni^{2+} and Cd^{2+} -ions. The adsorption isotherms, kinetics models, and thermodynamic parameters were also analyzed, manifesting that the adsorption is a spontaneous and endothermic process. The phase composition of the pristine and activated cordierite was analyzed by the X-ray diffraction method (XRD), Fourier transformation infrared (FTIR) spectroscopy and scanning electron microscopy (SEM). This work has shed light on the mechanism of heavy metals removal from the
aquatic medium using the novel hybrid (nano)syn-
thesized material.

**Keywords:** synthesis, surface coating, nanomateri-
al, toxic metals, adsorption.

**Correspondence:** jrusmirovic@tmf.bg.ac.rs

---

**Oleic Acid Double Coated Iron Oxide Nanoparticles as New Relevant Biocompatible Nanoparticles with a Particular Mechanism of Activity**

Elena-Alina Moacă¹, Dorina Coricovac¹, Cristina Dehelean¹, Cornelia Pâcuraru²

¹“Victor Babeş” University of Medicine and Pharmacy of Timişoara, Faculty of Pharmacy, Department of Toxicology, Timişoara, Romania;
²Politehnica University of Timişoara, Faculty of Industrial Chemistry and Environmental Engineering, Timişoara, Romania

Iron oxide nanoparticles have gained an increased interest in recent years due to their unique features, like: superparamagnetism, biocompatibility and stability in aqueous solutions. However, a lack of knowledge concerning the toxicity associated to their administration confines their use. This study was aimed to offer relevant information about the cytotoxicity induced by oleic acid double coated magnetic iron oxide nanoparticles (MIONPs) to a panel of healthy (keratinocytes, fibroblasts) and tumor (human and murine melanoma, lung carcinoma and breast carcinoma) cell lines. The MIONPs were obtained by combustion method followed by coating with a double layer of oleic acid. The physico-chemical properties of the biocompatible colloidal suspension were evaluated by means of suitable techniques, such as: optic microscopy (TEM and SEM), magnetic measurements (VSM) and dynamic light scattering (DLS). The cytotoxicity was detected with colorimetric cell-viability bioassays like MTT and Alamar blue. The dimensions of the coated MIONPs were in the range of 30 nm, size considered non-toxic for in vivo administration. The nanoparticles exerted a significant cytotoxic effect on all the tumor cell lines even at low concentrations (10 µM), whereas in the case of healthy cells the viability was affected only at the highest concentration tested (50 µM). The MIONPs induced a different kind of cell death, a particular enucleation process that was not described for other types of nanoparticles. These results show that MIONPs displayed a high stability in aqueous solutions (mandatory criteria for in vivo administration), and anticancer properties, making them suitable as nanoplatforms for chemotherapeutic agents.

*This work was supported by a grant of Minister of Research and Innovation, CNCS - UEFISCDI, project number PN-III-P4-ID-PCE-2016-0765, within PNCDI III.*

**Keywords:** magnetic iron oxide nanoparticles, oleic acid, cytotoxicity, melanoma, breast carcinoma

**Correspondence:** dorinacoricovac@umft.ro

---

**Effects of Silver Nanoparticles on Neurodevelopment Using C57BL/6 and A/J Primary 3D Organotypic Mouse Midbrain Cultures**

Brittany A. Weldon¹², Julie Juyoung Park¹², Sungwoo Hong¹², Tomomi Workman¹², Russell Dills², Ji Hyun Lee², William C. Griffith¹², Terrance J. Kavanagh², Elaine M. Faustman¹²*

¹Institute for Risk Analysis and Risk Communication, University of Washington, Seattle, WA;
²Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, USA

Many consumer, commercial, and medical products have been increasingly using silver nanoparticles (AgNPs) for their antimicrobial properties. Observations of silver in adult and fetal brain following in vivo AgNPs exposures have also led to concerns about the potential of AgNPs as neurotoxicants. In this study, we investigated effects of gold-cored AgNPs of differing sizes and coatings (20nm AgCitrate, 110nm Ag-Citrate, and 110nm AgPVP) on neurodevelopment across two mouse strains using our 3D organotypic embryonic midbrain micromass cultures. Primary cells from gestational day (GD) 11 C57BL/6 or GD 12 A/J mouse embryos were used. After 24-hour AgNP exposures at three different time points of development (days in vitro (DIV) 7, 15, and 22), cytotoxicity was assessed by both nominal and dosimetric dose. Dosimetry of silver and gold was evaluated in cultures, where gold acted as a tracer for uptake of intact gold-cored AgNPs and silver as a tracer for dissolved particles. Results by nominal and dosimetric dose demonstrated significantly increased cell death in a dose-dependent manner at DIV 15 and 22, which represents differentiation stages of neurodevelopment in both strains. When assessed by dosimetric dose, cultures were more sensitive to smaller particles in both strains despite less uptake of Ag. The extent of AgNP dissolution in the micromass cultures across
two mouse strains was different, suggesting potential genetic differences in AgNP uptake mechanisms. Future research is needed to elucidate uptake mechanisms for AgNPs with various sizes and coatings and to validate the effects of genetic background on AgNP uptake mechanisms.

**Keywords:** developmental neurotoxicity, *in vitro* nanotoxicology, dosimetry, genes x environment

**Correspondence:** faustman@u.washington.edu

---

**Impact of Sample Preparation of MWCNT for Developmental Toxicity by Intratracheal Instillation**

*Akihiko Hirose*¹, *Motoko Hojo*², *Norihiro Kobayashi*³

¹National Institute of Health Sciences, ²Tokyo Metropolitan Institute of Public Health

Some studies reported that the intratracheal or intraperitoneal administration with multi-walled carbon nanotube (MWCNT) to pregnant mice caused reproductive and developmental toxicity. We have recently suggested that the reproductive and developmental toxicity could depend on the fiber length of MWCNTs, by comparison of the intraperitoneal administration with various typed MWCNTs. Recently, we preliminary found that the strength of developmental toxicity was varied depending on the preparation methods of the dispersed solution of MWCNT. We postulated that the inflammation caused by the MWCNT administration may be related with preparation methods as well as the fiber length of MWCNT. In order to evaluate the reproductive and developmental toxicity based on various sample preparations of MWCNT, we conducted repeated intratracheal instillation studies in pregnant mice. Three types of MWCNT dispersions (bulk, heat-treatment, single dispersion by Taquann method) were administered to pregnant mice on gestational days 6, 9, and 12 at dosages of 4.0 mg/kg/day. The pregnant mice were dissected on the gestational day 15, and then reproductive and developmental parameters were evaluated. Body weights of the heat-treatment MWCNT exposed mice significantly decreased. Body weights of fetuses were significantly decreased in the bulk MWCNT exposed groups and decreased in the heat-treatment MWCNT exposed groups, although the change was not statistically significant. The increased numbers of neutrophil and eosinophil cells and the increased LDH activities in lavage of dam’s lung were correlated with the body weight effects. These suggested that the developmental toxicity of MWCNT could depend on inflammation in dams.

**Keywords:** nanomaterials, MWCNT, intratracheal instillation, inflammation

**Correspondence:** akihikoh@dranihs.net

---

**Demonstration of the Uptake of Gold Nanoparticles Using CytoViva Technology and Transmission Electron Microscopy**

*Melissa Vetten*¹², *Mary Gulumian*¹²

¹National Institute for Occupational Health, South Africa, ²University of the Witwatersrand, South Africa

Gold nanoparticles (AuNPs) have a wide range of potential applications which necessitates the need for toxicity studies prior to their commercialization. The uptake and intracellular fate of nanoparticles will influence their ability to cause toxicity and therefore needs to be determined. In this study, 14 nm AuNPs were found to be non-toxic to the human bronchial epithelial cell line BEAS-2B for up to 24 hours incubation. The uptake of these particles was then assessed using two approaches, namely through the CytoViva HSI system and Transmission Electron Microscopy (TEM). The CytoViva HSI system allowed for the acquisition of dark field microscopy images and the confirmation of the presence of AuNPs using hyperspectral imaging and spectral angle mapping. In addition, their 3D imaging technology confirmed the uptake and identified nanoparticles within the cells without any fluorescent labelling. This newly developed technology can locate non-labelled nanoparticles within a three dimensional space relative to their surroundings; however, staining with fluorescent markers is necessary to locate nanoparticles relative to subcellular organelles. Since AuNPs are known to interfere with some fluorescent dyes, the use of dyes must be validated prior to use in nanoparticle studies. TEM imaging was used to confirm the uptake and the presence of AuNPs in vesicles/vacuoles and in the cytoplasm. Both of these techniques require the availability of specialized equipment, however the sample preparation for TEM can be somewhat tedious. On the other hand, the sample preparation for CytoViva is quick and relatively easy once validated.

**Keywords:** gold nanoparticles, uptake, CytoViva, TEM, nanotoxicology

**Correspondence:** melissa.vetten@nioh.nhls.ac.za
Interference of Gold Nanoparticles (AuNPs) in Molecular Biology Assay Systems

Natasha Sanabria¹, Mary Gulumian¹,²
¹National institute for Occupational Health,
²University of the Witwatersrand, Johannesburg South Africa

It has now been established that intracellular nanomaterials interfere with different toxicity and genotoxicity assay systems. There is, however, a lack of validation when conducting routine tests for nucleic acid isolation, quantification, integrity and purity analyses, as well as, in the verification of qPCR-related gene expression analyses. Investigations were, therefore, conducted to assess the interference of gold nanoparticles (AuNPs) in these assay systems.

Results have indicated that the introduction of AuNPs to BEAS-2B cells produced absorbance peak shifts, which indicated changes in the quality of the isolated RNA. Although the RNA isolated from the 24 h AuNP-treated samples was considered to be suitable for RNA-based techniques when using the traditional methods, additional screening identified changes that are associated with structural alterations of functional groups. The wavelength shift observed was most probably due to these AuNPs interacting with the amines found in nitrogenous bases of the nucleic acid. Results have also indicated that AuNPs have the potential to interfere with the amplification and detection within the RT-qPCR assay mechanism, which relies heavily on the quantification of stably expressed reference genes.

In conclusion, caution is advised when only assessing DNA/RNA quantity, since structurally altered or damaged nucleic acids could be falsely interpreted as simply a low yield and, subsequently, produces false genetic expression data. Moreover, AuNPs have the potential to interfere with the assay mechanism of RT-qPCR, thus, assay verification is required for AuNP-related gene expression studies used to evaluate toxicity.

Keywords: qPCR, assay-interference, engineered nano-materials, gold nano-particles (AuNPs)

Correspondence: nmsanabria@yahoo.co.uk

Antitumoral Activity of MTX-II, a Basic Myotoxic Phospholipases A2, Isolated from Bothrops asper Snake Venom from Panama

Aristides Quintero¹,², Sulamita S. Setúbal³,⁴, Leonardo A. Calderón¹,⁴, Rodrigo G. Stábeli³,⁴, Juliana P. Zuliani³,⁴, Andreimar M. Soares³,⁴
¹Center for Information and Toxicological Research and Applied Chemistry (CEIITOXQUIA), UNACHI, Chiriqui, Panama,
²Department of Chemistry of the Autonomous University of Chiriquí (UNACHI), Chiriqui, Panama,
³Centre for Studies of Biomolecules Applied to the Health (CEBio), Rondônia, Brazil,
⁴Oswaldo Cruz Foundation (Fiocruz Rondônia), Rondônia, Brazil

Cancer is one of the leading causes of morbidity and mortality worldwide. The major drawback of the current methods of cancer treatment is that patients often do not respond or eventually develop resistance after initial treatment. This has led to the increased use of anticancer drugs developed from natural resources. Phospholipases A₂ (svPLA₂) are abundant components of snake venoms that have been extensively studied due to their pharmacological and pathophysiological effects on living organisms. This study aimed to assess the antitumor potential of MTX-II, a basic myotoxic PLA₂ isolated from crude Bothrops asper snake venom by a single-step chromatography using a CM Sepharose ion-exchange column (1.5 × 15 cm). The 2D SDS-PAGE analysis revealed that the protein has a single chain and molecular mass next to 14.2 kDa, confirmed by MALDI-TOF mass spectrometry. The isoelectric focusing revealed that the protein has pI value approximately to 8.2. Analysis of the N-terminal sequence demonstrated that MTX-II belongs to the enzymatically inactive Lys49 PLA₂-like subclass. It was observed that MTX-II does not affect the viability in vitro of J774.1 macrophages and induces your activation to start phagocytic activity and superoxide production. The MTX-II showed cytotoxic activity tumor cell lines of JURKAT cells (T cell leukemia) and SK-BR-3 (human breast adenocarcinoma) of 70%and
40%, respectively. The results obtained revealed that in vitro antitumor activity is independent of the enzymatic activity. However, further studies must be carried out to ensure the safety and efficacy of MTX-II for the development of anticancer drugs.

**Keywords:** snake venom, toxins, anticancer agents, apoptosis inducer, cancer

**Correspondence:** aristidesq@gmail.com

---

**Development and Application of Method for Analysis of Ochratoxin A in Grapes**

**Bojana Špirović Trifunović**, Ljilja Torović, Vojislava Bursić, Dragica Brkić, Sanja Lazić, Gorica Vuković

1. Faculty of Agriculture, University of Belgrade, Belgrade, Serbia; 2. Institute of Public Health Belgrade, Belgrade, Serbia; 3. Institute of Public Health of Vojvodina, Novi Sad, Serbia; 4. Faculty of Agriculture, University of Novi Sad, Novi Sad, Serbia

The aim of the study was development of a method for determination of ochratoxin A, a mycotoxin produced by *Aspergillus* and *Penicillium* fungi, in grapes, and its application for analysis of grapes harvested in Serbia in 2016.

QuEChERS procedure was applied for extraction of ochratoxin A from grape in several variations: acetonitrile or acidified acetonitrile extraction, without further treatment or followed by dispersive solid phase extraction clean-up on different sorbents used for pesticide residue analysis. LC-MS/MS analysis confirmed straightforward acetonitrile extraction as the most efficient. Optimization of LC-MS/MS conditions, performed using Mass Hunter Optimizer Software, based on quantification/qualification ion 239/221, resulted with fragmentation and collision energy of 105/120 and 20/33 V, respectively. Linearity of detector response was investigated based on solvent (acetonitrile), matrix match and standard addition calibrations, for black and white grape matrices. Black grape exhibited substantial matrix effect (29%), as opposed to white (-11%), demanding matrix calibration. Limit of detection of ochratoxin A was determined using Qualitative Mass Hunter program, and 1 µg/kg was established as limit of determination. Accuracy of the method was studied through recovery assay on several concentration levels, using black (81-104%) and white grape (94-117%). Precision, in terms of RSD, was 2.7% for black, and 2.6% for white grape. Following successful fulfilment of the relevant criteria of SANTE/11945/2015, optimised method was applied for the analysis of 250 grape samples (black and white 1:1), including 10 black and 25 white grapes from organic production. Fortunately, presence of ochratoxin A in analysed samples was not detected.

**Keywords:** ochratoxin A, grape, LC-MS/MS, method development

**Correspondence:** spirovic@agrif.bg.ac.rs

---

**The First Report on Ochratoxin A Concentrations in the Kidneys of the European Brown Bear (Ursus arctos L.)**

**Dubravka Rašić**, Maja Lazarus, Đuro Huber, Slaven Reljić, Maja Peraica

1. Toxicology Unit, 2. Analytical Toxicology and Mineral Metabolism Unit, Institute for Medical Research and Occupational Health, 3. Department of Biology, Veterinary Faculty of the University of Zagreb, Zagreb, Croatia

Ochratoxin A (OTA) is a nephrotoxic and carcinogen mycotoxin that contaminates different food commodities. Its toxicokinetics and accumulation in plasma, kidneys and liver are species-specific. The European brown bear (Ursus arctos L.) is the largest terrestrial mammal in Croatia and most its diet consists of plant food. Maize from supplemental feeding sites makes up an important part of the bears’ diet, especially in periods and years of low natural food resources. This supplemental maize is often of poor quality to begin with and is further adulterated by weather conditions, which enhance the production of OTA. We hypothesized that the OTA level in kidneys would reflect the intake of OTA-contaminated maize. A total of 56 kidney samples were collected (19 samples in 2013 and 37 samples in 2015) from bears hunted according to the Brown Bear Management Plan in Croatia. After liquid-liquid extraction, OTA concentration was measured using HPLC with a fluorescent detector. Regression analysis revealed that OTA concentration was significantly lower (13.82 ± 12.55 ng g⁻¹ tissue; median 12.25) in samples collected during 2013 than in samples collected during 2015 (142.30 ± 237.53 ng g⁻¹ tissue, median 53.31), while controlling for age, sex and season of collection (b=0.60, p<0.001, R²=0.46). These results are the first report on OTA concentrations in the kidney of the European brown bear. OTA in bear kidneys depends on the specific diet habits, bears’
Correlation Between Na+/K+ ATPase Isoforms and the In Vitro Cells Sensitivity to the Algal Toxin Palytoxin

Marco Pelin, Valentina Brovedani, Chiara Florio, Silvio Sosa, Aurelia Tubaro
Dept. of Life Sciences, University of Trieste, 34127 Trieste, Italy

Palytoxins (PLTXs) are highly toxic compounds identified in marine Palythoa zoanthids, Ostreopsis dinoflagellates and Thricodesmium cyanobacteria, and involved in adverse effects in humans after different exposure routes. Epidemiological and molecular evidences suggest a variable inter-individual sensitivity to PLTXs, possibly related to genetic-dependent differences in the Na+/K+-ATPase expression, the molecular target of these toxins.

To identify the specific Na+/K+-ATPase isoforms correlated with the in vitro cells sensitivity to PLTX, 9 cell lines (from skin, liver, breast, intestine and pancreas) were used evaluating PLTX cytotoxicity (EC50, concentration reducing cell viability by 50%; MTT assay) and its cells binding (Kd, binding affinity, and Bmax, maximum PLTX binding; cell-based ELISA). The results were then correlated with the Na+/K+-ATPase protein expression (flow cytometry) and the gene expression for the isoforms of the α (α1-4) and β (β1-3) subunits (real time PCR).

Among the 9 cell lines, a significant variability of the sensitivity parameters was recorded (median EC50=5.7x10^{-10} M; interquartile range=1.5x10^{-10}-1.9x10^{-9} M; median Kd=8.1x10^{-10} M; interquartile range=2.2x10^{-10}-2.4x10^{-9} M; median Bmax=0.015; interquartile range=0.0095–0.02738). Even though cell sensitivity to PLTX was not related with Na+/K+-ATPase protein expression, a significant correlation was observed with gene expression of specific Na+/K+-ATPase α and β isoforms: a significant positive correlation was found between Kd values and β2 gene expression (r=0.8052, p value=0.0159; Pearson correlation) and between Kd values and the ratio of α1/α2 gene expressions (r=0.7225, p value=0.0279; Pearson correlation). These data, for the first time, suggest a significant role of these isoforms in PLTX binding to Na+/K+-ATPase.

Keywords: palytoxin, Na+/K+-ATPase, genetic variants, Ostreopsis
Correspondence: mpelin@units.it
CAR-mediated Expression of CYP2B1 in Primary Rat Hepatocytes After Isolation by Means of EDTA Perfusion

Marc Wollenweber1, Dunja Dimitrijevic1, Bennard van Ravenzwaay2, Dieter Schrenk1
1University of Kaiserslautern, Food Chemistry and Toxicology, Kaiserslautern, Germany, 2BASF SE, Experimental Toxicology and Ecology, Ludwigshafen am Rhein, Germany

The constitutive androstane receptor (CAR) is an orphan nuclear receptor. In rodent liver, CAR is involved in the phenobarbital-mediated tumor promotion. However, due to the constitutive activity of CAR in several immortalized cell lines, it is difficult to measure in vitro CAR activation above baseline. In this study, we compare two different perfusion methods for the isolation of primary rat hepatocytes (pRHs) to establish an alternative and sensitive method for measuring CAR activation in vitro.

For the isolation of pRHs we used two perfusion methods, a two-step collagenase perfusion and an EDTA-based perfusion. CAR activation was determined by RT-qPCR of CYP2B1 mRNA after exposure to phenobarbital (PB), phenytoin (PHY) or TCPO-BOP (TC).

PB, PHY and TC significantly increased CYP2B1 expression in pRHs (preliminary data). In collagenase isolated pRHs expression levels peaked at 120-fold (PB, 0.5 mM), 85-fold (PHY, 50 mM) and 75-fold (TC, 10 µM) relative to the solvent control. In comparison, EDTA isolated pRHs showed similar expression patterns but with up to 7 times higher CYP2B1 induction compared to collagenase-treated pRHs.

In this study, we showed that pRHs isolated by EDTA perfusion showed significantly higher CYP2B1 induction than pRHs obtained by collagenase perfusion after exposure to CAR activators. As a possible reason it has been suggested that the collagenase perfusion may also digest structures on the cell surface of pRHs and thus disrupts cell signaling. Our findings provide a basis for further refinement of an in vitro assay for CAR activation.

Keywords: constitutive androstane receptor, primary rat hepatocytes, EDTA perfusion, CYP2B1, phenobarbital

Correspondence: wollenweber@chemie.uni-kl.de

Lessons Learned: Altetox Academy Hands-On Trainings in Non Animal Testing

Ilija Prachkovski, Francois Busquet
Altetox Academy

Over the last thirty years, dozen of validated alternative test methods exist in the EU and even more thanks to ICATM collaboration. Nevertheless, when one looks at the number of testing proposals submitted to REACH it is clear these methods are not being put to sufficient use. While ad-hoc events, tailor-made training, webinars, and scientific meetings regularly provide training in these new methods, more efforts should be invested into “after-sales” services to disseminate the emerging technologies and reach new audiences. The European Commission and the member states are actively filling the gaps in training via EU research programs such as Horizon2020, and the innovative medicines initiatives.

Altetox Academy has a mission to increase the use of validated alternative methods among researchers and toxicologists in Europe. Altetox Academy two-day training sessions are made up of 20% lectures and 80% hands-on-training in the lab. This study proves that there is an added and unique value of Altetox Academy training format to address its mission. Since its creation in 2012, twenty hands-on trainings took place and gathered in total approx. 200 participants in the lab and via webinars. This presentation intends to share feedback and lessons learned on reaching out the participants and how to perform successful trainings. Last, the sustainability of such initiatives will be described and the objectives of the medium-term listed.

Keywords: education, training, 3Rs, in vitro, in silico

Correspondence: ilija.prachkovski@altertox.be

New In Vitro Toxicity Pathway-based Bioassays for Toxicity Screening of Chemicals

A’edah Abu-Bakar1,3, Hao Hu2, Ting Yu3, Matti A Lang4
1Faculty of Pharmacy, Universiti Teknologi MARA Selangor, 42300 Puncak Alam, Malaysia,

Keywords: constitutive androstane receptor, primary rat hepatocytes, EDTA perfusion, CYP2B1, phenobarbital

Correspondence: wollenweber@chemie.uni-kl.de
With the implementation of the 3Rs principle (Replacement, Reduction, Refinement) in animal use for toxicity testing of xenobiotics, the demand for \textit{in vitro} toxicity bioassays is on the rise, especially those that are based on toxicity pathways. Existing commercial toxicity pathway-based bioassays were developed on the principle of one toxicity pathway working in isolation. In reality, various transcription factors are activated in response to toxic stimuli to express various toxicity pathways in defence. Thus, there is dire need for more accurate \textit{in vitro} bioassays that can measure various toxicity pathways. One strategy is to develop recombinant human cells that express gene reporter plasmid carrying specific binding sites for the various stress activated transcription factors. We have successfully constructed luc gene promoter-based bioassays that are driven by an entire gene promoter containing \textit{cis}-elements for various transcription factors, including AHR/Arnt dependent-XRE and Nrf2 dependent StRE. Three bioassays were constructed by stably transfected human breast cancer cell line (MCF-7) with: (i) wildtype bioassay containing the full length (2kb) promoter region of the \textit{Cyp2a5} gene; (ii) XRE-MUT bioassay with the AHR/Arnt \textit{cis}-element mutated; and (c) StRE-MUT bioassay with the Nrf2 \textit{cis}-element mutated. The bioassays were able to detect responses of various environmental contaminants at concentration range below LC$_{50}$. Additionally, concurrent application of the three bioassays would determine the predominant toxicity pathway of a given compound. The bioassays could potentially analyse and predict toxicity of environmental contaminants, which will benefit regulators and researchers in the field of risk assessment, as well as mining and remediation industries.

\textbf{Keywords:} in-vitro toxicity testing, toxicity pathway-based bioassay, chemical risk assessment, alternatives to animal testing, luc gene promoter-based bioassay

\textbf{Correspondence:} aedah_abubakar8902@utim.edu.my; a.abubakar@uq.edu.au; aedahabubakar@gmail.com

**Effect of Metformin on Doxorubicin Induced Cytotoxicity in Hep2 and Hepg2 Cells**

\textbf{A. Zeynep Ünal}¹, \textbf{Suna Sabuncuoğlu}¹, \textbf{FA Kaya}²

¹Hacettepe University, Faculty of Pharmacy, Department of Toxicology, Ankara, Turkey

²Hacettepe University, Faculty of Medicine, Department of Pediatrics, Division of Hematology-Bone Marrow Transplantation Unit, Ankara, Turkey

Doxorubicin (DOX) is a medication in a class of anthracyclines used in cancer chemotherapy including leukemia, breast and liver cancer. DOX is considered as cell-cycle specific and acts during multiple phases of the cell cycle. Metformin, a commonly used oral anti-hyperglycemic agent of the biguanide family, also activates AMPK. In the present study, the effect of MET on cytotoxicity of DOX have been evaluated in vitro. In order to do present study, drugs were applied on HepG2 and Hep2 cell lines for 24, 48 and 72 hours in different concentrations. In cells, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and lactate dehydrogenase release assays were performed for analysis of cell viability. Apoptotic and necrotic cell ratios were determined by flow cytometry and real-time cell motions were evaluated at XCELLigence. Before the main study, ideal doses were determined (in the Hep2 cell line doxorubicin 100-0.8 μM, metformin 1-10 mM; in the HepG2 cell line doxorubicin 0.08-3.2x10$^{-3}$ μM, metformin 10-0.04 mM). According to the results, MET synergise the cytotoxic effect of DOX when the drugs were used together at different doses in 24, 48 and 72 hours. At the high concentration of MET and DOX combinations, cell viability ratio decreased and DOX induced apoptotic cell counts increased. Furthermore, XCELLigence analysis (120 hours) results showed that 1-10 mM MET combinations increased the cytotoxic potential of DOX. In conclusion, cell properties and assay principals may affect the response for the interactions between DOX and MET. Cancer types and administered drug doses have to be considered for interactions.

\textbf{Keywords:} doxorubicin, metformin, cytotoxicity, interaction

\textbf{Correspondence:} a.zeynep.u@gmail.com
In Vivo Reactivating Efficacy of Oximes K203 and K027 Against a Direct Acetylcholinesterase Inhibitor: Dose-response Modeling

Evica Antonijević1, Kamil Musilek2, Kamil Kuca3, Danijela Džukić-Cosić1, Marijana Ćurčić1, Zorica Bulat1, Biljana Antonijević1
1University of Belgrade, Faculty of Pharmacy, Department of Toxicology “Akademik Danilo Soldatović”, Serbia, 2University of Hradec Kralove, Faculty of Science, Department of Chemistry, Czech Republic, 3University Hospital in Hradec Kralove, Biomedical Research Center, Czech Republic, 4National Poison Control Center, Military Medical Academy, Serbia

Reactivation of organophosphate(OP)-inhibited acetylcholinesterase (AChE) as specific endpoint is used in efficacy testing of experimental oximes, antidotes in OPs poisoning. According to our best knowledge, the majority of in vivo studies tested only one or two oxime doses resulting in qualitative oxime efficacy evaluation. However, quantitative analysis of in vivo dose-response data would improve identification and quantification of the effect as well as rigorous comparison of different oximes efficacies. Thus, we have evaluated in vivo dose-response relationship for two promising experimental oximes, K203 and K027, concerning reactivation of AChE inhibited by dichlorvos (DDVP). To compare the oximes effects, benchmark (BMD) covariate approach was used to estimate oxime dose (with 90% confidence intervals) that elicits a pre-specified effect size of 100% (2-fold increase in AChE activity compared to DDVP-treated group). Wistar rats (5/group) were treated with oxime (1.25%, 2.5%, 5%, 25% and 50% LD50 im) immediately after DDVP challenge (75% LD50 sc). Activity of AChE was measured in erythrocytes by Ellman’s method 60 min after the treatment. Dose-response and BMD modeling was done in PROAST software (version 64.13, RIVM, Nederlands). Exponential model m5-b \( y=a[-(c-1)\exp(-bx^2)] \) was selected as best estimate with parameters: \( a=0.8019, b_{K203}=0.0015, b_{K027}=0.003355, c=2.662 \) and \( d=1.218 \). Derived BMD100 were K203=194 (153, 243) and K027=100 (81, 125) µmol/kg bw, indicating that oxime K027 induces the same effect size with 2-times lower dose compared to oxime K203. Moreover, obtained confidence intervals of BMDs did not overlap allowing the conclusion that more potent dose-response relationship belongs to experimental oxime K027.

Keywords: benchmark dose, effect size, potency, erythrocytes, rat

Correspondence: evica.antonijevic@pharmacy.bg.ac.rs

The Effect of Thiamine on Activity of Enzymes (with a special emphasis on MAPK) in the Brain of Japanese Quails Treated with Chlorpyrifos

Dejana Ćupić Miladinović1, Sunčica Borozan2, Sanja Peković3, Sanja Đacić4, Danijela Džukić-Cosić5, Vitomir Ćupić1, Saša Ivanović1
1Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Belgrade, Serbia, 2Department of Chemistry, Faculty of Veterinary Medicine, University of Belgrade, Serbia, 3Department of Neurobiology, Institute for Biological Research “Sinisa Stankovic”, University of Belgrade, Serbia, 4Department for Physiology and Biochemistry, Faculty of Biology, University of Belgrade, Serbia, 5Department of Toxicology “Akademik Danilo Soldatović”, Faculty of Pharmacy, University of Belgrade, Serbia

The aim of this study was to investigate the influence of vitamin B1 (thiamine) on biochemical changes in the brain tissue of Japanese quail (Coturnix japonica) treated with chlorpyrifos. The following parameters were examined: cholinesterase activity (acetylcholinesterase – AChE and butyrylcholinesterase – BChE), nitrite concentration – NO2 (parameter of oxidative/nitrosative stress), activity of inducible nitric oxide synthase – iNOS, arylesterase – ARE, cyclooxygenase – COX and extracellular signal–regulated kinase – ERK (MAPK).

The study was conducted on eighty male Japanese quails (2 controls and 6 experimental groups, n= 10), 3-4 weeks old. One control group was treated only with vitamin B1, while the second one received pure corn oil. CPF dissolved in corn oil was administered to three groups of quails by gavage for 7 consecutive days at doses of 1.5 mg/kg BW, 3 mg/kg BW and 6 mg/kg BW. Another three groups were treated with 10 mg/kg BW of vitamin B1 i.m. 30 min after CPF administration (in above mentioned doses) for 7 consecutive days. Our studies have shown that CPF...
significantly inhibited both cholinesterases and ARE in brain, while vitamin B1 increased activity of enzymes in a dose dependent way. Also CPF has led to increase in the concentration of NO\textsubscript{2}-, activity of iNOS and COX, but after thiamine treatment there has been a decrease of these parameters. There has been a decrease of ERK expression after CPF treatment that demonstrates an increase of apoptotic vulnerability of cells exposed to CPF.

Overall these results confirm that CPF causes oxidative/nitrosative stress and apoptosis, but also support the hypothesis that thiamine belongs to the group of “antistress vitamins”.

Keywords: acetylcholinesterase, butyrylcholinesterase, oxidative/nitrosative stress, arylesterase, apoptosis

In relation to the total number of calls, enquiries regarding biocidal products are relatively rare and the number of human intoxications seems to be small. Only 10 cases with severe symptoms, which had to be treated medically, were recorded. No deaths were recorded in the local PIC.

Keywords: biocide, consultation, intoxication

Correspondence: dexc.vet@gmail.com

Evaluation of Biocidal Products Enquiries to the Austrian Poisons Information Centre 2015

Angelika Holzer, Tara Arif, Kinga Bartecka-Mino, Helmut Schiel, Dieter Genser
Poisons Information Centre, Austria

A biocidal product is any substance or mixture intending to destroy, deter, render harmless, prevent the action of, or exert a controlling effect on any harmful organism by any means other than mere physical or mechanical action. Biocidal products are divided into 4 main groups: disinfectants, preservatives, pest control and other biocidal products.

On behalf of and funded by the Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management the local Poison Information Centre (PIC) evaluated retrospectively enquiries regarding exposures to biocidal products in 2015.

PIC Austria received in total 25718 telephone enquiries in 2015. Regarding biocidal product exposure the PIC was contacted in 643 cases: 341 (53%) under the age of 15, 302 (47%) persons over 15 years of age. In 542 cases a poisoning could be excluded due to minor exposure. In 54 cases the risk of intoxication could not be estimated due to lack of sufficient information at the time of consultation. In 37 cases intoxication was suspected and medical observation was recommended. In only 10 patients an intoxication could be verified due to the severity of the symptoms. The causative substances were disinfectants (industrial n=5, household n=3) and chlorine gas (n=2).

Place of Oximes in the Management of Acute Poisoning with Cholinesterase Inhibitors: Experience of the Pharmacology Toxicology Department of University Hospital of Oran

Haciba Rezk-kallah\textsuperscript{1,2,3}, Bilel Chefirat\textsuperscript{1,2,3}, Sameh Benzerga\textsuperscript{1,2}, Anissa Zergui\textsuperscript{1,2}
\textsuperscript{1}Department of Pharmacology Toxicology, University Hospital of Oran, Algeria,
\textsuperscript{2}Department of Pharmacy, Faculty of Medicine, University of Oran 1, Algeria,
\textsuperscript{3}Environmental Health Research Laboratory, University of Oran 1, Algeria

Acute poisoning with pesticides is a serious public health problem. The indication of oximes in the treatment of poisoning by anticholinesterase pesticides is still controversial in practice, although it seems theoretically interesting because of the reversibility of the toxic cholinesterase binding and the atropine-like effect. The aim of this work is to instigate, through our experience, a rational and scientific approach of the use of oximes in the treatment of acute anticholinesterase poisoning. This is a descriptive study of cases of acute pesticide poisoning received at Oran University Hospital during the last twelve years. Data was collected prospectively, using a pre-established information sheet, accompanying the samples. Diagnosis and monitoring were performed by the determination of cholinesterase activity. The interest of the oximes will be discussed through the analysis of some observations and a review of the literature. A total of 944 cases of acute pesticide poisoning were recorded, representing 10% of total acute intoxications. They occupy second place after drug poisoning. Among our patients, the indication of oximes, when available, has been justified in organophosphate poisoning, life-threatening carbamate poisoning, and in the presence of a severe cholinergic table where the pesticide is not identified but cholinesterase activity is collapsed.
Oximes keep their place in the treatment of acute intoxication with cholinesterase inhibitors. Their indication should be discussed for each case by taking into account the anamnesis, clinical picture and rate of cholinesterase activity.

**Keywords:** pesticides, pralidoxime, organophosphorate, carbamate, cholinesterase activity

**Correspondence:** haciba_rezkkallah@yahoo.fr

---

**S-metolachlor: Acute and Subacute Effects on Common Carp (Cyprinus carpio L.)**

Božidar Rašković1, Vesna Poleksić1, Gorica Vuković2, Dejana Ćupić-Miladinović3, Gavrilo Božić1, Zoran Marković1, Draga Brkić1

1Faculty of Agriculture, University of Belgrade, Belgrade, Serbia;
2Institute of Public Health Belgrade, Belgrade, Serbia;
3Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia

Toxicological assessment of S-metolachlor, synthetic organic herbicide frequently used for efficient weeds control, was the subject of the present study. Two exposure experiments (acute - 4 day and subacute - 28 days) were conducted on five months old common carp originated from the same family from the selective breeding program. 96h LC50 was calculated to be 16.31 mg L−1, which is generally high value, since environmental concentrations of S-metolachlor are usually determined to be below 1 mg L−1. Concerning this, three concentrations were set for a subacute test, carried out in triplicates: C0 - 0.0 mg L−1, C1 - 0.5 mg L−1, C2 - 1.4 mg L−1, C3 - 4.1 mg L−1. Biomarkers used for the assessment of fish health status were: histopathology of gills and liver, as well as nuclear abnormalities on erythrocytes. Histological assessment found a number of alterations in both sampled tissues: hyperaemia, presence of eosinophilic granular cells, epithelial lifting, hyperplasia of epithelial cells and focal necrosis in gills; leukocyte infiltration, hyperaemia, fibrosis of blood vessels and focal necrosis in liver. Only two of all mentioned alterations (epithelial lifting and gills hyperemia) had higher levels in the control, while majority were absent from control group. Different morphological abnormalities were noticed on the erythrocyte nuclei: micronucleus, nuclear buds, fragmented-apoptotic, and bi-nucleated cells. Almost all alterations, either histopathological or nuclear, were higher compared to control, but due to the high variation in between groups, statistical significance was not established.

This study confirms low toxicity of S-metolachlor to common carp.

**Keywords:** herbicide, histopathology, gills, LC50,
erythrocytes

**Correspondence:** dragica.brkic@agrif.bg.ac.rs

---

**Post-exposure Treatment with the Oxime RS194B Rapidly Reactivates Brain Acetylcholinesterase Activity in Mice Exposed to Sarin and VX**

Nikolina Maček Hrvat1, Carol Green2, Suzana Žunec1, Zoran Radić3, Palmer Taylor3, Zrinka Kovarik1

1Institute for Medical Research and Occupational Health, Zagreb, Croatia;
2SRI International, Palo Alto, CA, USA;
3Skaggs School of Pharmacy & Pharmaceutical Sciences, University of California at San Diego, La Jolla, CA, USA

Acetylcholinesterase (AChE) has a vital function in cholinergic neurotransmission but is irreversibly disrupted after exposure to organophosphate (OP) nerve agents, resulting in onset of toxicity symptoms which may lead to death. Currently used therapy consists of quaternary pyridinium aldoximes as reactivators of inhibited AChE, given along with atropine. The permanent cation precludes these reactivators rapidly crossing the BBB in appreciable concentrations to reactivate synaptic AChE, thereby restricting their activity to the periphery. Alternatives encompass oximes lacking a permanent cationic charge or presenting a tertiary amine as found in the zwitterionic hydroxyiminoacetamido alkylamines (RS194B). We have shown RS194B to be an effective *in vitro* reactivator of human AChE inhibited by VX, sarin, other methylphosphonates and various alkylphosphorates. Here we examine the pharmacokinetic properties, oral bioavailability and antidotal efficacy of RS194B against OP exposure in mice. The results show that 2 h sequential administrations out to 10 h result in steady-state plasma and brain levels of the oxime. Moreover, within the 40 min period brain concentrations of RS194B exceed the plasma concentrations prior to the next administration. Also, RS194B substantially protected mice when administered by gastric lavage prior to OP exposure, whereas 2-PAM exhibited no protection when similarly administered. Furthermore, the observed recovery of the mice brain activity after administering RS194B after exposure to both, VX and sarin is consistent with its rapid tissue disposition and BBB penetration. Those results, along with low...
toxicity of RS194B in mice, make this oxime a lead candidate for analyzing efficacy, tissue disposition and pharmacokinetics in other animal species.

Acknowledgements: This work was supported by the Croatian Science Foundation (4307) and National Institutes of Health (NS U01-058046)

Keywords: CNS-active antidote, nerve agents, pharmacokinetics, tissue disposition

Correspondence: nmacek@imi.hr

---

Genotoxicity Evaluation of Chlorpyrifos, Imidacloprid and α-cypermethrin in Low Concentrations on Human Peripheral Blood Lymphocytes by Cytokinesis-block Micronucleus Assay

Vedran Mužinić, Davor Željezić
Institute for Medical Research and Occupational Health, Mutagenesis Unit, Zagreb, Croatia

The use of insecticides is increasing in modern society, but their toxic effects in low concentrations relevant for real scenario exposure have not received large attention. We have treated whole peripheral blood in vitro individually with three active insecticidal substances chlorpyrifos (a conventionally used organophosphate), imidacloprid (a novel neonicotinoid) and α-cypermethrin (a pyrethroid). Applied concentrations were estimate equivalents of acceptable daily intake (ADI), residual exposure level (REL), occupational exposure level (OEL) and a fourth common concentration (3 μg/mL). After treatment according to cytokinesis-block micronucleus assay, binucleated lymphocytes were analyzed for presence of micronuclei (MN), nuclear buds (NB) and nucleoplasmic bridges (NPB), and ratios of mono-, bi-, tri- and tetranuclear cells were calculated for estimation of cytokinesis-block proliferation index (CBPI). The results have shown that none of the tested substances exhibited significant induction of any markers of secondary DNA damage or effect on cell proliferation rates. We conclude that chlorpyrifos, imidacloprid and α-cypermethrin induce no significant secondary DNA damage nor affect proliferation across the tested concentrations.

This work has been supported by Croatian Science Foundation under the project 8366.

Keywords: chlorpyrifos, imidacloprid, α-cypermethrin, micronucleus assay, genotoxicity

Correspondence: vmuzinic@imi.hr

---

Neurotoxic Disorders Caused by Organophosphorus Insecticides: An Overview

Milan Jokanović
Faculty of Medicine, University of Nish, Nish, Serbia

In this presentation the neurotoxic disorders appearing in patients poisoned with organophosphorus pesticides and known mechanisms involved are reviewed. Organophosphorus compounds cause four main neurotoxic effects in humans: the cholinergic syndrome, the intermediate syndrome, organophosphate-induced delayed polyneuropathy and chronic organophosphate-induced neuropsychiatric disorder. Compared to the cholinergic syndrome, that causes millions of cases of poisoning with fatality of more than 15% each year, other disorders involve much smaller number of patients. Possible association of exposure to organophosphorus pesticides with neurodegenerative diseases, Parkinson’s disease and dementia will also be reviewed. This presentation is focused on neurotoxic disorders appearing after acute and chronic exposure to organophosphates with emphasis on molecular mechanisms, clinical presentation, pathogenesis, and possibilities for prevention/medical treatment.

Keywords: organophosphorus; neurotoxicity; cholinergic syndrome; acetylcholinesterase; intermediate syndrome; organophosphate-induced delayed polyneuropathy

Correspondence: milan.jokanovic@gmail.com

---

Effect of Bifenthrin on TNF α and Interleukin 1β in Mice Kidneys

Barbara Nieradko-Iwanick, Andrzej Borzęcki
Chair and Department of Hygiene, Medical University of Lublin

Pyrethroids, including bifenthrin, are used as insecticides of neurotoxic properties. Their use in pest control increases. There is data that pyrethroids can also induce organ toxicity, immunotoxicity and inflammation.

The aim of the study was to find out if 28-day exposure to bifenthrin affects TNF α and interleukin 1β levels in mice kidneys.

32 female mice were divided into 4 groups of 8. The experiment was accepted by Local Ethical Committee. Group 0 were controls. Groups 1, 2 and 3 received bifenthrin intraperitoneally at the dose of 1.61
mg/kg, 4.025 or 8.05 for 28 days. On day 29 they were anesthetized and kidneys were collected. TNF α and interleukin 1β were measured with use of ELISA kits (Cloud-Clone Corp., USA).

Mean TNF α level in the kidneys of mice from group 0 was 6 pg/ml, in group 1 = 5.4 pg/ml, in group 2 = 6.3 pg/ml and in group 3 = 9.5 pg/ml. Statistical significance was obtained for Group 3 vs 0 (p<0.05). Bifenthrin increased the level of interleukin 1β in a single dose proportionate manner in comparison to the control group. Mean interleukin 1β values were: in group 0 3.9 pg/ml, group 1 6.8, group 2 = 9.8, and group 3 11. There was a statistically significant difference between Group 3 and 0 (p<0.05).

Bifenthrin increases the level of interleukin 1β and TNF α in mice kidneys in a single dose proportionate manner which might disrupt cytokine regulatory processes and lead to disease.

Keywords: pyrethroid bifenthrin, TNF α, interleukin 1β, kidney, female mice

Correspondence: bnieradko wanicka @wp.pl

Porsulan, an Ancient Medicinal Herb, Could Have Protective Effects on Paraquat-induced Cytotoxicity on Isolated Mice Splenocytes

Seyed Hadi Mousavi1, Vafa Baradaran Rahimi2, Vahid Reza Askari2
1Medical Toxicology Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran 2Department of Pharmacology and Pharmacological Research Center of Medicinal Plant, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Paraquat is an herbicide that has a high toxicity to various organs of the body, especially lungs and kidneys. Because of its structural similarity to the active metabolite MPTP, it is thought to be the cause of neurodegenerative diseases, including Parkinson’s. The main mechanism of this combination is the induction of oxidative stress and apoptosis in various tissues and cells. Induced oxidative stress and apoptosis have been observed by paraquat in various tissues and cells including peripheral blood lymphocyte cells. It has also been shown that Porsulan has antioxidant and anti-apoptotic effects.

In this study, the protective effect of aqueous extract of Porsulan on cytotoxicity and oxidative stress by paraquat in mice splenocytes were investigated. Cell viability was assessed using MTT dye at 24 and 48 h after incubation with paraquat as well as MDA, ROS and NO, and TNF-α measured as oxidative and inflammatory markers, respectively.

Results indicated that all tested concentrations of the extract (20-160 µg/mL) significantly increased cell viability in concentration and time dependent manner (p<0.05 to 0.001). Also, inflammatory markers (TNF-α) and oxidative stress, especially MDA were significantly decreased after both 24 and 48 of pretreatment with the extract (p<0.05 to 0.001).

Our study could suggest that this plant is useful for reduction of cytotoxicity and inflammation induced by paraquat. Moreover, complementary therapy with this extract may have protective effect on farmers to avoid or delay the neurodegenerative diseases, such as Parkinson’s.

Keywords: paraquat, porsulan, Portulaca oleracea L, cytotoxicity

Correspondence: mousavih@mums.ac.ir

Primary Toxicological Assessment of the Fungicide Formulation Based on Azoxystrobin

Alina Popel, Marina Vasilyeva, Elena Yurkevich
Republican Unitary Enterprise «Scientific-Practical Centre of Hygiene», Belarus

The study of the toxic properties of plant protection products is conducted with a view to preventing or minimizing potential adverse effects on the human body and the environment. Therefore, it is now advisable to offer manufacturers of agrochemicals a wide range of promising plant protection products that will minimize the negative impact on public health, the environment and the associated economic damage.

The purpose of this work is to carry out a primary toxicological evaluation of a fungicide based on azoxystrobin.

In the course of the experiment it was found that LD50 formulation of fungicide with intragastric intake of more than 5000 mg/kg body weight. At a single exposure to the skin of LD50 more than 2000 mg/kg of body weight. When determining the parameters of acute inhalation toxicity, no cases of death of animals were recorded during the whole observation period. Doesn’t irritate the skin of rabbits, has a weak irrigation effect on the mucous membranes of rabbit eyes. Doesn’t have a sensitizing effect when applied to the test in guinea pigs. The cumulative coefficient is greater than 5.

When assessing the means of plant protection data from scientific literature, international databases, as
The results of the work done will allow increasing the range of applied plant protection products, use the drugs that are less hazardous to health and the environment in the agro-industrial complex.

Keywords: fungicide, primary toxicological evaluation, acute toxicity, plant protection, azoxystrobin.

Correspondence: vasmm11@gmail.com

New Psychoactive Substances (NPS): The Results of Toxicology Laboratory, Serbia, Nis in UNODC International Quality Assurance Programme (IQAP) and International Collaborative Exercises (ICE) in the Period 2015-2017

Maja Vujovic1,2, Biljana Milosavljevic2, Jovana Simic2
1University of Niš, Faculty of Medicine, Department of Pharmacy, Toxicology, Serbia,
2Institute of Forensic Medicine Niš, Toxicology Laboratory, Serbia

Illegal production and use of new NPS represent a worldwide health risk problem. The synthetic NPS are often labeled as “legal” and sold on the drug markets and darkened as substitutes for international controlled drugs. To struggle against illicit drugs, UN, through Drug Control Programme (UNDCP) and Office on Drug and Crime (UNODC), collaborate with many countries, governments, and institutions, continuously monitor and research global illicit drug markets. To participate in solving the drug problem, the Toxicology Laboratory at the Institute of Forensic Medicine in Nis, joined the UNODC International Quality Assurance Programme (IQAP) and International Collaborative Exercises (ICE) in 2015.

In this assay, we present our results covering a three-year period between 2015 and 2017. Methods: GC/MS, UHPLC-MS/MS, HPLC/PDA and immunoassay tests. In 2015, we identified the following drugs in BS1: Ketamine, Norketamine, Buprenorphine, Norbuprenorphine; BS2: Blank, MDA; BS3: MDA, Methadone; BS4: 6-MAM, Morphine, Nordazepam, SM-1: Caffeine, Cocaine, Lidocaine, Procaine; SM2: Amphetamine, Caffeine; SM3: Blank; SM4: Heroine, Morphine. In 2016, BS1: Morphine; BS2: Benzoylecgonine, Methylecgonine, Morphine; BS3: 3,4 MDMA; BS4: 2CB; SM1: Cocaine; SM2: JWH-073; SM3: Ketamine; SM4: Heroin. In 2017, BS1: Blank; BS2: Amphetamine, Nordazepam, Oxazepam, Temazepam; BS-3: GHB; BS4: Morphine; SM1: MDPV; SM2: Cocaine; SM3: 3,4 MDMA; SM4: Blank. In each of the two rounds per year the laboratory receives test samples that contain controlled drugs and their metabolites in urine (BS) and/or drugs in seized materials
Detection of Designer Drug - 4-bromo-2,5-dimethoxyphenylethylamine (2CB) in Urine Using GC-MS Method

Lazar Grahovac¹, Danijela Đukić-Ćosić², Vera Lukić³, Snežana Đorđević⁴, Biljana Antonijević², Marijana Ćurčić²

¹University of Belgrade-Faculty of Pharmacy, PhD student, modul Toxicology, Belgrade, Serbia,
²University of Belgrade-Faculty of Pharmacy, Department of toxicology “Akademik Danilo Soldatović”, Belgrade, Serbia,
³University of Belgrade, Institute of Forensic Medicine “Milovan Milovanović”, Belgrade, Serbia,
⁴National Poison Control Center, Military Medical Academy, Belgrade, Serbia

4-bromo-2,5-dimethoxyphenylethylamine (2CB) is psychoactive substance from the family of phenylethylamines. 2CB is a popular and recreational psychostimulant phenylethylamine, known among other street names as ‘nexus’ or ‘cyber’. It can act as partial agonist or antagonist of serotonin 5-HT2 receptors in central nervous system. Increase in recreational use of 2CB to achieve entactogenic effect indicates need for proposing adequate method for its detection in humans.

The objective of this analysis was to develop method for urine sample preparation for 2CB detection by GC-MS method. Urine samples spiked with the 2CB were prepared by liquid-liquid extraction (LLE) or solid phase extraction (SPE). Liquid-liquid extraction was done by using ethyl acetate and sodium hydroxide, while SPE was done by elution with the mixture of 2-propanol/methylene chloride/ammonia (74:24:2). Derivatization of 2CB in extracts was done with 2,2,2-trifluoro-N-methyl-N-(2,2,2-trifluoroacetyl)acetamide (MBTFA) during 20 minutes at 80 °C. Prepared samples were analyzed by GC-MS method. Obtained retention times for derivatized 2CB were 11 min while principal ions (m/z) for its identification after GC-MS analysis were 262 and 240. Chromatography peak areas imply equal efficacy of the both used sample preparation methods. Set conditions for urine sample preparation and derivatization show that developed GC-MS method is adequate for 2CB detection.

Keywords: 2CB, liquid-liquid extraction, solid-phase extraction, derivatization, GC-MS
Correspondence: lazargrahovac@gmail.com

Can Passive Inhalation of Cannabis Smoke Affect Someone’s Driving Abilities?

Ljubiša Božić, Saša Bovan
Department of theory, sociology and philosophy of law, Faculty of Law, University of Belgrade

Cannabis is one of the most frequently encountered illicit drugs in Serbia; therefore it poses a significant risk factor for traffic accidents. Serbia is one of European countries where legal limit, for presence of drugs in body fluids, is set at zero. Still, defense lawyers sometimes argue that the presence of cannabinoid metabolites in the defendant biological specimen, is result of passive unintentional inhalation of environmental cannabis smoke.

Several authors have studied passive exposure to cannabis smoke under extreme conditions (small, unventilated room, high smoke exposure, etc.) in order to demonstrate that passive inhalation affects blood or urine drug testing. Concentrations of THC (Δ9-tetrahydrocannabinol) and its metabolites, gained in such unrealistic circumstances, are in most cases lower than legal cannabinoids thresholds for drivers in some European states. Also, majority of measured cannabinoids concentration are below cutoff concentration of various immunoassay techniques, which implies possibility of staying undetected in routine drug screening. Nevertheless a real-life conditions experiment was conducted, where non-smoker participants spent three hours in well-attended coffee shop in Netherlands. Absorbed concentration of THC were measured and obtained result indicate that passive exposure to cannabis smoke in such circumstances may only lead to trace amounts of THC in serum.

All results given, obtaining such concentrations of cannabinoids, which would lead to impairment of driver, is possible only at extreme smoke exposure, for long period of time in confined space, and making it impossible for a person to be unaware of his/hers environment and potential consequences.

Keywords: Cannabis, impaired, driving, traffic, accidents
Correspondence: lj.bozic2608@gmail.com
POSTERS

Review of Analyzed Confiscated Illicit Substances. Uncommon Combination of Drugs of Abuse

Dragana Stojkov, Branislava Zdrale, Kristina Denic, Vera Lukic
Institute of Forensic Medicine, Faculty of Medicine, University of Belgrade

During 2017 substances of abuse, earlier confiscated on the territory of Republic of Serbia, were submitted to Reference Laboratory for identification of controlled psychoactive substances of Institute of Forensic Medicine. Gas chromatography with mass and liquid chromatography with tandem mass spectrometry were used for qualitative and quantitative determination of all samples (123). In marijuana samples (60), tetrahydrocannabinol, cannabidiol and cannabinol were detected and quantified. According to the amount of tetrahydrocannabinol (0.32 - 11.67 %) all marijuana samples were defined as substances of abuse. In 43 brown powder samples was detected heroin with its concomitant fillers like acetaminophen, caffeine, thebaol, meconin. Average heroin content in samples was 16.01 % (0.25–75.70 %). Lidocaine, levamisole, caffeine and cocaine as main component were identified in 11 analyzed samples. Cocaine content was from 0.95 % up to 67.80 %. Beside these, only 5 from all of analyzed samples were positive on amphetamine and 4 on MDMA. One of MDMA positive samples was with extremely specific content. It was capsule fulfilled with white and red small crystals. After analysis, it was proved that analyzed crystals contained mixture of MDMA (35.00 %), amphetamine (0.75 %) and cocaine (0.40 %). This is rare and unusual composition among the most common “ecstasy” form. This combination has pronounced cardiotoxicity which makes it extraordinary dangerous.

According to these results, marijuana and heroin are probably the most present “street” drugs. However new mixtures of known drugs may be extremely dangerous and of special concern.

Keywords: illicit substances, drugs of abuse, MDMA, cocaine

Correspondence: stojkovdragana@gmail.com

Use of Mephedrone During Pregnancy Induces Neurotoxicity in Offspring and Increases the Risk of Stillbirth

Ahmad Ghorbani1, Gholamreza Naseri2, Alireza Fazel3, Mohammad Jafar Golalipour4, Hossein Haghi2,4, Hamid Sadeghian2

Mephedrone is a synthetic derivative of cathinone, the natural psychostimulant alkaloid present in the Khat plant. Recently, regular and recreational use of mephedrone has increased among young adults in several countries. No experimental study has yet evaluated the toxicity of mephedrone in the gestational stage. This study was aimed to evaluate the effects of mephedrone exposure in pregnancy on newborn outcomes, focusing on hippocampal damage. The pregnant mice received mephedrone (50 mg/kg, sc) as regular schedule (once daily, day 5 to 18 of gestation) or repeated schedule (thrice daily at 5th, 6th, 11th, 12th, 17th, 18th day of gestation) to simulate regular or recreational uses of mephedrone, respectively. In both regular and repeated mephedrone groups, the percent of weight gain in pregnant mice was significantly lower than control group. Mephedrone significantly decreased the weight and number of delivered pups and increased the rate of stillbirth. Results of immunohistochemistry and TUNEL assays showed an inhibition of cell proliferation and an increase of apoptosis in the hippocampus of delivered pups of repeated mephedrone group. This apoptotic effect was associated with increased expression of the proapoptotic gene Bax and reduced the expression of antiapoptotic gene Bcl-2. Data of Morris water maze showed an impairment of the spatial learning and reference memory in offspring (60-day-old) born from mephedrone treated mothers. In conclusion, this study demonstrated that regular and repeated exposure to mephedrone during pregnancy increased the risks of low birth weight, stillbirth, and neurotoxicity in offspring.

1Pharmacological Research Center of Medicinal Plants, Mashhad University of Medical Sciences, Mashhad, Iran; 2Department of Anatomy and Cellular Biology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; 3Gorgan Congenital Malformations Research Center, Golestan University of Medical Sciences, Gorgan, Iran; 4Medical Genetics Research Center, Mashhad University of Medical Sciences, Mashhad, Iran; 5Department of Laboratory Sciences, School of Paramedical Sciences, Mashhad University of Medical Sciences, Mashhad, Iran; 6Division of Neurocognitive Sciences, Psychiatry and Behavioral Sciences Research Center, Mashhad University of Medical Sciences, Mashhad, Iran; 7Department of Basic Science and Neuroscience Research Center, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran
Mephedrone - “White magic”. Qualitative and Quantitative Analyse in Urine by LC-MS/MS

Branislava Zdrale, Dragana Stojkov, Kristina Denic, Vera Lukic
Institute of Forensic Medicine, Faculty of Medicine, University of Belgrade

Mephedrone, also known as “white magic” is a designer stimulative drug from cathinone class, present in Catha edulis plant leaves. It is usually in the form of white powder, tablets or capsules. The users can take it by mouth, inject, smoke, snort or use rectally. Onset of the effects and their duration depends on the way mephedrone is used. Mephedrone causes effects similar to the effects of cocaine, amphetamine and MDMA such as stimulation, euphoria, elevated mood, improved mental function. Because of its structure similar to biogenic amines mephedron influences cardiovascular and neurological system provoking thus side effect.

In Laboratory of toxicology, Institute of Forensic Medicine, urine sample from ICE program organized by scientific department of UNODC was analyzed. Qualitative and quantitative analyze of mephedrone was performed in urine by LC-MS/MS. Its identification was also confirmed by GC-MS.

Solid phase prepared sample using Strata-XC column was derivatized with MBTFA and analyzed by GC/MS system. It was identified on 8.25 min with target ions 119, 154 and 91.

Alkaline liquid-liquid extracted sample was analyzed by LC-MS/MS system with a C18 column (Kinetex 100Å, 2.6 µm, 75×4.6 mm). Assay was performed using 10mM ammonium formate (A) and methanol (B) as a mobile phase with a flow rate 0.8 ml/min. Target ions were 178, 160, 145. Mephedrone retention time was 0.98 min.

For quantitative analyze mephedrone standard (purity ≥ 99.8 %) was used. In submitted urine sample mephedrone is detected in concentration of 966 ng/mL.

LC-MS/MS is fast and precise method for reliable mephedrone identification and quantification in biological sample.

Keywords: mephedrone, white magic, mephedrone detection, synthetic cathinone, LC-MS/MS
Correspondence: branislavarusic@gmail.com

Testing of Tobacco and Tobacco Products in Croatia Before Transposing the 2014/40 / EU Tobacco Directive in May 2017

Ivona Vidić Štrac, Nino Dimitrov, Buga Kovacić, Rina Oliver Grbavec, Bernarda Damianić
Croatian Institute of Public Health

In December 2016, cigarettes and related products were bought at random. The tests were carried out in accordance with the requirements of the Ordinance on Health Safety of Consumer Items (OG 125/2009), REACH requirements for lead, cadmium and nickel, and the requirements for nicotine in e-liquids prescribed by the new tobacco directive.

Molds were detected in four samples of cigarettes or 20%. In 10% of cigarette samples, the carbon monoxide content in the smoke condensate is higher than maximum emission levels, while in one sample the tar content is higher than the stated value. Organochlorinated pesticides examination was complied with requirements. In fine-cut tobacco, molds were found in 50% of samples. Rolling paper complies also with the requirements prescribed in the Ordinance. The total content of lead in the mouthpiece was more than 0,05 % by weight, as prescribed by the requirements for products Commission Regulation (EU) 2015/628 of 22 April 2015 coming into contact with skin. The nicotine content was found to be greater than the maximum permissible value of 20mg/mL in 50% e-liquid samples.

There is few references on tobacco testing from the market. Values for nicotine in e-liquids declared and measured content are in accordance with the literature data that indicates the results deviations. The results for mouthpiece samples are of particular concern since no research has been done so far and there is no legislation on the matter, instead analogue regulations are applied.

Keywords: tobacco and related products, market, legislation
Correspondence: ivona.vidic-strac@hzjz.hr
Application of Physiologically-Based Pharmacokinetic Modelling to Pesticides in South Africa: Requirements, Feasibility, and Challenges

Turgay Celik¹, Wells Utembe², Mary Gulumian²,³
¹School of Computer Science and Applied Mathematics, University of the Witwatersrand, Johannesburg, South Africa,
²Department of Toxicology at the South African National Institute for Occupational Health, South Africa,
³School of Pathology, University of the Witwatersrand, South Africa

Physiologically-based pharmacokinetic (PBPK) models use physiologic properties of organisms and biophysical properties of substances to describe the absorption, distribution, metabolism and elimination (ADME) of substances. This information is very important for, inter alia, prediction of internal dose at target organs as well as interspecies dose and route-to-route extrapolation necessary for the risk assessment process in the registration of pesticides. PBPK modelling is especially invaluable in cases where specific target organ toxicity (STOT) is anticipated, and also for the determination of toxicokinetically and toxicodynamically derived chemical-specific adjustment factors (CSAFs) that are important in the derivation of parameters such as the acceptable daily intake (ADI) and reference dose (RfD).

Publicly available generic PBPK models, such as the USEPA’s Exposure Related Dose Estimating Model (ERDEM), as well as the Canadian Centre for Environmental Modelling and Chemistry’s generalized PBPK model make it possible for their applications by developed and developing countries. However, despite their availability, PBPK modelling is conspicuously underutilized in South Africa where numerous challenges may have hampered this possibility. Steps are therefore taken to urgently remedy the situation in South Africa by establishing a unit specializing in much needed computational toxicology in collaboration between the Department of Toxicology at the South African National Institute for Occupational Health (NIOH) and the School of Computer Science and Applied Mathematics at the University of the Witwatersrand. This in turn, will be able to deliver more adequately on the risk assessment requirements for the registration of pesticides in South Africa.

Keywords: PBPK modelling, dose
Correspondence: Turgay.Celik@wits.ac.za

TARGET ORGAN TOXICITY

New Insight into the Toxicity of Polychlorinated Biphenyls: Study on Animal Model

Aleksandra Buha¹, Vesna Milovanovic², Sasa Jankovic³, Zorica Bulat¹, Biljana Antonijevic¹, Vesna Matovic¹
¹Department of Toxicology “Akademik Danilo Soldatović”, University of Belgrade-Faculty of Pharmacy, Serbia,
²University Children’s Hospital, Belgrade, Serbia,
³Institute of Meat Hygiene and Technology, Belgrade, Serbia

Polychlorinated biphenyls (PCBs) are synthetic chemicals widely spread in the environment. Due to PCBs chemical stability and lipophilic nature, i.e. ability to accumulate and biomagnify in the food chain, humans interact with these chemicals on a daily bases. This study was aimed to investigate the effects of exposure to different doses of PCBs on body weight gain, hematological parameters, liver, kidney and thyroid function in animal model.

Six groups of male albino Wistar rats (7 animals/group) were receiving commercial PCBs mixture Aroclor 1254 dissolved in corn oil in six different doses (chosen to reflect the environmental exposure) by oral gavage during 28 days while controls were receiving corn oil only.

PCBs produced profound effects on body weight gain suggesting possible developmental toxicity. Critical effects of PCBs in blood was the diminishing effect on white blood cells count while platelets number was lowered only in group receiving the highest dose of PCBs. The study demonstrated both hepato- and nephrotoxic effects of PCBs with oxidative stress as an important mechanism of their toxicity. Namely, PCBs were shown to affect enzymatic and non-enzymatic components of antioxidant defence system and to cause both lipid and protein degradation in liver and kidneys. The obtained results also gave the confirmation of the thyroid disrupting effects of PCBs with profound effects on T4 hormone levels presumably as the result of PCBs direct effect on thyroid gland.

It can be concluded that PCBs impose significant adverse effects in even in relatively low doses that correspond to environmental ones.
Cadmium Levels in Rats Blood and Testes after Acute Oral Treatment with Two Doses

Milena Andjelković1,2, Simona Tatović2, Danijela Đukić-Ćosić2, Aleksandra Buha Đorđević2, Vesna Matović2, Zorica Bulat2
1Health Center Kosovska Mitrovica, 2University of Belgrade – Faculty of Pharmacy, Department of Toxicology “Akademik Danilo Soldatović”

Cadmium (Cd) affects reproductive organs by still unclear mechanisms of toxicity. Disruption of the blood-testis barrier, followed by permeability changes, is one of them. The aim of the study was to investigate Cd level in the blood and testes after low and moderate acute oral administration.

Male Wistar rats were randomly assigned to non-treated (control) and treated (Cd15 and Cd30) groups, administered by oral gavage 15 and 30 mgCd/kg body weight, respectively. After 24 hours, animals were sacrificed and blood and testes were used for Cd analyses. Mineralisation was carried out by microwave digestion system and Cd levels were measured by atomic absorption spectrophotometer with graphite tube. Nonparametric ANOVA, post hoc Mann Whitney test and Spearman’s rho correlation coefficient have been used for statistical analyses.

Blood Cd levels were 14.47 μgCd/L (controls), 51.60 μgCd/L (Cd15) and 52.03 μgCd/L (Cd30), indicating significantly higher Cd in treated groups compared with controls, but with no difference between the treated groups. In testes, Cd levels were 0.0024 μgCd/g, 0.0354 μgCd/g and 0.0798 μgCd/g in control, Cd15, and Cd30 groups, respectively. Treated groups have significantly higher Cd level than control one, and in contrast to blood Cd levels, higher Cd dose induced significantly higher Cd concentration in testes, compared with lower one (p<0.05). Furthermore, correlation analysis has showed significant correlation between levels of Cd in blood and testes (p< 0.01).

This study demonstrated that oral treatment with low and moderate Cd doses induced significant increase in both blood and testes, although the dose dependence was observed for testes only.

Keywords: reproductive organs, blood-testis barrier, correlation

Correspondence: millena.andjelkovic@gmail.com

Stability of Mitochondrial Intactness for Further Toxicological Studies

Ege Arzuk, Hilmi Orhan
Ege University, Faculty of Pharmacy, Pharmaceutical Toxicology Department, 35100 Izmir, Turkey

Mitochondria are critical subcellular organelles as they provide more than 95% of the energy for biochemical and physiological functions, as well as they play a critical role in lipid metabolism, steroidogenesis and programmed cell death. Recently we have shown their metabolic capacity for various drugs as well. Nevertheless, mitochondria from different species, as well as from different organs in the same organism exhibit diverse properties, which entails special attention in working on structures and functions of this organelle. Intactness of mitochondria as the whole organelle must be provided and maintained along in vitro studies, since these varieties may significantly influence both toxic pathways and toxic end-points in in vitro studies. In the current study, therefore, we have explored mitochondrial stability in isolated organelle and in frozen tissue by mitochondrial pore transition (MPT). Mitochondria were isolated from fresh tissue and either mitochondria or whole tissue were kept in various temperatures (room temperature, +4 °C and -86°C) for a day, for a week, for 3 months, respectively. MPT pore opening was assessed as mitochondrial swelling by a rapid decrease in absorbance at 540 nm. It has been found that mitochondria and tissue maintain functionality for limited time due to the ambient temperature. However, mitochondria keep stability much longer when frozen.

This study is supported by The Scientific and Technical Research Council of Turkey (TUBITAK, SBAG-114S310).

Keywords: subcellular isolation, integrity, mitochondrial pore transition

Correspondence: horhan@gmail.com

Exposure of Cigarette Smoke Extract May Induce Apoptosis and Autophagy in Human Placenta Choriocarcinoma JEG-3 Cells

Hae-Miru Lee, Kyung-Chul Choi
Laboratory of Biochemistry and Immunology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk, Republic of Korea

In this study, the effects of cigarette smoke (CS) on the induction of apoptosis via reactive oxygen species
(ROS) production and endoplasmic reticulum stress (ER-stress) of JEG-3 human choriocarcinoma cells were examined to confirm the relationship between CS and placenta development. Upon TUNEL assay, CS extract (3R4F; 0.3 μM and 2.1 μM) increased JEG-3 apoptosis. Western blot assay revealed that the protein expressions of p53, Bax and CCAAT-enhancer-binding protein homologous protein (CHOP) increased, while the levels of Bcl-2 were reduced following CS extract treatment. Moreover, DCFH-DA assay revealed increased ROS production. Upon MTT assay, isoprene (IP), one of ingredients of CS, deceased JEG-3 cell viability (10^{-11} M to 10^{-6} M). After based on the MTT assay, two IP concentrations of 10^{-11} M and 10^{-8} M were selected and the protein expressions of cyclin D1, cyclin E1, p21 and p27 decreased in response to IP. Furthermore, IP showed the greatest increase in autophagy at 24 h and further induction of cell death at 72 h upon MDC and TUNEL assay. Western blot analysis confirmed the increase in autophagy markers, LC3β and p62, as well as the increase or decrease of apoptosis markers p53, Bax, CHOP and Bcl-2 in response to its treatments. In addition to confirming increases in ROS through DCFH-DA, we also confirmed the expression of Nrf2, an antioxidant marker, and the expression of Kelch-like ECH-associated protein 1 (KEAP1), which specifically degrades Nrf2, by Western blot. Taken together, these results indicate that CS may inhibit the development of placenta via activation of ROS by inducing apoptosis and autophagy by affecting the expression of KEAP1, which regulates Nrf2 expression. [This research was supported by a grant (14182MFDS977) from the Ministry of Food and Drug Safety in 2017.]

**Keywords:** cigarette smoke, isoprene, placenta choriocarcinoma, cell cycle, EMT

**Correspondence:** kchoi@cbu.ac.kr

---

### TOXICOLOGY OF DRUGS

#### Harmful Effects of the Misuse of Diclofenac in the Geriatric Population

Bojana Petrović2, Predrag Vukomanović2, Saša Ivanović3

1Medical Sanitary School of Applied Sciences “Visan”, Belgrade, Serbia,
2Faculty of Veterinary Medicine University of Belgrade, Serbia

The geriatric population is affected by multiple physiological deficiencies, polymorbidity, poliotherapy and polypragmasy. Based on a detailed overview of relevant literature, theoretical and pharmacoepidemiological studies, diclofenac is one of the most frequently prescribed medications for patients over 65 years for painful conditions and rheumatic ailments in the Republic of Serbia. In addition, elderly very often takes diclofenac at the recommendation of an incompetent person or at his own discretion. This fact is particularly alarming, due to the known pharmacovigilance of diclofenac, especially in uncontrolled usage and overdosage. Diclofenac achieves analgesic, antiinflammatory and antipyretic activity by non-selective inhibition of cyclooxygenase: physiological COX1 (which contributes to harmful effects) and pathological COX2. The most common harmful effects of diclofenac are gastrointestinal haemorrhage and ulcer perforation, which are caused by various pathogenetic mechanisms, such as: weakening and disorders in the regeneration of the mucous barrier of the stomach, decreased bicarbonate and mucus secretion, disinhibition of HCl secretion, reduced circulation in the mucous membrane. Diclofenac can lead to nephrotoxicity, cardiotoxicity, hepatotoxicity, anaphylactic reactions. Furthermore, aging leads to changes in pharmacodynamics and pharmacokinetics, which affects the choice, dosage and frequency of the use of many medicines, and therefore diclofenac. Diclofenac can lead to an increase in the serum concentration of cardiac glycosides, and lowering effect of β-blockers, ACE inhibitors and thiazide diuretics, drugs that are often used in the therapy of elderly. Based on the above facts, it can be concluded that diclofenac is widely used in any pain symptom and is very commonly misused.

**Keywords:** chronic pain, elderly, diclofenac, pharmacovigilance.

**Correspondence:** bojanapetrovich@yahoo.com
Bile Acid Potentiates Apoptosis of Human Breast Adenocarcinoma Cells Induced by Doxorubicin

Bojan Stanimirov, Karmen Stankov, Nebojša Pavlović, Maja Đanić, G. Bogdanović, Vesna Kojić, Momir Mikov
1Faculty of Medicine, University of Novi Sad, Novi Sad, Vojvodina, Serbia,
2Oncology Institute of Vojvodina, Sremska Kamenica, Vojvodina, Serbia

The use of doxorubicin (Dox) is limited by cumulative, dose-dependent toxicity. Ursodeoxycholic acid (Udca), as a bile acid with amphipathic structure, has propensity to modulate transport of xenobiotics across biological membranes but also to modulate cell metabolism, proliferation and cell death. The aim of this study is to evaluate whether Udca potentiates apoptosis-inducing effects of DOX in MCF-7 cell line by measuring expression of apoptosis-regulating genes.

MCF-7 cells were treated either with 250 nM of Dox or with combined treatment by adding 0.05 mM Udca. Cell viability was determined by a colorimetric assay using MTT whereas gene expression was analysed using qRT-PCR.

The co-incubation of MCF-7 cells with Dox and UDCA resulted in significant inhibition of cell growth (p<0.05) compared to Dox. Relative expression of gene of pro-apoptotic Bax was increased in both Dox-treated cells (p=0.001) and co-treated cells (p=0.007), compared to untreated control. Relative quantitation of Bcl-2 mRNA revealed that expression of this anti-apoptotic marker was significantly reduced in cells co-treated with Udca (p=0.0002) compared to Dox-treated cells. The values of Bax to Bcl-2 ratio indicated that co-treatment with Udca induced apoptosis in higher level than Dox alone compared to control (p=0.004, p=0.008).

Udca potentiates mitochondrial pathway of Dox-induced apoptosis in MCF-7 cells, suggesting that Udca may be further investigated as a novel agent with primary aim to improve therapeutic index of DOX.

Supported by HORIZON2020 MEDLEM project No.690876, Project for Scientific and Technological Development of Vojvodina No.114-451-2072-/2016-02.

Keywords: chemotherapy, toxicity, mitochondria
Correspondence: bojan.stanimirov@mf.uns.ac.rs

Current Issues of Reglementation of Air Pollution in the Production of Cytotoxic Drugs

Vasilkevich Vadzim, Pavel Liapioshka
Republican unitary enterprise “Scientific Practical Centre of Hygiene”

For the treatment of patients with tumors in the Republic of Belarus are widely used cytotoxic drugs. Actual hygiene issue is the prevention of air pollution of the working area and the environment of products of the synthesis of cytotoxic drugs. This task is solved through the establishment of safe limits of occupational exposure, for example, in the study of bendamustine and erlotinib.

Erlotinib is a tyrosine kinase inhibitor, causes cell cycle arrest in G1 phase. Prolonged intragastric intake of toxic doses in the organism of laboratory animals (15 mg/kg/day for rats) led to changes that were most pronounced in critical organs – liver, kidney, female individuals also ovaries. Erlotinib has reproductive toxicity (rats, rabbits).

Bendamustine is an alkylating substance, which is capable of forming covalent bonds with DNA of cells. It was found that prolonged intravenous intake in laboratory animals (rats, dogs) critical organs and systems are the immune (immunosuppression), hematopoietic (lymphocytes in the blood), reproductive (testes) and urinary (kidney) system. Experiments on cell cultures revealed the presence of genotoxic (chromosomal aberrations), carcinogenic and teratogenic effects (rats, mice).

The possibility of developing late effects (genotoxic, carcinogenic and teratogenic effects) and toxic effects in critical organs are the scientific basis to restrict contact of workers by inhalation or through the skin of many cytotoxic drugs (as in our example, bendamustine and erlotinib) with strict control of working zone air.

Keywords: cytotoxic drugs, bendamustine, erlotinib
Correspondence: sabas2004@mail.ru

High Postmortem Clozapine Concentration: Reliable Cause of Death or Postmortem Redistribution – Case Report

Kristina Denic, Branislava Zdrale, Dragana Stojkov, Slobodan Nikolic, Vera Lukic
Institute of Forensic Medicine, Faculty of Medicine, University of Belgrade

Clozapine is atypical antipsychotic which is used in treatment of schizophrenia. It shows moderate first pass metabolism which is catalysed by CYP1A2 isoenzyme. The most important and pharmacological
active metabolite is N-desmethylclozapine. Clozapine is lipophilic drug with large volume of distribution, high protein binding and half-life of 12h. It has narrow therapeutic range (0.35-0.6 mg/L) whilst it is necessary to provide titration schedule in patient treatment and keep them in licensed doses.

There are cases with blood concentration of clozapine above toxic level (1mg/L) with no side effects. Also, there are registered cases of high postmortem level of this drug. The question is whether can we be sure that high postmortem concentration is the cause of death.

In the laboratory of toxicology, Institute of Forensic Medicine the postmortem blood and urine of female aged 53, were submitted. She has been treated with clozapine for many years under advisor of psychiatrist. She was found unresponsive in her bed. An autopsy was performed 7h after death.

Samples were analyzed by LC-MS/MS and GC-MS. Clozapine was detected at concentration of 2.18 mg/L in blood and 8.01 mg/L in urine. Desmethylclozapine levels were 0.96 mg/L and 4.71 mg/L, respectively. Detected concentrations were above the toxic one and may lead to the wrong conclusion of the cause of death. This finding can be explained by pronounced postmortem redistribution of clozapine, resulting in increased antemortem concentrations (for 400%).

According to this phenomena, it can’t be claimed that high postmortem clozapine concentrations are reliable cause of death.

Keywords: clozapine overdose, postmortem redistribution, fatal poisoning

Correspondence: kristinadenic@yahoo.com

In Vitro Evaluation of Hepatotoxicity by Amiodarone in Micropatterned Cocultured Hepatocytes (HepatoPac) using Liver-specific Biomarkers

Zsuzsanna Nereda, Roelof de Wilde, Zsuzsanna Gáborik
SOLVO Biotechnology, Budaörs, Hungary

Drug induced liver toxicity (DILI) is a major reason for discontinuing drug development programs and identifying potential risk for DILI is therefore important. Several in vitro tests have been developed to assess hepatotoxicity risk. Models using polarized primary hepatocytes with functional drug metabolizing enzymes and transporters, such as sandwich cultured hepatocytes, spheroids, randomly cocultured hepatocytes and micropatterned cocultured hepatocytes (HepatoPac) are because of their in vivo-like properties considered to be among the more physiologically relevant models. A drawback of many in vitro tools however is lab-to-lab variability. Micropatterned co-cultures (HepatoPac) have a tightly controlled architecture and because of this we hypothesized its lab-to-lab variability might be minimal. In addition, the model has a life-span of several weeks in culture, allowing repeat dosing of test compounds. We therefore evaluated the known hepatotoxicant amiodarone in HepatoPac and compared results to published data. Using albumin, urea, adenosine triphosphate (ATP) and glutathione (GSH), we found TC_{50} (the concentration that decreases a response by 50%) values close to published values. After single dosing, similar TC_{50} values were obtained on day 5 and 9 using intracellular ATP and GSH levels. After dosing every 2 days, we measured TC_{50} values of increasing potency over time, using albumin and urea levels in the medium. No toxicity was observed for negative control acetylsalicylic acid (aspirin) at any of the conditions. In conclusion, we successfully used the HepatoPac toxicity assay in our lab and demonstrated low variability between our and published data, indicating the robustness of this method between labs.

Keywords: liver toxicity, albumin, urea, ATP, glutathione

Correspondence: roelof.dewilde@solvo.com

Possible Interactions Between Metformin and Cisplatin

A. Zeynep Ünal¹, Suna Sabuncuoğlu¹, FA Kaya²
¹Hacettepe University, Faculty of Pharmacy, Department of Toxicology, Ankara, Turkey
²Hacettepe University, Faculty of Medicine, Department of Pediatrics, Division of Hematology-Bone Marrow Transplantation Unit, Ankara, Turkey

Cisplatin (CIS) is a chemotherapeutic drug which is the first member of a class of platinum-containing anti-cancer drugs, and it is used for treatment of various types of cancer like ovarian, bladder and cervical cancer. CIS is an alkylating agent which is cell-cycle non-specific and most active in the resting phase of the cell. Metformin (1,1-dimethylbiguanidehydrochloride, MET) is an oral biguanide antidiabetic drug that is widely used for the treatment of type 2 diabetes. MET and CIS have been applied as alone and combinations on HepG2 and Hep2 cell lines for 24, 48 and 72 hours in order to evaluate the interactions between the two drugs. In cells, real-time monitoring of cell viability, apoptosis and cell proliferation
has been evaluated. Cell viability and damage was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and lactate dehydrogenase release assays. Apoptotic and necrotic cell ratios were determined by fluorescence-activated cell sorting in flow cytometry and real-time cell motions were evaluated at XCELLigence. In the preliminary studies, the doses to be applied (in the Hep2 cell line cisplatin 100-0.8 μM, metformin 1-10 mM; in the HepG2 cell line cisplatin 2.0-0.08 μM, metformin 10-0.04 mM) and the duration of exposure (48 hours) were determined for cytotoxic evaluations. In general, it has been observed that the combination of metformin and cisplatin may increase cytotoxic effect, in spite of, the differences in the results depending on the method of analysis, the cell line and the dosage applied. In conclusion, interactions between cisplatin and metformin must be considered in diabetic cancer patient.

**Keywords:** cisplatin, metformin, drug interaction.

**Correspondence:** sunaatasayar@gmail.com

---

**Manifestations of Chronic Inhalation Toxicity of Zoledronic Acid**

**Yury Sobal, Pavel Liapioshka**

*Republican unitary enterprise “Scientific Practical Centre of Hygiene”*

Zoledronic acid is a bisphosphonate of the third generation with selective effect on bone tissue. Used for the treatment of osteoporosis and prevention of bone metastases in conjunction with standard chemotherapy.

Manifestations of chronic toxicity were investigated on white rats treated by inhalation daily 5 times a week with aqueous solution of zoledronic acid in doses of 0.01 mg/m3, 0.05 mg/m3, 0.1 mg/m3, while control group of animals was administered solvent (OECD Handbook No. 452, “Chronic toxicity studies”). After 4 months of inhalation the following were changed: the volume of the erythrocytes, the hemoglobin content in the erythrocytes, urinary pH decreased, content of urea in blood and urine increased, chlorides in the urine, the activity of alanine aminotransferase in serum decreased, the activity of aspartate aminotransferase in serum increased as well as the relative ratio of the weight of the buds. Fluctuations in these indicators were beyond the fluctuations of physiological standards for laboratory animals.

Based on the analysis of the obtained data of morpho-functional indices in albino rats subjected to 4-month inhalation receipt of zoledronic acid, the threshold of chronic action of zoledronic acid on the chloride content in urine was established at the level of 0.01 mg/m3.

**Keywords:** zoledronic acid, bisphosphonate, chronic toxicity

**Correspondence:** y_sobol@mail.ru

---

**Morphine Aggravates Cisplatin-induced Nephrotoxicity and Oxidative Stress in Rats**

**Atefeh Aminian1,2, Shiva Javadi1, Reza Rahimian1, Ahmad Reza Dehpour3, Fahimeh Asadi Amoli2, Payman Moghaddas2, Shahram E. Mehr1**

1Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran,
2Department of Pharmacology, School of Medicine, Arak University of Medical Sciences, Arak, Iran,
3Department of Pathology, Tehran University of Medical Sciences, Tehran, Iran

Cisplatin as a chemotherapy agent can cause nephrotoxicity. Morphine is widely used in various types of cancer for the clinical management of pain. Moreover, morphine has been reported to possess potential immunomodulatory and antioxidant properties. In this study we evaluated the effects of morphine in a rat model of cisplatin-induced nephrotoxicity. Following intraperitoneal (i.p) injection of a single dose of cisplatin (5mg/kg), animals received morphine (5 mg/kg/day, i.p) and/or an opioid antagonist, naltrexone (20 mg/kg/day, i.p), for 5 days. Cisplatin-induced nephrotoxicity was detected by alterations in kidney tissue morphology in addition to a significant increase in plasma creatinine and urea levels. In cisplatin group, levels of IL-1β and TNF-α were significantly increased in the renal tissue. Moreover, superoxide dismutase activity and glutathione (GSH) concentration were significantly reduced in renal tissue in cisplatin group compared with control animals. Treatment with morphine worsened the deleterious effects of cisplatin at clinical, biochemical and histopathological levels; whereas naltrexone diminished the detrimental effects of morphine in animals receiving morphine and cisplatin. Morphine or naltrexone alone had no effect on the mentioned parameters. Our findings indicate that concomitant treatment with morphine might intensify cisplatin-induced renal damage in rats. These findings suggest that opioid analgesics should be cautiously administered in patients receiving cisplatin chemotherapy.

**Keywords:** cisplatin, morphine, naltrexone, antioxidant, nephrotoxicity

**Correspondence:** atefeh.aminian@yahoo.com
Hydrocortisone Reduces Vesication by Mechlorethamine In Vivo
Hemanta C Rao Tumu, Benedette Cuffari, Blase Billack
Department of Pharmaceutical Sciences, St. John’s University, Jamaica, NY, United States

Extravasation reactions (EVRs) occur in approximately 6% of cancer patients receiving intravenous chemotherapy, with dermatotoxic effects ranging from tissue swelling and localized inflammation, to blistering (vesication) and ulcer formation. Current treatments aimed at reducing undesired EVRs remain grossly inadequate. Our long-term goal is to decipher the molecular mechanisms that regulate vesication and cutaneous inflammatory responses to cytotoxic chemotherapy drugs such as mechlorethamine (MEC), and to identify novel medical interventions to reduce EVRs. The purpose of the present study was to determine the vesicant countermeasure potential of hydrocortisone (HC). To this end, the mouse ear vesicant model (MEVM) was used, with male Swiss Webster mice serving as the test strain. Compared to control ears, mouse ears exposed to a single dose of MEC (0.500 μmol/ear) showed an increase in wet weights, ear thickness, edema, hyperplasia, vesication and inflammatory cell infiltration after 24 h. Tissue expression of inducible nitric oxide synthase (iNOS) and matrix metalloproteinase-9 (MMP-9) were up-regulated in response to MEC. Fluorescence microscopy of TUNEL stained sections showed that the occurrence of apoptosis extended from the epidermis of the MEC treated side all the way to the contralateral epidermis. In contrast, MEC exposed ears treated topically with HC at a test dose of 0.031 mg/ear showed a significant decrease in wet weight (12.6% less than MEC alone), morphometric thickness (16.5% less than MEC alone) and vesication (60.0% for MEC reduced to 33.3% after HC). Taken together, our studies suggest that lose-dose HC may serve as an effective countermeasure to chemotherapy EVRs.

Keywords: MMP-9, iNOS, extravasation, mouse ear vesicant model, hydrocortisone

Correspondence: billackb@stjohns.eduż

TOXICOLOGY OF MIXTURES/MIXTURE RISK ASSESSMENT

Integrated Toxicological and Chemical Evaluation of Complex Mixtures of Drinking Water Disinfection Byproducts
Alena Drazdova, Siarhey Sychik, Girina Veranika, Emelianova Olga, Buraya Valiantsina, Firago Anna
Republican Scientific-Practical Centre of Hygiene, Minsk, Republic of Belarus

Disinfection of water is a great public health concern. Use of water chemical disinfection allowed to reduce significantly level of waterborne diseases. The other side of this success are disinfection byproducts (DBPs) in the treated water. All methods of chemical disinfection (more widely used – chlorination and ozonation) are accompanied by the formation of a huge number of DBPs through reaction of the chemical disinfectant with naturally occurring inorganic and organic matter in the source water. In some cases number of DBPs exceed 400. As a result population is chronically exposed to a very large number of DBPs with potential carcinogenic effects or causing target-organ toxicity, such as reproductive and developmental toxicity in the low levels compared to levels found in drinking water. Many of them are volatile and express hazard also through inhalation and percutaneous exposure while bathing, showering and housework. As usual less than 10 DBPs are routinely monitored. The purpose of our research was to develop a research scheme for evaluation integrated toxicity of complex DBPs mixtures of drinking water.

Keywords: disinfection byproducts, integrated toxicity, genotoxicity, complex mixtures, low-level exposure.
Correspondence: water@rspch.by

Features of the Biological Effect of Multicomponent Mixtures Containing Styren
Bogdanov Ruslan, Bondarenko Ludmila
Republican unitary enterprise «Scientific Practical Center of Hygiene», Minsk, Belarus

In real conditions, a large number of different chemical substances enter the body at the same time causing
harmful effects. The combined effect of a mixture can lead to increased toxic effects if compared to the isolated effects of single chemicals.

In experimental studies on white rats, the combined action of styrene with formaldehyde, methyl methacrylate and acrylonitrile was studied after single inhalation and gastric intake. At the initial stage, the parameters of toxicometry of each substance DL_{16} (CL_{16}), DL_{50} (CL_{50}), DL_{84} (CL_{84}) were determined. The data obtained were used to establish the character of the combined action of styrene with the chemical substances studied by setting up experiments in 9 different variants of the ratios of sublethal doses of the components of the mixtures. Dependence of the mortality of experimental animals on the action of isolated substances and their combination was used to compile multiple regression equations.

Interpretation of the regression equation with inhalation intake of styrene and formaldehyde made it possible to establish the potentiating nature of the action of the mixture. The combination of styrene with acrylonitrile and styrene with methyl methacrylate at gastric intake also induced more than additive effect (potentiation of effects).

Thus, the combined action of styrene with formaldehyde, methyl methacrylate and acrylonitrile in experiments on white rats at the level of sublethal doses under the indicated exposure conditions is characterized by an increase in the toxic effect compared to their isolated effect on the organism of laboratory animals.

Keywords: combined effects, styrene, formaldehyde, methyl methacrylate, acrylonitrile

Correspondence: 7_rus@tut.by

Mixture of Cadmium and Decabrominated Diphenyl Ether: Target Tissue Doses and Hepatotoxicity in 28 days Exposed Wistar Rats

Marijana Ćurčić¹, Saša Janković², Sanja Stanković², Vesna Milovanović², Aleksandra Buha Đorđević¹, Evica Antonijević¹, Zorica Bulat¹, Slavica Vučinić², Vesna Matović³, Biljana Antonijević¹
¹University of Belgrade-Faculty of Pharmacy, Department of toxicology “Akademik Danilo Soldatović”, Belgrade, Serbia,
²Institute of Meat Hygiene and technology, Belgrade, Serbia,
³Clinical Center of Serbia, Laboratory for Medical Biochemistry, Belgrade, Serbia,
⁴Biochemistry Laboratory, Pediatrics Clinic, Belgrade, Serbia,
⁵National Poison Control Center, Military Medical Academy, Belgrade, Serbia

The objective of this study was to examine differences between toxic liver effects of external and internal doses of Cd+BDE-209 mixture. The activity of liver function enzymes AST, ALT, ALP, γ-GT, was measured in Wistar rats (8/group) receiving by gavage, for 28 days, either a single substance or their combination. Three groups were receiving Cd alone in the doses of 2.5, 7.5, or 15 mg/kg/day (doses ratio 1:3:6), three groups were receiving BDE-209 in the doses of 1000, 2000, or 4000 mg/kg/day (doses ratio 1:2:4), while nine groups were receiving different mixtures of Cd+BDE-209 (3x3 design). For the assessment of internal dose–response relationship and external dose-response relationship lower confidence limit of Benchmark dose (BMDL) was calculated using PROAST software.

Mixtures of Cd+BDE-209 more potently disrupted liver function than these toxic substances alone. After application of Cd+BDE-209 mixtures, different levels of Cd or BDE-209 were measured in liver than after application of single Cd or single BDE-209. After application of Cd+BDE-209 mixture liver tissue doses of Cd ratio was 1:3.8:8.5, while BDE-209 tissue doses ratio was 1.2:3:3. Difference between external and internal doses ratios pointed to interrelationship between Cd and BDE-209 on the absorption rate and/or distribution in liver. When applied as mixture Cd decreased BDE-209 level in liver, while BDE-209 increased Cd level in liver. The results of present work indicate advantage of target tissue dose use for dose response modeling and deriving reference points in toxicology.

Keywords: metals, POPs, internal dose, hepatotoxicity, mixture

Correspondence: makitox@pharmacy.bg.ac.rs
The Role of Pon1 Variants in Disease Susceptibility

Abudayyak Mahmoud1,2, Boran Tugce1, Oztas Ezgi1, Tükel Rumeysa1, Özhan Gül1
1Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Istanbul University
2Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Karadeniz Technical University

In today’s world, diseases such as cancer, diabetes, hypertension, dyslipidemia, dyspnea and apnea, obesity are among the most important health problems. As it is well known, oxidative stress plays role in the development of diseases. Furthermore, there are many reports about the relationship between genetic structure and development of diseases. Paraoxonase (PON) plays a role in antioxidant defense and protects the cells against reactive oxygen species. PON1 gene, of the PON family, contains two coding region polymorphisms: Q192R (rs662) and L55M (rs854560), which are responsible for an up to 13-fold interpersonal variation in PON1 enzyme activity and concentration. Different distribution of PON1 gene polymorphism makes it important in disease susceptibility of different ethnic groups. Even if several case-control studies have investigated the association between PON1 gene polymorphisms and diseases, the results have not been consistent. Therefore, in the preliminary studies, it was aimed to determine if PON1 Q192R and L55M genotypes were associated with the development of colorectal cancer, pancreatitis and hypothyroid. The genotyping analyses were performed on real-time PCR platform in a hospital based case-control study of 150 patients and 50 healthy controls in Turkish population. Statistical analysis showed no association between the development of the diseases and PON1 genotypes (OR≤1.46; p≥0.05). However, further studies with a larger number of participants are required.

Keywords: PON1 gene polymorphism, pharmacogenetics, paraoxonase activity

Correspondence: boranntugce@gmail.com

A Systematic Review of Assessment Approaches in Pharmacoeconomic Analyses in Turkey

Eren Ozcagli, Ebru Aksan
Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Istanbul University, Beyazit, 34116, Istanbul, Turkey

Health as a fundamental human right creates a legal obligation on governments to ensure access to health care. Availability, accessibility, acceptability and quality are the core components of the right to health. According to OECD Health Statistics 2017 data, it has been reported that health care-related expenditures showed a significant growth in years for many countries. The discipline of pharmacoconomics has been defined as the description and analysis of the cost of drug therapy to health care systems and society for reaching optimum results by using available resources. In this study, comprehensive review of the pharmacoeconomic studies in Turkey was investigated and a total of 40 studies met our inclusion criteria. These studies were divided into groups as prophylactic treatment analysis, diagnostic methods, treatment and illness analysis according to study subject. We aimed to achieve a result explaining which analyzing method is more efficient and the reason for preferring these methods. According to our results, it was seen that cost-effectiveness analyzes had the highest incidence (65.62%) among all studies. Cost-effectiveness results evaluated by comparing the cost of providing a pharmaceutical product or service with the adverse outcomes occurred by using this product or service and the main aim is to determine which alternative yields the optimal outcome for each cost spent and a good insight while determining reimbursement strategies and also to clinical decision makers for choosing the most cost-effective treatment options. The analysis of cost–minimization, cost–benefit and cost-utility also gives important data for pharmacoconomics.

Keywords: pharmacoeconomy, cost effectiveness, healthcare systems, literature review

Correspondence: erenozcagli@gmail.com

Cyanide Poisoning Associated with Domestic Violence. A Case Report

Maja Vujovic1, Stevan Todorovic2, Ivan Stojanovic2, Aleksandra Antovic2
1University of Nis, Faculty of Medicine, Department of Pharmacy, Toxicology, Serbia,
2University of Nis, Faculty of Medicine, Institute of Forensic Medicine, Serbia
Domestic violence was reported to the police by a 34-year-old female, allegedly attacked by her husband. When he opened the door to the police officers, he abruptly fell down in front of them with no signs of life. He often threatened to poison himself with cyanide if he was reported to the police. The investigative authorities ordered a judicial autopsy under the suspicion of suicidal poisoning. Autopsy findings: At the autopsy, external examination of the body showed no signs of trauma. Internal examination showed general signs of sudden death, an intensive unpleasant smell of gastric content, and swollen, red-colored gastric mucosa. Tissue specimens, gastric content, femoral blood, vitreous humor and urine samples were collected for toxicology analysis. Methods: The Prussian blue color reaction and gas chromatography-flame ionization detector with headspace extraction (HS - GC/FID) were applied for the identification of cyanide. Results: Toxicological analyses showed positive results of cyanide in all samples. Conclusions: According to the police investigation, toxicology results, and histopathology findings, it was unequivocally concluded that the cause of death was suicidal cyanide poisoning.

Keywords: GC-MS, cyanide, poisoning, domestic violence

Identification and Understanding Air Pollution-gene-cancer Relationship Using Comparative Toxicogenomics Database: Serbia as a Case Study

Dragica Jorgovanović1, Katarina Baralić1, Danyel Jennen2, Danijela Đukić-Ćosić1
1Department of Toxicology “Academic Danilo Soldatović”, University of Belgrade – Faculty of Pharmacy, Serbia
2Department of Toxicogenomics, Maastricht University, The Netherlands

The detrimental effects of air pollution on human health, including respiratory diseases and cancer development, have been demonstrated worldwide. The aim of this study was to examine a possible relation between the increased air pollutants and development of cancer in Serbia using a toxicogenomic approach.

Air pollutants were identified from the annual reports of air quality in the Republic of Serbia issued by the Ministry of Environmental Protection, Serbia, while the report on the most common cancer types were obtained from the Institute of Public Health of Serbia "Dr Milan Jovanović Batut", Cancer incidence and mortality in Central Serbia, 2014. Curated interactions between selected contaminants, genes and cancer types were downloaded using Comparative Toxicogenomics Database (CTD).

Suspended particles (PM10) were constantly increased in the air of the Republic of Serbia according to annual reports from 2010 to 2016. The daily limit (50 μg/m³) was exceeded at least once a year at all measuring points (n=35). The most common types of cancer were breast in women (99.7/100 000) and lung cancer in men (104.5/100 000). Obtained set (57) contained PM10 interacting genes associated with both breast and lung cancer. PM10 increased the expression of proto-oncogenes, such as FOS or KRAS. In addition, PM10 led to overexpression of JAG1 gene and therefore, activation of Notch pathway which is directly implicated in tumor growth.

These toxicogenomic findings support an association between PM10 and cancer development and should be confirmed by epidemiological and genome research.

Keywords: PM10, cancer, proto-oncogenes, toxicogenomic approach

An Overview of Exposure to Cosmetics/Personal Care Products in Children: the Experience of the Institute for Mather and Child Healthcare “Dr Vukan Ćupić”, Belgrade, Serbia

Snežana Ristić1, Vesna Matović2, Danijela Đukić-Ćosić2
1Institute for Mather and Child Healthcare “Dr Vukan Ćupić”, Belgrade, Serbia
2Department of Toxicology “Academic Danilo Soldatović”, University of Belgrade – Faculty of Pharmacy, Serbia

Cosmetics and personal care products are on the list of substances implicated in pediatric exposures all around the world. The aim of this study was to evaluate the frequency and distribution of acute exposure and intoxication by cosmetics/personal care products in children aged 0 to 14 years treated at the Institute for Mother and Child Healthcare “Dr Vukan Ćupić” (the only child institution in Serbia that has a clinical toxicologist) as a result of exposure to toxic substances. All data collected (age, sex, time and date of the admission to the Institute, type of substance
ingested, symptoms manifested, laboratory tests conducted, therapies, outcome and the eventual need for hospitalization) were placed in a singular electronic database during the period 2000-2017. Among all the children exposed to toxic substances (about 300-400 per year), exposure to cosmetics/personal care products was observed in about 1% of cases. The highest incidence occurred in the group of children 1-4 years old, with no sex difference. Most admissions at the Institute occurred in the evening hours, at about 19:00 h. The frequency of exposure was above 1% from 2000 to 2007, mostly related to the acetone, nail polish, hydrogen and hair dyes which induced mild symptoms such as nausea/vomiting and erythema/edema. For the period from 2007 to 2017 the frequency was even below 1% of all exposures, mainly related to shampoos and creams. Clinical symptoms were not observed in children exposed to cosmetic/personal care products during this period of time and there was no need for hospitalization.

Key words: pediatrics exposure, cosmetics/personal care products, the 17-year period

Correspondence: risticsn@gmail.com

The Assessment Of Potential Toxic Chemicals Present n Water From Springs And Wells In Gorobilje, Zlatibor District

Tomislav Tosti¹, Katarina Karlijković-Rajić², Nikola Horvacki³
¹Faculty of Chemistry University of Belgrade, ²Faculty of Pharmacy University of Belgrade

In the modern analytical chemistry, due to considerable insight into human's health, environment water analysis is fast developing. Water monitoring and chemical analysis of water requires careful planning to achieve valid results.

The framework of this project was to develop fast, precise and reproductive method for monitoring potential toxic anions in water from specific location in Serbia.

In this project we paid special attention to sampling because we thought that's the most important phase for valid analysis and also lots of mistakes can be made due to non-adequate sampling procedure.

Water samples for chemical analysis must be collected in well washed bottle (1 L volume), leave water to flow for 5 minutes after that time take the sample but leave 25 ml of free space. For fountain and ground water take 10 random samples and make representative one. Filter samples through 0.45 and 0.20 µm PTFE membrane filter and degas it on vacuum and keep it in refrigerator for further analysis.

The results obtained lead us to the conclusion that designed method can be used for precise, reproductive, rapid, and economic analysis of fluoride, chloride, nitrite, sulphate, bromide, nitrate, and phosphate in the investigated water. The concentration range for quantification was from 0.1 to 10 ppm for fluoride whereas the other anions were in range from 1 to 100 ppm. The well in the field exhibits highest nitrate (above 50 ppm) and phosphate concentration (~1 ppm); on the other hand isolated spring in the hill exhibits the best water quality.

Keywords: ion chromatography, nitrate, phosphate, nitrite, bromide

Correspondence: tosti@chem.bg.ac.rs

Toxicology and Anthropology: Understanding Poisons in Cultural Context

Tom Widger
Durham University, Department of Anthropology, University of Durham, South Road, Durham, DH1 3LE, United Kingdom

This poster introduces the Durham Critical Toxicologies Research Group, a collaboration of anthropologists, biological and medical scientists, and humanities scholars exploring the history and practice of toxicology, and the origins and effects of chemical pollution, especially in South Asia and Latin America. Anthropology is the study of human beings in sociocultural diversity. Anthropology can contribute to toxicology by extending toxicologists' understanding of how people understand toxic dangers in everyday contexts. The poster describes anthropological research carried out in Sri Lanka in 2015/16, which explored local perspectives on an epidemic of chronic kidney failure of unknown aetiology (CKDu), widely believed to be caused by agrochemical (glyphosate) poisoning. The research involved 12 months' qualitative, ethnographic study of how people in rural communities affected by CKDu theorized the origin of the disease in glyphosate exposure, described visible and invisible effects of environmental pollution in personal narrative terms, and changed water consumption habits and farming practices as a result. The research findings highlighted how sociocultural, historical, and political factors, amounting to an 'ethno-toxicology' around pesticides and CKDu, shaped public understandings of poisons and their effects, in turn influencing the ways medical toxicological information
and public health interventions were also understood and acted upon. Crucially, the research suggested that ethno-toxicological approaches suggested other possible causes of CKDu, including chronic dehydration and heat stress caused by heightened fears around water. The poster concludes that one way anthropologists and toxicologists can collaborate to help promote health in the developing world is through interdisciplinary dialogues on poisons.

**Keywords:** anthropology, culture, nephrotoxicity, pesticides, water

**Correspondence:** tom.widger@durham.ac.uk

---

**Macrolides – Monitoring in Surface Water by LC-ESI-MS/MS and In Silico Prediction of ADMET Parameters Relevant to Ecotoxicity**

Milena Jadrijević-Mladar Takač¹, Irena Žuntar¹, Adela Krivohlavek², Tin Takac³

¹Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, Zagreb, Croatia, ²Andrija Stampar Teaching Institute of Public Health, Miragajška 16, Zagreb, Croatia, ³Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 19, Zagreb, Croatia

The aim of study was to monitor macrolides in surface water by LC-ESI-MS/MS and to predict toxic potential by ADMET Predictor™ in order to elucidate their impact on environment and health. ADMET-related descriptors (ADMET Predictor 8.1, Simulation-Plus, USA) relevant to environmental toxicity were computed using four models: the fathead minnow acute toxicity model based on lethal effects on Pimephales promelas (Minnow LC₅₀, TOX FHM), the concentration needed to inhibit 50% growth in protozoan species Tetrahymena pyriformis (Th pyr plGC₉₀), the lethal concentration that results in death of 50% of Daphnia magna (water fleas) (Daphnia LC₅₀, TOX DM) and bioconcentration factor (BCF). Erythromycin (ERY) was detected in 14 samples out of 148 (9.5%) in concentration 0.3 – 5.3 μg L⁻¹, while azithromycin (AZY) in 6 of 148 samples (4%) and concentration 0.2 – 1.1 μg L⁻¹.

The results of this in silico study revealed that investigated macrolides are non-biodegradable molecules (TOX BIODEG 96%). Predicted BCF for ERY and AZY were 0.155 and 0.234, TOX DM 170.1 and 1140.503 and TOX FHM 3.948 and 1140.503, respectively. Based on predicted scores for BCF, TOX DM and TOX FHM ERY has been shown as more ecotoxic substance comparing to AZY.

**Keywords:** macrolides, LC-ESI-MS/MS, ecotoxicity, ADMET predictor analysis

**Correspondence:** jmtmilenamc@gmail.com, izuntar@pharma.hr

---

**Possible Radical Scavenging and Antioxidant Activities of New Class Norcantharimide Derivatives**

Gözde Girgin¹, Saziye Sezin Palabiyik², Suna Sabuncuoglu³, Ayse Tan³, Özlem Gündogdu¹, Nurhan Kishali³, Yunus Kara³, Terken Baydar¹

¹Department of Toxicology, Faculty of Pharmacy, Hacettepe University, Ankara, ²Department of Toxicology, Faculty of Pharmacy, Ataturk University, Erzurum, ³Department of Chemistry, Faculty of Science, Ataturk University, Erzurum, ⁴Department of Chemistry, Faculty of Science, Mus Alparslan University Mus, Turkey

Cantharidin and its analogues have been found to have inhibitory effects against protein phosphatases. N-derivatives of norcantharimide, analogues of cantharidin, have been found to show effect on a number of cancer types. Oxidative stress is the imbalance between oxidants and antioxidants in biological systems and may trigger various pathologies and disorders. Maintaining the balance between ROS and antioxidants in the cell is very crucial. Reactive oxygen species (ROS) are the most reactive free radicals including hydroxyl, superoxide (O₂⁻) and nitric oxide (NO•) radicals. On the other hand, superoxide dismutase (SOD) and catalase (CAT) are two main enzymes of the antioxidant defense system against ROS. In the present study, new synthesized six norcantharimide derivatives were evaluated for their possible radical scavenging effects at various concentrations with different in vitro methods by NO•, O₂•⁻, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assays. It has been found that some of the synthesized norcantharimide derivatives have radical scavenging activities at different concentrations in vitro. Hence, antioxidant effects of all the synthesized compounds have further been evaluated ex vivo by measuring two main antioxidant enzyme activities,
SOD and CAT. SOD activity was found to range from 86% to 141% while CAT ranged from 93% to 119% of the positive control ascorbic acid. It has been observed that these derivatives have radical scavenging activities and altered both antioxidant enzyme activities though they follow no pattern. This is the first study to show that these norcantharimide derivatives can cause distinctive changes on the balance between oxidant and antioxidant status.

**Keywords:** norcantharimide, free radicals, superoxide, nitric oxide, catalase

**Correspondence:** gzdgirgin@gmail.com and tbaydar@hacettepe.edu.tr

---

**Microreactors: Hazard Reduction in an Efficient Alternative to a Batch Synthesis of Organic Compounds**

Julijana Tadić¹, Marina Mihajlović¹, Mića Jovanović²

¹Innovation Center of Faculty of Technology and Metallurgy ltd. in Belgrade, ²Faculty of Technology and Metallurgy University in Belgrade

Organic synthesis has traditionally been performed in batch reactors, but nowadays microreactors have gained a lot of attention. In microreactors the reaction takes place under strongly controlled conditions in a restricted space, which allows very efficient heat and mass transfer and improved productivity compared to the batch reactors. Moreover, due to the small volumes of reagents many safety issues can be overcome. The handling of potentially hazardous or toxic materials is limited and thus safer for the operator. It is possible to perform reactions that involve transient and reactive intermediates that could not be otherwise handled or stored in traditional batch mode. Furthermore, the volume of reagents and solvents is reduced by far, so the screening of reaction conditions becomes simple and time- and cost-efficient. The safe manufacturing of potentially toxic or explosive organic intermediates or performing of reactions run under high pressures, or above the boiling point of the solvent can be carried out in microreactors with minimum risk. Continuous flow synthesis using microreactors can be combined with other technologies, such as microwave irradiation or photochemistry, leading to improved efficiency. For these reasons microreactors can be seen as a novel technology that opens the way for new synthetic routes of valuable molecules.

**Keywords:** continuous flow synthesis, hazardous chemistry

**Correspondence:** jtadic@tmf.bg.ac.rs
INDEX OF AUTHORS

A
Abu-Bakar, A‘edah 150
Abudayyak, Mahmoud 83
Achard, Sophie 90
Afandiyev, Ismayil 78
Afzalaghaee, Monavar 110
Aguilar-Garduño, Clemente 30
Ahamava, Nastassia 84
Ahel, Marijan 137
Ahmed, Shaheda S. 91, 133
Ahonen, Merja 15
Akinsegun, Akinsuyi 80
Aksan, Ebru 169
Alavantić, Dragan 50
Alegakis, Athanasios 17
Ammol, Fahimeh Asadi 166
Anđelković, Milena 141, 162
Antonijević, Biljana 18, 112, 122, 126, 128, 130, 138, 152, 158, 161, 168
Antonijević, Evica 18, 122, 126, 128, 130, 138, 141, 152, 168
Antovic, Aleksandra 169
Apostolov, Marko 149
Aràoz, Romulo 22
Arif, Tara 153
Arman, Ilyna 134
Arri, Nuray 100, 118
Arzuk, Ege 162
Aschner, Michael 20
Atilla, Koray 29
Aydm, Ahmet 31, 32, 140
Aydm, Sevtap 100, 118, 135
B
Babic, Gordana 128
Babić, Olivera 137
Babulovska, Aleksandra 85, 103
Bacanli, Merve 32, 100, 118
Bačun-Družina, Višnja 42
Badea, Madalina Andreea 136
Balu, Kate 120
Banin, Eahud 14
Baradaran Rahimi, Vafa 156
Baralić, Katarina 105, 122, 125, 130, 141, 170
Batkeča-Mino, Kinga 153
Basaran, Arif Ahmet 32, 100, 118, 135
 Başaran, Nurşen 32, 69, 100, 118, 135
Batsh, Yasser 140
Baydar, Terken 172
Benc, Ivana 110
Becit, Merve 135
Becker, Kathrin 81
Bekjarovski, Niko 85, 103
Belfield, Samuel 104
Belsčak-Cvitano, Ana 42
Benderitter, Marc 104
Bengalli, N. 49
Bennett, Breana 98
Benzeraga, Sameh 153
Berlang de Moraes Barros, Silvia 69
Berrada, Houda 111
Bianchi, Fernando 91
Bibby, Louis 133
Bjelog, Sanja 89
Billack, Blase 69, 167
Bingol Ozakpinar, Ozlem 115
Blagojević, Zorica 108
Bobić, Stanka 89
Bogdano, G. 164
Bojadžieva, Irena 98
Bojačić, Janja 110
Bojanić, Ljudica 110
Bojović, Tavljar, Mateja 131
Boley, Scott 73
Bolognesi, Claudia 42
Bonassi, Stefano 42, 97, 142
Boobis, Alan 46, 47, 74
Bopp, Stephanie 47, 130
Borčić, Vladan 117
Borzan, Sunčica 152
Borščki, Andraž 155
Bosak, Anita 114
Botha, Anna-Maria 140
Boukerma, Khaled 90
Bovan, Saša 158
Boyarst, Tatiana 34
Božić, Aleksandra R. 103
Božić, Gavril 154
Božić, Ljubica 158
Bozkurt, Edibe Nurzen 99
Brajenović, Nataša 40, 94
Braković, Gordana 95, 128
Brajković, Zorica 95
Brankovic, Marija 37
Brauer, Simone 97, 142
Brčić Karićonj, Irena 40, 94, 112
Brkić, Dragica 148, 154
Broukhuisen, Pieter van 58
Brouwers, Olaf 13
Browedani, Valentina 149
Brown, Richard 52, 70
Bubalo, Volodymyr 114
Bucur, Ilk İndeşer 118, 135
Buha Dordević, Aleksandra 54, 122, 126, 128, 141, 161, 162, 168
Bulat, Petar 31, 59, 69
Bulat, Zorica 31, 59, 122, 126, 141, 149, 152, 161, 162, 168
Burić, Petra 77
Burić, Vojislava 148
Bušić, Arijana 42
Busquet, Francois 150
Buženna, Alice 90, 91

C
Cáceres, Nicolás 118
Caglayan, Aydan 77
Çakmak, Gonca 42, 130
Calderón, Leonardo A. 147
Çal, Tuğba Göl 100, 118, 135
Camatini, M. 49
Cameán, Ana Maria 120
Carballo, Dionisia 111
Castaño, Argelia 34
Cavaiers, Maria Fernanda 118, 135
Cayr, Elif Aslıhan 140
Celik, Turgay 161
Cesarethi, Yıldırım 99
Cetin, Tugce 139
Cevdi, Katlan, Doruk 77
Chang, X-R 138
Chaparoska, Daniela 85, 103
Chirafe, Bilal 153
Cheng, Yu-Wen 113
Chen, Si 33
Choi, Kyung-Chul 162
Cohen, David P.A. 104
Çok, İsmet 99
Colosio, Claudio 8, 16, 17, 59, 69, 70
Cordier, Werner 127
Coricovac, Dorina 145
Corsini, Emanuela 26, 27, 69, 70
Corvaro, Marco 26
Corvi, Raffaela 130
Cosic, Vladan 93
Creuzet, Sophie 22
<table>
<thead>
<tr>
<th>Name</th>
<th>Page(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crijns, Francy</td>
<td>13</td>
</tr>
<tr>
<td>Cronin, Mark</td>
<td>104</td>
</tr>
<tr>
<td>Cuffari, Benedette</td>
<td>167</td>
</tr>
<tr>
<td>Curling, Linda</td>
<td>120</td>
</tr>
<tr>
<td>Cvjetović, Anka</td>
<td>106</td>
</tr>
<tr>
<td>Cvetković, D.</td>
<td>127</td>
</tr>
<tr>
<td>Cvetkovska, Anita</td>
<td>98</td>
</tr>
<tr>
<td>Cvijetić, Ilija N.</td>
<td>103</td>
</tr>
<tr>
<td>Čakar, Uroš</td>
<td>128</td>
</tr>
<tr>
<td>Četojević-Simin, Dragana</td>
<td>107</td>
</tr>
<tr>
<td>Čebović, Tatjana</td>
<td>92</td>
</tr>
<tr>
<td>Čirković, Maja</td>
<td>89</td>
</tr>
<tr>
<td>Čupić Miladinović, Dejana</td>
<td>152, 154</td>
</tr>
<tr>
<td>Čupić, Vitomir</td>
<td>152</td>
</tr>
<tr>
<td>Čurčić, Marijana</td>
<td>122, 126, 128, 130, 152, 158, 168</td>
</tr>
<tr>
<td>Čakar, Uroš</td>
<td>128</td>
</tr>
<tr>
<td>Četojević-Simin, Dragana</td>
<td>107</td>
</tr>
<tr>
<td>Čebović, Tatjana</td>
<td>92</td>
</tr>
<tr>
<td>Čirković, Maja</td>
<td>89</td>
</tr>
<tr>
<td>Čupić Miladinović, Dejana</td>
<td>152, 154</td>
</tr>
<tr>
<td>Čupić, Vitomir</td>
<td>152</td>
</tr>
<tr>
<td>Čurčić, Marijana</td>
<td>122, 126, 128, 130, 152, 158, 162, 170</td>
</tr>
<tr>
<td>Čakar, Uroš</td>
<td>128</td>
</tr>
<tr>
<td>Četojević-Simin, Dragana</td>
<td>107</td>
</tr>
<tr>
<td>Čebović, Tatjana</td>
<td>92</td>
</tr>
<tr>
<td>Čirković, Maja</td>
<td>89</td>
</tr>
<tr>
<td>Čupić Miladinović, Dejana</td>
<td>152, 154</td>
</tr>
<tr>
<td>Čupić, Vitomir</td>
<td>152</td>
</tr>
<tr>
<td>Čurčić, Marijana</td>
<td>122, 126, 128, 130, 152, 158, 168</td>
</tr>
<tr>
<td>Čakar, Uroš</td>
<td>128</td>
</tr>
<tr>
<td>Četojević-Simin, Dragana</td>
<td>107</td>
</tr>
<tr>
<td>Čebović, Tatjana</td>
<td>92</td>
</tr>
<tr>
<td>Čirković, Maja</td>
<td>89</td>
</tr>
<tr>
<td>Čupić Miladinović, Dejana</td>
<td>152, 154</td>
</tr>
<tr>
<td>Čupić, Vitomir</td>
<td>152</td>
</tr>
<tr>
<td>Čurčić, Marijana</td>
<td>122, 126, 128, 130, 152, 158, 168</td>
</tr>
<tr>
<td>Dacić, Sanja</td>
<td>152</td>
</tr>
<tr>
<td>Daljevic, Radoslav</td>
<td>93</td>
</tr>
<tr>
<td>Dalla Costa, Silvia</td>
<td>53</td>
</tr>
<tr>
<td>Daly, Ann K.</td>
<td>36</td>
</tr>
<tr>
<td>Damianić, Bernarda</td>
<td>160</td>
</tr>
<tr>
<td>Dänick, Sven</td>
<td>25</td>
</tr>
<tr>
<td>Davidović, M.</td>
<td>51</td>
</tr>
<tr>
<td>Davuljigari, Chand Basha</td>
<td>143</td>
</tr>
<tr>
<td>Dehelean, Cristina</td>
<td>145</td>
</tr>
<tr>
<td>Dehpour, Ahmad Reza</td>
<td>166</td>
</tr>
<tr>
<td>Demir Akts, Ayse</td>
<td>115</td>
</tr>
<tr>
<td>Demirbügen, Merve</td>
<td>29</td>
</tr>
<tr>
<td>Denic, Kristina</td>
<td>127, 159, 160, 164</td>
</tr>
<tr>
<td>Dickinson, Anne</td>
<td>91, 133</td>
</tr>
<tr>
<td>Diderich, Bob</td>
<td>58, 63, 70</td>
</tr>
<tr>
<td>Dijk, Frank van</td>
<td>58</td>
</tr>
<tr>
<td>Dills, Russell</td>
<td>145</td>
</tr>
<tr>
<td>Dimitrijevic, Dunja</td>
<td>150</td>
</tr>
<tr>
<td>Dimitrov, Nino</td>
<td>160</td>
</tr>
<tr>
<td>Dinischiotu, Anca</td>
<td>136</td>
</tr>
<tr>
<td>Dishovský, Christoph</td>
<td>116</td>
</tr>
<tr>
<td>Dive, Vincent</td>
<td>22</td>
</tr>
<tr>
<td>Djordjevic, Dragana</td>
<td>128</td>
</tr>
<tr>
<td>Dorne, Jean-Lou</td>
<td>27</td>
</tr>
<tr>
<td>Dragasevic, Natasa</td>
<td>37</td>
</tr>
<tr>
<td>Dragićević, Igor</td>
<td>35</td>
</tr>
<tr>
<td>Dragić, Nataša</td>
<td>89</td>
</tr>
<tr>
<td>Dragojević, Jelena</td>
<td>135</td>
</tr>
<tr>
<td>Dragomanova, Stela</td>
<td>116, 117</td>
</tr>
<tr>
<td>Drakulić, Dunja</td>
<td>116</td>
</tr>
<tr>
<td>Drazdova, Alena</td>
<td>167</td>
</tr>
<tr>
<td>Drofenik, Jernej</td>
<td>131</td>
</tr>
<tr>
<td>Dumitrache, Florian</td>
<td>136</td>
</tr>
<tr>
<td>Durgo, Ksenija</td>
<td>42, 130</td>
</tr>
<tr>
<td>Dzoljic, Eleonora</td>
<td>37</td>
</tr>
<tr>
<td>Džudovč, Jelena</td>
<td>149</td>
</tr>
<tr>
<td>Danić, Maja</td>
<td>164</td>
</tr>
<tr>
<td>Dedibegović, Jasmina</td>
<td>124</td>
</tr>
<tr>
<td>Dermanović, Mirjana</td>
<td>110</td>
</tr>
<tr>
<td>Dolić, Maja B.</td>
<td>144</td>
</tr>
<tr>
<td>Dordević, Snežana</td>
<td>39, 92, 95, 128, 149, 158</td>
</tr>
<tr>
<td>Dukić-Ćosić, Danijela</td>
<td>105, 122, 123, 124, 125, 126, 128, 130, 138, 152, 158, 162, 170</td>
</tr>
<tr>
<td>Dukić, Mirjana</td>
<td>122, 126, 128</td>
</tr>
<tr>
<td>Edler, Lutz</td>
<td>25</td>
</tr>
<tr>
<td>Egbe, Edmund</td>
<td>114</td>
</tr>
<tr>
<td>El-Abssawy, A.A.</td>
<td>49</td>
</tr>
<tr>
<td>El-Mekawy, A.</td>
<td>49</td>
</tr>
<tr>
<td>Elmi, Sam</td>
<td>110</td>
</tr>
<tr>
<td>Embry, Michelle</td>
<td>74</td>
</tr>
<tr>
<td>Emeljanova, Olga</td>
<td>167</td>
</tr>
<tr>
<td>Enoch, Steve</td>
<td>104</td>
</tr>
<tr>
<td>Erkekoglu, Pinar</td>
<td>101</td>
</tr>
<tr>
<td>Escrivá, Laura</td>
<td>82</td>
</tr>
<tr>
<td>Estrada, Carolina Campos</td>
<td>133</td>
</tr>
<tr>
<td>Ezgi, Oztas</td>
<td>169</td>
</tr>
<tr>
<td>Faletti, AG</td>
<td>108</td>
</tr>
<tr>
<td>Fang, Mingzhu</td>
<td>102</td>
</tr>
<tr>
<td>Fan, Qiuyan</td>
<td>142</td>
</tr>
<tr>
<td>Fan, Ximin</td>
<td>142</td>
</tr>
<tr>
<td>Fatur, Tanja</td>
<td>131</td>
</tr>
<tr>
<td>Faustman, Elaine</td>
<td>69, 74, 98, 145</td>
</tr>
<tr>
<td>Fazel, Alireza</td>
<td>159</td>
</tr>
<tr>
<td>Ferrer, Emilia</td>
<td>111</td>
</tr>
<tr>
<td>Filipović, A.</td>
<td>51</td>
</tr>
<tr>
<td>Filipović Trčković, Jelena</td>
<td>116</td>
</tr>
<tr>
<td>Firman, James</td>
<td>104</td>
</tr>
<tr>
<td>Fleaca, Claudiu</td>
<td>136</td>
</tr>
<tr>
<td>Florio, Chiara</td>
<td>149</td>
</tr>
<tr>
<td>Font, Guillermina</td>
<td>111</td>
</tr>
<tr>
<td>Foth, Heidi</td>
<td>32</td>
</tr>
<tr>
<td>Franke, Katrin</td>
<td>84</td>
</tr>
<tr>
<td>Fuchs, Dietmar</td>
<td>81</td>
</tr>
<tr>
<td>Fukumura, Masao</td>
<td>64</td>
</tr>
<tr>
<td>Gaborik, Zsuszanna</td>
<td>165</td>
</tr>
<tr>
<td>Gačaša, Bojana</td>
<td>117</td>
</tr>
<tr>
<td>Gadaga, Louis</td>
<td>61</td>
</tr>
<tr>
<td>Gad, Marwa</td>
<td>84</td>
</tr>
<tr>
<td>Galarza, RA</td>
<td>108</td>
</tr>
<tr>
<td>Gallegos, Ana</td>
<td>39</td>
</tr>
<tr>
<td>Gambacorta, Lucia</td>
<td>24</td>
</tr>
<tr>
<td>Gao, Lan</td>
<td>111</td>
</tr>
<tr>
<td>Garcia-Rayero Vinas, Natalia</td>
<td>140</td>
</tr>
<tr>
<td>Gedanken, Aharon</td>
<td>14</td>
</tr>
<tr>
<td>Genser, Dieter</td>
<td>153</td>
</tr>
<tr>
<td>Georgieva, Almira</td>
<td>117</td>
</tr>
<tr>
<td>Georgieva, Marieta</td>
<td>116</td>
</tr>
<tr>
<td>Georgieva, Stanislava</td>
<td>132</td>
</tr>
<tr>
<td>Gerasimova-Peneva, Anelia</td>
<td>132</td>
</tr>
<tr>
<td>Ghanous, Hanan</td>
<td>69, 73</td>
</tr>
<tr>
<td>Ghorbani, Ahmad</td>
<td>159</td>
</tr>
<tr>
<td>Ghorbani, Samira</td>
<td>134</td>
</tr>
<tr>
<td>Gilbert, Steven</td>
<td>123</td>
</tr>
<tr>
<td>Gil, Fernando</td>
<td>30</td>
</tr>
<tr>
<td>Girgin, Gözde</td>
<td>139, 172</td>
</tr>
<tr>
<td>Goessler, Walter</td>
<td>97, 142</td>
</tr>
<tr>
<td>Golocorbin-Kon, Svetlana</td>
<td>29</td>
</tr>
<tr>
<td>González-Alzaga, Beatriz</td>
<td>30</td>
</tr>
<tr>
<td>Gostner, Johanna M.</td>
<td>81</td>
</tr>
<tr>
<td>Gottschalk, Christoph</td>
<td>25</td>
</tr>
<tr>
<td>Gou, P-H</td>
<td>138</td>
</tr>
<tr>
<td>Grahovac, Lazar</td>
<td>158</td>
</tr>
<tr>
<td>Green, Carol</td>
<td>154</td>
</tr>
<tr>
<td>Griffith, William C.</td>
<td>98, 145</td>
</tr>
<tr>
<td>Guillermina, Font</td>
<td>120</td>
</tr>
<tr>
<td>Gulumian, Mary</td>
<td>7, 69, 81, 127, 146, 147, 161</td>
</tr>
<tr>
<td>Gündogdu, Özlem</td>
<td>172</td>
</tr>
<tr>
<td>Guo, Lei</td>
<td>33</td>
</tr>
<tr>
<td>Gü, Özhan</td>
<td>169</td>
</tr>
<tr>
<td>Gurer-Orhan, Hande</td>
<td>45, 101, 106</td>
</tr>
<tr>
<td>Hadi Mousavi, Seyed</td>
<td>156</td>
</tr>
<tr>
<td>Haghir, Hossein</td>
<td>159</td>
</tr>
<tr>
<td>Hales, Barbara F.</td>
<td>8</td>
</tr>
<tr>
<td>Hara, Shuntaro</td>
<td>19</td>
</tr>
<tr>
<td>Hartung, Thomas</td>
<td>28</td>
</tr>
<tr>
<td>Hassan, S.K.</td>
<td>49</td>
</tr>
<tr>
<td>Heikal, Tarek</td>
<td>80</td>
</tr>
<tr>
<td>Hernández, Antonio F.</td>
<td>18, 30</td>
</tr>
<tr>
<td>Hilbeck, A.</td>
<td>83</td>
</tr>
<tr>
<td>Hin, Taufiq Yap Yun</td>
<td>137</td>
</tr>
<tr>
<td>Hirose, Akihiro</td>
<td>146</td>
</tr>
<tr>
<td>Hogervorst, Jelena</td>
<td>117</td>
</tr>
<tr>
<td>Ho, Han Kiat</td>
<td>85</td>
</tr>
<tr>
<td>Hojo, Motoko</td>
<td>146</td>
</tr>
<tr>
<td>Holzer, Angelika</td>
<td>153</td>
</tr>
<tr>
<td>Hong, Sungwoo</td>
<td>145</td>
</tr>
<tr>
<td>Horvacki, Nikola</td>
<td>171</td>
</tr>
</tbody>
</table>
Tsvetanova, Elina 117
Tubaro, Aurelia 149
Tugce, Boran 169
Tuijthof, Gabrielle 13
Tulah, Asif S. 91
Tumu, Hemanta C Rao 167
Turkalj, Mirjana 112
Turna, Burak 29
Tzatzarakis, Manolis 17

U
Ubavić, Milan 117
Ulutaş, Onur Kenan 99
Umicević, Nina 138
Ünal, A. Zeynep 151
Ündeğer Bucurgat, Ülkü 100
Uslu, Duyusal 106
Usoro, Chinyere 114
Utembe, Wells 81, 161

V
Vadzim, Vasilkevich 164
Vahle, John 73
Vakonaki, Elena 17
Valdivia-Flores, Arturo G. 99
Valiantsina, Buraya 167
Vasile, Eugenia 136
Vasilyeva, Marina 156
Veranić, Girina 167
Verbić, Tatjana 94
Vetten, Melissa 127, 146
Vicini, Riccardo 90, 91
Vidaković, Aleksandar 119, 125
Vidli Štrac, Ivona 160
Vinković Vrček, Ivana 15, 97, 142
Vićtorović-Todorović, Maja D. 103
Voronina, Alla 109
Vračko, Marjan 126
Vranac, Sanja 131
Vučinić, Slavica 38, 95, 128, 168
Vujanović, Dragana 122, 126, 128
Vujatović, Tamara 103
Vujović, Maja 93, 143, 169
Vukčević, Sežana 95
Vukomanović, Predrag 119, 120, 121, 129, 163
Vuković Ercegović, Gordana 102, 128
Vuković, Gorica 148, 154
Vuković, Nenad 106

W
Wang, D 138
Wang, J-L 138
Wang, Tao 69
Weideman, Patricia 73
Weldon, Brittany A. 145
Wexler, Philip 57, 123
Widger, Tom 171
Wilde, Roelof de 165
Wilks, Martin F. 16
Wium, Cherylynn 60, 61, 70, 120, 127
Wollenweber, Marc 150
Workman, Tomomi 98, 145
Worth, Andrew 47, 130
Wyk, Johannes H van 140

Y
Yao, Y-L 138
Yemitan, Omoluyi 80
Yeniyiay, Levent 29
Yome, Julie 97
Yüce, Kunter 77
Yurkevich, Elena 156
Yu, Ting 150

Z
Zakarian, Armen 22
Zanjani, Bamdad Riahi 110
Zarbl, Helmut 102
Zastenskaya, Irina 34, 52
Zdralj, Branislava 127, 159, 160, 164
Zec Petković, N. 112
Zekiolu, Osman 29
Zekiri-Keka, Fljamure 98
Zerboni, A. 49
Zergui, Anissa 153
Zeynep Ünal, A. 165
Zheng, Wei 142
Zhminko, Petro 53
Ziegler-Skylakakis, Kyriakoula 121
Zivanovic, Dragan 102
Zivkovic, V. 127
Zlatić, Katarina 114
Zubko, Olena 114
Zuliani, Juliana P. 147
Zurita, S. 108
Zyl, Robyn van 127

Ž
Želježić, Davor 112, 155
Živadinović, Emil 89
Živković, M. 51
Živković Semren, Tanja 94
Žunec, Suzana 154
Žuntar, Irena 172
CONGRESS of Toxicology in Developing Countries (10; 2018; Beograd)

Book of Abstracts / 10th Congress of Toxicology in Developing Countries (CTDC10) [and] 12th Congress of the Serbian Society of Toxicology (12th SCT), April 18-21, 2018, Belgrade, Serbia; [editor Vesna Matović]. - Beograd : Serbian Society of Toxicology, 2018 (Beograd : Dosije studio). - 184 str.; 30 cm

Na vrhu nasl. str.: IUTOX International Union of Toxicology. - Tekst štampan dvostubačno. - Tiraž 350. - Registar.

ISBN 978-86-917867-1-7

1. Udruženje toksikologa Srbije. Kongres (12; 2018; Beograd)

a) Toksikologija - Apstrakti

COBISS.SR-ID 261499916