



11th INTERNATIONAL CONGRESS OF THE TURKISH SOCIETY OF TOXICOLOGY

02-05 November 2022, Kemer, Antalya-Türkiye

For a safe and healthy world

ABSTRACT BOOK



**11th International Congress of the
Turkish Society of Toxicology
02-05 November 2022 - Antalya, Turkey.**

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WELCOME

It is my great pleasure to invite all of you to the 11th International Congress of the Turkish Society of Toxicology (TST), which will be held in Antalya, Turkey between 02-05 November, 2022.

Our congress with the theme of “For a safe and healthy world” will highlight emerging developments in numerous areas of toxicology. It has been over a year since the COVID-19 pandemic was first reported, and despite precautionary measures and vaccines there are still new waves occurring. COVID-19 has alerted the world to the fact that health and wellbeing is a priority and has also heightened the respect and recognition of the heroic work of healthcare personnel. Through our convening, we hope to share lessons and effective strategies for containing the pandemic. The scientific programme will also consist of plenary lectures, and sessions covering many topics such as, ecotoxicology, environmental toxicology, endocrine disrupters, metal toxicity, molecular toxicology, genotoxicity, food safety, regulatory toxicology, alternative methods, and risk assessment. Many short communications, oral and poster presentations will be also held for early career researchers. The scientific programme will provide the opportunity for all kind of attendees to learn about recent developments in toxicology. The Congress is a continuation of the series of traditional TST meetings, including meetings in Ankara (1987 and 2009) in Antalya (1997, 2006, 2012) and in Izmir (2015). Our Congress will provide an important national and international platform for sharing and discussing the latest developments with professionals scientifically.

During the meeting, you will have the opportunity to learn more about Antalya, which is the most visited touristic province of Turkey’s Mediterranean coast. The area has witnessed many historical changes; you can enjoy the charming atmosphere and the stunning view of the sea.

We invite you to attend TST2022 in the hope of carrying out a scientifically and socially satisfying, congruent congress. On behalf of the Organizing and the Scientific Committees, I wish to express our gratitude to all the speakers, oral and poster presenters who will share their knowledge with us and also to all of you who will participate in advance.

Looking forward to meeting you all at TST2022!

Prof. Dr. Nursen BAŞARAN
President of TST



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The Organising Committee of TTD2022 is thankful to the above organizations and sponsors for their contribution and support



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SCIENTIFIC PROGRAMME

02 NOVEMBER 2022 – WEDNESDAY

Hall A - Biyoteknolojik İlaçlarda Klinik Çalışmalar-Güvenlik Değerlendirmeleri (This session will be in Turkish)

Chairs: Semra Şardaş, Gül Özhan

13.30-14.00	Biyoteknolojik İlaçlar ve Üretim Süreçleri Devrim Demir Dora
14.00-14.30	Biyobenzer İlaçlarda Klinik Çalışmalar ve Karşılaştırılabilirlik İrfan Çiçin (Online)
14.30-15.00	Biyobenzer Ürünlerde Ekstrapolasyon, Değiştirilebilirlik M.Cem Ar (Online)
15.00-15.30	Biyoüstünler Alper B. İskit
15.30-16.00	Biyolojik ve Biyobenzer İlaçlarda Farmakovijilans, İzlenebilirlik ve Risk Yönetimi Semra Şardaş
17.00-18.00	Opening Ceremony

Hall A - Opening Lecture

Chair: Nurşen Başaran

17.30-18.15	The History of the Advancement of Toxicology in Turkey Ali Esat Karakaya
18.00-20.00	Opening Reception
20.00	Dinner

03 NOVEMBER 2022 – THURSDAY

Hall A - Plenary Lecture

Chair: Ali Esat Karakaya

09.00-09.45	Non-Pharmacological Mechanism in Substance-Based Medical Devices/Challenges in Regulatory Definition Marco Racchi
09.45-10.00	Coffee Break

Hall A - Session 1, Genotoxic Impurities in Pharmaceutical Compounds

Chairs: Lutz Mueller, Ahmet Aydın

10.00-10.30	The staged TTC concept in the evaluation of genotoxic impurities in drugs Lutz Mueller
10.30-11.00	Strategies on the assessment of genotoxic impurities in pharmaceuticals Andreas Czich (online)
11.00-11.30	Regulation of Genotoxic Impurities – Guidelines and Experiences since 2006 Roland Froetschl
11.30-12.00	Genotoxic impurities and other possible impurities in pharmaceutical products: From the application perspective Ahmet Aydın
12.00-13.30	Lunch



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SCIENTIFIC PROGRAMME

03 NOVEMBER 2022 – THURSDAY

Hall A - Plenary Lecture

Chair: José E. Manautou

13.30-14.15 Chemical Stewardship through Sustainability Lenses, **Salmaan H Inayat-Hussain**

14.30-16.30 Hall C - Industry Sponsored Workshop / MAT-TEK

16.45-17.00 Hall C - Industry Sponsored Presentations / Redoks
Orbitrap (HR)-MS Applications in Toxicological Drug Screening

Hall A - Session 2, Vaccine Safety-Toxicity and Clinical Trials

Chairs: Asuman Karakaya, Pınar Erkekoğlu

14.30-15.00 Vaccine Safety and Toxicity. What have We Learned from COVID-19 Pandemic
Pınar Erkekoğlu

15.00-15.30 Clinical Trials of COVID-19 Vaccines
Serhat Ünal

15.30-16.00 Vaccine Hesitancy
Levent Akın

16.00-16.30 Pharmacoeconomic Aspects of COVID-19 Vaccines
Zafer Çalışkan

16.30-17.00 Characterization and Quality Control Procedures for Vaccines: A Special Focus on COVID-19 Vaccines
Emirhan Nemutlu

17.00-17.15 Coffee Break

Hall A - Session 4, Scientific Approach to Chemical Warfare and Fourth Generation Nerve Agents_Novichoks

Chairs: Onur Erdem, Sermet Sezigen

17.15-17.45 A generic presentation on fourth generation agents and their risks – novel therapeutic options for nerve agents/organophosphates poisoning
Ahmet Aydın

17.45-18.15 Case studies for Novichok incidents in UK and Russia
Sermet Sezigen

18.15-18.45 Review-based evaluation of decontamination techniques after Novichok exposure
Denis Josse (online)

18.45-19.15 In-vitro and Pre-Clinical Studies of Decontamination Efficacy of Chemical Warfare agents from the skin
Laura Cochrane (online)

20.00 Dinner

Hall B - Session 3, Current approaches on detecting the DNA damage and repair of chemicals

Chairs: Benu Karahalil, Neslihan Aygün Kocabaş

Genetic risks from heat-damaged DNA in food
Miral Dizdaroğlu (online)

Mode of action (MOA) based threshold for genotoxic carcinogens: Benzene case
Neslihan Aygün Kocabaş

Mitochondrial DNA damage as genotoxicity marker
Nadja Cristhina de Souza Pinto (online)

The impacts of prominent detoxifying and repair gene polymorphisms on neurological diseases
Benu Karahalil

In vitro genotoxicity of two graphene oxides before and after prolonged mild thermal treatment
Sanin Haveric

Hall B - Session 5, Environmental Biomonitoring of Hazardous Substances

Chairs: Anja Haveric, Yalçın Duydu

Air pollution context and biomonitoring in Sarajevo, Bosnia and Herzegovina
Anja Haveric

Does research in the field of Occupational Toxicology of Metals have any Future in Developed Countries?
Petar Bulat

Tobacco during pregnancy: looking at self-reported tobacco exposure, urinary cotinine levels and perinatal outcomes, **Carla Costa**

Monitoring air pollution and the health-related biomarkers: lessons from HUMNap
Katarina Matkovic



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SCIENTIFIC PROGRAMME

04 NOVEMBER 2022 – FRIDAY

Hall A - Plenary Lecture

Chair: Gülden Omurtag

09.00-09.45 Safety Evaluation of Food Additives
Yalçın Duydu

09.45-10.00 Coffee Break

Hall A - Session 6, Application of Comet Assay in Human Biomonitoring

Chairs: Mirta Milic, Joao Paolo Texeira

10.00-10.30 The comet assay in human biomonitoring: results from the COST Action hCOMET
Andrew Collins

10.30-11.00 Suitability of salivary leucocytes to assess DNA damage and repair in human biomonitoring studies using the comet assay
Blanca Laffon

11.00-11.30 Characterization of Portuguese Wildland firefighters before a Wildfire Season: Looking at the cytogenetic effects
Joao Paolo Texeira

11.30-12.00 Effect of a meat-based diet on fecal water genotoxicity: a randomized clinical trial in healthy subjects
Lisa Giovannelli

12.00-12.30 The damage and protective effects on DNA after single use combination of volatile anaesthetics and gamma radiation radiotherapy of 1 or 2Gy in vivo: preliminary results
Mirta Milic

12.30-13.45 Lunch

Hall A - Session 7, In Silico Toxicology

Chairs: Hande Sipahi, Kristie Sullivan

13.45-14.15 Regulatory Applications of Computational Toxicology: Progress and Strategies to Ensure Wider Use
Kristie Sullivan

14.15-14.45 *In silico* approaches to assess the potential environmental effects of diverse chemicals
Melek Türker Saçan

14.45-15.15 Filling data gap for toxicological evaluation of pharmaceuticals: In Silico approach
Muhammed Hamitoğlu

15.15-15.45 *In silico* toxicological evaluation of drug impurities aligned with ICH M7. A case study for methylprednisolone aceponate impurities
Gülçin Tuğcu

15.45-16.00 Coffee Break

Hall B - Session 8, Current Concerns about Endocrine Disrupting Chemicals

Chairs: Hande Gürer Orhan, Belma Koçer Gümüsel

Causality Between Prenatal Exposure to Pharmaceuticals and Childhood Obesity
Hande Gürer Orhan

Epigenetic Effects of Endocrine Disrupting Chemicals
Belma Koçer Gümüsel

Zebrafish as a Model for Chemical Induced Adipogenesis and Related Metabolic Diseases
Jorke Kamstra

Risk Assessment of Endocrine Disruptors
Johanna Zilliacus



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04 NOVEMBER 2022 – FRIDAY

Hall A - Session 9, Organ-specific Toxic Pathways: recent progress in drug-induced adversities		Hall B - Session 10, Conceptual models in immunotoxicology: alternative approaches and computational strategies	
Chairs: Hilmi Orhan, Mathieu Vinken		Chairs: Özlem Atıl Eklioğlu, Marco Racchi	
Hall 1		Hall 2	
16.00-16.30	Paracetamol-induced kidney damage occurs by different mechanism from hepatotoxicity Hilmi Orhan	Key characteristics of immunotoxicants: the way forward? Dori E. Germolec	
16.30-17.00	Adverse outcome pathways for in vitro prediction of liver toxicity Mathieu Vinken	In vitro tests addressing immunotoxicity with a focus on immunosuppression Emanuela Corsini	
17.00-17.30	The role of mitochondria in cell death and regeneration during acetaminophen hepatotoxicity Anup Ramachandran	Integrated testing strategy for immunotoxicity: the agrochemical example Marco Corvaro	
17.30-18.00	Advanced <i>in vitro</i> models for nephrotoxicity testing: as complex as possible, but simple in use Silvia Mihaila	The Universal Immune System Simulator: an in silico model to predict the risk associated with exposure to immunotoxicants Francesco Pappalardo (Online)	
20:00	Gala Dinner		

05 NOVEMBER 2022 – SATURDAY

Hall A - Plenary Lecture			
Chair: Emanuela Corsini			
09.00-09.45	Mutidrug Resistance Protein-4 (Mrp4): A New Member in the Constellation of Factors Contributing to Non-alcoholic Liver Disease and Metabolic Syndrome José E. Manautou		
09.45-10.00	Coffee Break		
Hall A - Session 11, The safety of Nanomaterials		Hall B- Session 12, Lipids in health and diseases	
Chairs: Heidi Foth, Murat Özmen		Chairs: Suna Sabuncuoğlu, Petar Bulat	
10.15-10.45	Possible interactions and risks between nanoparticles and chemical pollutants in aquatic environments Murat Özmen	Sterol markers of cholesterol and bile acid metabolism Dieter Luetjohann	
10.45-11.15	Effect of BaSO ₄ nanoparticles on human lung cells under different culture conditions Heidi Foth	Sphingolipid metabolism in toxicological exposures Suna Sabuncuoğlu	
11.15-11.45	Genotoxicity of engineered nanomaterials: New approaches to hazard and risk assessment Maria Dusinska	Role of Ceramides in Insulin Resistance and Diabetic Dyslipidemia From a Biochemical Point of View Mutay Aslan	
12.00	Closing Remarks		



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03 NOVEMBER 2022 – THURSDAY

Oral Presentations 1 - Hall C

Chairs: Ülkü Ündeğer Bucurgat, Bülent Ergun

17.00-17.10	Comparison of EU and TR safety guidelines on the use of nanomaterials in cosmetics Yağmur Emre Arıcan
17.10-17.20	In silico prediction of ADME and toxicity profiles of the drug candidates Tuğçe Yeşil
17.20-17.30	Evaluation of neopterin levels and kynurenine pathway in acute coronary syndrome patients Sonia Sanajou
17.30-17.40	Modifying Effects of Gender on the Toxicity of Methyl Mercury and Thimerosal Selinay Başak Erdemli-Köse
17.40-17.50	Evaluation of Possible Toxic Effects of Preservatives and Adjuvants Used in Vaccines Anıl Yirun
17.50-18.00	Comparison of possible cytotoxic effects in healthy and cancer cells and evaluation of antioxidant capacities of polymeric nanoparticles encapsulated with naringin and berberine Jülide Secerli
18.00-18.10	Hepatotoxicity induced by low doses of Pb: subacute toxicity study in rats and Benchmark modeling Dragana Javorac
18.10-18.20	The protective role of UMCA on bleomycin-induced lung fibrosis in rats Sezgin Aydemir
18.20-18.30	Are rare earth elements safe for use in zootechny?; trophic transfer and effects of lanthanide nanoparticles in a simple food web Daniel Mark Lyons
18.30-18.40	Microplastics: How aware are we? Özge Sultan Zengin
18.40-18.50	Medroxyprogesterone Acetate And Ethodolac Exposure During Pregnancy - Elif Onat
18.50-19.00	Cerebral oxidative stress and histological changes induced by di (2-ethyl hexyl) phthalate exposure in early adolescent and adolescent periods in rats - Deniz Özkan Vardar

04 NOVEMBER 2022 – FRIDAY

Oral Presentations 2 - Hall C

Chairs: Sinem Ilgın, Sibel Özden

13.45-13.55	Role of Ahr/CYP1A1 signaling in the interaction between benzo[a]pyrene and chlorpyrifos Burcu Ünlü Endirlik
13.55-14.05	Determination of exposure to organophosphate flame retardants in preschool children: a human biomonitoring study in three Turkish provinces Hadi Attaran
14.05-14.15	Cell response to N-alkyl quaternary quinuclidine oxime treatment Suzana Zuneć
14.15-14.25	Biomonitoring of Glyphosate-Based Herbicide Among Farmers in Northern Cyprus: A Pilot Study Kumsal Kocadal
14.25-14.35	The nematode Caenorhabditis elegans in Toxicity Testing: A pioneering study on the utility of this model organism Fehmi Burak Alkas
14.35-14.45	Investigation of the presence of volatile organic pollutants (VOCs) in tap water in Ankara provincial districts Onur Kenan Ulutaş
14.45-14.55	Redox status changes in rat plasma induced by phthalate and bisphenol A mixture after subacute exposure Katarina Baralić



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14.55-15.05	Determination of aflatoxin m1 levels in milk samples consumed in the Turkish Republic of N. Cyprus Cengiz Bereket
15.05-15.15	Evaluation of the apoptotic and antioxidant effects of pitavastatin in cervical cancer cell line Aysun Ökçesiz
15.15-15.25	Assessment of organophosphate and pyrethroid pesticides exposure in preschool children in Türkiye Ayca Aktas Sukuroglu
15.25-15.35	Microplastic Pollution in Plant Ecosystems Hakan Özden
15.35-15.45	Effects of COVID-19 disease on DNA damage, oxidative stress and immune responses Merve Hazar
15.45-16.00	Coffee Break
Oral Presentations 3 - Hall C	
Chairs: Emre Durmaz, Sevtap Aydın	
16.00-16.10	Apoptotic, genotoxic and immunotoxic evaluation of bisphenol derivatives exposure in HepG2 cells Aylin Balcı Ozyurt
16.10-16.20	Determination of in vitro genotoxic effects of pine resin and Aronia melonacarpa on prostate and breast cancer cell lines by using comet assay technique Ayfer Beceren
16.20-16.30	Investigation of the cytotoxic and genotoxic effects of royal jelly on healthy and cancer lung cell lines Gökçe Taner
16.30-16.40	Investigation of the Effects of Zearalenone in the Genes Related to Energy Metabolism In Vitro: Relation with the Epigenetic Alterations. Ecem Fatma Karaman
16.40-16.50	Promising novel bioinspired materials for theragnostic applications: Is the synthesis of non-cytotoxic and non-genotoxic dipeptide structures possible? Merve Bacanlı
16.50-17.00	The impact of DNA methylation on the expression of CYP3A4 gene Seher Karslı
17.00-17.10	Investigation of the Protective Effect of Walnut Oil in an in vitro Alzheimer's Disease Model Göksun Demirel
17.10-17.20	Effect of melphalan and epigallocatechin gallate combinations on cytotoxicity in neuroblastoma cell line Cağatay Oltulu
17.20-17.30	Investigation of the apoptotic properties of combined use of lonidamine and doxorubicin on In229 human glioblastoma cell line Ayşenur Bilgehan
17.30-17.40	Cytotoxic effect of abemaciclib in HepG2/THP-1 co-culture model Tugce Boran
17.40-17.50	In vitro evaluation of copper ferro (II) oxide nanoparticles induced neurotoxicity Mahmoud Abudayyak
17.50-18.00	Comparative toxicity responses of THS derived from conventional cigarette and heated-tobacco products in human bronchial epithelial cells Rengin Reis
18.00-18.10	Human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM) model as an assessment tool for cardiotoxicity Zehra Şeker



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Lectures





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PL-01. Use of Adverse Outcome Pathways for The In Vitro Prediction of Liver Toxicity

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Adverse outcome pathways (AOPs) are pragmatic tools in toxicology and human risk assessment with broad potential. AOPs are designed to provide a clear-cut mechanistic representation of toxicological effects that span over different layers of biological organization. Because of its unique location and function in the organism, the liver is a frequent target for systemic toxicity. Not surprisingly, several AOPs related to liver toxicity have been introduced over the fast few years. Focus will be put in this presentation on liver toxicity triggered by bile acid accumulation. This so-called cholestatic hepatotoxicity underlies many drug-induced liver injury cases and has also been associated with various other types of chemicals, including food additives, cosmetics and biocides. The present presentation will give an overview of the validation of this AOP on cholestatic liver injury. In particular, testing of the specificity and applicability domain will be presented along with its potential to predict cholestatic liver toxicity *in vitro*.



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PL-02. The Staged TTC Concept in The Evaluation of Genotoxic Impurities in Drugs

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The "threshold of toxicological concern" (TTC) concept was elaborated some 30 years ago by the FDA to allow for a toxicological residue limitation eg in food in case insufficient data are available to make a fully data-driven assessment. When genotoxic impurities in pharmaceuticals emerged as an issue needing more strategic control some 20 years ago, this concept was started to be used by the industry and health authorities for impurities with such suspected properties of genotoxic and carcinogenic potential. In the meantime, the concept has been successfully introduced into the ICH M7 guideline to control mutagenic impurities in drugs. It continues to be used successfully and is probably the one key argument together with structure-activity correlations to limit exposure of patients to such impurities to below toxicologically concerning levels. An expansion of its use is the use for less than lifetime exposure situations as often occurring with use of drugs to treat diseases. More recently, new evidence for exposure to mutagenic and carcinogenic nitrosamines via drug intake emerged. In this context, the TTC is challenged for extremely potent carcinogens such as nitrosamines. The state of the art on this topic is evolving and the presentation will include more recent developments around this topic.

Keywords: Drugs, genotoxic impurities, risk assessment



PL-03. Zebrafish as a Model for Chemical Induced Adipogenesis and Related Metabolic Diseases

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Humans are widely exposed to endocrine disrupting chemicals (EDCs) and current research has indicated that developmental exposure to specific EDCs, so-called metabolism disrupting chemicals (MDCs), may play a role in the latent onset of obesity and other metabolic disorders like type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD). Within the European H2020 project GOLIATH (Generation Of NoveL, Integrated and Internationally Harmonised Approaches for Testing Metabolism Disrupting Compounds), we aim to get a better understanding of molecular mechanisms of MDCs, and work towards integrated testing and assessment of this class of chemicals.

Zebrafish (*Danio rerio*) is emerging as a valuable model organism to study human metabolic diseases. The goal of this study was to analyze the effects of early developmental exposure to chemicals on adipogenesis, pancreas and liver development in the zebrafish. We have developed a transgenic fish line to examine effects of MDCs on the endocrine and exocrine pancreas (via insulin (Ins) and elastin fluorescent reporters, respectively), as well as lipid metabolism in the liver (via fatty acid binding protein 10a, *Fabp10a*), in combination with a lipid stain to assess alterations in adipogenesis. With this model, we have analyzed different environmental chemicals for their potency to disrupt metabolism. Furthermore we have performed in depth transcriptomics analysis to pinpoint the mechanisms of action of the chemicals in the fish.

Preliminary results indicate ectopic expression of Ins producing cells as the most sensitive endpoint at 5 days post fertilization. Furthermore, some chemicals induced liver size and/or higher expression of *Fabp10a*, whereas exocrine pancreas development and adipogenesis at 16 dpf seemed less sensitive. With the transcriptomics analysis we will attempt to anchor these changes to affected mechanisms which is ongoing work.

Our preliminary results indicate the use of zebrafish as a model to screens chemicals for their potency to disrupt metabolism and might be considered as a novel testing strategy when further characterized.

This project (GOLIATH) has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 825489



**PL-04. DNA Damaging and Protective Effects After Single Combined
Radiotherapy Use of Volatile Anaesthetics, and 1 or 2 Gy Gamma-Irradiation
In Vivo: Preliminary Results**

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Although it was demonstrated that both can cause DNA damage not only *in vitro*/developing animals or humans but also in adult organisms and humans, the combined impact of volatile anaesthetics (VAs) halothane(H)/sevoflurane(S)/isoflurane(I) and ionizing radiation used in radiotherapeutic (RT) exposure *in vivo* has not previously been analysed. In this study, we wanted to check the impact of single exposure in concentrations of both agents used in RT *in vivo* conditions in adult male animals since both agents can affect genders differently, with females more damage-prone. Healthy Swiss albino male mice (240 in total, 48 groups) were exposed to either: H/S/I therapeutic doses alone (2 h); 1 or 2 Gy γ - radiation alone; or to combined exposure. Peripheral blood, frontal lobe brain, liver, and kidney cortex samples from five animals per group were taken immediately, 2, 6, and 24 h after exposure. DNA damage and cellular repair index (CRI) were analysed using alkaline comet assay and the tail intensity (TI) parameter. Elevated TI levels for S/H were usually the highest at 6 h, and within 24 h were usually repaired for S but not H, while I treatment usually caused lower TI than in control groups. Combined exposure demonstrated usually a slightly H/S protective and I protective effect, which was stronger for 2 Gy than 1 Gy. Although preconditioning protection should be VA similar, CRI and TI histograms indicated different modes of action in DNA damage creation for each VAs and each organ. I/S/H preconditioning demonstrated in general protective *in vivo*, but in some cases with damage not repaired in total during a 24-hour period from the single exposure. Due to the constant increase in the use of RT+VA, further studies should explore the mechanisms behind these effects, including longer and multiple exposure treatments and *in vivo* brain tumour models.^{1,2,3,4}

Keywords: cellular DNA repair index, DNA damage; ionizing radiation; volatile anaesthetics, tail intensity, the protective effect



**PL-05. Effect of a Meat-Based Diet on Fecal Water Genotoxicity: a
Randomized Clinical Trial in Healthy Subjects**

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Diet is known to alter the gut microbiota as well as the cytotoxic and genotoxic activities of the luminal colonic content. Red and processed meat enriched diet is connected to increased formation of mutagens during cooking, but also of lipid peroxidation derivatives, whose formation is catalyzed by heme iron in the colon, and N-nitroso compounds. We evaluated the impact of three diets differing for their associated cancer risk [a meat-based diet (MBD: high-risk), a meat-based diet with alpha-tocopherol integration (MBD-T: medium-risk), and a pesco-vegetarian diet (PVD: low-risk)] on fecal microbiota and colorectal cancer risk (CRC) biomarkers.

A 3-month randomized parallel open clinical trial was conducted at the University Hospital of Careggi, Florence, Italy. Of the 113 enrolled patients, 85 concluded the three-month intervention providing biological samples at the beginning and at the end of the dietary intervention. Fecal water (FW), a mixture of all water-soluble harmful substances and metabolites in the faeces, was prepared from each sample. FWs were used to evaluate cyto- and genotoxicity induced on cultured HT-29 human cells and to evaluate mutagenicity induced on the yeast model *Saccharomyces cerevisiae* D7 strain.

FWs samples from the MBD group at the end of the intervention showed reduced cell viability (-13%, $p=0.076$), whereas no change was found in the MBD-T or PVD groups. An increase in FWs genotoxicity was also found in the MBD group (from 30.5 ± 8.1 to 55.2 ± 7.8 , mean \pm SE, $p=0.028$) and not in the MBD-T or PVD groups. No significant effect of any diet was found in the *S. cerevisiae* mutagenesis test.

These results indicate that the MBD diet was associated with increased genotoxicity of fecal water, a marker of colon cancer risk. Alpha-tocopherol integration of the MBD (MBD-T) protected from this effect.



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PL-06. Suitability of Salivary Leucocytes to assess DNA Damage and Repair in Human Biomonitoring Studies Using The Comet Assay

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Peripheral blood leucocytes (PBL) have been traditionally used to assess DNA damage and repair by the comet assay, but validating alternative non-invasive samples would expand the application of this assay in human biomonitoring. The objectives of this study were to test the validity of salivary leucocytes (both fresh and cryopreserved) as a proper biomatrix for the comet assay, and to evaluate the ability of this approach to detect (i) diverse types of primary and oxidative DNA damage, and (ii) repair activity of DNA damage induced by different action mechanisms. Salivary leucocytes were exposed to four genotoxic agents and showed dose-dependent increases of primary and oxidative DNA damage, similar to those obtained in PBL, demonstrating the suitability of these samples to detect genetic damage from different origin. Cells were also challenged with three genotoxic agents acting by diverse action mechanisms. In order to study repair kinetics, comet assay was performed just after treatment and at other three additional time points. Saliva leucocytes were as suitable as PBL for assessing DNA damage of different nature that was efficiently repaired over the evaluated time points, even after 5 months of cryopreservation (and after 24 h stimulation with PHA). This study demonstrates that salivary leucocytes can be effectively employed in comet assay as an alternative or complement to blood samples. Frozen salivary leucocytes were proved to be a very convenient sample in large biomonitoring studies.

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PL-07. Characterization of Portuguese Wildland Firefighters Before a Wildfire Season: Looking at The Cytogenetic Effects

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Biological monitoring represents a valuable tool for assessing the potential health effects related to hazardous exposures. The increasing risk of wildfires episodes and longer fire seasons highlight the need for occupational studies enrolling wildland firefighters. Woodsmoke is a complex mixture of pollutants, some of which are listed as probable or known carcinogens. IARC has classified firefighting as potentially carcinogenic to humans. Still, few studies have been focused on the health effects associated with wildland firefighter exposure. Bio4Fox aims to establish a panel of biomarkers for the surveillance of firefighters' occupational health during different phases (pre-, during and post- fire season). Around 173 northern Portuguese wildland firefighters (144 males and 29 females; mean age of 38.13 ± 10.93 and 36.55 ± 10.37 , respectively) were enrolled in the pre-fire season. Data on sociodemographic factors, lifestyle, health status and occupational history were collected through a validated questionnaire. We observed statistically significant results within cytogenetic effects accessed through the Buccal Micronucleus Cytome assay (BMCyt) and some self-reported variables. The frequency of cells with condensed chromatin (a marker of cell death) and binucleated cells (failed cytokinesis) was higher among females. No correlation between age, alcohol consumption, time of service and BMCyt data were found. Some related occupational factors increased the risk of cytogenetic effects (i.e., being part of Permanent Intervention Teams and having past occupational exposures). A weak, though statistically significant, positive association with DNA damage (i.e., nuclear buds) and smoking years, in former smokers, was found. Lower %micronuclei and %pynotic were observed among subjects taking vitamin supplements and consuming vegetables, respectively. Other important diet and health status variables were related with BMCyt outputs. Our findings furnish a better characterization of Portuguese wildland firefighters before a wildfire season. We expect to contribute to the implementation of health and safety measures highly needed in this sector.

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**PL-08. Monitoring Air Pollution and The Health-Related Biomarkers:
Lessons from HUMNap.**

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Exposure to outdoor air pollution is related to severe health effects, especially in urban areas, due to higher concentrations of pollutants caused by urban agglomeration, traffic, and industrial activities. Additionally, the emission of BTEX (benzene, toluene, ethylbenzene, and xylene) compounds becomes higher, mainly due to intensive industrialization and urbanization. Hence, we aimed to investigate possible effects of air pollution and BTEX exposure on DNA damage detected by the alkaline comet assay and micronucleus assay on human buccal and peripheral blood cells. The first part of the study was conducted during the colder period of the year 2021 and the follow up part during the warmer period of the year 2022. It involved 60 healthy volunteers aged 18-55 years with BMI < 30 kg/m² living in Zagreb (Croatia). To evaluate potential impact of air pollution on comet and micronucleus assay results, association was made using different time windows of measured air pollution parameters prior to sampling, while BTEX were analysed in whole blood using headspace solid-phase micro extraction (SPME) followed by gas chromatography-mass spectrometry (GC-MS). All measured outdoor air pollution parameters were in agreement with previously reported values, which were below regulatory limit, except for PM₁₀ particle fraction (particles with aerodynamic diameter less than 10 µm) and benzo[a]pyrene bound to PM₁₀, that exceeded regulatory limit levels. Statistical analysis was used to investigate correlation between the measured biomarkers of exposure and DNA damage along with their association to different sampling periods, using Spearman's correlation and Linear regression modelling. Future research will include same study design for populations living in other Croatian cities, where we expect exposure to different set of air pollutants.

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PL-09. The Key Characteristics Approach as Applied to Immunotoxicants.

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The concept of chemical agents having properties that confer potential hazard called Key Characteristics (KCs) was first developed for carcinogens using mechanisms as an organizing principal to evaluate the hazards of novel chemicals. The ability of this framework to complement or replace existing approaches has also been recognized for multiple target organs and toxicity, including endocrine disruption, hepatotoxicity and reproductive toxicants. Many of the mechanisms through which therapeutics and environmental chemicals modulate immune function are well-understood and the cellular and molecular targets of these agents have been described. Recently, an international group of experts with knowledge of the immune system and expertise in immunotoxicity hazard evaluation and risk assessment was assembled with the goal of identifying KCs of immunotoxic agents, defined as substances that can alter one or more immune functions resulting in an adverse effect for an organism. The 10 KCs identified encompass the spectrum of immune function, from initiation to regulation, and are applicable to immune suppression, hypersensitivity and autoimmune responses, recognizing that some substances may demonstrate multiple characteristics and may impact more than one aspect of immunotoxicity. In addition to describing the KCs, this presentation will demonstrate their application using the environmental contaminant 2,3,7,8-tetrachlorodibenzo dioxin and the immunosuppressive drug cyclosporine. An understanding of how substances cause changes in immune function earlier in the drug discovery or hazard assessment process will allow us to develop better methods to evaluate immunotoxicity in humans, and to enable a more comprehensive mechanistic understanding of the adverse effects of chemicals on immunity.



PL-10. Does research in the field of Occupational Toxicology of Metals have any Future in Developed Countries?

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The development of industry in developed countries has led to a significant reduction in the occupational exposure of employees to metals. Current levels of occupational exposure are very close to the levels of exposure in the environment. This raises the question of the future of the research in the field of occupational exposure to metals in developed countries.

Material and Methods: In order to quantify the research trend in the field of occupational toxicology of metals we searched the Medline database using PubMed Medical Subject Headings (MeSH) terms composed into a search string to find articles describing studies dealing with occupational exposure to heavy metals from Europe or North America. The proposed search string was ("Europe"[Mesh] OR "North America"[MeSH]) AND "Metals, Heavy"[Mesh] AND "occupational exposure"[MESH]. In addition, we searched for studies dealing individually with cadmium, chromium, copper, iron, lead, and mercury; together with occupational exposure and Europe or North America. The number of studies in total and by each metal was analyzed descriptively through time, from 1967 to 2022.

Results: A total of 1827 studies were found dealing with occupational exposure to heavy metals in Europe and North America. A steady increase in studies is seen from the end of 1980s to 2007, when there were 78 studies published. The decade starting with 2007 shows steady numbers of between 50 and 70 studies per year, and since 2017 there has been a decline in these studies to numbers below 50. After 2019, when 54 studies were published, only 35 and 29 studies were published in 2020 and 2021, respectively. A similar pattern denoted by an increase in studies until the mid-2000s, and a decrease since then is seen also for individual heavy metals.

Conclusion: The declining trend in research in the field of occupational toxicology of metals in developed countries is causing a dose of concern. Especially, having in mind grooving the use of metals in nanotechnologies.



PL-11. Tobacco During Pregnancy: Looking at Self-Reported Tobacco Exposure, Urinary Cotinine Levels and Perinatal Outcomes

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Despite the widespread evidence that tobacco use and exposure to environmental tobacco smoke (ETS) during pregnancy adversely affects the health of women and their offspring, this is still an important global public health concern, namely to guide health promotion planning. This study, carried out under the frame of the NeoGene project, aimed to: i) assess smoking prevalence using self-reported tobacco exposure data, ii) validate self-reported smoking with biochemical indicators (maternal urinary cotinine (UC) levels), and iii) investigate the association between tobacco exposure with adverse perinatal outcomes.

From a sample of 595 pregnant women, the following were analysed: i) information on smoking and ETS-related variables via a validated questionnaire; ii) the levels of UC on maternal urine collected on the day of the delivery, by solid-phase competitive ELISA; and iii) the correspondent data on perinatal outcomes, retrieved from the hospital electronic medical records.

Self-reported smoking prevalence was 27.9% and 12.9% before pregnancy and at birth, respectively. Amongst the self-reported non-smokers, 31.7% reported exposure to ETS prior to pregnancy, decreasing to a prevalence of 28.6% in the third trimester. UC levels were found to be in very good agreement with maternal self-reported smoking status in the third trimester ($k=0.919$), but not with self-reported ETS exposure ($k=0.386$; also in the third trimester). Maternal active smoking in the third trimester of pregnancy was associated with a decrease in birth weight, length and head circumference; these indicators were found to increase in cases where smoking cessation occurred during pregnancy. Results demonstrate that it is essential to promote tobacco consumption cessation and decrease ETS exposure during pregnancy, through either tobacco control interventions or new/updated legislation, in order to improve perinatal outcomes.

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Keywords: tobacco, smoking, pregnancy, questionnaires, urinary cotinine, perinatal outcomes



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PL-12. Safety Evaluation of Food Additives

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Food safety organizations continuously evaluate the safety of foods. These organizations can be global (JECFA), regional (EFSA), and national (ANSES, BfR). In the European Union, food safety is ensured by EFSA in collaboration with the European Commission, European Parliament, and the Member States. Within this interaction, EFSA serves as the risk assessor while European Commission, European Parliament, and the Member States are serving as the risk managers. The safety evaluation process of EFSA will be reviewed through the example of aspartame.

All food additives authorized in the European Union undergo a thorough safety assessment as required by EU legislation. In accordance with this practice, EFSA has initiated a program to re-evaluate the safety of Aspartame and completed it in 2013. This re-evaluation is based on the risk assessment of aspartame. To this end, short-term and long-term effects of aspartame in experimental animals, including carcinogenicity, neurotoxicity, reproductive/developmental toxicity, and genotoxicity have been examined by the scientists of the relevant expert panel of EFSA.

In re-assessing the carcinogenic potential of aspartame the biggest challenge was that many of the studies dated back to the 1970s. Even though standards in the design and conduct of studies have markedly developed since the 1970s the quality and number of available animal studies were considered sufficiently high to conclude by the EFSA's experts. After a comprehensive assessment of available studies, the experts have not identified scientific evidence supporting the carcinogenicity of aspartame.

Studies in experimental animals and humans have shown that after oral ingestion, aspartame is fully hydrolyzed within the gastrointestinal tract. Phenylalanine (50%), aspartic acid (40%), and methanol (10%) are the resulting breakdown products of aspartame. Due to the very efficient hydrolysis in the gastrointestinal tract, the amount of intact aspartame that enters the bloodstream has been reported as undetectable in several studies conducted on various animal species and humans. This finding forms the basis for the risk assessment of aspartame conducted by EFSA in 2013. Accordingly, the expert panel focused on the hazard identification/characterization and exposure assessment of the breakdown products.

The exposure assessment of aspartic acid shows that the daily aspartic acid exposure in highly exposed age groups of the population was lower than the aspartic acid exposure originating from aspartame consumption at the Acceptable Daily Intake (ADI) level (40 mg/kg/day). According to this assessment the daily aspartic acid exposure released from aspartame has considered to be safe by the expert panel.



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The reproductive and developmental toxicity database on methanol was limited and the available studies were not adequate to derive a NOAEL. Therefore a NOAEL of approximately 560 mg/kg/day for oral methanol exposure was derived by the experts from the NOAEC of 1300 mg methanol/m³ from an inhalation study in mice as the point of departure (POD). Accordingly, the experts of EFSA concluded that the calculated NOAEL (560 mg/kg/day) for methanol by oral exposure is 140 - fold higher than the maximum amount of methanol that could be released when aspartame is consumed at the ADI level.

In essence, in EFSA's (2013) last evaluation the risk assessment of aspartame is based on plasma phenylalanine levels in patients with phenylketonuria (PKU). The expert panel of EFSA has estimated a phenylalanine plasma concentration of 120 µM as the maximum safe plasma phenylalanine concentration that can be released from aspartame. According to the kinetic studies the peak plasma phenylalanine levels have not exceeded the clinical target threshold (240 µM) when a normal individual (or an individual heterozygous for PKU) consumed aspartame at levels below the current ADI level. Based on these assessments the ADI level of 40 mg/kg/day for aspartame has been approved again by EFSA. However, the criticisms about the safety of aspartame still continue.

Keywords: Food additives, safety evaluation, aspartame



PL-13. Paracetamol-Induced Kidney Damage Occurs by Different Mechanism from Hepatotoxicity

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Paracetamol (PAR) overdose is the primary cause of fatal acute liver injury in western world. The mechanism of this toxicity has been well defined. Because of the fatal nature of hepatotoxicity and relatively much lower incidence of PAR-induced nephrotoxicity, both clinical and scientific attention has been given to liver until recently. However, further analyses revealed that the incidence of kidney toxicity is much higher. While mechanisms of PAR-induced liver injury have been well studied, molecular mechanisms involved in PAR-induced kidney injury are not characterized yet. We recently showed the potential of 4-methylpyrazole (4MP) as an antidote against PAR-induced nephrotoxicity. Data of this study revealed that PAR-induced nephrotoxicity does not follow a similar cascade of molecular events as in PAR-induced hepatotoxicity ^[1]. Further we hypothesized that cysteine conjugate of PAR may be activated to reactive metabolites via renal *cysteine conjugate β -lyase*, which is abundant in proximal tubular cells where kidney injury occurs. *Cysteine conjugate β -lyases* also have mitochondrial forms, and possible bioactivation of PAR-Cys may occur in this critical organelle, as many other drugs or chemicals ^[2]. We demonstrated that PAR-Cys serves as a substrate for renal *cysteine conjugate β -lyase* in *in vitro* mouse and human kidney cytosolic, as well as mitochondrial fractions ^[3]. Further incubations in the presence of nucleophilic trapping agents revealed that electrophilic intermediates from PAR-Cys are formed upon both incubations with cytosolic and mitochondrial *β -lyases*. The differences in the mechanisms of PAR-induced liver and kidney injuries together with this alternative mechanism will be discussed in the symposium.

Acknowledgements: This study was supported by Ege University Scientific Research Grants (TOA-2021-22922) and by Aliye Uster Foundation.



PL-14. The Impacts of Prominent Detoxifying and Repair Gene Polymorphisms on Neurological Diseases

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There are many neurological system disorders that require clinical care. According to the U.S. National Library of Medicine there are more than 600 neurologic diseases. I will focus on Parkinson disease (PD), stroke, multiple sclerosis (MS) of these diseases that I am working on. A stroke, or brain attack, happens when blood flow to your brain is stopped. The brain needs a constant supply of oxygen and nutrients in order to work well. If blood supply is stopped even for a short time, this can cause problems. MS is primarily a neurodegenerative and an inflammatory disease. PD is also a chronic and progressive neurodegenerative disease of the brain, is associated with the loss of dopaminergic neurons. The pathogenesis of these diseases remain unclear; however, oxidative DNA damage due to reactive oxygen species (ROS) is believed to play a major role in their etiology. Environmental and genetic factors have been implicated in their pathogenesis. Environmental factors induce oxidative stress, which has been defined as principle pathological cause of neurodegeneration. The ROS toxicity is due to direct damage of macromolecules, such as DNA, that contributes to inflammation and tissue damage. Antioxidant defense and multiple DNA repair systems protect cells from oxidative stress. All these factors have been co-existed in MS, which look as if only one of them alone cannot be responsible for the induction of MS. Studies showed that *IL-18* which was known as interferon-gamma inducing factor proinflammatory cytokine, *IL-18 607C/A* gene promoter polymorphism was a major genetic factor for determining individual susceptibility to MS, where smoking status also increases the risk of MS. In our previous study, it was demonstrated that a repair enzyme, *XRCC1*, its variant genotypes had a two to three-and-a-half-fold higher risk of PD than controls. *Glutathione S transferase (GST) (M1, T1, P1)* have major roles in detoxification of the products of oxidative stress and they are polymorphic. DNA damages can also be repaired by repair enzymes such as *OGG1* and *XRCC1* which are highly polymorphic. In a study conducted with stroke patients, *OGG1 Ser326Cys* and *XRCC1 Arg399Gln* gene polymorphisms had impacts on the development of stroke. Polymorphisms in DNA-repair and detoxifying genes may be important in the etiology of these neurological diseases. Data on gene polymorphisms encourage researchers to work on detoxifying and repair gene polymorphisms in neurological disease.

Keywords: Neurological disorders, Multiple sclerosis, Parkinson disease, DNA repair, gene polymorphism



PL-15. Air Pollution Context and Biomonitoring in Sarajevo, Bosnia and Herzegovina (Antalya 2021)

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Bosnia and Herzegovina (B&H) is among the European countries with the poorest air quality. Solid fuels, traffic, and the lack of efficient air-pollution reduction policies are recognized as the main contributors. As a result of temperature inversion, citizens of Sarajevo, the capital of B&H, additionally witness longer periods of fog with sustained air pollution in autumn and winter months. Air pollution is characterized as human carcinogen; therefore, it is a significant global public health issue extending from subclinical effects to the consequence of premature death. Till recently, biomonitoring of air pollution in Bosnia and Herzegovina was poorly performed. Since good health and well-being, along with the environmental protection, are accepted as sustainable development goals, we aimed to implement various approaches towards biomonitoring of air pollution. *In vivo* human biomonitoring, using comet assay on oral leukocytes and buccal micronucleus cytome assay, was performed in regards to the seasons. *In situ* plant biomonitoring using plant comet assay has been established in *Ligustrum vulgare* L. as a robust model sensitive to seasonal and sampling site variations. Additionally, characterization of ambient air and *in vitro* genotoxicity testing of water-soluble fraction of PM₁₀ samples has been comprised. Observed genotoxic effects generally correspond to the air quality data. By clarifying biological effects of air pollution in Sarajevo area, we expect to affect policy makers towards establishment of relevant regulations supporting the use of safer fuels, and air pollution responsible stakeholders.

Keywords: comet assay, oral leukocytes, plant comet assay, *in vitro* genotoxicity



PL-16. *In vitro* and Pre-Clinical Studies of Decontamination Efficacy of Chemical Warfare Agents from the Skin.

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High toxicity of nerve agents and other classes of chemical agents emphasize the need for effective protection of front-line emergency responders and medical personnel. Lessons learned from historical events involving nerve agents, clearly indicate how decontamination efficacy is time-dependent and how toxicologically relevant nerve agent concentrations may arise when patients are exposed to even comparably low doses without adequate medical countermeasures.

There has been interest from emergency responders on the decontamination efficacy of RSDL® (Reactive Skin Decontamination Lotion Kit) on nerve agents, and other toxic compounds. Their primary goals are to reduce the potential exposure to chemicals and provide post exposure decontamination to prevent toxic compounds from entering the body.

An overview of *in vitro* and *in vivo* studies will be shared and discussed around select toxic compounds including nerve agents, vesicant agents, and other chemicals or toxins of concern.

The effectiveness of decontamination of toxic agents as described in *in vitro* investigational studies that developed analytical and validation procedures using liquid chromatography–mass spectrometry (LC-MS), gas chromatography–mass spectrometry (GC-MS), and ion chromatography, depending on the compound of interest, will be reviewed.

Pre-clinical investigations will also be discussed, to further enhance the agent profiling studies, to demonstrate the correlation between *in vitro* and *in vivo* efficacy, and to provide operational doctrine considerations for percutaneous decontamination of chemicals of concern.



PL-17. A Generic Presentation on Fourth Generation Agents and Their Risks – Novel Therapeutic Options for Nerve Agents/Organophosphates Poisoning

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Fourth generation agent (FGAs) is a kind of chemical warfare agent (CWA). Their chemical structure is similar to organophosphorus compounds. They can stay in the applied area more than other nerve agents due to low volatility. Their toxicity is similar to VX. These chemicals are very popular among terrorists and mass destruction properties. Russian scientists developed this new series of neurotoxic agents derived from the G-series and V-series organophosphate agents, this was named as the A-series (A-230, A-232, and A-234).

These agents block the acetylcholinesterase enzyme in blood and cause cholinergic toxidrome including salivation, excessive sweating, runny nose, lacrimation with small (often pinpoint) pupils, cough, bronchospasm, bronchorrhea, labored breathing, wheezing, respiratory failure, excessive urination, diarrhea, abdominal cramps, nausea, vomiting, bradycardia, confusion, drowsiness, twitching, fasciculations, cramps, fatigue, weakness, tremors, slurred speech, ataxia, seizures/convulsions, unconsciousness, coma.

There is no specific information about the protection and decontamination for FGAs. These compounds can be destroyed by basic solutions with more than 10% NaOH or NaCO₃, or household bleach. Water and soap is also capable of decontaminating contaminated area including human body parts. Along with supportive care and decontamination, anticholinergics (e.g., atropine), oxime preparation (AChE reactivators (e.g., obidoxime, 2-PAM)), and anticonvulsants (e.g., the benzodiazepines - diazepam, midazolam, and lorazepam) are the main elements for treating poisoned patients.

Even if there is not available high volume data about these new chemical agents, personnel protection equipments, decontamination procedures and medical treatment is similar to other classical organophosphate type nerve agents. There are many uncertain points on these chemicals. After clarification of uncertain points including their chemical structures and properties, effective protection and treatment regimen against them will be possible.

Keywords: Chemical weapons, fourth generation agents, nerve agents, organophosphates, atropine, oxime.



PL-18. Genotoxic Impurities and Other Possible Impurities in Pharmaceutical Products: From the Application Perspective

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Genotoxic and other possible impurities in medicinal products is one of the critical topics for human medicinal product productions in recent years. To protect human health, impurity levels should conform safe limits. Impurities can come from starting materials, chemical synthesis, production steps, residual solvents, production equipments, filtering apparatus, packaging materials, and storage conditions etc. Thresholds and evaluation processes of impurities are included by different international guidelines. These are ICH Q3A, ICH Q3B, ICH Q3C, ICH Q3D, EMA and FDA guidelines for specific topics like nitrosamine impurities.

Isolation and characterization of impurities is mandatory to obtain data for doing further safety evaluation of them. High technique equipments are used to isolate and characterise impurities. These are capillary electrophoresis, electron paramagnetic resonance, gas-liquid chromatography, gravimetric analysis, high performance liquid chromatography, UV Spectrometry, IR spectroscopy, mass spectrometry, nuclear magnetic resonance (NMR) spectroscopy, and RAMAN spectroscopy. If the impurity levels is below the threshold limits which are stated in related guidelines, there is no need to further actions. When the impurity levels exceeds the threshold limits, toxicological evaluation should be carried out to clarify their safety. Necessary steps for the safety evaluation of them is followed. It does not mean that all of the limit exceeding impurities should be avoided. After toxicological evaluation of them necessary measures is taken. First step is the application of computational techniques to predict their toxicological profile. If this prediction gives us a toxicological risk, toxicological testing is carried out including genotoxicity testing and general in vivo toxicological analysis.

In this review, special application of guidelines related with different impurities will be discussed and informed from the viewpoint of toxicological and regulatory purposes.

Keywords: Impurities, genotoxic, elemental impurities, nitrosamines, safety evaluation.



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PL-19. Genetic Risks from Heat-Damaged DNA in Food

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Consumption of foods prepared at high temperatures has long been associated with numerous health risks, including colorectal cancer, metabolic disease, and aging. To date, the chief identified source of risk in food has been small molecules produced by cooking and activated into forms that react with healthy DNA upon consumption, leading to damage and mutations. Here, we considered whether the DNA in food itself might present a hazard. Heat is reported to accelerate chemical modification to isolated DNA such as deamination and oxidation, and human cells take up canonical nucleosides via salvage pathways. These facts led us to hypothesize that high-temperature cooking might cause significant damage to the DNA in food, and if the damaged components can be salvaged by cells and incorporated into DNA via polymerase enzymes, this damage might find its way into cellular DNA. To test this, we evaluated the effects of cooking on DNA in foods, and we report finding very high levels of hydrolytic and oxidative damage to all four DNA bases. We further surveyed a range of damaged 2'-deoxynucleosides for their ability to be taken up into DNA in cell culture, and observed evidence of substantial uptake in cellular DNA, activating DNA repair systems and promoting double-strand chromosomal breaks. Feeding a deaminated 2'-deoxynucleoside to mice resulted in its incorporation into the DNA of intestinal tissues, ultimately leading to elevated levels of double-strand breaks in the tissue. The results suggest a new and direct pathway whereby high-temperature cooking may contribute to genetic risks.



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PL-20. Multidrug Resistance Protein-4 (Mrp4): A New Member in the Constellation of Factors Contributing to Non-alcoholic Liver Disease and Metabolic Syndrome

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Hepatobiliary transporters are not only determinants of drug pharmacokinetics, pharmacodynamics, but also important contributors of drug-induced liver injury (DILI). Our laboratory has shown that paracetamol-induced DILI confers resistance to subsequent paracetamol toxic insult. Furthermore, this acquired tolerance to paracetamol hepatotoxicity is associated with hepatocellular regeneration and enhanced expression of the hepatobiliary transporter multidrug resistance protein 4 (Mrp4 or ABCC4). To begin deciphering this relationship, we performed partial hepatectomy in mice with genetic ablation of Mrp4. To our surprise, lack of Mrp4 did not alter hepatocyte compensatory proliferation rates or liver injury following partial hepatectomy, but instead led to regeneration of liver tissue with a steatotic phenotype. Further studies showed that Mrp4 function regulates adipogenesis and that Mrp4 knockout mice exhibit a metabolic syndrome phenotype. Collectively, our studies have established Mrp4 as a genetic factor that contributes to the development of hepatic steatosis and metabolic syndrome.



PL-21. *In Vitro* Genotoxicity of Two Graphene Oxides Before and After Prolonged Mild Thermal Treatment

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Introduction: The important properties of graphene nanomaterials, such as surface chemistry, play a significant role in their biocompatibility and potential use in biomedicine. Graphene oxide, derivative of graphene, has oxygenated groups on the surface of the molecule and it is produced by exfoliation of graphite oxide.

Aim: The aim of this study was to evaluate graphene oxide biocompatibility by measuring levels of induced DNA damage in peripheral blood mononuclear cells (PBMCs).

Material and methods: Genotoxic effects of two graphene oxides, exposed or unexposed to prolonged mild thermal treatment, were assessed by alkaline comet assay in PBMCs after 3h treatment. Effects of graphene oxides were compared according to the applied concentrations (2.5, 5 and 10 mg/ml) and the size of nanoparticles (larger and smaller than 200 nm). DNA damage was analysed using Comet Assay IV software and measuring tail intensity (%) in at least 200 cells for each treatment.

Results: Observed results indicate a significant increase of DNA damage in the concentration-dependent manner for all tested graphene oxide materials. However, larger particles (>200 nm) show higher toxicity at lower concentrations compared to smaller particles (<200 nm) that induced significant increase of DNA damage at higher applied concentrations. Thermal treatment resulted in lower DNA damage for >200 nm graphene oxide at concentration of 5 mg/ml and for <200 nm graphene oxide at concentration of 2.5 mg/ml.

Conclusion: Genotoxicity of thermally treated and untreated graphene oxides in vitro depends on their size and concentration. Thermal treatment is a promising method for the surface modification of graphene oxides and shows potential to inhibit DNA damage in vitro. Further correlation studies between surface characteristics and biocompatibility of graphene oxides are needed.

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Keywords: graphene nanomaterials, comet assay, tail intensity, PBMCs



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PL-22. Strategies on the assessment of genotoxic impurities in pharmaceuticals

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The assessment of potential genotoxic impurities under ICH M7 in pharmaceuticals is a complex process which starts at candidate selection and covers the full value chain including life cycle management. The initial step is the evaluation of the synthesis route by chemists, listing synthesis compounds, known and observed impurities and reasonably predictable impurities. This is also true for synthesis changes during development and for marketed products that are part of the optimization of synthesis routes.

With this list of compounds often consisting of up to 100 compounds an automated in silico analysis is performed and the result will be assessed by a well experienced in silico toxicologist. For this an automated system was developed and established, which also covers a thorough data base and literature research to identify available genotoxicity results.

The thorough assessment of the genotoxicity results by an experienced Genetic Toxicologist is key. There are cases where a positive Ames result is caused by an impurity of the impurity or an bacteria specific mutagen.

Based on the results of this analysis, the impurities will be classified into 5 groups. The different groups will determine if a compound needs to be controlled (group 1 and 2), tested and/or controlled (group 3) or can be handled under ICH Q3. Control can be performed at the TTC or at a compound specific AI calculated from carcinogenicity studies.

N-Nitrosamines (NAs), as part of the Cohort of concern under ICH M7 have been of recent concern as impurities in pharmaceuticals. This concern is mainly based on a subgroup of Nitrosamines, which are highly potent mutagenic carcinogens in rodent bioassays. Concern has been raised that the Ames test may not be sensitive enough to detect the mutagenic potential of Nitrosated Drug Substance Related Impurities (NDSRI). Current status and case studies will be presented.



PL-23. Regulatory Applications of Computational Toxicology: Progress and Strategies to Ensure Wider Use

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Computational toxicology plays an essential role in worldwide efforts to implement New Approach Methodologies (NAMs) to assess the potential hazards of drugs, chemicals, and other products. Computational approaches can fulfill a wide range of functions in toxicology, including chemical structure and property similarity analysis to facilitate read-across, literature compilation and sorting using text recognition, modeling of chemical behavior in organisms or ecosystems, analysis of massive cheminformatics databases, predictive modeling to fill data gaps, and more. Computational approaches can in many cases provide actionable information about chemical risks with fewer resources—time, money, and animals—than traditional animal tests. They can also be useful when compounds haven't yet been synthesized. While computational approaches have been used in toxicology for several decades, renewed interest in replacing animals in toxicology testing in recent years has inspired a scientific and regulatory revolution, increasing the development of many kinds of computational models. Strategies to combine biological (e.g., in vitro) and computational evidence streams together to replace animal tests are being explored more frequently, especially in light of the first Organization for Economic Cooperation and Development Test Guideline using (Quantitative) Structure Activity Relationship predictions and in vitro data in a Defined Approach for skin sensitization. In order to realize the promise of computational approaches, regulatory acceptance and use must be increased. Prospective users of models (researchers/toxicologists) and their outputs (regulators) can gain confidence through transparent documentation, establishment and sharing of best practices, training and education, and collaborative case study exercises relevant to a variety of decision-making contexts.



PL-24. The role of mitochondria in cell death and regeneration during acetaminophen hepatotoxicity

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Acetaminophen (APAP) overdose is the most common cause of acute liver failure in Western countries, and the role of mitochondrial dysfunction in APAP-hepatotoxicity has been well recognized. Early studies focused on the role of mitochondrial oxidant stress in activating MAP kinases like JNK and its role in amplifying mitochondrial injury through generation of peroxynitrite and induction of the mitochondrial permeability transition. Our recent studies now clarify nuances of early mitochondrial changes after NAPQI-induced protein adduct formation, where directional superoxide formation towards the cytosol initially preserves mitochondrial function but activates JNK in the cytosol. Studies have also revealed early adaptive responses, where mitochondria alter morphology to accommodate loss of mitochondrial membrane potential but ultimately undergo mitochondrial fission in the face of sustained amplification of mitochondrial oxidative stress after JNK translocation to the organelle. In addition to these critical roles of mitochondria in mediating APAP-induced injury, it is now also becoming clear that the organelle plays an important role in liver recovery as well. Mitochondrial biogenesis is an adaptive response to maintain metabolic homeostasis after mitochondrial dysfunction. Evaluation of liver recovery after APAP-induced centrilobular necrosis revealed that this involves an upregulation of mitochondrial biogenesis in the layer of cells surrounding the areas of necrosis, which are critical for successful liver recovery and regeneration. These central roles of the mitochondria in APAP pathophysiology will be the focus of this talk.



PL-25. Genotoxicity of Engineered Nanomaterials: New Approaches to Hazard and Risk Assessment

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Hazard assessment of engineered nanomaterials (ENMs) depends on an intimate knowledge of their physicochemical properties and their interactions with target biological materials. Principles established for standard chemicals apply also to ENMs, but in addition there are nanospecific considerations. First, pristine ENMs must be characterised in terms of size, shape, chemical composition, coating, crystallinity, charge, dissolution, ion release, etc. The dispersion method used and characterization of ENMs in exposure medium must be assessed as ENMs tend to agglomerate and this can influence results.

The testing strategy for ENM genotoxicity needs to cover key endpoints – mammalian gene mutation (as ENMs cannot penetrate bacterial cell walls), chromosomal damage and aneugenicity. Additionally cellular uptake needs to be demonstrated. Though ENMs do not need to enter cells to have effect, proof of uptake confirms that ENMs are in contact with cells - especially important when negative results are obtained. Uptake depends on ENM size, shape, shell structure, surface chemistry, corona formation, etc. Also, cells vary in their uptake ability, which needs to be considered when selecting a cell type for genotoxicity testing of ENMs. For example, lymphocytes and related cell lines have a lower uptake than THP-1 cells (monocytes). To ensure that the NM actually reaches the DNA (during mitosis, when the nuclear membrane is absent), a prolonged exposure period (24-48h) is recommended.

It is impossible to test all ENMs in vivo and thus new approach methodologies (NAMs) that include advanced in vitro and in silico models are under development, to facilitate fast and efficient hazard assessment. Thus, the development of specific OECD test guidelines (TGs) for hazard assessment of ENMs (or modification of existing TGs) is a priority.

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PL-26. Mitochondrial DNA as a Target for Environmentally Relevant Genotoxins

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Mitochondria play essential roles in cellular homeostasis, as the main cellular site for ATP generation, control of cellular fate and intermediary metabolism. Mitochondrial function depends on the integrity of the mitochondrial DNA (mtDNA), as it codes for 13 essential subunits of 4 of the five complexes of the oxidative phosphorylation, complexes I, III, IV and the ATP synthase. Nonetheless, the mtDNA is prone to damage, and it is known to accumulate more damage than the nuclear DNA in physiological conditions. However, damage to the mtDNA is often overlooked in environmental genotoxicology studies. A comprehensive review of available literature on mtDNA damage by environmentally relevant DNA damage agents revealed that only a few have characterized mitochondrial DNA effects. Thus, we investigated whether known environmental genotoxins also affect mitochondrial function and mtDNA stability, and whether these effects occur at the same concentration range as those observed for nuclear DNA. Polycyclic aromatic hydrocarbons (PAH) can arise from natural sources, but are among the most common anthropogenic environmental pollutants, mainly due to fossil fuel burning. PAHs are known nuclear DNA genotoxins and several of them are known human carcinogens. We investigated the effects of PAH exposure to mitochondrial function and mtDNA stability in A549 human lung fibroblasts exposed to a standard mixture of 10 common PAHs. We found a dose dependent inhibition of mitochondrial function, as measured as whole cell respiration rate. Expression of key mitochondrial proteins was also impaired in PAHs-treated cells. Finally, we observed extensive mtDNA damage, as measured by a long extension PCR assay. Together, our results suggest that mitochondria are important cellular targets of environmentally relevant toxins and should be included in regular assessment of cytotoxic and genotoxic potential of chemicals.

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PL-27. Risk assessment of endocrine disruptors

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Endocrine disruptors are a well-recognized concern for public health. Endocrine disruptors are chemical substances that interfere with the hormonal system and cause harmful health effects in humans and in wildlife. Endocrine disruptors can act by binding to hormone receptors or affecting the hormone levels and thereby disrupting the normal endocrine signalling in the body. A special concern is exposure to endocrine disruptors during sensitive windows of development such as prenatal and early postnatal periods leading to permanent changes that appear later in life. Endocrine disruptors have been associated with e.g., male and female reproductive disorders, thyroid related disease, neurodevelopmental and metabolic effects. Risk assessment of endocrine disruptors in the European Union is focused on identifying endocrine disruptors using criteria based on the WHO definition of endocrine disruptors. The criteria are today applied in the legislations for pesticides and biocides and activities are ongoing to apply them in other EU legislation such as REACH. The presentation will present the process for hazard identification of endocrine disruptors based on the criteria and discuss the progress and challenges.

Keywords: endocrine disruptors, risk assessment



PL-28. Vaccine Safety and Toxicity. What Have We Learned From COVID-19 Pandemic?

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Immunization with vaccines provide active acquired immunity to a particular infectious disease. Vaccines mainly contain antigens as active ingredients and other ingredients like adjuvants, preservatives and excipients. The most highly used adjuvants are aluminum salts. However, aluminum, a toxic metal, may cause harmful effects after acute or chronic exposure. Rarely, aluminum adjuvants may cause granulomas forming at the injection site. Some researchers suggest that Alzheimer's disease can be due to chronic aluminum intoxication. Others report that breast cancer is aggravated by the topical application of an aluminum salt. Moreover, autism is suggested to result from an immune cascade initiated by aluminum adjuvants. These reports should be discussed in the light of scientific evidence. Preservatives, such as thimerosal, protect the vaccine from bacterial or fungal contamination. Today, thimerosal is only used in multi-dose vials, like flu vaccines. Thimerosal is added to various cosmetic products and medical preparations for the same purpose. Although thimerosal contains ethyl mercury, not methylmercury, it is associated with autism in children due to the fact that cumulative mercury exposure can be risky for the first 6 months of life. COVID-19 is a viral disease that has claimed the life of over 6.5 million patients. Several vaccines have been developed against this disease. Among those, an inactivated vaccine applied in Turkey is a formaldehyde inactivated vaccine with aluminum hydroxide (0.225 mg) and with no preservative. Other vaccines on the market do not contain adjuvants or preservatives. However, long term effects need to be evaluated for DNA/mRNA vaccines and there are concerns for viral vector vaccines as their effectiveness might be compromised by pre-existing immunity to adenoviruses. Therefore, the safety profiles of all the vaccines should be evaluated carefully and possible toxic effects should be discussed, particularly for susceptible populations like children or for patients with preexisting pathologies.

Keywords: vaccine, safety, toxicity, COVID-19, adjuvant, preservative



PL-29. In Vitro Tests Addressing Immunotoxicity with a Focus on Immunosuppression

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A well-functioning immune system is essential for maintaining the integrity of an organism. Exposure to immunotoxic compounds can have serious adverse health consequences affecting responses to both communicable and non-communicable diseases. It is therefore important to understand the immunotoxic potential of xenobiotics and the risk(s) they pose to humans. While animal testing has been the gold standard for hazard identification of immunotoxic compounds, a lot of efforts and successes have been made to develop non-animal-based test systems. When using in vitro assays for screening purposes, it is clear that one assay alone will not be able to cover all of the potential adverse effects of chemicals on the immune system and that several assays will be required to identify immunotoxicants. A tiered testing strategy currently represents a valuable approach to assess immunotoxicity in vitro. Pre-screening for direct immunotoxicity in vitro should begin by evaluating myelotoxicity. If not myelotoxic, chemicals should be tested for leukotoxicity, compounds should then be tested for immunotoxicity at non-cytotoxic concentrations using various approaches, e.g. cytokine production, lymphocyte proliferation assay, and natural killer cell assay, etc. The term “new approach methodologies” (NAMs) refer to any non-animal-based approaches that can be used to provide information in the context of chemical hazard identification and characterization, including integrated approaches to testing and assessment (IATAs) and defined approaches for data interpretation (DAs). The aim of this presentation is to present the state of the art of available NAMs in to assess immunotoxicity, focusing on immunosuppression.



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PL-30. Chemical Stewardship Through Sustainability Lenses

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Chemical stewardship is key in managing 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. This is aligned with the UN Sustainable Development Goals (SDGs), the world's comprehensive plan of action, which includes goals to ensure good health and well-being and responsible consumption and production. From the 17 SDGs, there are 4 SDGs with specific targets which are directly linked to chemicals and waste management. For example, under SDG Goal 3, Target 3.9 is on the reduction of the no of deaths and illnesses from hazardous chemicals by 2030 and Goal 6 has a target to improve water quality by reducing pollution of chemicals. Target 12.4 is very important as it is to achieve the environmentally sound management of chemicals and wastes throughout the life cycle and we have under SDG 14 on reducing marine pollution of all kinds. In order to achieve these goals, industries need to integrate chemical stewardship to protect workers and community members against adverse health outcome from chemical exposure. This talk will discuss the current approaches and examples of chemical stewardship and regulatory toxicology implementation especially through the lenses of sustainability.



PL-31. The Universal Immune System Simulator: an In Silico Model to Predict The Risk Associated with Exposure to Immunotoxicants

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The Universal Immune System Simulator (UISS) is a simulation framework to model the immune system. It integrates simulation engines, optimization techniques and other prediction models. UISS is then capable to reproduce general immune system behavior connected to several immune system responses (to viruses, bacteria, tumors and auto-immune disease) and drug-induced immune system responses. The UISS model is used in several funded projects to model knowledge on immunological aspects of different pathologies and support in silico trials, with a special attention on those regulatory aspects that are still not addressed. Immunotoxicity risk assessment of chemicals is an evaluation of the potential for unintended effects of chemical exposure on the immune system. These effects manifest as following principal types of immunotoxicity: immunosuppression involving infection and carcinogenesis, immunoaccentuation involving sensitisation and autoimmunity, or immunostimulation. This immune dysregulation may lead to a variety of illnesses. Included among them are illnesses that are associated with a dysfunctional immune system, such as infections, inflammatory diseases, allergic diseases, autoimmune diseases. For example, exposure to chemicals is associated with immunosuppression manifesting the reduction of resistance to infections, development of autoimmune disease and hypersensitivity responding directly as an allergen or enhancing the induction of allergic sensitisation. In this context, is interesting to understand the conditions under which a mathematical model developed for one purpose and application (e.g. in the pharmaceutical domain) can be successfully translated and transferred to another (e.g. in the chemicals domain) without undergoing significant adaptation.



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PL-32. *In Silico* Approaches to Assess the Potential Environmental Effects of Diverse Chemicals

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The European Union (EU) has designed a framework to analyze chemicals globally which is called REACH and was legislated in 2007. There are millions of chemicals registered under the REACH. These chemicals have diverse structures and application areas. Depending on the production volume and considering their effects on the environment, these chemicals are categorized in many ways, such as high production volume (HPV), contaminants of increasing concern (CEC), endocrine disrupting chemicals (EDC), etc. Since many studies in the literature reveal that the removal of many of these chemicals in treatment systems is low, after their production and/or application, these chemicals are released into the four basic environmental compartments (air, water, soil/sediment, biota) in one way or another. In this context, the assessment of some parameters, such as physicochemical properties, persistence, bioaccumulation, toxicity, biodegradation potential, etc., is crucial to understanding the distribution, impact, and fate of chemicals in the environment. Data on chemicals are still extremely scarce. *In silico* approaches, which are also employed to assess chemicals' safety for human health and risks, are used to fill the existing data gaps. Grouping, docking, and read-across techniques as well as simulating the fate and transportation of chemicals in the environment, are all examples of *in silico* tools.

In July 2022, there were roughly 21.500.000 Google search results for the keywords "*in silico* approach and environment". *In silico* approaches refers to quantitative structure-activity/property/toxicity relationship (QS-A/P/TR). These techniques include QSAR based on regression and classification, machine learning, toxicophore, read-across, interspecies, docking, and numerous expert systems. In this presentation, an overview of *in silico* approach scheme and techniques used for the modeling and/or predicting various chemical properties/responses which are used in the assessment of potential environmental effects will be provided. Additionally, the existing challenges and potential future directions of *in silico* ecotoxicological research will be discussed.



PL-33. Role of Ceramides in Insulin Resistance and Diabetic Dyslipidemia From a Biochemical Point of View

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Changes in the metabolism of sphingomyelins (SM), which are among the most abundant circulating lipids, have been associated with obesity. Patients with obese type 2 diabetes (T2DM) have increased plasma ceramides (CER), and this increase is associated with insulin resistance and the degree of inflammation. A persistent CER elevation results in excessive ceramide deposition in the muscles of obese individuals with T2DM. Ceramide accumulation in human tissues inhibits insulin action and subsequent glucose uptake through inactivation of protein kinase B (PKB), also known as Akt. The production of metabolites such as ceramide-1-phosphate (C1P), sphingosine and sphingosine-1-phosphate (S1P), which are important regulators of inflammation, have also been associated with an increase in CERs.

We have reported a significant decrease in serum levels of very-long-chain C24 SM, very-long-chain C22-C24 CERs, insulin resistance (HOMA IR) and C1P in obese patients following laparoscopic sleeve gastrectomy (LSG) after postoperation day 1 and day 30 compared to preoperation levels. At 30 days postsurgery, mean body mass index (BMI) was reduced by 11%, fasting triglycerides were significantly decreased, and insulin sensitivity was increased compared to presurgery values. A significant positive correlation was found between HOMA-IR and serum levels of C22-C24 CERs in LSG patients.

In a separate study we have found that C16-C24 SM, C16-C24 CER and C16 CER-1P levels were significantly increased in T2DM patients with LDL-C above 160 mg/dL compared to those with LDL-C below 100 mg/dL. **C16-C24 SM, C22-C24 CER, C16 CER and C16 CER-1P levels** showed a statistically significant increase in T2DM patients with non HDL-C above 130 mg/dL compared to those with non HDL-C below 130 mg/dL. C24:C16 SM and C24:C16 CER ratio showed a significant correlation with both LDL-C and non HDL-C levels. Obese T2DM patients (BMI>30) had significantly higher circulating levels of C24 SM, C18-C24 CER compared to those with BMI<30.

In **conclusion**, plasma sphingomyelins and ceramides are elevated in obese dyslipidemic T2DM patients. Serum long chain CERs, C24:C16 SM, C24:C16 CER ratio may serve as prognostic and diagnostic markers in type 2 diabetic dyslipidemia.

Keywords: sphingolipid, obesity, dyslipidemia, type 2 diabetes.



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PL-34. Sphingolipid Metabolism in Toxicological Exposures

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Lipids have crucial roles in biological systems such as energy metabolism, signaling, and barrier function. Lipidomics is a recently developed field that makes extensive use of analytical chemistry concepts and modern techniques, particularly mass spectrometry, to study cellular lipids. Toxicologists have a significant role to play in this scientific area. Transcription, translation, enzyme activity, and lipid composition are all affected by harmful xenobiotics or nontoxic mediator reactions. Sphingolipids are one of the most important lipids and present in all eukaryotic cells. They have been linked to a wide range of biological processes, including the development of structural domains, polarized cellular trafficking, and signal transduction. Sphingolipids have attracted a lot of attention in recent years as a result of the discovery of their participation in a variety of biological processes. Sphingolipid metabolism can be associated with many diseases, and it can also be disrupted by toxic compounds and play an important role in the toxicity mechanisms of these compounds. The idea of targeting these pathways for therapy against cancer, Alzheimer's disease, and a number of significant human diseases has increased interest in recent research that has identified and characterized many of the main enzymes involved in sphingolipid metabolism. Studies on the mechanistic toxicity of hazardous substances and lipidomic research in toxicology can both help to understand the function of lipids in disease including sphingolipids.



PL-35. *In Silico* Toxicological Evaluation of Drug Impurities Aligned With ICH M7: A Case Study for Methylprednisolone Aceponate Impurities

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Identification of the genotoxic potential of chemicals during the processes of risk and safety assessment for human health is a significant concern. However, due to resource constraints, *in silico* methods have been on rising for genotoxicological endpoint estimations. Many regulatory documents and guidelines, such as The International Conference on Harmonization (ICH) M7 (R1) guidance document, allow and describe *in silico* methods for the evaluation of genotoxicity in the absence of adequate experimental data. ICH M7 specifies that two complementary methods may be used to evaluate impurities that are present above the level specified in the guideline, one statistical-based and one expert rule-based. For the final decision, impurities are fallen in one of the five classes described in the guidance.

Methylprednisolone aceponate is a corticosteroid for the treatment of various diseases as an anti-inflammatory agent. Due to the lack of experimental data, four impurities of Methylprednisolone aceponate were evaluated for their genotoxic potential *in silico* using multiple software. The molecular structures were used to identify structural alerts related to protein binding, DNA binding, mutagenicity, and micronucleus test. In addition, the predictions for mutagenicity were made via software employing read-across and QSAR models. The presence of common structural alerts for all substances under evaluation indicated that any experimental data belonging to the active substance could be used to estimate genotoxicity-related endpoints.



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PL-36. The History of the Advancement of Toxicology in Turkey

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Considering the increasing importance of toxicology in the protection of human health and the environment, there have been tremendous developments in the last decades. These developments were achieved both in toxicity tests used in the safety assessment of chemicals and in their rational management. Today, in addition to established traditional toxicity tests, we have advanced methods based on omics technology and in silico methods based on artificial intelligence. Also we have very functional and effective organizations for management of chemicals including toxicological societies. The subject of “The History and The Advancement of Toxicology in Turkey” cannot be handled independently of these global developments. In this talk, the advancement of toxicology in the last century and explain where toxicology is in Turkey will be briefly reviewed.

Keywords: Toxicology in Turkey



PL-37. Possible Interactions and Risks Between Nanoparticles and Chemical Pollutants in Aquatic Environments

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Nanomaterials have become important elements of our daily life, given their increasing use in nanotechnological applications. Today, the annual global nanoparticle production is estimated to be well over 60,000 tons. The increase in production and use of nanomaterials, however, poses also environmental risks and threats. In this context, studies evaluating the effects of nanomaterials on the environment and human health have also gradually increased. Although the first reports on nanoparticle toxicity were appeared in the early 1990s, only recently nanotoxicology has become an important study topic and now each year hundreds of articles are published on the topic. As the importance and usage areas of nanoparticles expanded, so the questioning of their environmental hazards. Since the final destination of nanoparticles is generally aquatic environment where accumulation and organismic interaction is readily achieved, they pose more danger to aquatic organisms than to organisms in other environments. Aquatic organisms are generally exposed to nanoparticles through ingestion, contact, and the gills. Although, some nanoparticles have been recognized as being safe, some studies have also shown that many can be toxic to environment. Moreover, the vast majority of reports on nanoparticles have focused on the effects of a single nanoparticle exposure, while most nanoparticles are not usually found alone but may in complex with others or with different kind of pollutants in aquatic ecosystem. Thus, nanoparticles may cause ecotoxicological risks previously unforeseen. Findings from current studies suggest that multiple interactions with nanoparticles and/or chemicals may be in the form of additive effects rather than synergistic or antagonistic effects. Furthermore, in the presence of nanoparticles such as Ag₂O and TiO₂ with high photocatalytic degradation ability, secondary degradation products of various pollutants can also lead to unexpected toxic effects. In addition, nano-pollutants can affect the bioavailability of essential nutrients for the organism or cause a change in the microstructure of the environment. In this regard, toxicological research should not only be limited to individual nanomaterials, but should also investigate possible interactions and risks with other nanoparticles and chemical pollutants, especially in aquatic ecosystems.

Keywords: nanoparticle, nanometal, toxicity, environmental risks, aquatic environment



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PL-38. Case studies for Novichok incidents in UK and Russia

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Nerve agents (NA) including tabun, sarin, soman, cyclosarin, and VX are banned chemical warfare agents which are classified under Schedule I chemicals in Chemical Warfare Convention (CWC) and they have similar physical, chemical, and toxicological properties to organophosphorus (OP) compounds. Novichok agents are fourth generation of nerve agents which were developed by the Soviet Union during the cold war. Novichok NA was used against former GRU officer Sergei Scripal and his daughter in Salisbury, United Kingdom on March 04, 2018. Both Mr. Scripal and his daughter and a police officer who checked their home after the incident were exposed to Novichok. All three of them stayed in the intensive care unit for months due to systemic effects of acetylcholinesterase inhibition. After this chemical terrorist attack, the Organisation for the Prohibition of Chemical Weapons (OPCW), which is the executive body for the CWC added the Novichok agents to "list of controlled substances" of the CWC.

On 30 June 2018, two civilians were found unconscious in Amesbury, United Kingdom. They were poisoned with Novichok. One of them died and other one was transferred to intensive care unit. It was found that they were accidentally exposed to Novichok which was used in Mr. Scripal assassination.

Finally, Russian opposition leader Mr. Alexei Navalny fell ill during a flight from Tomsk to Moscow on August 20, 2020. He was evacuated to a hospital in Berlin, Germany and he also stayed in intensive care unit for than one month.

Current chemical detectors could not detect and identify Novichok agents. Besides, there is limited data about decontamination and antidote treatment of chemical casualties who may be exposed to Novichok.

Keywords: Chemical terrorism, nerve agents, chemical weapons



PL-39. Review-Based Evaluation of Decontamination Techniques After Novichok Exposure

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Exposure to highly hazardous toxic chemicals, either in accidental, natural or malicious contexts, is of concern both for the civilians and military. The chemical warfare agents (CWA) are among the most toxic chemicals which have been used in military or terrorist acts. In recent years, although major international efforts have been made to ban their synthesis and use, CWA, including the organophosphorus nerve agents (OPNA), still made the headlines in the international news. The OPNA « Novichoks », which were secretly developed in the former Soviet Union and which belong to the A-series of OPNA, are the least well characterized of these agents. They are a group of dozen of compounds sharing similar chemical backbones : phosphonates or phosphates containing amidine or guanidine fragments in the molecule. Their physicochemical properties, mostly based on predictive models, are only partially established. Of low volatility and high persistency, these agents are likely to be encountered as liquids. According to the first experimental data, including their skin permeation, preliminary guidelines have been established on how to perform the decontamination of victims with currently available emergency decontamination kits such as RSDL and dry absorbents.

Keywords: decontamination, nerve agent, novichok



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PL-40. Mode of action (MOA) based threshold for genotoxic carcinogens: Benzene case

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It has been common practice that direct acting genotoxic carcinogens are modelled using linear non-threshold approaches. However, in recent decades increasing recognition has emerged that chemicals which damage DNA, but by an indirect mechanism, can act by a threshold mechanism. The threshold model leads to the expectation that only those exposures greater than the threshold of effect contribute to risk and can accommodate dose rate. Thus, if the dose rate is a significant factor in the risk for an effect, it suggests the threshold model is more likely. The “mode of action” (MOA) concept is based on the understanding that experimental data very often result from a dose range at which the induction of non-linear “tumour promotion” mechanisms, the saturation of defense mechanisms are likely to play an important role. In case of benzene, Occupational exposure to benzene at high levels has been associated with an increased risk of acute myeloid leukaemia (AML). The MOA for AML development leading to mortality is anticipated to include multiple earlier key events, which can be observed in hematotoxicity and genetic toxicity in the peripheral blood of exposed workers as they are observed at lower doses and earlier time points than leukemia mortality. Prevention of these early events would lead to prevention of the apical, adverse outcomes, the morbidity and mortality, caused by the myelodysplastic syndromes (MDS) and AML. Considering benzene carcinogenicity is based on threshold mediated MOA, a health-based benzene OEL can be set with high quality data from genotoxicity and hematotoxicity endpoints as they are the most sensitive and relevant to the proposed MOA. Evaluation of the database of high-quality studies for both hematotoxicity and genetic toxicity suggests effects are observable at exposures of 2 ppm. In this presentation, the details will be presented as case example of benzene.

Keywords: Benzene, mode of action, genotoxic carcinogen, occupational exposure limit



PL-41. Filling Data Gap For Toxicological Evaluation of Pharmaceuticals: In Silico Approach

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Predictive toxicology plays an important role in Filling data gap for toxicological evaluation of pharmaceuticals. In this presentation, toxicological endpoints of vitamin D2, D3, and their metabolites were evaluated and afterwards the data gaps in the toxicological endpoints were filled by forming a category. The read-across was justified by the structural and metabolic similarities. Molecular similarity and mechanistic relevance were found to be substantial, resulting in low uncertainty. Based on the current existing data, vitamin D and the metabolites do not present a concern for genotoxicity. Additionally, there is no indication for skin sensitization. Carcinogenicity, teratogenicity, developmental, and reproductive toxicity data on the studied compounds are insufficient to assert them as toxic. The untested vitamin D analogs within this category (calciferol, ercalcidiol, ercalcitriol, calciol, calcidiol, and calcitriol) can be read across to complete the data gaps for the toxicological endpoints. The experiments performed in the cited literature works are not intended for children and infants. Therefore, our conclusions are applicable to adults, and we cannot comment on the toxicity of this compounds on children and infants.

Keywords: Category approach, human toxicology, read-across, safety assessment,



PL-42. Integrated Testing Strategy for Immunotoxicity: The Agrochemical Example

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An assessment of the immunotoxicity potential is required in some geographies for agrochemical active substances. Requirements may include:

- 1) Direct testing of immunotoxicity potential,
- 2) review of the overall toxicity profile of effects on the immune system, and
- 3) consideration Classification & Labelling (in systems like the GHS or CLP where effects on the immune system can be considered as part of the Specific Target Organ Toxicity classification).

The presence of immunotoxic effect in adults may trigger additional requirements (such as the presence of an extra cohort in an Extended one-generation study for assessment of developmental immunotoxicity. Testing for immunotoxicity effects currently requires animal models. Despite there are no internationally agreed Test Guidelines, testing for certain aspects of immune toxicity (e.g. TDAR) can be conducted under local test guideline (e.g. OPPTS 870.7800), often in integrated form with other toxicity studies. The Purpose of this presentation is to provide an overview of the progress in the area of immunotoxicological assessments for agrochemicals. Specifically, the EPA recently reviewed the ability of this test to be predictive of adverse outcomes relevant to risk assessment. This facilitated greater acceptance of waivers for rodent immunotoxicity studies based on a tiered approach including an integrated assessment of immune function in standard toxicology studies followed by and assessment of the potential impact on risk assessment, before immunotoxicity test is triggered and conducted. Finally an opportunity is given by in vitro assays and the generation of an OECD IATA strategy which may allow for early screening of immune function in the future, using relevant (human) test systems.

Keywords: Immunotoxicity; agrochemicals



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PL-43. Causality Between Prenatal Exposure to Pharmaceuticals and Childhood Obesity

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Over the past 30 years enhanced exposure to endocrine disruptors has been reported to increase infertility, hormone-related cancers, reproductive and developmental anomalies. Recently exposure to some endocrine disruptors, named "obesogens", are suggested to cause lipid accumulation and obesity by modulating adipogenesis. Childhood obesity is dramatically elevated in the last decade. Prenatal exposure to obesogens is suggested to induce obesity later in life. Although obesogenic effects of industrial and environmental chemicals have been investigated extensively, endocrine modulating pharmaceuticals can also potentially be obesogenic. In this scope, both endocrine related adverse effects and obesogenic effects of an estrogenic drug, diethylstilbestrol, have been revealed in detail, but these effects of many drugs have not been investigated yet. Therefore, our hypothesis is that prenatal exposure to some pharmaceuticals which are considered relatively safe and prescribed during pregnancy such as paracetamol, alpha-methyldopa, and indomethacin can cause endocrine modulation which may lead to obesogenic effects later in life. The data from literature and our preliminary findings related to the endocrine modulating effect of these drugs as well as their obesogenic potential will be discussed in detail.



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PL-44. The Comet Assay in Human Biomonitoring: The COST Action Hcomet

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The COST Action hCOMET lasted from 2016 until 2020. COST Actions are essentially networking projects, with training of young scientists a priority. We achieved our two main scientific aims: first, compiling and analysing a database of human comet assay results, and second, helping to reduce the variability in the assay by preparing standard procedures and guidelines. hCOMET had 25 full member countries, covering most of Europe, plus six observers. Results from the database analysis have been impressive, demonstrating the valuable contributions the comet assay can make to molecular epidemiology. We have established that measurements of DNA damage have predictive value, correlating with mortality. Retrospective studies are planned, using biobank samples of blood cells in nested case-control studies. Our understanding of the nature and causes of intra- and inter-laboratory variations in comet assay results has been enhanced, and the standard protocols prepared during the Action (currently being published) will, we hope, be widely adopted. Although now officially ended, hCOMET survives as a scientific community (www.hcomet.eu).



PL-45. Epigenetic Effects of Endocrine Disrupting Chemicals

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Endocrine disrupting chemicals (EDCs) disrupt the normal endocrine functions and alter the endocrine phenotype of the exposed individual through various mechanisms. They mimic or inhibit the effects of hormones that disrupt the normal functioning of the body. Humans are exposed to EDCs very widely and from different sources in everyday life. Bisphenol A (BPA) and di (2-ethylhexyl) phthalate (DEHP) are chemical substances that are widely present in the environment and known to have endocrine disrupting properties. BPA is widely used in the production of polycarbonate plastics. DEHP is one of the most widely used phthalic acid esters. Both of these compounds are used as plasticizers. It is also possible to be exposed to those chemicals throughout life, especially in critical developmental periods such as pregnancy, lactation, and puberty. Early life exposure to EDCs has been associated with later life adversities such as infertility, obesity, cancer, and diabetes. Mechanisms underlying such associations are unknown but are likely to be mediated by epigenetic alterations induced by EDCs. The changes in gene expression that are independent of alterations in DNA sequence are called epigenetic alterations. Increasing evidence showed that epigenetic mechanisms including DNA methylation, histone modifications, and miRNAs play an important role in pathologies as a result of estrogenic, anti-estrogenic, and anti-androgenic effects of these chemicals. Although epigenetic effects of EDCs have been well documented, the exact mechanisms by which they interfere with epigenetic marks are not well studied. Understanding the mechanistic links between EDC-induced epigenetic changes and phenotypic endpoints will be critical for providing improved regulatory tools to better protect the environment and human health from exposure to EDCs.



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PL-46. Advanced in Vitro Models for Nephrotoxicity Testing: as Complex as Possible, But Simple in Use

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Adverse effects caused by exposure to foreign compounds, including drugs, often involve the kidney because of its role in maintaining body homeostasis. Early prediction of those effects, such as drug-drug interactions and renal toxicity, is imperative for the development of new and safe drugs by the pharmaceutical industry. Current in vitro assays do not accurately allow such prediction, predominantly due to inadequate preservation of the organs' microenvironment. The kidney epithelium is highly polarized, and the maintenance of this polarity is critical for optimal functioning and responsiveness to environmental signals influencing cell proliferation, migration and differentiation. This presentation will provide an overview of advances in 3D cultures of human renal cells and organoids in microfluidics, and in particular kidney tubules, thereby improving physiological performance of the tissue. These kidney-on-a-chip platforms have great potential for drug screenings and provide novel alternative strategies for prediction of renal drug disposition and safety assessment in a human-specific context.

Keywords: in vitro models, nephrotoxicity, organoids, drug screenin



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PL-47. Sterol Markers of Cholesterol and Bile Acid Metabolism

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Regulation of cholesterol (Ch) homeostasis is controlled by three main fluxes, i.e. intestinal absorption, de novo synthesis (ChS) and catabolism, predominantly as bile acid synthesis (BAS). High serum total Ch and LDL-Ch concentrations in particular are considered risk factors and markers for the development of atherosclerosis or neurodegenerative diseases. Pharmaceutical treatments to lower serum Ch have focused on reducing Ch absorption or ChS and increasing BAS. Monitoring of these parameters is complex involving isotope techniques, cholesterol balance experiments and advanced mass spectrometry based analysis methods. Nowadays surrogate markers were explored that require only one single fasting blood sample collection. These markers were validated in specific, mostly physiological conditions and during statin treatment to inhibit ChS or during ezetimibe treatment to inhibit cholesterol absorption. We retrospectively evaluated the use of serum campesterol (Camp), sitosterol (Sit) and cholestanol (Cholol) as markers for cholesterol absorption, lathosterol (Lath) as marker for ChS and 7 α -hydroxycholesterol (7 α -OH-Ch) and 27-hydroxycholesterol (27-OH-Ch) as markers for BAS under conditions of Chol absorption restriction. Additionally, their values were corrected for Ch concentration (R_sterols) in order to harmonize for common changes in serum lipoproteins, the transporter proteins of sterols and oxysterols in the periphery. Additionally cholesterol precursors such as lanosterol, desmosterol and 7-dehydrocholesterol are used as indicators of genetically inherited disturbances in cholesterol synthesis. Increased levels of bile acid precursors such as 7 α - and 27-OHC or others can be used to diagnose and to monitor disturbances in bile acid synthesis and metabolism.

Keywords: cholesterol absorption, cholesterol synthesis, bile acid synthesis, statins, ezetimibe inherited diseases



PL-48. Regulation of Genotoxic Impurities – Guidelines and experiences since 2006

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In 2006 the EMA published a first guidance document “Guideline on the limits of genotoxic impurities” to provide recommendations on the control of genotoxic impurities in pharmaceuticals for human use. In 2008 the US FDA published the “Draft Guidance for Industry on Genotoxic and Carcinogenic Impurities in Drug Substances and Products”. These guidances formed the basis for the initiation of the ICH process to develop ICH M7. This guideline has been adopted in 2014 and is now considered as the global standard to regulate and limit genotoxic impurities in pharmaceuticals for human use. ICH M7 focuses on DNA reactive (mutagenic) impurities as those of highest concern because they are considered problematic with respect to application of threshold approaches. For non-DNA-reactive genotoxic impurities a threshold approach is considered possible and applicable for establishing limits.

The key element of ICH M7 is the classification of the impurity based on a hazard assessment using available data and the implementation of a generic limit, the TTC, for potential mutagenic impurities. Impurities are classified into 5 classes with classes 1 -3 classifying the mutagenic impurities. Classification is based on the available data on the mutagenic and carcinogenic potential. Class one is reserved for carcinogenic mutagens with sufficient carcinogenicity data to establish a substance specific limit. Classes two and three define impurities with either only in vitro (Ames) or in silico data as evidence for a mutagenic potential. For impurities classified in class two or three the generic limit (TTC) would apply. Exceptions however apply for impurities belonging to the Cohort of Concern.

The implementation of ICH M7 has led to a harmonized regulation of mutagenic impurities in most markets around the globe. It prevents manufacturers from having to deal with different limits for the same impurity in different regions. It also guides regulatory agencies to a harmonized assessment for the same impurity. In conclusion ICH M7 has greatly facilitated and harmonized the regulation of mutagenic impurities in pharmaceuticals.

Keywords: regulation, genotoxic impurities



PL-49. Effect of BaSO₄ Nanoparticles on Human Lung Cells Under Different Culture Conditions

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Purpose of the study: BaSO₄ and TiO₂ nanoparticles are part of the ingredient list in many products of daily life, such as toothpaste, suncreme, textiles, cosmetics or nano-functionalized plastics. Our studies were focussed on the effect of BaSO₄ or TiO₂ NP in human lung cell cultures and aerosol application of NP.

Methods: A human lung tumor cell line (A549), normal human peripheral lung cells (PLC) and a human endothelial cell line (EA.hy929) were cultured either in normal submerge systems or on polycarbonate inserts (MatriGrid = MG) shaped like alveoles. The confluency on MGs was detected by TEER-measurement (transepithelial/transendothelial electric resistance). The size-distribution of BaSO₄ or TiO₂ NP in the aerosol was determined by Scanning Mobility Particle Sizer (SMPS) and Optical Particle Sizer (OPS).

The effect of NP was determined by cell viability, genotoxicity, ROS production, GSH content and IL-8 release by appropriate assays.

Results: BaSO₄ NP cause toxic effects after 24 h exposure in A549 at 0.5 mg/ml. PLC were 10fold more sensitive under submerged conditions for 24 h. After 72 h of exposure toxic effects were higher in all cell cultures. Significant H2A.X expression after exposing to BaSO₄ was found in A549 at 0,005mg/ml after 24 and 72h but not in PLC. The NP-aerosols show a 2.5-fold (BaSO₄) and a 10-fold (TiO₂) induction of ROS after (0.9 g/l) for 1 h. The applied NP aerosols of BaSO₄ and TiO₂ cause no relevant toxic effects in A549 cultures for 24 or 72 h.

In Resazurin-assay exposure of Co-cultures (PLZ/EA.hy926) to aerosols of BaSO₄ with averagely 5 x 10⁴ particles/cm³ decreases viability to 40-60 % (depending on patient) when cultures were washed before exposure. NP-aerosols with BaSO₄- and TiO₂ show a reduction cellular GSH-levels for both NP when cultures were washed before exposure.

Keywords: Nanoparticles lung cell aerosol



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PL-50. Vaccine Hesitancy

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People retard or refuse vaccines for themselves or their children. World Health Organization (WHO) has declared that vaccine hesitancy is globally one of the leading health threat This is an expanding challenge for mankind and countries.. Studies showed that people have concerns about vaccine safety which is one of the factors. A wide variety factors may cause vaccine refusal or hesitancy. These include: negative beliefs based on myths, such as infertility, misinformation; mistrust in the health personnel and health system, geographic barriers, the role of community leaders; costs. But there is no single intervention strategy that prevent vaccine hesitancy. The problem varies and must be well-understand by conducting studies and the health authority should develop tailored strategies to improve vaccine acceptance by considering studies' results. Vaccine hesitancy is a complex, global problem that varies widely. Remote and rural communities were affected highly; in some areas concerns are related to subgroups of religious or philosophical objectors. Fear of needles also can be a factor for vaccine refusal. A high level of education does not predict vaccine acceptance. Even sometimes higher education can cause vaccine hesitancy although well education is a promoter of vaccine acceptance in the many studies. Effective communication is key to dispelling fears, addressing concerns and promoting acceptance of vaccination.



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PL-51. Pharmacoeconomic Aspects of COVID-19 Vaccines

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Vaccines and vaccination throughout life holds significant benefit at an individual, population, and socioeconomic level. Without any vaccine or insufficient vaccination coverage can create harmful effect on population health status and socioeconomic level. One of the best example is Covid-19 pandemic. The COVID-19 pandemic is a major challenge for not only health systems but also social and economic aspects of societies worldwide. According to the latest estimates, there have been confirming 813 million death and 20.5 million years of life lost in globally. Therefore, an increase in vaccination rates per capita has significant health status and economic effects. Vaccines have a high probability of reducing healthcare costs and increasing DALYs and QALYs compared to doing nothing. Pharmacoeconomic analysis helps in decision making and policy makers, which will significantly impact the health acer expenditure and country's economy. Pharmacoeconomic studies for COVID-19 vaccine involves the vaccine process, its safety and efficacy, its cost-effectiveness and economic burden.

The aims of study are present cost-effectiveness Covid-19 vaccines using recently literature and investigate burden of Covid-19 treatment in Turkey. To estimate the burden of COVID-19 treatment, the data is obtained from The MoH published data source on COVID-19 cases and experts. The overall treatment cost is estimates as between 30 billon TYR and 35 billion TRY.

Keywords: Covid-19, vaccine, cost-effectiveness, expenditure



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PL-52. Characterization and Quality Control Procedures for Vaccines: A Special Focus on COVID-19 Vaccines

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The vaccines, biopharmaceutical products, represent a fast-growing rate in the global pharmaceutical market. The challenge starts with enhanced complexity and variability that comes from the size of biopharmaceuticals compared to conventional pharmaceuticals. Therefore, physicochemical characterization of biopharmaceuticals must be done using multiple, complementary, as well as orthogonal and state-of-the-art analytical methods. The selected methods for characterization must be cover primary amino acid sequence (e.g., truncation, deamidation, oxidation), posttranslational modifications (e.g., glycosylation), higher-order structural changes (e.g., secondary folding, aggregation), and impurities. Besides the physicochemical characterization of the vaccines, preclinical safety evaluation of vacancies, predicting the metabolism and toxicity of a vaccine, the biological processes underlying individual variations in response, and identification of vaccine-related alterations in metabolic pathways also is very critical for vaccine development. Today the detailed knowledge converged in the integrated “omics” (genomics, transcriptomics, proteomics, and metabolomics) holds immense potential for understanding the mechanism of diseases, facilitating their early diagnostics, selecting personalized therapeutic strategies, assessing their effectiveness, and offering a revealing route in the development of vaccine candidates. In this speech, state-of-the-art analytical methods used in the characterization and quality control of vaccines and omics studies for vaccine development and safety evaluation will be discussed.

Keywords: Vaccine, characterization, quality control, omics, safety



PL-53. Non-Pharmacological Mechanism in Substance-Based Medical Devices/Challenges in Regulatory Definition”

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Medical devices represent a broad category of products intended to be used in the prevention, diagnosis, monitoring, treatment or alleviation of a disease or injury and their most recent evolution has led to an increasing number of products which include "substances" and which, due to their presentation and site of application, are similar to medicinal products.

In the new regulation 2017/745/EC that governs medical devices, the role of the so-called "medical devices made of substances" has been strengthened and made explicit, which include those devices that contain substances that can also be absorbed. Among these, many are composed mostly of substances of natural origin. Products of this type are often referred to as "borderline" and in many cases require a complex analysis and discussion of their mechanism of action. In fact, the mechanism of action of a medical device is the discriminating element with respect to medicinal products, in that it must be non-pharmacological (immunological or metabolic). This difference is often not intuitive and is based on the correct interpretation of essential terms such as "pharmacological, immunological and metabolic mechanism of action", which have important regulatory implications. The correct interpretation of the terms is the first step to be able to give a correct classification to many products that fall into the so-called "borderline" category.

The main problem of products that contain substances of natural origin starts from the fact that natural substances can follow different regulations, from food to therapies. As far as their constituents are concerned, they are characterized by a complexity that is not easy to deal with both from a scientific and regulatory point of view, but above all from a therapeutic point of view. The experimental approach for determining the mechanism of action of an isolated molecule can follow complex but well-regulated rules for demonstrating quality, efficacy and safety. The same rules must be adapted for complex substances in order to find appropriate methods and define a correct regulatory framework for them.

From a scientific point of view, it is likely that new methods, such as those proposed by systems biology, are applicable to the definition of the mechanism of action of complex substances. However, the Directive 2001/83 / EC on medicines seems to be modeled on single molecule products (or combinations of molecules), while the Regulation 2017/745 / EC on medical devices while introducing for the first time the official term "medical devices made of substances" does not yet allow us to define the methods and experimental evidence to be able to define the concept of non-pharmacological mechanism of action of complex substances. Pharmacologists are essential in this open discussion to bring the correct interpretation of the terms associated with these decisions and open up to the opportunities that the regulation can offer for innovation and therapeutic improvement with complex natural substances.



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OP-01. Effects of COVID-19 Disease on DNA Damage, Oxidative Stress and Immune Responses

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease 2019 (COVID-19), was emerged in China and spread rapidly around the World including our country. Outbreak declared by the World Health Organization (WHO) as a pandemic on March 11, 2020. COVID-19 poses a great threat and concern for public health with its fatal consequences. A specific drug has not been developed against this virus to date. Most people with COVID-19 disease show mild to moderate symptoms and recover without the need for special treatment, while others become seriously ill and need medical attention. One of the most important unknown questions in this disease is why some people develop serious / severe disease, while others do not. It is suggested that this may result from individual variability in immune response. The overproduction of pro-inflammatory cytokines due to this infection is associated with lung tissue damage and COVID-19 pathogenesis. There are limited studies on how SARS-CoV-2 infection affects some molecular pathways including oxidative stress, DNA damage, and apoptosis. In our study, we aimed to evaluate DNA damage using alkaline Comet assay and their relationship with oxidative stress and immune response using ELISA technique in SARS-CoV-2 positive patients and healthy controls. Our results showed that DNA damage and oxidative stress parameters increased with cytokine increases in SARS-CoV-2 positive patients when compared to control. In conclusion, the effect of SARS-CoV-2 infection on DNA damage, oxidative stress and immune responses may be crucial in the pathophysiology of the disease. It is suggested that Illumination of these pathways will contribute to the development of clinical treatments in COVID-19 disease in the future.



OP-02. Role of AhR/CYP1A1 Signaling in The Interaction Between Benzo[a]Pyrene and Chlorpyrifos

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Humans are exposed to various environmental contaminants as mixtures rather than individual compounds. Two such groups of chemicals are polycyclic aromatic hydrocarbons and pesticides, whose potential mixture interactions are not well-studied. As representatives of these groups, we studied mixture effects of benzo[a]pyrene (B[a]P) and chlorpyrifos on human liver HepG2 cells. We observed antagonistic interaction on cell viability and ROS production and synergistic effects on *CYP1A1* expression. Using microsomes, both compounds showed inhibition on CYP1A1 enzyme. To confirm *in vitro* results, zebrafish embryos (ZFEs) were used to further assess mixture interactions of these compounds on several developmental endpoints. The results showed synergistic interactions on non-inflated swim bladder, yolk-sac and pericardial edema and spinal deformation. In addition, synergistic interactions on *CYP1A* expression was observed in transgenic Tg(cyp1a:EGFP) ZFEs. Functional knockdown of aryl hydrocarbon receptor (AhR) partially rescued developmental toxicity, pointing to significant AhR-dependence of the synergistic effects. These ZFE results were likely due to inhibition of CYP1A by chlorpyrifos which led to sustained levels of B[a]P and AhR activation, which caused excessive toxicity. Both AhR activation and CYP1A1 inhibition were suggested as the underlying mechanisms responsible for the synergistic interaction between B[a]P and chlorpyrifos in *in vivo* and *in vitro* systems.

Acknowledgements: This study was funded by Karolinska Institutet. B.Ü.E. was supported by the TÜBİTAK 2219 scholarship.

Keywords: benzo[a]pyrene, chlorpyrifos, mixture effects, zebrafish embryos, HepG2 cells.



OP-03. Comparison of EU and TR Safety Guidelines on the Use of Nanomaterials in Cosmetics

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Purpose: The regulation on the use of nanomaterials in cosmetic products are provided by SCCS in EU and by TMMDA in TR. In order to see the differences between EU and TR regulations, a comparison must have been made.

Method: For comparison, SCCS and TMMDA guidelines on safety of nanomaterials in cosmetics have been reviewed and the results have been given in a chart.

Results: So far, the results have demonstrated that TR regulation is outdated and needs an urgent update in the manner of harmonization with EU in order to access to EU common market. In this manner, the result chart might prove helpful in updating TR regulation to the EU standards. Some of the outcomes of this study are as following:

- definition of nanomaterials is outdated in TR,
- chart 1 of TR regulation “Safety Assessment Chart for Nanomaterials in Cosmetics” is outdated,
- producing *in vivo* data on the use of nanomaterials in cosmetic products is prohibited in EU,
- EU now gives priority to the use of BMDL in the calculation of a PODsys while calculating margin of safety,
- Risk assessment is more comprehensive in EU regulation,
- Some of the structural parameters of nanomaterials have been addressed separately in SCCS notes,
- In the same “...parameters for identification and characterization of nanomaterials...” chart of the two guidelines, SCCS guideline has more parameters which proves TR guideline is outdated,
- About dermal exposure, SCCS has developed some equations while TR guideline has no equations yet,
- SCCS has developed a “Scoring system with key questions to assess a selected signal for prioritization on risk potential for human health” for being able to give priority to some nanomaterials for assessment.

Keywords: Regulatory Toxicology, Safety Guidelines, Nanomaterial, Cosmetics, SCCS, TMMDA



OP-04. Evaluation of the Apoptotic and Antioxidant Effects of Pitavastatin in Cervical Cancer Cell Line

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Pitavastatin (PITA) is the newest member of the statin family that widely used to treat hypercholesterolemia. In the recent years, it has been recommended to use in cancer treatment however the mechanism of anticancer effects has not been fully clarified. This study was aimed to evaluate the possible apoptotic and antioxidant effects of PITA in cervical cancer (HeLa) cells. In this study, the effects of PITA and cisplatin on apoptosis were determined by cell cycle analysis. Also, the “FITC Annexin V Apoptosis Detection Kit I” protocol was followed for cell apoptosis detection. The “ROS Detection Assay Kit” protocol was followed to examine the formation of reactive oxygen species (ROS) for the possible antioxidant/oxidant effect of PITA.

In cell cycle analysis, cells was monitored after 48 hours of exposure to PITA. As a results of this study, SubG1 peak was assessed as a mark of late apoptosis, and apoptosis was observed only at a concentration of 1 µM, and at a combination of PITA 1 µM and cisplatin 20 µM. According to the protocol for the examination of apoptosis, all concentrations were found to be significant compared to the control ($P<0.001$). Early apoptosis was seen at all concentrations of PITA, with the highest percentage of early apoptosis at the 0.1 µM concentration. It was determined that the ROS levels generally increased in concentration and time. The combination of 100 µM PITA and cisplatin significantly increased the ROS level compared to the positive control.

In conclusion, PITA induced apoptosis in HeLa cells and caused the generation of ROS. Investigating whether PITA is effective in cervical cancer cell line will help to identify new therapeutic strategies for treatment. Our study shows that PITA may be a promising option in the treatment of cervical cancer in combination with cisplatin.

Keywords: apoptosis; cervical cancer; combination therapy; pitavastatin; ROS

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OP-05. Assessment of Organophosphate and Pyrethroid Pesticides Exposure in Preschool Children in Turkey

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Children's environmental health is influenced not only by traditional environmental hazards such as tobacco smoke, air pollution, pesticides but also new emerging threats such as endocrine disruptor chemicals and climate change. Pesticides, which are among these threats, are products developed for the benefit of humanity and are one of the biggest environmental risk factors in the world which leads to increased mortality and a wide variety of diseases in both wildlife and humans today. Organophosphate pesticides (OPs) and pyrethroids (PYRs), which are important groups of pesticides, are widely used in Turkey as well as all over the world. The aim of this study was to determine the OP and PYR exposure levels in urine samples obtained from preschool Turkish children (3-6 years old) living in two cities, one with high agricultural production (Mersin, n:54) and the other with high consumption (Ankara, n:132). Three non-specific metabolites of PYR insecticides and four non-specific and one specific metabolite of OPs were analyzed using liquid chromatography-mass spectrometry (LC-MS/MS). The non-specific PYR metabolite 3-Phenoxybenzoic acid (3-PBA) (87.1%; n=162) and the specific OP metabolite 3,5,6-Trichloro-2-pyridinol (TCPY) (60.2%; n=112) were the most frequently detected metabolites in all samples. When two provinces in the study were considered, the mean concentrations of 3-PBA and TCPY were 0.3 ± 0.8 and 0.11 ± 0.43 ng/g creatinine respectively. Although due to the large individual variation no significant differences were found between 3-PBA ($p=0.9969$) and TCPY ($p=0.6558$) urine levels determined in the two provinces, significant exposure differences were determined both between provinces and within the province in terms of age and gender. This study is an important contribution to the limited information about childhood exposure to OPs, PYRs and also a risk assessment, the estimated daily intake (EDI) and hazard quotient (HQ) were determined for urine concentrations.

Keywords: Organophosphate pesticides; pyrethroid pesticides; preschool children; liquid chromatography-mass spectrometry



OP-06. Determination of Exposure to Organophosphate Flame Retardants in Preschool Children: A Human Biomonitoring Study in Three Turkish Provinces

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Flame retardants (FRs) have been included in consumer products in different chemical structures since the 1960s, both to prevent combustion and to reduce their flammability. They are member of endocrine disrupter chemicals family and present all around us, in our homes, schools, workplaces and leisure venues including furniture, electronics, wall coverings, floor finishes and paints. The prohibition of the use of polybrominated diphenyl ether (PBDE) formulations under the Stockholm Convention has led to the introduction and use of new alternative FRs. Organophosphate FRs (OPFRs) are the leading ones among these. Increasing knowledge of FR-related toxicity in children indicates that exposure to FR leads to health risks, especially in sensitive developmental periods. It is obvious that determination of the exposure to FR through biomonitoring, specifically in children, is very important for public health. The aim of this study is to provide the first information about exposure levels of OPFRs in preschool children in Turkey. For this purpose, we determined the exposure levels of preschool children to some OPFRs living in Ankara (n=118; male= 62, female= 56), Mersin (n=54; male=33, female=21) and Balıkesir (n=30; male=14, female=16). Two specific metabolites of OPFRs, bis(1,3-dichloro-2-propyl) phosphate (BDCIPP) and diphenyl phosphate (DPHP), were analyzed in urine collected from preschool children (3-6 years old) using liquid chromatography-mass spectrometry (LC-MS/MS). In the samples taken from Ankara, Balıkesir and Mersin, the mean levels of BDCIPP were determined as 0.78±1.46 ng/ml, 2.28±2.71 ng/ml and 0.72±0.95 ng/ml, respectively. Mean DPHP levels were determined as 0.45±0.33 ng/ml for Ankara, 0.46±0.21 ng/ml for Balıkesir and 0.52±0.53 ng/ml for Mersin residents. These first preliminary results of our study reveal the existence of OPFRs exposures of preschool children in Turkey, even though they live in different regions. These findings are extremely important as they are the first findings obtained from humans in Turkey.

Keywords: Flame retardants, organophosphate, children, urine, Liquid chromatography–mass spectrometry, Turkey



OP-07. Cell Response to *N*-alkyl Quaternary Quinuclidine Oxime Treatment

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Quinuclidine derivatives are recognized as compounds with diverse biological and pharmacological activities. Their oxime analogues have been evaluated as antidotes for organophosphorus compounds (OPCs) poisoning. Although globally used as pesticides or misused as chemical weapons, there is still no efficient treatment for OPCs. According to our previous studies, it is advisable to perform cell-based assays for antidote candidates prior to other studies of their efficiency, especially *in vivo*, since many of them are excluded from advanced testing due to possible adverse effects. With that in mind, in a present study, we tested seven newly synthesized 3-hydroxyimino quinuclidinium bromides with different alkyl chain lengths (C_nQNOH; *n* = 8 – 16). Human hepatocyte (HepG2) cell line was used to determine their influence on cell viability and homeostasis, membrane integrity and oxidative status. We used standard methods for evaluating specific targets: activity of mitochondrial succinate dehydrogenase by the tetrazolium salt MTS, lactate dehydrogenase (LDH) leakage by fluorescent resazurin, mitochondrial membrane potential assay through TMRM signal and induction of reactive oxygen species (ROS) by fluorescent dye DCFDA, respectively. Our results showed that quinuclidinium oximes with a long side alkyl chain (C12, C14 and C16) were the most toxic to cells with IC₅₀ values in micromolar range. Furthermore, compound with C12 alkyl chain displayed a notable time-dependent toxicity. More detailed analysis revealed that all three cytotoxic compounds triggered significant LDH release and decrease in membrane mitochondrial potential, indicating a necrotic-like impact. In concentrations corresponding to IC₅₀ values, tested oximes also significantly induced ROS generation. Such results indicate that these oximes induced negative effects and could not be considered for further evaluation as antidotes. However, their observed influence on cells opens up a new perspective to investigate them as drugs for specific conditions and diseases. Acknowledgment: This work was supported by the Croatian Science Foundation (project UIP-2017-05-7260).

Keywords: antidotes, quinuclidine oximes, cytotoxicity, necrosis, ROS



OP-08. Biomonitoring of Glyphosate-Based Herbicides Among Farmers in Northern Cyprus: A Pilot Study

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N-(phosphonomethyl) glycine, commonly known as glyphosate (GLY) is an organophosphate herbicide that inhibits the 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase) in shikimate biosynthetic pathway of plants. Following its marketing in 1974, it became the most widely used herbicide worldwide. Since its use is ubiquitous, possible health outcomes of GLY and glyphosate-based herbicide (GBH) exposure are always in the scope of studies. In 2015 the International Agency for Research on Cancer (IARC) classified the GLY as “Group 2A – probably carcinogenic to humans”. This classification directed some countries to completely ban or restrict the use of GLY and thus, sparked a debate. Among carcinogenicity, it is known that exposure to GLY is linked to many other health problems such as kidney damage, mental and neurological disorders, reduction of sperm motility in men and an increased rate of miscarriages in women. Thus, biomonitoring of GLY exposed individuals is crucial to protect their wellbeing. The objectives of this study were as follows; a) conducting exposure and risk assessment, b) measuring possible oxidative damage of GBH in the urine sample of exposed farmers residing in North Cyprus. To do so, a total of 60 urine samples (30 pre-, and 30 post-application) were collected from 30 farmworkers. Creatinine analyses were conducted by a fully automated device, Roche Cobas Integra 400. The GLY and its major metabolite, AMPA levels were measured using LC-MS/MS. To measure the oxidative damage, a commercially available 8-OHdG ELISA kit was used. The obtained results were the very first human biomonitoring of the GLY conducted in North Cyprus and this study was a pioneer to study the link between GLY exposure and 8-OHdG levels. The efforts are ongoing to expand the number of participants and prepare a report to allow regulatory bodies to act.



OP-09. The Nematode *Caenorhabditis Elegans* in Toxicity Testing: A Pioneering Study on the Utility of This Model Organism

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Introduction: *Caenorhabditis elegans* is a robust testbed particularly suited to the determination of neurotoxicity, for which it serves as a model organism. In addition, its use has slowly expanded to genotoxicity in this organism, as it shares many homologous pathways to humans in genotoxic response.

Astragalus species have traditionally been used as immune system enhancers, urinary and respiratory disease ailments, among others. This genus, while comprising one of the largest, have been traditionally underrepresented in the discipline of toxicology, with especially their cytotoxicity and genotoxicity scarcely properly evaluated in model organisms. One class of major secondary metabolites, cycloartenol glycosides isolated from these species, in particular, have been individually under-evaluated as well.

Aims: The current study aimed to attain 2 goals: The establishment of *C. elegans* as a reliable and repeatable experimental testbed in the determination of cytotoxic, neurotoxic/chemotactic and genotoxic experimental methods for materials and to establish a safety profile for isolated and purified *Astragalus* metabolites. These were isolated from *Astragalus* species by the authors, but have not undergone appreciable cytotoxicity, genotoxicity or neurotoxicity testing.

Methods: *C. elegans* WT strain was cultivated in accordance with the protocols established by the CGC. Alkaline Comet Assay was utilised to assess genotoxicity. Neurotoxicity was assessed by the application of the Nematode Chemotaxis Assay and cytotoxicity was determined by the combination of nematode survival ($\geq 95\%$) and the frequency of death fluorescence events. All methods were specifically modified for efficacy.

Results: Our study demonstrated that the toxicity testing utility of *C. elegans* is demonstrable and robust, therefore suited to study a variety of toxicities in a dependable manner. The results indicate that cytotoxicity, neurotoxicity/chemotaxis and genotoxicity is detectable and evaluable in *C. elegans*, therefore there exists great potential for the expansion of this organism as an alternative to human *in vivo* studies, as well as *in vitro* experimentation.

Keywords: *Caenorhabditis elegans*, Genotoxicity, Chemotaxis, Cytotoxicity, *Astragalus*



11th INTERNATIONAL CONGRESS OF THE TURKISH SOCIETY OF TOXICOLOGY

For a safe and healthy world

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OP-10. *In silico* Prediction of ADME and Toxicity Profiles of the Drug Candidates

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Computational methods present an advantage of estimating the bioavailability and safety of drug candidates and selecting better lead compounds even before they are synthesized and undergo preclinical tests and clinical trials. The computational models that can predict ADME/Tox profiles have become an alternative and complementary approach to existing *in vitro* and *in vivo* toxicity tests, thereby minimizing the need for animal testing, reducing the cost and time of relevant tests, and improving bioavailability/toxicity prediction and efficacy/safety assessments. *In silico* drug design and development studies that focus on the treatment of COVID-19 increase day by day. Some old drugs and newly designed molecules are investigated *in silico* for the therapeutic potential against SARS-CoV-2 and their safety by different research groups. In this presentation, our *in silico* ADME/Tox profile studies of anti- SARS-CoV-2 agents will be discussed in detail based on different safety parameters including structural alerts, carcinogenicity, mutagenicity, and target organ toxicity.



OP-11. Evaluation of Neopterin Levels and Kynurenine Pathway in Acute Coronary Syndrome Patients

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Coronary atherosclerosis is the most important cause of coronary artery disease. Rupture in atherosclerotic plaque is the main cause of the acute coronary syndrome. Neopterin and indolamine 2,3-dioxygenase [IDO, which is shown by the ratio of kynurenine (Kyn) to tryptophan (Trp)], are important biomarkers of cellular immune responses. They are elevated in various pathologies, including inflammation, infections, and malignancies. A total of 70 participants were included in this presented study. The measured values were compared between the patient and control groups. The determined parameters for the patient group and control group were, troponin (2 ± 5 and 0.1 ± 0 $\mu\text{g/L}$), mb form of creatine kinase (CK-MB) (73 ± 108 and 29 ± 20 U/L), pro-brain natriuretic peptide (pro-bnp) (3083 ± 6365 and 126 ± 65 ng/L), lactate (2 ± 1 and 1.5 ± 0.4 mmol), low density lipoprotein (LDL) cholesterol (124 ± 43 and 103 ± 32 mg/dL), urea (40 ± 23 and 28 ± 9 mg/dL), creatinine (1.2 ± 1.3 - 0.7 ± 0.2 mg/dL), Kyn (2.4 ± 0.6 and 1.8 ± 0.3 $\mu\text{mol/L}$), Kyn/Trp (45 ± 12 and 31 ± 5 $\mu\text{mol/mol}$), serum neopterin (14 ± 5 and 8 ± 3 nmol/L), urinary neopterin (220 ± 61 and 147 ± 29 $\mu\text{mol/mol creatinine}$) and biopterin (245 ± 74 and 137 ± 37 $\mu\text{mol/mol creatinine}$), respectively. The detected values of the patient group were higher than the control group. The heart ejection fraction percentage of the patient group was lower than the control group (47 ± 10 and 64 ± 2 , respectively; $p < 0.05$). Thus, the determination and use of neopterin and IDO parameters as biomarkers in patients with acute coronary syndrome can be meaningful and valuable. On the other hand, further studies with larger patient-based studies are needed to support these results. This study was the first author's doctorate thesis, partially supported by the Hacettepe University Scientific Research Foundation Unit (#TDK-2019-17670).



OP-12. Determination of *In Vitro* Genotoxic Effects of Pine Resin and *Aronia Melonacarpa* on Prostate and Breast Cancer Cell Lines by Using Comet Assay Technique.

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The cancer burden continues to grow globally and places extraordinary physical, emotional and financial pressures on individuals, communities and health systems. Studies continue without slowing down to establish new targets and treatment strategies for cancer, which still remains an important problem in the clinic and does not have a definitive cure yet. Natural compounds obtained from different sources stand out as new therapeutic agents in cancer due to their more effective and selective properties and lower toxic effects. Thus, we aimed to clarify the possible anticancer effects of *Aronia melonacarpa* on human breast cancer cell line (MCF-7) and pine resin on prostate cancer cell line (PC-3).

Our target plants, pine resin and *Aronia melonacarpa* were collected from different locations from Aegean and Blacksea region of Türkiye, respectively. The genotoxic effect of the methanol extracts was determined by alkaline comet assay. 100, 200 and 400 µg/ml extract concentrations, which have the high cytotoxic effect, were used. The examined groups were control negative, plant extract and positive control groups. Comparison of the anti-cancer and genotoxic effect of different concentrations on MCF-7 of *Aronia melonacarpa* and pine resin on PC-3 was evaluated by SPSS 20.0 program. Comet assay results showed that the both *Aronia melonacarpa* and pine resin extract exhibited genotoxic effects against MCF-7 and PC-3 cell lines in a dose-dependent manner by increasing the mean percentage of DNA damage after 24hrs of treatment ($p < 0.001$).

However, the observed toxicity of this two plant extract needs to be confirmed by additional studies. Based on our results, further examination of the potential anticancer properties of two plant species and the identification of the active ingredients of these extracts is warranted. This preliminary study is important for future studies and we anticipate that it will contribute to the development of a new drug in cancer treatment.



OP-13. Investigation of The Cytotoxic and Genotoxic Effects of Royal Jelly on Healthy and Cancer Lung Cell Lines

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The use of products obtained from honey bee hives, such as honey, bee bread, bee venom, pollen, propolis and royal jelly, for protecting and maintaining health, has attracted considerable attention in recent years. There is widespread use of various bee products in both traditional and modern medicine. Many scientific studies aim to investigate the beneficial effects of these bee products on pharmacological properties due to their biological activities, which leads to an increase in the production of nutraceuticals and functional foods containing these products. Among the bee products royal jelly is gaining more importance and has been reported that it has many biological activities such as antioxidant, antitumor, anti-inflammatory, antimicrobial and wound healing. In this study the cytotoxic, genotoxic and antigenotoxic effects of royal jelly on BEAS-2B (healthy epithelial lung cell) and A549 (cancer cell) cell lines were investigated. Cytotoxic effects of royal jelly obtained from Bursa region was evaluated by both neutral red uptake (NRU) and 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) tests, and its genotoxic/antigenotoxic effects were evaluated by comet assay. According to the results of NRU and MTT cytotoxicity tests performed on BEAS-2B and A549 cells, it was concluded that royal jelly did not show a cytotoxic effect on both cell lines at concentrations of 12.5-1600 µg/ml. According to the genotoxicity results using the Comet test, it was determined that royal jelly did not cause DNA damage in BEAS-2B and A549 cells. On the other hand, when its antigenotoxic effect was evaluated in cells exposed to DNA damage with H₂O₂, it is shown that royal jelly reduced the damage at all treatment concentrations. The results obtained revealed that royal jelly showed activity reducing cell and DNA damage due to its strong antioxidant activity.

Keywords: royal jelly, cytotoxicity, genotoxicity, antigenotoxicity, comet assay



OP-14. Modifying Effects of Gender on the Toxicity of Methyl Mercury and Thimerosal

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Exposure to organic mercury compounds has been associated with various neurotoxic effects, depending on the structure of compound and exposure route. Humans are extensively exposed to methylmercury, an organic mercury compound with well-known neurotoxicity. Methylmercury is bioaccumulated mainly in sea organisms and general population is exposed to methylmercury by consuming fish. On the other hand, ethyl mercury is also present in fish, however to a lesser extent. Moreover, ethyl mercury is present in thimerosal, which is used as a preservative in vaccines. Gender-based differences are observed in neurodevelopmental disorders as testosterone and estradiol can alter the toxic effects of compounds. In the present study, we aimed to investigate the toxic effects of methylmercury and thimerosal on SH-SY5Y cell line in cellular environments containing high testosterone/low estradiol and high estradiol/low testosterone and determine whether male and female brains respond differently to the toxic effects of methylmercury and thimerosal. Study groups were designed as: i. control; ii. thimerosal; iii. methylmercury; iv. high testosterone/low estradiol + thimerosal; v. high estradiol/low testosterone + thimerosal; vi. high testosterone/low estradiol + methylmercury and vii. high estradiol/low testosterone + methylmercury. Cells were exposed to inhibitory concentrations 20 (IC₂₀s) of methylmercury and thiomersal along with testosterone and estradiol, whose chosen doses did not cause cell proliferation and did not reduce cell viability. The results showed that methylmercury and thiomersal have significant toxic effects on SH-SY5Y cells. These effects were demonstrated by the evaluation of antioxidant enzyme levels, oxidative stress parameters, oxidative DNA damage and apoptosis. We observed that testosterone had significant exaggerating effects especially on thimerosal-induced toxicity, while estradiol did not enhance the toxic effects of organic mercury compounds; indeed, estradiol led to a reducing effect in their toxicity. Although the models do not fully reflect *in vivo* conditions, we can suggest that testosterone and estradiol at physiological concentrations can alter the toxic effects of organic mercury compounds and neurotoxic agents may exert different effects in different genders.

Keywords: apoptosis, estradiol, methylmercury, neurotoxicity, oxidative stress, testosterone, thimerosal



OP-15. Apoptotic, Genotoxic and Immunotoxic Evaluation of Bisphenol Derivatives Exposure in HepG2 Cells

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Purpose: Bisphenols belong to a group of chemicals known as “diphenylmethanes” that are used for hardening plastics, epoxy resins and polycarbonates. These chemicals are used in the manufacturing of polycarbonate plastics and resin coating of the inner walls of food and beverage cans. Bisphenol A [4,4'-(propane-2,2-diyl)diphenol; BPA] has become one of the most widely used plasticizers throughout the globe. Reproductive and developmental problems, metabolic diseases such as diabetes and obesity, thyroid disorders, various organ (testicle, brain, liver, kidney, heart) damages, behavioral disorders and fetotoxicity are suggested to be caused by BPA exposure. With the search for alternative substances to BPA, the use of other bisphenol derivatives namely bisphenol F (4,4'-dihydroxydiphenyl-methane; BPF) and bisphenol S (4,4'-sulfonylbisphenol; BPS) has increased. In this study, we aimed to evaluate the possible apoptotic, genotoxic and immunotoxic effects of widely used bisphenol derivatives on human hepatocellular carcinoma cells (HepG2).

Methods: The apoptotic effects of bisphenols were evaluated by detecting different caspase activities that have role in the extrinsic and intrinsic caspase pathways. Besides genotoxic effects of these chemicals were evaluated by measuring the levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) and 8-oxoguanine glycosylase (OGG1). To determine the immunotoxic effect of bisphenol derivatives, the levels of interleukin 4 (IL-4) and interleukin 10 (IL-10), transforming growth factor beta (TGF- β) and tumor necrosis factor alpha (TNF- α), which are known to be expressed by HepG2 cells, were measured.

Results: The results indicate that both BPF and BPS may also cause apoptotic, genotoxic and immunotoxic effects. Comprehensive *in vitro* and *in vivo* research is needed to further examine the toxic effects of alternative bisphenol derivatives. Moreover, safer alternatives for bisphenol derivatives should be developed as our results show that BPF and BPS have similar toxic effects of BPA.

Keywords: bisphenols, HepG2, genotoxicity, apoptosis, DNA base damage, immunotoxicity



OP-16. Evaluation of Possible Toxic Effects of Preservatives and Adjuvants Used in Vaccines

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There have been serious concerns about the safety of vaccine adjuvants like aluminum compounds and vaccine preservatives like thiomersal for many years. In this study, we aimed to evaluate the possible toxic effects of thimerosal and/or aluminum on the SH-SY5Y cell line. The cytotoxic effects of thiomersal and aluminum were evaluated. The inhibitory concentration 20 (IC₂₀) for thiomersal was 1.15 µM and 362 µM for aluminum. Intracellular reactive oxygen species (ROS) levels, oxidative stress parameters, DNA damage, nuclear factor erythroid 2-related factor 2 (Nrf 2), dopamine β-hydroxylase (DBT) and dopamine transporter (DAT) protein levels and neurotransmitter levels (dopamine, norepinephrine), were measured after IC₂₀ concentration of these compounds were applied to neuroblastoma cells for 24 hours. ROS levels increased significantly in cells treated with aluminum and aluminum+thiomersal. Total glutathione (GSH) levels decreased in the aluminum administered group vs. control. Malondialdehyde levels as biomarkers of lipid peroxidation increased in aluminum and aluminum+thiomersal groups. Total antioxidant capacity (TAOC) and protein oxidation levels increased significantly in the aluminum and aluminum+thiomersal groups vs. control. Nrf2 levels and DNA damage were found to be higher in all treatment groups. While dopamine levels increased significantly in cells treated with thiomersal and aluminum+thiomersal, DAT levels were found to be higher in all experimental groups compared to the control and DBH levels were markedly higher in the combined. Norepinephrine levels did not change significantly in the experimental groups. These findings show that both thiomersal and aluminum can change the oxidant/antioxidant status in neuroblastoma cells and can cause DNA damage. In addition, thiomersal and/or aluminum have a significant modifying effect on dopamine and DAT levels in neuroblastoma cells. We can state that the changes observed in cells treated with aluminum+thiomersal are more pronounced. In some vaccines, including vaccines for influenza, both compounds may be present. Therefore, safer alternatives of thiomersal and aluminum compounds should be developed and both *in vitro* and *in vivo* mechanistic studies should be performed on the neurotoxic effects of thiomersal and aluminum.

Keywords: aluminum, thiomersal, oxidative stress, neurotransmitter, dopamine.



OP-17. Investigation of the Effects of Zearalenone in the Genes Related to Energy Metabolism *In Vitro*: Relation with the Epigenetic Alterations.

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Zearalenone (ZEA), produced by various *Fusarium* species, is a non-steroidal estrogenic mycotoxin. It contaminates cereals such as corn, wheat, oat and soybean and water, therefore it has negative effects on human health. In present study, the effects of ZEA on epigenetic modifications and metabolic pathways have been investigated in HepG2 cells in order to elucidate the possible relationship between metabolic dysfunctions. For this purpose, cytotoxicity tests (MTT and NRU) were performed and 5-methyl cytosine (5-mC%) levels were measured using the Elisa kit. Global histone modifications were analyzed using western blot. Gene-specific DNA methylation levels of *PPAR γ* were determined by pyrosequencing. According to the MTT and NRU tests, the IC₅₀ values were determined as 143.35 and 60.45 for ZEA, respectively. ZEA caused an increase in global DNA methylation levels at 1.46 and 2.54 fold after 10 and 50 μ M of ZEA exposure. 1-50 μ M of ZEA exposure caused alterations in *PPAR γ* expression and promoter DNA methylation significantly. Besides, 1-50 μ M of ZEA exposure showed significant alterations in the expression levels of nuclear receptor genes (*AhR*, *LXR α* , *PPAR α*), and carbohydrate-lipid metabolism related genes (*L-fabp*, *LDLR*, *GAPDH*, *Glut2*, *Igf-1*, *Akt1*, *HK2*). In addition, 1-50 μ M of ZEA exposure resulted in significant changes in global histone modifications (H3K27me, H3K9me3, H3K9ac). Expression levels of the chromatin modifying enzymes *EHMT2*, *ESCO1*, *HAT1*, *KAT2B*, *PRMT6* and *SETD8* were upregulated by 50 μ M of ZEA exposure using PCR arrays, consistent with the results of global histone modifications. Taken together, the epigenetic mechanisms of DNA methylation and histone modifications may be key mechanisms in ZEA toxicity. Furthermore, effects of ZEA in metabolic pathways could be mediated by epigenetic modifications. It is expected that results will contribute to better understanding of key molecular events in the toxicity of endocrine disrupting chemicals for risk assessment process.



OP-18. Promising Novel Bioinspired Materials for Theragnostic Applications: Is The Synthesis Of Non-Cytotoxic and Non-Genotoxic Dipeptide Structures Possible?

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Purpose: The diagnosis and treatment of the chronic diseases such as cancer in a certain coordination is a subject that has been emphasized in recent years. Theragnostic approaches carry the drug and imaging agent in a single formulation and can be used for different purposes. This system can be used for pre-screening patients and enabling personalized medicine especially in cancer treatment. An ideal theragnostic should be biocompatible and can be used safely in humans. Although several types of theragnostics have been developed, none of yet satisfied these criteria. Bioinspired materials with noble metal centers encapsulating therapeutic and imaging agents were shown to possess theragnostic activities. In this study, it was aimed to synthesize, characterize, and evaluate the cytotoxic and genotoxic effects of self-assembly of diphenylalanine (Phe-Phe) dipeptides presence of mercury (Hg^{2+}) ions to be used for theragnostic.

Methods: Cytotoxicity and genotoxicity studies were done in mouse fibroblast (NIH/3T3) cells by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and single cell gel electrophoresis (Comet) assays, respectively.

Results: It was found that cell viability decreased in a dose dependent manner in 24-, 48- and 72-hours treatment. Also, Phe-Phe dipeptides did not cause any significant changes in DNA damage at the concentrations of 1, 2 and 5 mg/mL in 4- and 24-hours exposures. In the 48-hours exposure, Phe-Phe peptide exposure at concentrations of 2 and 5 mg/mL caused a significant increase in DNA damage and in the 72-hours of exposure, a significant increase in DNA damage was observed at all studied concentrations. According to the results obtained in our study, it is predicted that Phe-Phe dipeptides presence of Hg^{2+} ions are biocompatible. However, it is clear that further toxicological analyzes such as the determination of the possible molecular mechanisms are needed for the safe use of these compounds for theragnostic purposes.

Keywords: diagnosis, treatment, dipeptide, MTT, Comet



OP-19. The Impact of DNA Methylation on The Expression of *CYP3A4* Gene

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Epigenetic alterations involved in the expression of drug-metabolizing enzyme genes make a significant contribution to interindividual differences in drug response. Pharmacoepigenetic research has the potential to improve patient care by predicting adverse drug reactions. One of the most common epigenetic modifications is DNA methylation, which can occur in genes encoding drug-metabolizing enzymes and can affect the pharmacokinetics of drugs. The mechanisms of DNA methylation are likely to influence the interindividual expression of *CYP3A4*. This study aims to investigate the epigenetic factors altering the regulation of *CYP3A4* enzyme activity in chronic kidney disease (CKD).

The gene expression profiles of *CYP3A4* and the nuclear receptor *PXR* genes were evaluated in patients with CKD (n=67) and healthy controls (n=29) by RT-PCR analysis. The differentially methylated CpG promoter region of the *CYP3A4* gene was analyzed by bisulfite sequencing. The expression levels of *CYP3A4* and *PXR* genes were found low in CKD patients (1.92 ± 0.22 and 0.87 ± 0.07 , respectively) when compared to healthy controls (2.54 ± 1.08 and 1.42 ± 0.25 , respectively) and the association was found significant for *PXR* ($p < 0.05$). The demethylation rates of the *CYP3A4* promoter were associated with glomerular filtration rates ($p < 0.05$).

This study revealed the importance of analyzing DNA methylation profiles of genes involved in drug metabolism to predict adverse effects in patients with chronic kidney disease who are exposed to polypharmacy. Further studies in this area are promising for the development of new biomarkers.

Keywords: epigenetic; DNA methylation; *CYP3A4*; chronic kidney failure



OP-20. Comparison of Possible Cytotoxic Effects in Healthy and Cancer Cells and Evaluation of Antioxidant Capacities of Polymeric Nanoparticles Encapsulated with Naringin and Berberine

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Purpose: Phytochemical compounds obtained from plants have been used for many years due to their important therapeutic effects and today, they are still the source of many drugs. Among these phytochemical compounds, naringin and berberine have been reported to have various effects such as antimicrobial, anti-inflammatory, antioxidant and anticancer. In this study, it was aimed to evaluate the antioxidant properties of naringin and berberine substances and poly(methylmethacrylate) (PMMA) coated naringin and berberine nanoparticles (NP) and their possible cytotoxic effects on mouse fibroblast (3T3) and colon cancer (Caco-2) cells.

Methods: Antioxidant capacities of berberine, naringin, berberine-loaded PMMA NP and naringin-loaded PMMA NPs (25-2000 μ M) were investigated by 2,2-diphenyl-1-picrylhydrazil (DPPH) test. The cytotoxic effects of berberine, berberine-loaded PMMA NP and naringin-loaded PMMA NPs (2,5-100 μ M) and naringin (25-2000 μ M) were evaluated by 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazine (MTT) assay in 3T3 and Caco-2 cells after 24, 48 and 72 hours of exposure.

Results: It was found that DPPH inhibition of berberine, naringin, berberine-loaded PMMA NP and naringin-loaded PMMA NPs at 2000 μ M concentration reached 41.24%, 64.08%, 25.84%, 34.11%, respectively. As a result of the MTT assay; after 24, 48 and 72 hours of exposure, the determined IC₅₀ values in 3T3 cells were 79.20 μ M, 92.43 μ M, 60.97 μ M for berberine; 1891.20 μ M, 1955.34 μ M, 1931.37 μ M for naringin; 89.49 μ M, 91.77 μ M, 65.99 μ M for berberine-loaded PMMA NPs; 88.80 μ M, 95.67 μ M, 67.50 μ M for naringine-loaded PMMA NPs, respectively. After, 24, 48 and 72 hours of exposure, the determined IC₅₀ values in Caco-2 cells were 64.48 μ M, 66.39 μ M, 73.72 μ M for berberine, respectively; 1991.12 μ M, 1531.17 μ M, 1452.17 μ M for naringin; 93.24 μ M, 68.35 μ M, 78.82 μ M for berberine-loaded PMMA NPs; 76.04 μ M, 81.89 μ M, 82.24 μ M for naringin loaded PMMA NPs, respectively. Based on these data, it is thought that polymeric nanoparticles encapsulated with naringin and berberine may contribute to new treatment approaches for cancer, but further research is required.

Keywords: PMMA, Berberine, Naringin, DPPH, MTT



OP-21. Hepatotoxicity Induced by Low-doses of Pb: Subacute Toxicity Study in Rats and Benchmark Modelling

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In the liver Pb found to induce oxidative stress leading to oxidative damage of crucial molecules, proteins, nucleic acids, and lipids. As a divalent cation, chemical configuration allows Pb to mimic essential cations in physiological processes. Therefore, the purpose of the presented study was to simulate a low-dose Pb exposure scenario in animal model and to derived lower limit of Benchmark dose (BMDL) for hepatotoxic effects. Forty-two male Wistar rats were divided in 7 groups: one control group and six groups treated with Pb doses of 0.1, 0.5, 1, 3, 7, 15 mg/kg b.w./day respectively (oral gavage). After 28 days, rats were sacrificed and the liver were used for Pb and essential elements (Cu, Zn, Mn, Fe) levels, as well oxidative status parameters (superoxide dismutase, superoxide anion radical, malondialdehyde, total sulfhydryl groups, and advanced oxidation protein products) determination. Statistical evaluation was performed using Graph Pad Prism8 software (GraphPad Software Inc., San Diego, USA) while dose response modelling was performed using PROAST 70.1 software. The results have shown decrease Cu levels in the liver and increase in advanced oxidation protein products and superoxide dismutase activity. Benchmark dose-response modelling has shown dose-dependent decrease in Cu levels in the liver (BMDL: 2.7 e-06 mg/kg b.w./day) and the dose dependent increase of advanced oxidation protein products levels (BMDL: 0.00025 mg/kg b.w./day), indicating that those changes might be the most sensitive effects of low Pb dose hepatotoxicity. Our results might be useful in further consideration of point of departure of low-dose Pb exposure and observed BMDLs might be useful in assessing human health risk assessment of low-dose Pb exposure.



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For a safe and healthy world

OP-22. The Protective Role of UMCA on Bleomycin Induced Lung Fibrosis in Rats

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Bleomycin (BLM), an antibiotic drug, is used for the treatment of cancers like Hodgkin's lymphoma, Kaposi's sarcoma and cervical cancer. The most common adverse effects occurred during BLM treatment is lung toxicity, which manifest as fibrosis. UMCA® (Pelargonium sidoides root extract) is a commercial product to treat acute and chronic upper respiratory tract infections. UMCA® has antiviral, antibacterial, immunomodulatory and cytoprotective properties, which is related to its polyphenol, coumarin, anthocyanidin and flavonoid contents. In the present study, we aimed to investigate the possible protective effects of UMCA® on BLM induced lung fibrosis in rats. Wistar albino rats were included in this study and randomly divided into 4 groups (Control, BLM, BLM+UMCA and UMCA). The control group received physiological saline. UMCA® was orally applied at a dose of 200 mg/kg/day and single intratracheal administration of BLM was conducted at a dose of 10 mg/kg. All animals were decapitated after 10 days and lung tissues were removed for the biochemical and histopathological examinations. Malondialdehyde (MDA) and glutathione (GSH) levels and Myeloperoxidase (MPO) activity were determined. Chemiluminescence (CL) method using luminol and lucigenin probes, additional nitric oxide and peroxynitrite measurements were applied. Histopathological observations were analyzed with H&E and Gomori's one-step trichrome staining. Collagen contents of lung tissue were also determined with a spectrophotometric method in paraffin-embedded tissues. BLM elevated MDA levels and MPO activity and depleted GSH levels in the lung tissues ($p < 0.001$). CL measurement levels were also increased in BLM group respect to the control. Contrary to this, UMCA® treatment reversed these effects, significantly ($p < 0.001$). BLM caused alveolar structural disturbance and inflammatory cell infiltrations and collagen content was also significantly increased in BLM group compared to the control (65.7 ± 10.6 , $p < 0.001$). UMCA® administration reduced degenerations and decreased collagen content in the lung tissue. In conclusion, UMCA® has an antioxidant and protective effects on BLM induced lung fibrosis in rats.

Keywords: Bleomycin, lung fibrosis, UMCA®, Pelargonium sidoides, antioxidant



OP-23. Investigation of The Presence of Volatile Organic Pollutants (Vocs) in Tap Water In Ankara Provincial Districts

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The issue of cleanliness and reliability of water resources is a concern that is kept under regular and constant control in terms of public health. However, the list of regularly controlled chemical pollutants is limited, and it should not be forgotten that the presence of some pollutants that occur with regional or time-dependent changes may be missed. Volatile organic compounds (VOCs) are organic chemicals that both evaporate into air and dissolve in water. VOCs are common in everyday life because they are used in industry, agriculture, transportation and many processes in our normal daily life. Widely used VOCs are released into the environment, and when they reach water sources and groundwater, many VOCs become persistent and can be incorporated into the drinking water supply system. Many of these chemicals are toxic and may pose human health or ecological concerns in drinking water or the environment. Volatile organic pollutants also show a great diversity, and studies based on long-term and country-wide wide and widespread sources show that some VOCs are seen in water resources, for example, butylmethylether is still common while benzene and carbon tetrachloride tend to rise and should be monitored. In this study, a signal search will be made to determine whether there is a regional water pollution in the tap waters in Ankara. Tap water samples has been collected from the districts of Ankara, and the amount of VOCs will be measured with the Head Space Gas Chromatography method. The results of the ongoing study and whether there is a signal indicating pollution in any region of the province will be presented later.

* This study is supported by TUBITAK.

Keywords: drinking water, volatile organic compounds, pollution



OP-24. Investigation of The Protective Effect of Walnut Oil in an *In Vitro* Alzheimer's Disease Model

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Purpose: Recent studies suggest that there is a serious increase in Alzheimer's disease (AD) rates. Many factors play a role in the progression of AD. There is no known treatment for AD other than palliative treatment. For this purpose, many herbal products and dietary supplements are being studied by researchers as novel approaches for improving the symptoms of neurodegeneration. It is known that walnut oil is a good source of polyphenols, antioxidants and other chemicals that are suggested to improve general well-being. Therefore, walnut oil may be a potential supplementary treatment for neurodegenerative illnesses.

Methods: In the present study, human neuroblastoma cells (SH-SY5Y) were used to create an AD model by the application of brain-derived neurotrophic factor (BDNF) with retinoic acid to improve the neuronal properties of human neuroblastoma cells (SH-SY5Y cell line). Cytotoxicity, reactive oxygen species (ROS) levels, oxidative stress parameters, neurotransmitter levels and neurotransmitter-related protein levels were measured comparatively among the groups by treating all groups with the highest dose of walnut oil that does not cause cytotoxicity.

Results: Our findings show that walnut oil can lower oxidative stress ($p < 0.05$) in AD model by scavenging free radicals and maintaining an antioxidant-oxidant balance. The intracellular ROS levels of the AD group showed increases compared to the treatment of the walnut oil group ($p < 0.05$). Protein carbonyl levels of AD group was found to be significantly lower than control group (34%). The walnut oil group showed increases in dopamine serotonin and acetylcholine levels compared to the AD group. However, walnut oil has no significant effect on reducing lipid peroxidation ($p < 0.05$).

Conclusion: In conclusion, we can suggest that a walnut oil-rich diet may help reduce the risk of AD or may postpone the promotion and progression of AD by lowering oxidative stress.

Keywords: Alzheimer's disease, in vitro model, walnut oil

Acknowledgement: This research was supported by TUBITAK Project no: 118C483 and Cukurova University BAP Project ID: 14186



OP-25. Comparative Toxicity Responses of THS Derived from Conventional Cigarette and Heated-Tobacco Product in Human Bronchial Epithelial Cells

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Thirdhand smoke is a new toxicological concept that describes the residual nicotine and other chemicals left on indoor surfaces by tobacco smoke. People might be exposed to these chemicals by touching contaminated surfaces or breathing in the off-gassing from these surfaces. Due to its complex nature, exposure to THS might be a public health concern for people, especially those with chronic respiratory diseases. In the present study, we examined the cytotoxic, oxidative, and inflammatory responses of two different THS types derived from a conventional cigarettes (THS-C) and heated-tobacco products (THS-H). For this purpose, we exposed the terry clothes samples to tobacco smoke in a closed chamber and extracted cloth samples in a cell medium for 24h at 37°C. Following, the cytotoxicity of two types of THS was assessed by MTT assay in BEAS-2B human bronchial epithelial cells. MDA and intracellular GSH levels were determined in cell pellets to assess oxidative response. The AhR and IL-6 levels induced by THS were determined from cell lysates and cell supernatants by ELISA kit, respectively. According to our results, both types of THS led to dose-dependent cytotoxicity in cells, particularly with THS-C. Moreover, GSH depletion and MDA increase were observed, remarkably with THS-C. AhR activation and IL-6 were also elevated with THS slightly. To conclude, THS derived from different types of tobacco products might represent a public health concern for people, particularly who suffer from respiratory diseases.



OP-26. Effect of Melphalan and Epigallocatechin Gallate Combinations on Cytotoxicity in Neuroblastoma Cell Line

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Neuroblastoma is a malignant tumor most commonly seen in young children and originates from the sympathetic nervous system. Melphalan is an alkylating cytotoxic drug used in the treatment of neuroblastoma. Epigallocatechin gallate is a biologically active ingredient of green tea that has apoptotic, chemopreventive and chemotherapeutic properties. The aim of our study was to evaluate the cytotoxic effect of the combination of melphalan and epigallocatechin gallate on astrocyte and neuroblastoma cell lines.

Neuroblastoma (N1E-115) and astrocyte (C8D1A) IC₅₀ doses were determined by MTT test. The neuroblastoma IC₅₀ dose was applied to the astrocyte and neuroblastoma cell lines. Caspase 3 and caspase 9 mRNA expressions were determined by qRT-PCR method.

Neuroblastoma IC₅₀ values determined in MTT test were lower than astrocyte IC₅₀ values. Neuroblastoma cell line caspase 3 expression was increased in all groups. Although caspase 9 expression was increased in all groups, a significant increase occurred only in the melphalan+epigallocatechingallate group.

The fact that neuroblastoma IC₅₀ values were lower in the melphalan and epigallocatechin gallate and combinations group compared to the astrocyte IC₅₀ values showed a selective effect in all groups. The increase in caspase 3 can be interpreted as the onset of apoptosis. The increase in caspase 9 expression in all groups indicates that the triggered apoptosis will occur through the mitochondrial pathway. In conclusion, in our study, it has been shown that epigallocatechin gallate and melphalan have a selective effect on healthy astrocyte cells and neuroblastoma cancer cells and trigger apoptosis through the mitochondrial pathway.

Keywords: Melphalan, Epigallocatechin gallate, Neuroblastoma, Astrocyte, Apoptosis



OP-27. Human Induced Pluripotent Stem Cell-Derived Cardiomyocyte (Hpsc-CM) Model as an Assessment Tool for Cardiotoxicity

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Cardiotoxicity is an important cause of medicinal products withdrawal during the drug development process. Approximately 10% of drugs are withdrawn from the market because of cardiotoxicity problems eventually leading to a waste of time and money. In general, preclinical drug toxicity assessments include animal studies and immortalized cell lines. However; the toxicity assessments could not be interpreted correctly to extrapolate outputs for human due to the low specificity, consistency, and poor biological relevance of these experiment models. Hence, there is a need for new models with improved cell technologies which are also correspondent with high throughput screenings.

As it is well known, drug development is a long, costly and challenging process. The most important challenge during this process is to find the most effective novel drug candidates with minimizing the safety problems. One of the aim of a toxicologist during preclinical studies is to assess the toxicity of drug candidates with *in vitro* models which have the best extrapolation features to human. The human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM) model is a novel approach for testing the preclinical cardiovascular safety and efficacy of drugs. Since hiPSC-derived cells carry the donor's genetic information, it is a valuable drug toxicity tool which can mimic donor-specific drug response. hiPSC-CMs show features such as having the ability to maintain the stability of cell culture, having convenience for genetic engineering with gene editing tools, expressing plenty of cardiac-specific ion channels, including calcium handling properties. Herewith, hiPSC-CM is a unique approach for screening cardiotoxicity of drugs and evaluating the mechanism of toxicity. The aim of this review is to highlight the importance and advantages of hiPSC-CM model -which have the speciality to reduce the time from bench-to-bedside of new drugs with less cardiotoxicity- as a platform for the study of drug induced cardiotoxicity.

Keywords: cardiotoxicity, human induced pluripotent stem cells, cardiomyocytes



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OP-28. Microplastics: How Aware are We?

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Plastics are widely used in all areas of life. Inappropriate disposal and non-biodegradability of plastic waste cause them to accumulate at an uncontrolled rate in terrestrial and marine ecosystems. Plastics in the environment are naturally broken down by effects such as wind, air, sunlight, waves, currents, tides, salinity, and living activities in the waters, and turn into micro- (<5 mm) and nano- (<1000 nm) plastics. Environmental conditions carry nano- and microplastics from urban areas to non-urban areas or even far from residential areas. The threat posed to the environment and living things have reached an alarming level due to the features such as the presence of toxic compounds in the content of microplastics, acting as a transport vehicle for living things by adsorbing various toxic pollutants, and being ubiquitous at any time. Microplastic pollution, which is found in excessive amounts in natural fresh and saltwater resources, air, and soil, should be considered a major problem in terms of environment, biota, food, and health. Microplastics, as a new type of environmental pollutant, have attracted the attention of researchers around the world for the last few years. There are a lot of studies to indicate that nano- and microplastics penetrate cells and have potentially toxic effects such as cytotoxicity, DNA oxidative damage, and induction of reactive oxygen species. After chromosomal rearrangements in the human body, ROS induce mutagenesis by breaking the double helix of DNA and cause mechanical and genetic damage, leading to cancer formation. Despite the studies, the subject of microplastics is still full of question marks and the necessary consciousness hasn't been formed in people. This presentation is aimed to emphasize the risks and raise awareness about microplastics by explaining the sources of exposure to microplastics, the effects of microplastics on bodies, and what can be done to reduce exposure.



OP-29. Cytotoxic Effect of Abemaciclib in HepG2/THP-1 co-Culture Model

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Abemaciclib, a cyclin-dependent kinase inhibitor, is used for the treatment of advanced or metastatic breast cancer as a monotherapy or in combination with endocrine therapy. It has been reported that abemaciclib induced hepatotoxic effects in patients and the molecular mechanisms have not been known clearly. HepG2/THP-1 co-culture model has been used for the evaluation of hepatotoxicity in recent years to detect especially immune-mediated hepatotoxicity. In the co-culture model, HepG2 cells simulate hepatocytes and THP-1 macrophages kupffer cells. In the preliminary study, it was aimed to investigate abemaciclib-induced hepatotoxicity using HepG2/THP-1 co-culture model. Ketoconazole was used as a reference chemical to validate the co-culture model. THP-1 macrophages differentiated with phorbol 12-myristate 13-acetate were co-cultured with HepG2 cells in a system that the cells are separated by a porous membrane. Following ketoconazole exposure, cytotoxicity, reactive oxygen species (ROS) production and mitochondrial membrane potential (MMP) were evaluated in both single-culture and co-culture models. According to the findings, IC₅₀ values were determined as 60.88 µM and 57.46 µM for single-cultured and co-cultured HepG2 cells, respectively. IC₅₀ values were calculated as 64.04 µM and 45.44 µM for single-cultured and co-cultured THP-1 cells, respectively. ROS production was increased at 1, 5 and 10 µM of ketoconazole, and MMP was dissipated at 5 and 10 µM of ketoconazole in both culture conditions. In the co-culture model, the cells were more sensitive to ketoconazole-induced MMP loss than in the single-culture model. After HepG2/THP-1 co-culture model was validated, the cells were exposed to abemaciclib for 24 h, and then cytotoxicity and ROS production were evaluated in the co-culture model. The preliminary study showed that abemaciclib lead to cytotoxicity and ROS production in HepG2 cells. Underlying mechanisms of abemaciclib-induced hepatotoxicity will be evaluated by further studies.



OP-30. Investigation of The Apoptotic Properties of Combined Use of Lonidamine and Doxorubicin on Ln229 Human Glioblastoma Cell Line

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Purpose: Glioblastoma (GBM) is the most malignant and highly aggressive brain tumor. New drugs or drug combinations are developing to improve treatment of GBM. Hexokinase 2 (HK2) is a novel target which shows its effect by regulating glucose dependent energy metabolism and mitochondrial apoptotic pathways. Lonidamine (LND) is one of the HK2 inhibitors which has less side-effect potential compared to other anticancer drugs. Widely used chemotherapeutic agent doxorubicin (DOX) is a DNA topoisomerase-2 enzyme inhibitor. However, its use has been restricted due to its side effects. By combining LND and DOX for synergistic GBM treatment, we aim to reduce toxicity and prevent drug resistance.

Method: MTT was performed for the combined use of DOX and LND on the human GBM cell line LN-229. According to the results, combination index studies were carried out using the CompuSyn program. The combination index was used to determine the dose in western blot studies. Expression levels of cleaved- proliferator-activated receptor (c-PPAR) and HK2 were examined by western blot.

Results: In the MTT analysis performed on LN229 cells, IC50 values of DOX and LND were found as 15.87 μ M and 1915 μ M, respectively, after 24 hours of exposure. As a result of the combination index experiment, Fa was 0.9 and CI was 0.05 for our combination doses, and DOX was 0.35 μ M and LND 182 μ M. When DOX+LND was given in combination, a significant decrease was observed in HK2 values, while a significant increase was observed in cleaved-PPAR levels.

Discussion: In line with the findings obtained here, the toxicity of LND was reduced by decreasing effective dose. Also, it increased the sensitivity to DOX by inducing apoptosis and increasing environmental Ph. Thus, DOX-induced toxicity and drug resistance were reduced. In conclusion, the values found are promising for clinical application.

Keywords: GBM, HK2, LN229, LND, DOX, Apoptosis, Cancer



OP-31. Are Rare Earth Elements Safe for Use In Zootechny?; Trophic Transfer and Effects Of Lanthanide Nanoparticles in A Simple Food Web

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Rare earth elements have become a major topic of interest due to their potentially beneficial effects as feed additives in zootechny. For example, the European Food Safety Agency has recently suggested that such use of lanthanides in specific farm animals does not represent a cause for concern in terms of toxicity profile. In contrast, recent evidence suggesting that lanthanides, especially in fine particulate form, may give rise to deleterious effects in a range of organisms indicates more caution is warranted. It is in the context of such conflicting information that we report on lanthanide nanoparticles in an aquatic food web in terms of uptake, bioaccumulation, toxicity and biomagnification. Lanthanum fluoride nanoparticles (25 nm diameter) were synthesised and their impact on growth and photosynthesis in alga *Dunaliella biocculata* (concentration range 1-10 mg L⁻¹) was determined. While significant effects were not recorded, clear sequestration of the nanoparticles by the micro-algae indicated a potential route to trophic transfer, thus uptake of nanoparticle-treated algae by mussel *Mytilus galloprovincialis* was investigated. Mussels were exposed to nanoparticle (2 mg L⁻¹) -loaded algae for 24 h and accumulation of nanoparticles was detected in hemolymph, gills and hepatopancreas. It was noted that the uptake of nanoparticles was significantly greater in the presence of algae than for nanoparticles on their own. A significant reduction in mussel total haemocyte count, and accumulation in gills (25 µg g⁻¹) and hepatopancreas (50 µg g⁻¹), was noted after nanoparticle treatment. Depuration of the nanoparticles from the mussels was tracked and over 96 h lanthanide concentrations in the pseudofaeces returned to near background levels. Ultimately, these data indicate that trophic transfer is an efficient route to bioaccumulation and potentially biomagnification of lanthanide nanoparticles and indicates that broader toxicity testing be carried out in a range of non-target organisms when lanthanides are used in zootechny.



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OP-32. In vitro Evaluation of Copper Ferro (II) Oxide Nanoparticles Induced Neurotoxicity

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Copper-based (Cu-based) nanoparticles are frequently preferred nanomaterials due to their physicochemical properties. The use of copper as the metal in the spinel ferrite (copper Ferro (II) oxide, CuFe_2O_4) increases the electromagnetic properties of the composite, leading to wide applications in electronics and communication, medical devices, sensors, and catalyzers. Previous data approved the accumulation of Cu-based NPs in different organs such as the respiratory system, liver, and brain. Studies reported that Cu-based NPs can cause DNA damage, oxidative stress, cell death, and apoptosis in different cell lines. However, studies related to CuFe_2O_4 induced toxicity are still insufficient and no study evaluated their neurotoxicity. In the current study, SH-SY5Y human neuroblastoma cells and Neuro-2a mice neuroblastoma cells were treated with characterized (29.5 ± 8.5 nm) CuFe_2O_4 NPs at different concentrations for 24 hours. After the intake of NPs into the cell was detected (≥ 172.2 ng/ 10^6 cells), the cytotoxicity, genotoxicity, and oxidative damage endpoints were estimated by known assays. Results show that CuFe_2O_4 NPs induced cell death in dose dependent manner, that the IC_{50} calculated at 31.4 $\mu\text{g/mL}$ and 68.7 $\mu\text{g/mL}$, in SH-SY5Y and Neuro-2a, respectively, Annexin V assay results indicate necrosis as the main cell death pathway in Neuro-2a cells and the low concentrations in SH-SY5Y cells. Comet assay results show that the increase in the tail moment ratio was more than 2.5-fold in Neuro-2a and more than 8.5-fold in SH-SY5Y cells. While CuFe_2O_4 NPs significantly change glutathione (GSH) and malonaldehyde (MDA) levels and Catalase (CAT) and Superoxide dismutase (SOD) activities in all concentrations in SH-SY5Y cells, only the high concentration changes the MDA level and CAT activity in Neuro-2a cells. The results highlight the importance of scanning the toxicity of nanomaterials in general and CuFe_2O_4 NPs specifically; However, further studies are required to clarify the toxicity mechanisms.



OP-33. Medroxyprogesterone Acetate And Ethodolac Exposure During Pregnancy

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Studies in animals have shown that progestogens, including MPA, can have an adverse effect on the developing fetus, including teratogenicity and fetotoxicity. Nonsteroidal anti-inflammatory drugs (NSAIDs) can impair women's fertility; Discontinuation of NSAID therapy should be considered in women who have difficulty conceiving or are investigated for infertility. This case is a report describing the development of omphalocele in the fetus as a result of the use of medroxyprogesterone acetate (MPA) and etodolac during pregnancy. It was observed that omphalocele developed in the fetus in the curettage material taken. Although firm conclusions cannot be drawn, this case report may add to existing data on the use of MPA and etodolac in pregnancy.

The 25-year-old female patient with an unplanned pregnancy and a history of two live births used MPA for 12 days starting from the 3rd gestational week and 12 days from the 5th gestational week. At the same time, the patient used etodolac for 5 days while her pregnancy was 3 weeks and 5 days old. As a result of the literature review of the patient who was referred to us from the Gynecology and Obstetrics Clinic, it was found that MPA was FDA pregnancy risk category X and etodolac was FDA pregnancy risk category C. Curettage was recommended to the patient by the Gynecology and Obstetrics Clinic due to the possible risks of MPA on pregnancy. It was observed that omphalocele developed in the fetus in the curettage material taken.

When the current literature on the use of MPA and etodolac in pregnancy is examined, it is seen that this case report is a human pregnancy outcome data showing that developed omphalocele in the fetus as a result of exposure to these drugs in the first trimester of pregnancy.



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OP-34. Microplastic Pollution in Plant Ecosystems

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Microplastics, plastic particles of size less than 5 mm, has increased exponentially in the last decade and have toxic effects in ecosystems. The vast majority of the research has focused on marine species and the marine environment but the plant ecosystem is also highly affected. Mechanisms of particulate plastics' influence on plants including changing soil physicochemical properties, changing rhizosphere environment, nutrient (im)mobilization, direct toxicity, bioturbation and decomposition by earthworms. The interaction of particulate plastics with environmental pollutants (i.e., heavy metal(loid)s and organic chemicals) include two aspects; adsorption/desorption of heavy metal(loid)s and organic pollutants by particulate plastics, and release of chemically active substances inherently contained in particulate plastics. Although in low numbers, a wider range of terrestrial species has been investigated, including plants used for human consumption such as wheat, garden onion and green onion, lettuce or maize. We need to establish efficient and rapid quantitative and qualitative methods for particulate plastic analysis in the soil and plants. And also understand the distribution characteristics and pollution levels of particulate plastics in the soil-plant system. Research on plant uptake of particulate plastics in the future, and reveal the mechanisms of plastic uptake by plants, such as the uptake pathways and uptake kinetics across different plant species and different types of particulate plastics. In the light of all this information, there is a need for future research in order to best manage the plastic films used and discarded, especially in agricultural areas, in order to reveal possible problems that may arise.



OP-35. Cerebral Oxidative Stress and Histological Changes Induced by Di (2-Ethyl Hexyl) Phthalate Exposure in Early Adolescent and Adolescent Periods in Rats

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Endocrine disrupting chemicals (EDCs) were defined as ‘the exogenous agents that interfere with synthesis, secretion, transport, metabolism, binding action, or elimination of hormones that are responsible for reproduction and developmental process’. Early life exposure to EDCs may affect future generations' health and increase the risk of childhood diseases. Many consumer products contain EDCs such as di (2-ethylhexyl) phthalate (DEHP). In this study, preadolescent Sprague Dawley male rats were exposed to 30 mg/kg/day (D1) and 60 mg/kg/day (D2) DEHP until the end of adolescence period and the toxicity of DEHP on brain tissues were evaluated in adulthood by measuring oxidative stress parameters (total glutathione and lipid peroxidation) and histological alterations. A significant increase in glutathione levels in brain tissues was found in the D1 group (51.3%) and in the D2 group (98.5%) possibly in response to the oxidative stress caused by DEHP. As compared to control, neither group showed any increase in lipid peroxidation. Histological alterations were observed in DEHP groups compared to control: At low doses, shrinkage of neurons, vacuoles in the cytoplasm of pyramidal neurons, pycnosis in oligodendrocytes, microglia cells, and especially in the high dose group, damaged neuron remnants and more microglia cells were determined. A decrease in myelinated axons was observed in treated groups. In the cerebellums of DEHP treated groups, indistinguishable cell extensions in the molecular layer, edema and congested vessels were observed. Edema, microglia, and glial cells with pycnotic nuclei were observed in the hippocampus of D1 and D2 groups. Pyramidal neuron loss was determined in the CA1 region of D1 group and CA1, CA2 and CA3 regions of D2 groups. The results indicate that DEHP exposure during early development may have a significant impact on brain toxicity. These effects of phthalate exposure on the organism highlight the need for more detailed studies.

Keywords: Di (2-ethyl hexyl) phthalate, oxidative stress, adolescent, histological effect, brain tissue



OP-36. Redox status changes in rat plasma induced by phthalate and bisphenol A mixture after subacute exposure

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This study aimed to assess/compare the changes in redox status parameters in rat plasma after 28-day exposure to three single endocrine disruptors (bis (2 –ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), and bisphenol A (BPA)) and their mixture. Male rats were randomly divided into 5 groups (6 animals): Control group (corn oil), DEHP group (50 mg/kg b.w./day), DBP group (50 mg/kg b.w./day), BPA group (25 mg/kg b.w./day) and MIX group (50 mg/kg b.w./day DEHP + 50 mg/kg b.w./day DBP + 25 mg/kg b.w./day BPA). At the end of the experiment, animals were euthanized and blood was extracted into heparin vacutainers for plasma separation. Superoxide dismutase activity (SOD), superoxide anion ($O_2^{\cdot-}$), total oxidative status (TOS), pro-oxidant-antioxidant balance (PAB), malondialdehyde (MDA), and sulfhydryl (SH) groups were measured. Additionally, oxidative damage score (ODS), antioxidative protection score (APS), and global score of oxidative balance (OXY) were calculated. A significant decrease in SOD activity occurred in MIX compared to the control, but also compared to DBP and BPA groups. An increase in MDA could be noted in DEHP and MIX group, while more prominent in MIX. A significant increase in TOS level was present only in MIX group (compared to the control). There was no significant change in any of the treated groups for SH groups, PAB and $O_2^{\cdot-}$. APS decrease occurred in MIX compared to the control, as well as compared to DBP and BPA groups. On the other hand, ODS and OXY increase could be seen in both MIX and DEHP groups (compared to the control), but more prominent in MIX. The obtained results demonstrated that DEHP/DBP/BPA mixture lead to more pronounced changes in redox status parameters in comparison with the single substances in rat plasma after the subacute exposure, while, in some cases, the effect of the mixture was directed by DEHP.

Keywords: plasticizers, mixture toxicity, endocrine disruptors, oxidative stress, antioxidative protection



OP-37. Determination of aflatoxin m1 levels in milk samples consumed in the Turkish Republic of Northern Cyprus

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AFM1 is the hydroxylated metabolite of AFB1, which is formed in the liver by cytochrome P450 enzymes and can be secreted into the urine, feces, and milk of mammals. AFM1 is a carcinogenic, cytotoxic, teratogenic, mutagenic and genotoxic agent that poses a significant health risk to both humans and animals. This study was conducted to determine the presence of AFM1 in both raw and UHT cow milk consumed in the Turkish Republic of Northern Cyprus (TRNC), and to determine whether it poses a risk to public health. In this survey, a total of 20 UHT cow milk samples from 2 separate milk brands produced in the TRNC and distributed in the market, and 22 raw cow milk samples collected from the different dairies were analyzed for the presence of AFM1 by high performance liquid chromatography (HPLC) with fluorescence detector after immunoaffinity cleanup. AFM1 could not be detected in any of the analyzed raw and UHT cow milk samples. The LOD and LOQ values of the HPLC-FLD method were 1.038 and 3.145 µg/L, respectively. The mean recovery and repeatability values of the method were 95.6% and 4.9%, respectively. Although the presence of AFM1 in milk samples consumed in the TRNC poses no major risk to public health, more milk samples and animal feed must be monitored on a regular basis to decrease potential consumer exposure.

Keywords: Mycotoxin, aflatoxin M1, milk, High Performance Liquid Chromatography (HPLC)



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Poster Presentations





PP-01. Evaluation of The Cytotoxic, Genotoxic and Apoptotic Effects of Genistein and 5-Fluorouracil Combined with TRAIL in SW620 Cells

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Colorectal cancer continues to be a cause of morbidity and mortality with a 9% share of cancer incidence. It is the third most common cancer and the fourth most common cause of death worldwide (1,2). Although chemotherapeutic treatment regimens are the basic step in cancer treatment, the re-development and progression of the tumor due to drug resistance, the cellular damage of chemotherapeutic drugs not only in cancer cells but also in normal cells, and the side effects such as nausea, vomiting and fatigue have led to increased need of the development of different treatment principles (3-5). For this reason, it is aimed to evaluate the cytotoxic, genotoxic and apoptotic effects of genistein in combination with 5-fluorouracil and TRAIL ligand in SW620 cells to contribute to the solving the drug resistance problem in colorectal cancer treatment.

Methods: Cytotoxicity was investigated by MTT method and genotoxic effects were investigated by Comet method. Reactive oxygen species (ROS), mitochondrial membrane potential and caspase 3-8-9 activities were investigated to illuminate the mechanism. Additionally, the apoptotic effects were determined real time polymerase chain reaction (RT – PCR) method.

Results: Our results showed us that the cytotoxic, genotoxic and apoptotic effects of genistein, 5-fluorouracil and TRAIL in combination are more than they were alone and they show synergistic effects together. It has been observed that the increase in ROS, decrease in mitochondrial membrane potential and increase in caspase 3, 8, 9 activities may play a role in the occurrence of these effects. Also, it has been determined that the applied combinations can contribute to the resistance problem that may occur in the treatment of colorectal cancer, with the decrease in DcR1 and XIAP genes.

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PP-02. Determination of Deoxynivalenol in Commercially Available Cereals from Turkey By HPLC

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Purpose: Cereals, the first plant species cultivated by humans, have been a staple food for many human societies for over ten thousand years (1). However, some fungal species that can infect cereals from production to processing produce toxic by products called mycotoxins (2, 3). Mycotoxins, known mainly for their teratogenic, carcinogenic, hepatotoxic effects, are known as aflatoxins, ochratoxin A, fumonisins, deoxynivalenol and zearalenone (4). The aim of this study is to determine the deoxynivalenol (DON) levels in cereals sold in Turkey by using HPLC via UV/Visible detector. Thus, it has been ensured to contribute to the taking of measures to reduce consumer health risks.

Methods: 96 commercially available cereal samples collected from markets (branded products) and street bazaars in Istanbul between September and October of 2020 were analyzed. 24 of these samples are ashoura wheat, 24 of them are rice, 24 of them are corn flour and 24 of them are whole wheat flour. After determination of deoxynivalenol levels in these samples via HPLC-UV/Vis, LC-MS analysis was used for confirmation.

Results: In 25 samples, DON was detected by HPLC analysis (LOD-LOQ, 0.28-0.92 µg / kg). The recovery levels for the known amounts of DON (1 ppm) were found as 96% (SD 5.10, *n* = 5). In addition to these results, in the confirmation analysis done by LC-MS, DON could not be detected in these 25 samples.



PP-03. Effects of Acrylamide, Hydroxymethyl Furfural and Caffeic Acid on DNA damage in HepG2 Cells

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Acrylamide (2-propenamide, AA) and 5-hydroxymethyl furfural (HMF) are compounds that occur with heating process in foods with carbohydrate. Maillard reaction is the main reaction that causes these compounds. These two compounds have risks on human health according to findings of recent researches. According to the International Agency for Research on Cancer (IARC) (Group 2A) classification, AA is possible carcinogenic compound for humans. The results from some epidemiological studies showed that coffee consumption is critical for exposure to these two risky compounds. Despite high exposure, a causality between coffee consumption and various cancers has not been established. Although roasted coffee beans contain AA and HMF, some compounds that present in this plant naturally are thought as beneficial. Caffeic acid is one of the compounds that has been shown in studies that it has antibacterial, antiviral, anti-inflammatory, antiatherosclerotic, immunostimulant, antidiabetic, cardioprotective, antiproliferative, hepatoprotective, antioxidant and anticancer effects. Even if these positive effects, some studies have focused on harmful effects of caffeic acid, also caffeic acid has been classified as 2B (possible carcinogen) by the IARC.

In our study, it is aimed to investigate the possible genotoxic effect of AA and HMF with/without CA in human hepatocellular carcinoma cell lines (HepG2) by single cell gel electrophoresis (COMET) which is used to detect DNA damage. Tail intensity was used as genotoxicity marker. According to our results the doses of AA (1, 5, 10, 25 and 50 µM) and HMF (1, 25, 50 and 100 µM) did not increase DNA damage alone. DNA damage was decreased when CA is applied alone at the doses of 25 and 50 µM to HepG2 cells. The toxic effects of AA and HMF were potentialized when combined with CA.

Keywords: Acrylamide, 5-hydroxymethyl furfural, HepG2 cells, DNA damage, Comet Assay



PP-04. DNA Damage and Oxidative Stress Response in The Human Neuroblastoma SH-SY5Y Cell Line Exposed to Clinically Relevant doses of Ketamine

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Introduction: Ketamine is a dissociative anaesthetic used to induce general anaesthesia in certain medical procedures. Considering the dissociative and sedative properties of ketamine, it has also been used as a recreational drug in a variety of social settings. Ketamine abuse displays neurobehavioral alterations such as depression, anxiety, cognition deficits, and schizophrenia. While the exact mechanism is still unclear, some findings suggest that oxidative stress and genotoxic properties may be implicated in ketamine-induced neurological impairment.

Methods: The aim of this study was to explore the effects of exposure to ketamine depending on selected concentrations used during general anaesthesia, analgesia or drug-abuse available from the literature. Human neuroblastoma SH-SY5Y cells were treated with ketamine at concentrations of 0.09, 0.37 and 1.49 mg/L. Cell viability was studied using the MTS assay. We measured biomarkers of oxidative stress [malondialdehyde (MDA), reactive oxygen species (ROS) production and glutathione (GSH) levels] and primary DNA damage (using the alkaline comet assay) as the endpoints that can be disrupted following ketamine use.

Results: After a 24-hour treatment, the applied concentrations reduced cell viability up to 30 %. Although no changes in the concentration of MDA and GSH were observed, a significant decrease in ROS levels was determined after exposure to 0.37 and 0.09 mg/L of ketamine in comparison to control cells. Ketamine treatment at all of the tested concentrations resulted in a significant increase of primary DNA damage versus negative control. However, no significant differences between the applied concentrations were found.

Conclusion: Considering that SH-SY5Y cells originated from neural tissue, the findings regarding genotoxic potency of ketamine *in vitro* call for concern, especially in light of the possible neurotoxic effects that may be relevant in *in vivo* conditions. Their extent and outcomes could be further elucidated using other molecular biology and cytogenetic methods and experimental models.

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Keywords: ketamine, neurotoxicity, DNA damage, oxidative response



PP-05. Use of Human Neuroblastoma SH-SY5Y Cells to Evaluate Cytotoxic, Genotoxic and Oxidative Stress-Induced Effects of Codeine and Morphine

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Codeine and morphine are alkaloids from the opioid family widely used as pain relief drugs or antitussive cough suppressant. Both drugs are found naturally in the poppy plant, *Papaver somniferum*. If misused or abused, neurobehavioral alterations, respiratory depression, constipation, sedation, tolerance, nausea, vomiting, itch, dry mouth and addiction may occur. While the current knowledge related to toxic effects of these opioids is limited, the aim of this study was to determine if they could induce a DNA damaging effect and oxidative stress response in human neuroblastoma SH-SY5Y cells. 24-h treatment with morphine and codeine at 1.56 $\mu\text{mol/L}$ resulted with 80.84 ± 4.14 and 85.82 ± 3.85 % viable cells, respectively. These concentrations allowed genotoxicity testing using the alkaline comet assay, which resulted in a significant increase of primary DNA damage after both treatments versus negative control (median tail intensity 0.22 % DNA). At the same tested concentration, codeine exerted a slightly higher genotoxic potency towards SH-SY5Y cells (median tail intensity 2.25 %) than morphine (median tail intensity 1.07 % DNA). Differences in the observed DNA damage pattern could be attributed to specific interactions of the tested compounds with DNA, which affect their behavior during alkaline denaturation and electrophoresis. Codeine induced a significant decrease in glutathione (GSH) and a significant increase in the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), whereas morphine induced a significant decrease in reactive oxygen species (ROS) production and SOD, and a significant increase in GPx and CAT activity when compared to controls. There was no significant impact of the investigated drugs on the levels of lipid peroxidation (MDA). Our results warrant further investigation to obtain insights into the mechanisms of toxic effects of codeine and morphine.

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PP-06. Evaluation of The Use of Nanotechnology in Pharmaceutical Applications and The Toxic Effects of Nanoparticles

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Nanotechnology is a term refers to the use of nano-structures in technological fields. With the use of nanotechnology in medicine and pharmaceuticals, the concepts of nanomedicine and nanopharmaceuticals have entered our lives. Studies show that nanopharmaceuticals are promising in increasing the efficiency of diagnosis and treatment and reducing side effects. Though, the toxicity of nanopharmaceuticals is an important problem. The variable physicochemical properties of nanostructures have a direct effect on their toxic properties. In scope of this study, it was aimed to review the literature outcomes about toxic affects of certain types of nanoparticles commonly used as components of nanopharmaceuticals; additionally it was intended to detect the relationship between the nanoparticle types, certain physicochemical properties and their possible toxic affects. However, it was detected that, there are many researches about toxicities of a great number of nanoparticles and the physicochemical properties of these particles and testing conditions are also in a wide range, as a result the toxicity profiles vary. Additionally, it was determined that nanopharmaceutical-specific standards have not been defined by national or international regulatory authorities for characterization and toxicity tests. This explains the reason for the variations among the study results in the literature. As a result; based on the current data, to classify the nanoparticle types and to set a relationship between their physicochemical properties and possible toxic effects was not possible. On the other hand, it was detected that the number of the researches focus on the toxicity assessment of inorganic, organic and carbon-based nanoparticles decrease respectively and the most reported toxic effect of the inorganic particles was cytotoxicity. To have a reliable toxicity data, robust nanoparticle characterization and toxicity assessment methods should be designed and standardized by the regulatory authorities.

Keywords: nanomedicine, nanotechnology, toxic effects of nanoparticles, cytotoxic effects of nanoparticles.



PP-07. Toddlers' Overall Daily Exposure to Trace Elements in Indoor Dust

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The ubiquitous presence of trace elements in indoor and outdoor environments and their toxicity represent a threat to public health even at low levels of exposure and in particular to vulnerable population groups such as children. Indoor dust is an important part of daily exposure to toxic elements, especially given the amount of time spent indoors nowadays.

In dust samples obtained from kindergartens (n=10) and cars (n=21) in the city of Zagreb, Croatia concentrations of 18 elements using ICP-MS were determined followed by an estimation of element exposure rates for toddlers (2-6 years of age) through ingestion, inhalation and dermal absorption of dust at central and worst case scenarios considering three different microenvironments where children spend most of their time – the home, kindergarten, and car. Calculations were made based on a time-weighted model that integrates the time fraction spent in each microenvironment (14 h, 7 h, and 1 h a day, respectively).

In the central case scenario, daily intake of dust from homes contributes the most to the overall exposure to trace elements, with the largest proportion of 64 % calculated for Al, Se and Sr. In the worst-case scenarios, the highest contribution for Co, Cr, Cu, Mo and Sn was from intake of car dust (from 53 % for Cr to 95 % for Mo), because of the high concentrations measured in certain cars. Cars represent a microenvironment with potentially high levels of exposure to traffic-related pollutants. Oral intake was identified as the most important exposure pathway for trace elements from dust, except for Cr, Ni and Sb where dermal contact was the main route of exposure. A health risk assessment considering dust ingestion, inhalation and dermal absorption of analyzed dust indicated that no adverse health effects are expected from the overall exposure to trace elements.

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PP-08. Exposure of Breastfed Infants from Croatia to Polybrominated Diphenyl Ethers and Polychlorinated Biphenyls

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The benefits of breastfeeding are undeniable; however, it is known that human milk is a significant exposure route of newborns to persistent lipophilic chemicals, such as polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs). Despite bans on the use of both classes of compounds, they are still ubiquitous in the environment and can cause adverse health outcomes in humans, especially infants.

The levels of seven most frequently analyzed PBDEs and seven indicator PCBs were measured in human milk samples (N=18) collected in 2020 from primipara women living in Zagreb, Croatia. Microwave-assisted extraction was used as the extraction technique, and purified extracts were analyzed using GC-ECD.

All of the targeted PCBs were detected in all of the analyzed milk samples (\sum PCB ranged between 15.03 and 71.00 ng/g lw, median: 31.32 ng/g lw), while BDE-153 was the only PBDE compound detected in all samples, and BDE-28 and -183 were not detected at all. \sum PBDE ranged from 0.20 to 6.29 ng/g lw (median: 0.67 ng/g lw). The obtained mass fractions were used to calculate estimated daily intakes (EDI) for breastfeeding infants, and consequently, they were significantly higher for PCBs. The EDIs of \sum PCB ranged from 87.07 to 449.64 ng kg⁻¹ bw day⁻¹ (median: 165.59 ng kg⁻¹ bw day⁻¹), and from 0.77 to 40.84 ng kg⁻¹ bw day⁻¹ (median: 3.60 ng kg⁻¹ bw day⁻¹) for \sum PBDE. Additionally, the risk quotients for BDE-47, -99 and -153 were <1 in all samples, indicating that the breastfeed infants in this area were not at a health risk from exposure to PBDEs from human milk. \sum PCB > \sum PBDE clearly indicates a greater persistence and bioavailability of PCBs, compounds that were banned before any of the study participants were even born. Current PCB levels are very similar to those observed in the same area ten years earlier, which indicates the achievement of steady state concentrations of PCBs in human milk.

This work has been supported in part by the Croatian Science Foundation under project HrZZ-UIP-2017-05-6713.



PP-09. Developmental Parameters in Placentas of Female Rats Exposed to α -Cypermethrin During Gestation

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The placenta is a temporary multifunctional organ with a key role in the maintenance of pregnancy and necessary for foetal growth and development. Exposure to endocrine disruptive chemicals (EDCs) during vulnerable period of life such as pregnancy can affect placental development and function as well as pregnancy outcome. Due to the high abundance of hormone receptors, the placenta is a highly sensitive organ to EDCs. Pyrethroids are the fourth most used group of insecticides worldwide, with proven endocrine disruptive effects. Alpha-cypermethrin (α -cyp) is a type II synthetic pyrethroid insecticide whose effect as an EDC on placental development has still not been explored. Thus, the aim of this investigation was to evaluate the effects of gestational exposure to α -cyp on developmental parameters in placentas of female rats by determination and quantification of protein expression of proliferating and apoptotic markers using an immunohistochemical method and assessment of histopathological changes by classical histopathological analyses. Pregnant Wistar rats were orally exposed to α -cyp at 1, 10 and 19 mg/kg bw/day, diethylstilbestrol (positive control), corn oil (solvent control) and water (negative control) from the 6th to the 21st day of gestation (DG). The gravid uterus was dissected under general anaesthesia at 21st DG and placentas were sampled. Immunohistochemical analysis of phosphorylated histone H3 expression revealed a statistically significant decrease in cell proliferation in α -cyp treated groups at 10 and 19 mg/kg bw/day. Analysis of the expression of cleaved caspase-3 showed that, at the tested concentrations, α -cyp does not cause apoptosis. Numerous histopathological changes were detected in placentas of rats exposed to α -cyp, but their occurrence was not statistically significant in comparison with controls. Further research is needed to better understand the effects of α -cypermethrin as an endocrine disruptor on developmental parameters in placentas of female rats exposed during gestation.

Keywords: pyrethroid, endocrine disruptive chemicals, placental development, immunohistochemistry, histopathology



PP-10. Effects of Thymoquinone and Etoposide Combination on Cell Viability and Genotoxicity in Human Cervical Carcinoma Cells

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Thymoquinone might have an important role in preventing DNA damage, regulating DNA repair mechanisms, and inhibiting carcinogenesis. Studies on the cytotoxic and genotoxic effects of thymoquinone together with etoposide in cervical cancer are insufficient. Our study aimed to evaluate the effect of combinations with thymoquinone on etoposide cytotoxicity and genotoxicity in cervical cancer (HeLa) cells. Cytotoxicity and genotoxicity were evaluated by MTT assay and alkaline comet technique, respectively. The IC₅₀ values of thymoquinone were 233.6 µM and 145.5 µM, the IC₅₀ values of etoposide were 167.3 µM and 52.7 µM; for 24 and 48 h, respectively. Thymoquinone significantly decreased the approximate IC₅₀ value of etoposide in doses of 15.63 µM and above for 24 h and 31.5 µM and above for 48 h in a dose-dependent manner. 0.1-5 µM thymoquinone and 1 µM etoposide alone did not cause DNA damage, but at higher concentrations increased DNA damage significantly in a dose-dependent manner. Thymoquinone significantly reduced DNA damage induced by 10 µM etoposide at the concentrations of 0.1-10 µM. Our results show that thymoquinone might increase the cytotoxic and genotoxic effects of etoposide in HeLa cells at high doses and reduce DNA damage at low doses that are not cytotoxic, which suggests that etoposide may increase its anticancer effect at high doses, but comprehensive studies are needed on this subject. This study is a preliminary study and will contribute to the development of new treatment strategies.

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Keywords: *Thymoquinone; etoposide; cytotoxicity; genotoxicity; comet assay; HeLa cells*



PP-11. Optimising the Conditions For *S*-Phenylmercapturic Acid Analysis in Urine Using Gas Chromatography-Mass Spectrometry

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Benzene is an aromatic compound with cancerogenic properties to which people, except in professional settings where various exposures may be present, are exposed by cigarette smoke or motor vehicle exhaust. *S*-phenylmercapturic acid (*S*-PMA) is considered a specific marker of benzene exposure in the general and occupationally exposed population. For occupational exposure, the European Chemical Agency (ECHA) recommended an *S*-PMA biological limit value of 2 µg/g creatinine. The aims of this study were to optimise the conditions of *S*-PMA extraction from urine and to develop and validate a sensitive and specific analytical method for determining the mass concentration of *S*-PMA in urine using gas chromatography-mass spectrometry (GC-MS). Factors that affect the extraction efficiency (sample volume, pH value and volume of ethyl acetate) and the derivatisation of *S*-PMA (type and amount of derivatisation agent, temperature and time of reaction) were examined. Optimised conditions included extraction of 1 mL urine with 4 mL ethyl acetate at pH 2 and derivatisation with 1 mL of 1.25 M hydrochloric acid in methanol at 50 °C for 15 minutes. The proposed GC-MS method showed good linearity ($R^2 > 0.9996$) in the examined concentration range (0.5–100 µg/L), good precision (RSD < 7%), accuracy (> 95%) and sensitivity (limit of detection: 0.03 µg/L and limit of quantification: 0.09 µg/L). The method was applied to determine the mass concentration of *S*-PMA in the urine of 20 subjects who were not occupationally exposed to benzene. Smokers (N = 10, mean: 0.8 µg/g creatinine; range: 0.3–5.8 µg/g creatinine) had a significantly higher ($p = 0.014$) concentration of *S*-PMA in urine compared to non-smokers (N = 10, mean: 0.2 µg/g creatinine; range: 0.1–2.3 µg/g creatinine). The described method permits sensitive, fast and reliable determination of low µg/L *S*-PMA levels in urine.



PP-12. Evaluation of Oxidative Stress Response and Primary DNA Damage in HepG2 Cell Line Exposed to Ketamine

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Introduction: Ketamine is a dissociative anaesthetic widely used in clinical practice, while its sub-anaesthetic administration has been explored for treatment of depression and chronic pain. Due to its hallucinogenic and dissociative effects, ketamine is also used as a recreational drug. Agents used for the induction of anaesthesia have been shown to promote oxidative stress, which has been implicated in the aetiology of various chronic diseases. Research of oxidative/antioxidative properties of ketamine has so far produced ambiguous findings, while data on genotoxicity are insufficient to reach a conclusion on its genotoxic potential.

Methods: In order to investigate the unexplored effects of ketamine on cell level, hepatocytes HepG2 were exposed to ketamine at concentrations of 0.09, 0.37 and 1.49 mg/L for 24 h, relevant in case of analgesia, anesthesia or drug-abuse. Cell viability was studied using the MTS assay. Ketamine toxicity was further evaluated using markers of DNA damage and oxidative stress according to a specific method protocol. Lipid peroxidation level [malondialdehyde (MDA)], reactive oxygen species (ROS) production and glutathione (GSH) levels were measured as biomarkers of oxidative stress, while primary DNA damage was determined using the alkaline comet assay.

Results: The selected concentrations of ketamine did not decrease cell viability below 90 %. Although no changes in ROS levels were visible after the treatment, a significant decrease in concentration of MDA and GSH was observed at all three ketamine concentrations in comparison to control cells. Ketamine treatment at all of the tested concentrations resulted in a significant increase of primary DNA damage versus the negative control. We also found significant concentration-dependent differences in DNA damage after exposure to ketamine. The observed DNA damage pattern could be associated with specific interactions of ketamine and/or its metabolites with DNA (most likely intercalations), as well as with a repair-induced onset of additional breaks, which affected the amounts of lesions detectable by alkaline comet assay.

Conclusion: The findings regarding genotoxic potency of ketamine detected *in vitro*, together with the observed imbalance in the oxidative-antioxidant equilibrium detected in HepG2 cells call for additional studies on a wider range of doses and other experimental models. Future studies at DNA level should focus on the use of specific modifications of the comet assay and related methods that will prove the presumed mechanisms of interaction of the tested substance with the DNA molecule.

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For a safe and healthy world

PP-13. Genotoxicity of Metal-Based Nanoparticles in Human Salivary Leucocytes

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The use of metal-based nanoparticles (NP) is widely extended since they have a wide variety of applications in consumer products and biomedical practices. As a result, human exposure to these nanomaterials is frequent, becoming a concern to public health. Human salivary leucocytes have been proposed as a proper biological sample for the comet assay, becoming an adequate non-invasive alternative model to evaluate DNA damage *in vitro*. In the present study, the suitability of salivary leucocytes to be employed for *in vitro* nanogenotoxicity studies was addressed by testing some of the metal-based NP most frequently present in consumer products, namely titanium dioxide (TiO₂), zinc oxide (ZnO), and cerium oxide (CeO₂) NP. Alkaline version of comet assay was carried out to evaluate primary genetic damage, whereas hOGG1-modified comet assay was employed for oxidative DNA damage evaluation. Possible interferences of the NP with the methodological procedure or the hOGG1 activity were analysed prior to any additional experiments. Treatments with the three NP tested showed a dose-dependent increase of both primary and oxidative DNA damage. Together with contributing to increase the knowledge on the impact of metal-based NP on human health, data from this study support the use of salivary leucocytes as a proper and sensitive biomatrix for *in vitro* nanogenotoxicity studies, as well as reinforce the importance of testing the suitability of standard toxicology protocols for hazard assessment of nanomaterials.

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PP-14. Review and Comparison of EU and TR Safety and Testing Guidelines on Cosmetics

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Purpose: The EU regulations are accepted as the world standard on the testing and safety assessment of cosmetic ingredients. In order to provide harmonization between EU and TR regulation to access EU common market with no obstacle, a comparison was necessary.

Methods: In this study, 11th revision of the SCCS guideline have been reviewed, summarized and have been brought to a conclusion. Also, a comparison has been made by comparing and highlighting the differences between SCCS guideline and TMMDA guideline.

Results: As a result of this study, some points in TMMDA guideline that needs an update has been indicated. Some examples on these points are as following;

- TMMDA guideline lacks in silico methods on genotoxicity and carcinogenicity,
- TMMDA guideline does not mention New Approach Methodology and Next-Generation Risk Assessment,
- TMMDA guideline does not mention about properties of endocrine disruptors,
- Lack of information about Good Manufacturing Practice while providing information on the Microbiological Quality of the Finished Cosmetic Product in the TMMDA guideline,
- TMMDA guideline does not provide information on compliance with testing and marketing bans,
- Specifically, there is no information about nanomaterials in general cosmetic guideline of TMMDA in spite of there is a separate guideline on nanomaterial-included cosmetics. However, there is information about nanomaterials in both general cosmetic guideline of SCCS and specific guideline related to nanomaterial-included cosmetics,
- Lack of information on Threshold of Toxicological Concern and internal Threshold of Toxicological Concern in the TMMDA guideline,
- TMMDA guideline only mentions mathematical methods while SCCS guideline has been providing both deterministic and probabilistic methods on inhalation models.

Keywords: Regulatory Toxicology, Cosmetics, SCCS, TMMDA, Safety Guidelines, Cosmetic Safety Assessment



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PP-15. Evaluation of Vitamin D Prescribing and Consumption in Turkey

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Vitamin D plays several roles in keeping body's cells healthy and functioning the way they should. Most people don't get enough vitamin D, so supplements are common. However, it's also possible for this vitamin to build up and reach toxic levels in body. In this study the preparation containing Vitamin D and analogs prescription in primary care and outpatient consumption in Turkey between the years of 2015- 2018 of the situation was investigated. Also the number of protocols created with Hypervitaminosis D were evaluated.

In this study, drug consumption data evaluated by Prescription Information System (PIS) and ATC/DDD (Anatomical, Therapeutic and Chem- Defined Daily Dose) Methodology. Descriptive statistics were used for evaluation of the data, and numerical values were presented via tables and graphics. Statistical analysis of the data was performed using the SPSS 23 (Statistical Package for the Social Sciences) package program. Chi-square test was used to evaluate the relationship between the variables and the P value below 0.05 was accepted as statistical significance.

Between 2015 and 2018 the total number and the percentage of prescriptions containing A11CC (Vitamin D and Analogs) ATC coded drugs created by family physicians increased over the years. The consumption of the drugs with A11CC ATC code in outpatients increased over the years according to the ATC/DDD methodology. Also there was a statistically significant difference between the number of Hypervitaminosis D protocols over the years.

One of the limitations of the study was that the prescription of the drugs containing Vitamin D and its analogues was evaluated only in primary care physician in this study. In addition, in this study, no evaluation was made about the usage of the prescribed drugs by the patients.



PP-16. Evaluation of Metals and Organic Chlorinated Pesticide Residue Levels in Medicinal Herbal Products

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Medicinal herbal plants have a long history of use in therapy throughout the world and still make an important part of traditional medicine. In addition, The World Health Organization (WHO) states that the interest in medicinal herbal products continues to increase. On the other hand, consumption of these products contaminated with metals and/or some organic chlorinated pesticide (OCP) residues can cause serious consequences on human health. This situation is a major concern for traditional and herbal medicine. In the present study, we aimed to determine levels of metals and some organic chlorinated pesticide (OCP) residues in some products which are marketed in twenty medical herbal products in different regions within Ankara. For this purpose, levels of metals (As, Cd, Cr, Cu, Fe, Pb, Se, Zn) and OCP residues (α -BHC, β -HCH, γ -BHC, 2,4'-DDE, 4,4'-DDE, 4,4'-DDD, 2,4'-DDT, 4,4'-DDT) were analyzed by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Gas Chromatography Electron Capture Detector (GC-ECD), respectively. Our results showed that Cd, Cr and Fe levels were higher compared with EHO standards OCP residues were not detected in medicinal herbal plants. The present work implies that, regular and systematic screening of raw medicinal herbs is important for the determination and control of the levels of element or/and OCP residue contaminants.

Keywords: Herbal Medicine, Toxic Metal, Organochlorine Pesticide, GC-ECD, ICP-MS



PP-17. Investigation of Effects of Oral Anti-Diabetics on Lipid Peroxidation and Enzymatic Defense System.

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Aim: T2DM is a metabolic disorder which characterised by hyperglycemia and first-choice treatment procedure is application of oral anti-diabetics, such as metformin (MET) and vildagliptin (VILDA). The main purpose of this study is to find out anti-diabetics' effects in both monotherapy and combination therapy on oxidative stress and enzymatic defense system.

Method: Adult female Sprague-Dawley rats were used. T2DM was induced by single dose i.p. administration of 80 mg/kg STZ. Treatment group was treated by 100 mg/kg/day MET and 6 mg/kg/day VILDA for 8 days. Erythrocyte and tissue samples were used for determination of malondialdehyde (MDA), glutathione peroxidase (GPx) and catalase (CAT) activities by spectrophotometric method.

Results: Erythrocyte MDA in MET/VILDA were significantly higher than control, MET and VILDA however, in VILDA; were significantly lower than MET/VILDA and MET. Also, GPx and CAT in VILDA were significantly higher than control, MET and MET/VILDA; GPx in MET was significantly lower than VILDA and MET/VILDA.

Conclusion: VILDA was found the most effective to decrease lipid peroxidation by enhancing GPx and CAT enzymes. Also, it was observed that MET is more effective on CAT than GPx but lower than VILDA. The highest MDA was observed in combination which may caused by reduced activity of CAT and GPx. VILDA alert the GPx and CAT activity to reduce oxidative stress. Combining vildagliptin with another anti-diabetic may be more proper to reduce oxidative stress. In order to strengthen these conclusions, studies on CuZn-SOD activities should be continued.

Acknowledgement: This study supported by T.C. Sağlık Bakanlığı Pendik Devlet Hastanesi, İstanbul, Turkey.

Keywords: Antidiabetic, antioxidant, lipid peroxidation, combined antidiabetic



PP-18. Investigation of Effects of Oral Anti-Diabetics on Telomerase (TE) Activity in Rats.

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Aim: T2DM is a metabolic disorder which characterised by chronic oxidative stress and oral antidiabetics such as metformin (MET) and vildagliptin (VILDA) is the first-choice treatment. Recently, MET was reported as a possible anticatalyst for the aging process related to telomerase enzyme and telomere length. MET increased telomerase activity by decreasing the oxidative stress level caused by T2DM. Therefore, the main purpose of this study is to find out anti-diabetics' effects as in both monotherapy and combination therapy on aging process associated with oxidative stress.

Method: Adult female Spraque-Dawley rats were used. T2DM was induced by single dose i.p. administration of 80 mg/kg STZ. Treatment group was treated by 100 mg/kg/day MET and 6 mg/kg/day VILDA for 8 days. Telomerase enzyme (TE) activity was measured using Rat-Telomerase ELISA Kit in serum samples of rats.

Results: TE activity is significantly higher in MET and VILDA monotherapy groups than control group. Effects of MET monotherapy on TE activity is significantly higher than VILDA monotherapy. In combination therapy, TE activity is significantly lower than control and monotherapy groups.

Conclusion: MET was found more effective on TE activity by reducing the oxidative stress caused by T2DM. VILDA also increased the TE activity but lower than MET. Also, it has been observed that combination of these two oral antidiabetics significantly reduced TE activity compared to the both control and monotherapy groups. Therefore, it can be concluded that; VILDA increased TE activity as MET however, binary combination reduced TE activity.

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Keywords: antidiabetics, oxidative stress, aging, telomerase,



PP-19. Possible Effects of Hexahydroquinoline Derivatives on Cytotoxicity and Intracellular Ros Production On HEPG2 Cell Line

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Purpose: Recently hexahydroquinoline (HHQ) derivatives, which were obtained by condensing 1,4-dihydropyridine (1,4-DHP) ring and cyclohexane ring with the Hantzsch reaction in this study, were shown to exhibit antioxidant properties *via* inhibiting reactive oxygen species (ROS) (1). These compounds are also suggested to improve endothelial function and prevent atherosclerosis (2).

Methods: Seven compounds having alkyl 2,6,6-trimethyl-5-oxo-4-(1-phenylethyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate and alkyl 4-benzyl-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate structure were synthesized to determine the effect of the carbon chain between main structure and phenyl group at 4th position of HHQ ring on biological activity in contrast to previously synthesized HHQ derivatives. The structures of the compounds were elucidated by spectral methods (IR, 1H-NMR and HRMS). MTT proliferation and viability test was employed to identify the cytotoxic properties of these compounds in HepG2 cells as liver is the primary target organ for many drugs. The effects of seven compounds on intracellular ROS production were also investigated.

Results: For MTT assay, these compounds were applied to HepG2 cells at a concentration range of 5 - 200 µM. MTT results showed that inhibitor concentration 50 (IC₅₀) values of these seven compounds were between 76 – 317.3 µM. Two compounds having benzyl and phenylethyl groups at 4th position of the HHQ ring caused significant and similar increases in ROS production vs. control. We demonstrated that novel compounds have cytotoxic effects at very high doses compared to the human blood concentrations of their available drug analogues. Further studies on different cell types should be conducted to better understand their antioxidant, potential anti-inflammatory and anti-atherosclerotic effects.

Keywords: hexahydroquinoline, ROS, MTT, cytotoxicity, synthesis

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PP-20. Alterations on Global DNA Methylation In Sh-Sy5y Cells in Response to Neonicotinoid Insecticides of Imidacloprid and Thiamethoxam

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Neonicotinoid pesticides, an alternative to organophosphates, have been widely used in recent years. However, many studies showed neonicotinoids induced toxic effects in various tissues especially in the nervous system and the underlying mechanisms of the neurotoxic effects have not been elucidated yet. Therefore, we aimed to investigate the non-genotoxic effects of neonicotinoid insecticides of imidacloprid and thiamethoxam in human neuroblastoma (SH-SY5Y) cells. The cells were exposed to imidacloprid and thiamethoxam in concentrations of 100, 200, and 500 μ M for 24 hours, then global DNA methylation and expression of genes involved in global DNA methylation (*DNMT1*, *DNMT3a* and *DNMT3b*) were investigated. Global DNA methylation significantly increased after imidacloprid exposure at 100 μ M, and thiamethoxam exposures at 200 μ M and 500 μ M (>1.5-folds). Imidacloprid significantly decreased the expression of *DNMT1* and *DNMT3a*, whereas thiamethoxam did not cause any significant changes in the expression of *DNMT* genes. Our findings suggested that imidacloprid and thiamethoxam-induced neurotoxic effects may be a consequence of epigenetic alterations.

Acknowledgement: This work was supported by Scientific Research Projects Coordination Unit of Istanbul University (Project numbers: 35359).

Keywords: Imidacloprid; Thiamethoxam; Global DNA methylation; *DNA methyltransferase* genes; SH-SY5Y cells.



PP-21. Heavy Metal Content in Children's Finger Paint

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Introduction-Objective: Finger paint can be expressed as paints that are in a very fluid state and that children make by rubbing on their fingers and using their fingers on a paper or a floor. Heavy Metals are elements that can have a toxic effect even at low concentrations, and in general, toxic-effective elements taken with industrial and industrial products that are not required by the body are meant. Heavy metals cause adverse effects on neurological development and cognitive functions, especially in childhood. The aim of this study; to evaluate the safety of marketed finger paint products for children by investigating the heavy metal (Arsenic, Cadmium, Nickel, Lead, Mercury, Antimony) content in the composition and through toxicological risk assessment.

Materials-Methods: Red and yellow colored finger paints belonging to 12 different brands were included in the study. Metal levels were measured using the inductively coupled plasma mass spectrometry (ICP-MS) technique. The dyes were made ready for measurement by subjecting them to microwave acid digestion.

Conclusion: Metal levels of different brands in red paints were 0,680±0,648 ppm for mercury, 1,507±1,391 ppm for lead, 1,543±0,824 ppm for nickel, 6,790±2,805 ppm for arsenic, 0,047±0,050 ppm for cadmium, 1,447±0,893 ppm for antimony. Metal levels of different brands in yellow paints were 0,484±0,414 ppm for Mercury, 1.173±1.079 ppm for lead, 1.713±1,183 ppm for nickel, 6,682±2,319 ppm for arsenic, 0,088±0,070 ppm for cadmium, 1,467±0,654 ppm for antimony. It was concluded that it would be important to determine the risk status for children's health by making a safety assessment of these amounts detected in finger paints.

Keywords: Children's finger paints, heavy metal determination, safety assessment



PP-22. Alterations in Intercellular Communication/Gap Junctions After Fumonisin B1 Exposure in HEK-293 Cells

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Fumonisin B1 (FB1) is a mycotoxin produced by *Fusarium* species in maize and maize-based products. Although it is one of the most common mycotoxins that frequently contaminates corn products, it causes serious health problems in humans because it is the most important type in terms of toxicity among fumonisins. The International Agency for Research on Cancer (IARC) has classified FB1 as Group-2B (“possibly carcinogenic to humans”) regarding hepatocellular carcinomas and renal tubule carcinomas in experimental animals. FB1 causes toxic effects by causing accumulation of sphinganine, which plays an important role in the pathways associated with cancer development and apoptosis mechanisms, and disruption of sphingolipid biosynthesis. Also, little is known about the early molecular changes associated with FB1 carcinogenicity. Based on its non-genotoxic effect, it is thought that epigenetic mechanisms may play a role in the carcinogenic effect of FB1. Our study is planned to elucidate the key molecular mechanisms involved in the toxicity of FB1. For this purpose, the effects of FB1 on intercellular communications in human embryonic kidney cells were investigated. Cytotoxicity tests were performed and exposure concentrations were determined as 10, 50, and 100 μ M. Cell proliferation and 5-methyl cytosine levels were measured using the Elisa kit. Gene expression levels (*Cx43*, *Cx45*, *e-Cadherin*) and scrape loading/dye transfer assay were performed to analyze intercellular communication-gap junction functions. FB1 caused an increase in 5-mC% and significantly decreased gap junction functions. It is thought that FB1 may show toxic effects by affecting epigenetic modifications and intercellular communication. The elucidation of the mechanisms of chemical carcinogenesis also contributes to the development of biomarkers suitable for the early detection of carcinogenesis.

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PP-23. Comparative Anti-Cancer Potential of The Three *Cistus* L. Species Tested via 2D and 3D Cell Culture Systems

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3D (three dimensional) *in-vitro* cell culture models are becoming increasingly popular due to its advantage to mimic target organ microenvironment, specifically, the *in vitro* tumor models. As a therapeutic and biological discovery tool, they might exhibit more reliable results compared to the two-dimensional (2D) models for drug development. In this study, we aimed to determine the comparative anti-cancer potentials of the *C. salviifolius* L., *C. laurifolius* L. and *C. creticus* via both 2D cell cytotoxicity and 3D spheroid formation assay in MIA PaCa-2 and HDF cell lines. Firstly, the effect of *Cistus* sp. on cell viability was estimated by MTT assay and the results obtained were compared with those obtained in 3D Bioprinting method (Bioprinting Kit, Greiner Bio-One, Germany). The effect of *Cistus* sp. on spheroid growth was investigated using ImageJ software to measure the size change of spheroids (Image J.2.0 software, USA). Spheroids treated with different doses of *Cistus* sp. (0.125-1 mg/mL) showed a significant reduction in size in terms of diameter and area, while the area for control spheroids kept on increasing for up to 10 days. 1 mg/mL of *C. salviifolius* and *C. creticus* extracts showed inhibitory effect on spheroid growth after 10 days treatment similar to 10 µM doxorubicin (positive control) (95% reduction compared with the control). The most remarkable decline in spheroid size was observed by 1 mg/mL of *C. laurifolius* with a 98% reduction. As a conclusion, all *Cistus* sp. showed concentration-dependent anti-cancer activity against the pancreatic cancer cell line MIA PaCa-2 in both 2D and 3D models.



PP-24. Determination of the Genoprotective Effects of Rosmarinic Acid On Limbal Stem Cells by Comet Assay

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Oxidative, and especially photo-oxidative processes, are crucial factors for many ocular conditions, and there is growing evidence demonstrating that permanent oxidative stress contributes to the development of many ocular diseases. Especially increases in the accumulation of hydrogen peroxide and markers of the oxidative damage to DNA, lipids, proteins observed in several eye diseases emphasize the involvement of oxidative stress pathways. There is a need to investigate the therapeutic approaches considering topical/systemic use of antioxidants in ocular diseases. Limbal epithelial stem cells (LESCs) are tissue-specific multipotent stem cells located in the anatomical limbus region, located at the junction of the sclera and cornea layers of the eye. The aim of this study is to examine the effects of natural antioxidant rosmarinic acid (RA) on oxidative DNA damage using LESCs. After corneal transplantation, the unused portion of 3 healthy donor tissues was taken to the cell culture laboratory. Total 30 limbal explants were prepared, transferred to 12 well tissue culture polystyrene plates and cultured for 14 days in DMEM F12 medium containing human serum and antibiotics. Cells growth from explants were enzymatically disaggregated with trypsin/EDTA. RA (50, 100 and 200 µg/ml) was applied to the 1×10^5 cells/ml for 1h to determine its genotoxicity and all also cells were treated with H_2O_2 (50 µM) 5 min on ice after 1h pretreatment with RA to determine antigenotoxic effects. After applying basic alkaline technique of comet assay our results showed that different concentrations of RA applied to limbal stem cells were not genotoxic when compared with the untreated negative control groups. Also when compared to positive control group, RA treatment seemed to decrease the DNA damage induced by H_2O_2 . It is thought that RA may offer a treatment option for diseases caused by oxidative damage.



PP-25. Evaluation of Selected Metal Oxide Nanoparticles Toxicity on Chlamydomonas Reinhardtii

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The use of metal nanoparticles for various purposes has increased in recent years. This situation also brings environmental risks. Exposure of algae, which is an important element of the food chain in aquatic ecosystems, to metal nanoparticles may pose significant risks in terms of the food chain. In this study, the toxic effects of nanoparticles were evaluated in *Chlamydomonas reinhardtii*, a single-celled green algae exposed to TiO₂, ZnO and Fe₂O₃ nanoparticles at different concentrations. Of the NPs tested, titanium (IV) oxide (TiO₂, Sigma-Aldrich 718467) has a size of 21 nm. Other test materials ZnO NP (Sigma-Aldrich, 544906) and Fe₂O₃ NP (Sigma-Aldrich, 544884) have particle sizes <100 nm and <50 nm, respectively. The algae culture was cultured in sterile conditions with a ventilated mechanism providing illumination with a light intensity of approximately 2500 lumens with the help of a fluorescent lamp that provides daylight illumination in the 14:10 hour light:dark photoperiod. To determine NP toxicity, testing was initiated by incubating 1 mL of algae culture in 15 mL of TAP medium per vessel in sterile cell culture flasks. Studies were conducted with 105 living cells in each vial and exposing the cells to a range of nanometal concentrations. *Chlamydomonas* cell growth was checked by means of a automated cell counter at 24, 72 and 120 h for algal growth to determine the total cell number and viability (%) in the test medium. The findings showed that the tested nanoparticle concentrations had no toxic effect on *C. reinhardtii*. However, it is necessary to determine the risks for other living organisms in the environment with comprehensive studies.

Keywords: metal oxide nanoparticle, *chlamydomonas reinhardtii*, toxicity,



PP-26. *In Vitro* Comparison of the Potential DNA Damage Effects of H₂O₂ and Etoposide for 30 and 60 Minutes Exposures With The Comet Assay

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The comet assay providing the estimation of both DNA damage and repair is a fast, sensitive, and affordable technique. For long years, DNA damage and repair in individual cells and tissues have been evaluated by using the comet assay. Also, the comet assay is the standard method used in ecogenotoxicology, molecular epidemiology, human and environmental biomonitoring. Single/double DNA strand breaks, alkali labile sites, base-pair damages, and apoptotic nuclei can be determined by using this method.

Positive controls are chemicals known to induce DNA damage by the way of direct or indirect, and also they cause single/double DNA strand breaks identified by the comet assay. The examples of positive controls used in the comet assay are hydrogen peroxide (H₂O₂), methyl methane sulfonate (MMS), ethyl methane sulfonate (EMS), etoposide, and ethyl nitroso urea (ENU). The choice of a suitable positive control is an essential part of genotoxicity tests. However, it has been stated that the results obtained from different positive controls at discrete concentrations and time intervals varied. Accordingly, we aimed to compare H₂O₂, and etoposide, at different concentrations for 30 minutes, and 1h, respectively.

In our study, the alkali comet assay (pH>13) was performed to compare the results obtained from two different positive controls by using 3T3 cell lines. H₂O₂ was tested at 1, 5, 10, 25, 50, 75, 100 and 200 µM for 1h. For 30 minutes, up to 75 µM H₂O₂ concentrations were tested. As for etoposide, six different concentrations (0,05, 0,1, 0,5, 1, 5, 10 µM) were used for 30 minutes and 1h.

According to the comet assay results, the exposure of 25 and 50 µM H₂O₂ for 30 minutes significantly induced DNA damage more than for 60 minutes. DNA strand breaks resulted from the exposure of 1, 5, and 10 µM etoposide concentrations for 30 minutes were higher than for 60 minutes (p<0,05, one-way ANOVA).



PP-27. Comparative Evaluation of Protective Effects of Different *Tilia* Species Against Oxidative Stress and Inflammation

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Medicinal plants are sources of bioactive secondary metabolites which have been reported to exert beneficial effects for health including decreasing risk for the reactive oxygen species (ROS) related diseases. Inflammation is also linked to oxidative stress. Excessive and prolonged production of ROS may lead to chronic inflammation. Although nonsteroidal anti-inflammatory drugs (NSAIDs) are effectively used against inflammatory diseases, side effects such as gastro-toxicity create a need of safer alternatives. In the light of this knowledge, we aimed to investigate the protective effect of hydroalcoholic extracts of different plant parts of four *Tilia* species; flowers, bracts and inflorescences of *T. cordata*, *T. platyphyllos*, *T. rubra* and *T. tomentosa* against oxidative stress and inflammation which are widely used in Turkish folk medicine. The phenolic contents of extracts were qualified and quantified by HPTLC and HPLC-DAD methods with protocatechuic acid, hyperoside, isoquercitrin, astragalin, quercitrin and tiliroside as standards. Phenolic and flavonoid contents were determined by Folin Ciocalteu and aluminium chloride methods, respectively. For the antioxidant activities DPPH, CUPRAC and FRAP assays performed. The protective role of *Tilia* species against LPS induced inflammation was performed with *in vitro* nitrite assay on RAW 264.7 murine macrophage cell line. The highest phenolic content determined in *T. rubra* flowers which were dominated by protocatechuic acid and isoquercitrin, also showed the highest antioxidant activity with FRAP and CUPRAC assays. Highest flavonoid content determined in *T. rubra* bracts which has the highest hyperoside and quercitrin content also showed the highest antioxidant activity with DPPH assay. *T. tomentosa* bracts which were found rich in tiliroside showed highest inhibition of inflammatory mediator NO in a concentration-dependent manner. In overall, the phenolic and flavonoid contents were highly correlated with the antioxidant activities and all extracts inhibited LPS induced nitrite production by more than 50% at 0.125–1 mg/mL concentrations.



PP-28. Preliminary Result of Subacute Toxicity Study of Termiye (Lupen= Lupinus Albus L.) Plant in Sprague Dawley Rat Species

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The main purpose of this study is to determine the safety profile of Termiye (Lupen= Lupinus albus L.) plant products conducting repeated dose toxicity study of it, analyzing the effect of this repeated dose application on the antioxidant enzyme components and oxidative stress parameters of rat in blood and assessing toxicity profile of it with in silico methods. Repeat-dose toxicology study of treated (debittered) Lupen suspension and crude (non-debittered) Lupen suspension; low dose (666 mg/day) and high dose (2000 mg/day) in total 4 groups, 5 males and 5 females in each group, were performed on rat by oral gavage for 28 days. Blood biochemical analysis were done for every animal. At the end of the study, animals were sacrificed and blood samples, liver and kidney tissues were removed by euthanasia. After the tissues were weighed, and they were stored at -80 °C for histopathological analysis. Hemogram tests were performed AST/ALT, Creatinin (CRE) and Urea (URE) in blood samples. The weight gains of the animals were measured weekly.

According to URE and CRE values of the Termiye plant (both crude and treated) applied group's, it did not cause any change on the kidney. But for the liver, it was seen that the plant given as crude increased the AST values compared to the plant given as treated. In the hemogram test results, WBC, RBC, HGB and HCT values were found to be higher in the animals group given crude.

This study will continue with histopathological analysis of tissue samples, antioxidant and oxidative stress analysis of blood samples and in silico toxicity evaluation of it.



PP-29. Irritation Evaluation in Children's Finger Paints

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Introduction-Objective: Finger paint can be expressed as liquid (or jellylike) paints that children use for painting with their fingers on a paper or surfaces. Although the intended use is to paint any surface other than human skin, children can also use finger paints on their faces. For this reason it is important to evaluate the irritation potential of finger paints in terms of predicting and preventing irritation. The aim of this study is to monitor the irritation potential of finger paints for children and to evaluate their safety through a toxicological risk assessment.

Materials-Methods: A total of 24 paints, red and yellow of finger paints belonging to 12 different brands were included in the study. ICCVAM recommended Hen's Egg Test - Chorioallantoic Membrane (HET-CAM) Test Method protocol was applied for the irritation potential. Fresh fertile 50-60 g of Leghorn chicken eggs were collected and were prepared for the experiment according to the protocol. 0.3 mL of finger paint was applied directly onto the CAM, covering at least 50% of the CAM surface area. Their reactions on the CAM were observed over a period of 300 seconds (at fixed time intervals of 0.5, 2 and 5 minutes). The observation time of each of the endpoints; Hemorrhage (bleeding from the vessels), Vascular lysis (blood vessel disintegration, Coagulation (intra- and extra-vascular protein denaturation) were monitored and recorded, in seconds. This study was repeated three times for each sample for better evaluation of their irritation potential.

Results: As a result of the HET-CAM test procedure applied on 24 samples used in the general study, no irritation was observed in the samples. It was concluded that it is important to determine the risk status for children's health by making a safety assessment of the scores that show the irritation potential of finger paints.

Keywords: Children's finger paints, irritation potential, safety assessment



PP-30. Molecular Mechanisms of UR-144-Induced Cell Death in Cardiomyoblastic Cells

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The use of synthetic cannabinoids (SCs) has increased in recent years. UR-144 is, a synthetic cannabinoid receptor agonist, is used since 2012. UR-144 has been reported to induce cardiovascular adverse events which can be life-threatening. The data on the toxic potential of UR-144 is quite limited. Therefore, it is important to elucidate the underlying mechanisms of UR-144-induced cardiac adverse events. In the study, it was aimed to investigate molecular mechanisms of UR-144-induced cytotoxic effects using cardiomyoblastic cells.

Methods: Following 48 h exposure, the cytotoxic potential of UR-144 was evaluated with WST-1 and LDH assays. Apoptosis/necrosis, autophagy, and reactive oxygen species (ROS) level were assessed using flow cytometry. Total antioxidant capacity (TAC) was examined by spectrometry. Cytoplasmic Ca²⁺ level was evaluated with fluorescent-labeled dye, and released troponin T and death-associated kinase 1 (DAPK1) expressions were determined with western blot. The role of DAPK1 on UR-144-induced cell death was investigated using DAPK1 inhibitor.

Results: According to the findings, UR-144 induced cytotoxic effect starting from 50 µM dose in LDH assay. Apoptosis was induced at a very low rate after 50 and 200 µM UR-144 treatment. However, it was observed that autophagic cell death was more prominent than apoptosis and necrosis at the same concentrations. Both ROS and TAC levels were significantly decreased at 200 µM. Cytoplasmic Ca²⁺ and released troponin T levels showed a significant increase at 200 µM. DAPK1 protein expression was induced following 200 µM exposure, and DAPK1 inhibition alleviated UR-144-induced cytotoxicity at 200 µM.

Conclusion: The findings suggested that UR-144-induced cardiomyoblastic cell death might be associated with increased cytoplasmic Ca²⁺ levels and DAPK1 activation, and DAPK1 inhibitors can be a therapeutic option in UR-144 poisoning. However, further studies are needed to clarify the molecular mechanisms of UR-144-induced cardiotoxic effects in humans.

Keywords: UR-144, synthetic cannabinoids, cytotoxicity, cardiotoxicity



PP-31. The role of IL-13 and TNF- α Polymorphisms in Genetic Susceptibility to Asthma Disease

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Asthma is a very common disease, in which both genetic and environmental factors play a role. Studies on individual susceptibility in asthma have shown that Single Nucleotide Polymorphisms (SNP) in the IL-13 and TNF- α genes are important. The aim of this study is to determine the role of polymorphisms in IL-13 and TNF- α genes in genetic susceptibility to asthma. For this purpose, DNA samples of asthma patients (n=124) and control group (n=115) were included in the study and IL-13 C1923T and TNF- α G308A genotypes were determined by PCR-RFLP method. In our study, when adjustments were made for age, gender and body mass index, it was observed that the risk of developing asthma increased 2,879 times in patients with IL-13 TT genotype compared to those with IL-13 CC genotype (p=0,047). Similarly, the risk of developing asthma was increased 5,327 times in patients with TNF- α AA genotype compared to those with GG genotype (p<0,001). We conclude that the results of this study will be beneficial both in understanding the etiology of asthma and in improving the treatment strategy of the disease.



PP-32. Combined Effects of Lead and Polychlorinated Biphenyls on Reproductive System In Male Rats

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Lead (Pb) and Polychlorinated-biphenyls (PCBs) have oxidative stress as their mechanism of pathotoxicity. High level of oxidative stress cause damage to sperm cells and male reproductive organs. The objective of this study was to examine if the mixture of Pb and PCBs has any combined effect on the sperm cells and male reproductive organs in rats. Experiment was conducted in accordance with the approval of Ethical Committee No. 323-07-11822/2018-05. Wistar male rats were divided into 10 groups (6 rats/group) including a control group. In the 3 x 3 design, three doses of Pb (0.1 mg/kg/day, 0.5 mg/kg/day and 1 mg/kg/day) and three doses of PCBs (0.25 mg/kg/day, 0.5 mg/kg/day, and 1 mg/kg/day) were combined. After 28 days of oral exposure by gavage, rats were anesthetized by the combination of ketamine/xylazine. After sperm cells diffusion from the epididymal cauda in 37°C saline solution, the motility count was determined using a light microscope. Aliquot of the sperm suspension was fixated by the mixture of formalin and phosphate buffer and further used for a count and morphology analysis. Results of this study have shown that Pb in combination with the PCBs as a co-variate decreased the sperm motility and sperm count, and increased the percentage of abnormal sperm count. Relative mass of testes, epididymis and cauda in experimental groups did not show statistically significant differences from the control group. Comparing with the available data on Pb or PCBs influence on sperm count, motility and morphology, findings of this study confirmed the hypothesis that the mixture of the two environmental contaminants is more potent than the single chemical towards male infertility. Our ongoing research on oxidative stress parameters in male reproductive organs should support the understanding of the mechanisms involved in combined adverse effects of Pb and PCBs on reproductive system in male rats.

Keywords: male infertility; lead and PCBs mixture; redroductive toxicity; oxidative stress;

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PP-33. Cadmium and Prostate Cancer – A Possible Connection: Evidence From Studies in Humans and Animals

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An association between cadmium (Cd) exposure and increased risk of prostate cancer as well as the incidence of death from prostate cancer was observed. Nevertheless, IARC's latest report from 2018 links Cd with the occurrence of prostate cancer, but with limited evidence in human studies. The study aimed to determine the Cd distribution pattern in an *in vivo* model, with particular reference to the male reproductive organs, and establish the potential Cd implication in the prostate cancer occurrence/development in human study. Two experimental groups of male Wistar rats were acutely treated with Cd (15 mg Cd/kg b.w., Cd₁₅ group, and 30 mg Cd/kg b.w., Cd₃₀ group), and sacrificed 24h after treatment. Blood and prostate samples were mineralized, and Cd was determined. The human study encompassed 41 patients with prostate cancer and 61 healthy volunteers. Blood samples and prostate tissues were collected and Cd were determined. In animal study, experimental groups treated with Cd had significantly higher blood Cd levels than controls. Also, prostate tissue Cd levels were higher in treated groups than controls (Me: 43.4; IQR 16.9-44.1 µg Cd/kg of Cd₁₅ group and Me: 39.8; IQR 35.8-42.7 µg Cd/kg of Cd₃₀ group vs. Me: 12.0; IQR 9.0-15.8 µg Cd/kg of the control group, both $P < 0.05$). A human study determined significantly higher blood Cd levels in prostate cancer patients compared to control (Me: 1.294; IQR 1.011-1.874 µg Cd/L vs. Me: 1.010; IQR 0.724-1.374 µg Cd/L, respectively, $P < 0.001$). The average Cd level in tumor tissue was 5.09 ng/g (IQR 3.05-8.71). Blood Cd levels were recognized as a significant predictor of prostate cancer (OR=5.35). Presented results of human data support the potential Cd implication in prostate cancer occurrence and/or development since Cd is recognized as endocrine disruptors. Presented results undoubtedly indicate significant Cd distribution in prostate tissue and potential prostate cancer implications.



PP-34. Evaluation of The Effects of Different Formulations of Subunit Covid-19 Vaccine on Cytokine Levels in HepG2 Cells

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COVID-19 pandemic has been causing high rates of morbidity/mortality all over the world. To prevent the devastating effect of the disease, many vaccine research/development studies have been carried out. Cytokine storm causes life-threatening systemic inflammatory syndromes involving elevated levels of cytokines and immune-cell hyperactivation during COVID-19. Tumor necrosis factor beta (TNF- β) is a pro-inflammatory cytokine that plays an important role in acute infection response. Interleukin 10 (IL-10) is the main inflammatory cytokine that inhibits cytokine synthesis. Interferon alpha (IFN- α) enhances proliferation of human B cells, as well as being able to activate natural killer (NK) cells while interferon beta (IFN- β) balances expression of pro- and anti-inflammatory agents in the brain, and reduces number of inflammatory cells that cross the blood brain barrier. In this study, the effects of various formulations of a peptide vaccine on cytokine levels in HepG2 cells were evaluated. The subunit vaccine was formulated in two different ways as “peptide emulsion” and “peptide-loaded liposome”. Working groups are designed as follows: i. control; ii. control emulsion; iii. control liposome; iv. peptide emulsion; v. peptide loaded liposome. Cells were exposed to determined doses of emulsion, liposome, and their peptide-loaded forms for 24 h. Then the cytokine levels, TNF- β , IL-10, IFN- α and IFN- β were measured by using ELISA method. Different formulations affected cytokine levels at different rates. TNF- β and IL-10 levels were significantly decreased in control liposome, peptide emulsion and peptide-loaded liposome groups compared to control. IFN- α levels were also markedly lower in the control liposome and peptide loaded liposome groups compared to the control. IFN- β levels were significantly decreased only in the peptide loaded emulsion group. In summary, the effects of COVID-19 vaccines (developed with peptide-loaded emulsion and peptide-loaded liposome formulation) on cytokine, levels were markedly different than control. These results should also be confirmed with *in vivo* experiments.

Keywords: COVID-19, Vaccine, HepG2, Cytokine



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PP-35. Toxicological Risk Assessment in Pharmaceutical Industry

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Cross-contamination in the shared facility or equipment is a major concern occurring during the manufacture of medicinal products. In some cases it may not be possible to eliminate residual exposure with cleaning procedures. Any unintentional exposure through cross-contamination may lead to undesired effects on population and pharmaceutical industry should take protective measures for risk minimization or elimination. The main factors for cross-contamination depend on the properties of the substances and variables in the manufacturing environment. For many years, efforts have aimed to established to protect populations against any potential adverse health effects of residual active pharmaceutical ingredients. Derivation of limits with toxicological risk assessment is the methodology that enables an acceptable exposure limit for an ingredient. In the presence of cross-contamination, exposure levels that can be considered as “safe” for all populations are needed to manage the risks predicted by a comprehensive scientific evaluation. The derivation of health based exposure limit is a methodology created for determining a safe or acceptable exposure limit for a particular substance. In this manner, exposure limits that are considered as safe. Regulatory authorities, various committees, and scientific studies play a crucial role in determining limits for specific exposures. Permitted daily exposure determination is a multistep process involving extensive toxicological research. In accordance with the requirements of the regulations, the information to be provided for determining these values and the issues to be solved will be clarified step by step.



PP-36. Pharmacovigilance Awareness Among the Community Pharmacists and Pharmacy Technicians in Istanbul

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Pharmacovigilance is the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other drug-related problems. Community pharmacists and pharmacy technicians play an important role in the detection and reporting of adverse drug reactions. During their communication with the patients, they can easily access to the information of the drugs, nutritional supplements, herbal products used by the patients and the adverse effects developed against these products. However, the worldwide pharmacovigilance activities reported from community pharmacies are with poor contribution. This study was conducted in community pharmacies around Kadıköy and Fatih districts of İstanbul (Turkey) in order to detect pharmacovigilance awareness of pharmacists and pharmacy technicians. Therefore, a face to face questionnaire was used to investigate awareness in community pharmacists (n=108) and pharmacy technicians (n=87) in determined districts. The knowledge of pharmacovigilance practice, compliance in adverse drug reaction reportings, under reporting reasons and perceptions of the community pharmacists and pharmacy technicians on pharmacovigilance practice were evaluated. According to our search this is the first awareness study conducted on pharmacy technicians in Turkey, although they are not considered to be healthcare providers. In our study the pharmacovigilance awareness and adverse effect reporting rates were evaluated for both community pharmacists and pharmacy technicians. It has been detected that pharmacists in the Fatih and Kadikoy districts have 52.72% and 49.05% awareness for adverse event reportings. While pharmacy technicians have lower awareness 47.7% and 23.40% respectively. It has been observed that the most common reasons of drawbacks include main pharmacovigilance awareness metrics. We found that many adverse events could not be reported because the information could not be conveyed from the technician to the pharmacist. According to the results of our study, further activities are needed to raise awareness of pharmacy technicians as well as pharmacists in order to contribute pharmacovigilance system. Technicians also need to be trained for pharmacovigilance activities as they are in one-to-one communication with the patient in many cases. The observed results point out the urgent need of pharmacovigilance training and education for pharmacists and pharmacy technicians.



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PP-37. Evaluation of Low-Dose Naltrexone as a Possible Novel Anticancer Drug

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Naltrexone (NTX) is long acting and potent opioid antagonist that competitively block mu, delta, kappa receptors as well as opioid growth factor. NTX is approved for treatment of opioid dependence and alcoholism with standard dose range of 50-100 mg. Preclinical and clinical studies have reported that low dose naltrexone (LDN) can reduce tumor growth by interfering with cell signaling and modifying immune system. Recently, LDN with oral daily dose of 1 to 5 mg is used as an off-label treatment to alleviate cancer, chronic pain and autoimmune diseases. Intermediate blockage of opioid growth factor receptor by LDN lead to an upregulation of endogenous opioid growth factor and receptors which gives promising antitumor effect by impairing cancer cell proliferation, blocking tumor mitosis and prevent uncontrolled proliferation. Whereas, continuous receptor blockage by standard dose NTX produce opposite effect particularly enhanced cellular growth. This review aims to summarize the in vivo and in vitro studies conducted on cell culture, animal models and human subjects who receiving LDN. A systemic search on LDN in cancer therapy suggested that LDN alone or combine with other agents might be less toxic, low cost and safe approach to prevent and treatment of various cancer. This review emphasizes the value of potential future research on LDN as true novel anticancer drug or an effective adjunct therapy to conventional chemotherapy.

Naltrexone (NTX) is long acting and potent opioid antagonist that competitively block mu, delta, kappa receptors as well as opioid growth factor. NTX is approved for treatment of opioid dependence and alcoholism with standard dose range of 50-100 mg. Preclinical and clinical studies have reported that low dose naltrexone (LDN) can reduce tumor growth by interfering with cell signaling and modifying immune system. Recently, LDN with oral daily dose of 1 to 5 mg is used as an off-label treatment to alleviate cancer, chronic pain and autoimmune diseases. Intermediate blockage of opioid growth factor receptor by LDN lead to an upregulation of endogenous opioid growth factor and receptors which gives promising antitumor effect by impairing cancer cell proliferation, blocking tumor mitosis and prevent uncontrolled proliferation. Whereas, continuous receptor blockage by standard dose NTX produce opposite effect particularly enhanced cellular growth. This review aims to summarize the in vivo and in vitro studies conducted on cell culture, animal models and human subjects who receiving LDN. A systemic search on LDN in cancer therapy suggested that LDN alone or combine with other agents might be less toxic, low cost and safe approach to prevent and treatment of various cancer. This review emphasizes the value of potential future research on LDN as true novel anticancer drug or an effective adjunct therapy to conventional chemotherapy.



PP-38. The Effect of Acrylamide on Bax and Bcl-2 Gene Expression in The Pancreatic β -cell Model System

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Acrylamide (AA) is a toxic substance that can form in some foods during high-temperature cooking processes. The aim of this experiment was to examine whether AA treatment affects the transcription of the pro-apoptotic Bax and anti-apoptotic Bcl-2 genes, as well as the Bax / Bcl-2 ratio in pancreatic β -cells. Since most cells (> 80%) in rodent islets of Langerhans are β -cells, the rat pancreatic insulinoma cell line (Rin-5F) was used in the experiment, as a validated β -cell model system. Cells were treated with 10 mM (IC₅₀) AA concentration at various time intervals (0.5, 1, 3, 6, 12, 24 h). Bax and Bcl-2 gene expression in β -cells was tested by real-time PCR technique. Mann-Whitney U test was used for comparing differences between corresponding means. Values of $p < 0.05$ were considered as statistically significant. Analysis of relative Bax gene expression showed an increase in mRNA synthesis during treatments at all time points ($p < 0.05$) compared to control cells. Treatments at 0.5, 1, 3, 6, 12, and 24 h increased the relative mRNA transcription for Bax 3.13, 2.76, 2.8, 3.42, 3.98, and 3.89-fold, respectively. The relative expression of the Bcl-2 gene in treated Rin-5F cells showed a 2.75, 2.14 and 2.54-fold increase ($p < 0.05$) compared to control cells, only during 0.5, 1 and 3 h treatment, respectively. Treatments at 6, 12 and 24 h did not affect significantly ($p > 0.05$) Bcl-2 gene transcription. Treatment with acrylamide for 12 and 24 h has led to a rise in the value of the Bax / Bcl-2 ratio (3.5 times and 3 times, respectively) in the Rin-5F cell line. Increased Bax / Bcl-2 ratio could indicate potential occurrence of apoptosis in β -cells after prolonged exposure of cells to acrylamide.

Keywords: acrylamide, apoptosis, β -cells, Bax, Bcl-2, real-time PCR



PP-39. *In Silico* Toxicogenomic Case Study: Role of Lead and Polychlorinated Biphenyls Mixture in The Endocrine System Diseases

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Endocrine disrupting chemicals (EDCs) are mostly present in a variety of human-made products and cause adverse outcomes by interfering with the endocrine system. Even though it is very difficult to predict the cocktail effects of such chemicals, EDCs can cause more severe toxicity when exposed in combination. This *in silico* toxicogenomic data-mining approach was designed to: (I) shed light on how lead (Pb) and polychlorinated-biphenyls (PCBs) may contribute to endocrine system diseases (ESDs), (II) predict the possible mechanisms of the cocktail effects of these compounds in pathophysiology of ESDs. Comparative Toxicogenomics Database (CTD; <http://ctdbase.org/>), GeneMania (<https://genemania.org/>), and ToppGene Suite (<https://toppgene.cchmc.org/enrichment.jsp>) were used for toxicogenomic data mining. The results revealed that 69 genes were linked to the co-exposure of Pb and PCBs and the pathophysiology of ESDs. The leading interaction between these genes was co-expression (47.11%), followed by physical interactions (28.59%) and co-localization (13.19%). According to gene ontology analysis, signaling receptor binding, transcription factor binding, and oxidoreductase activity were among the most significant altered molecular functions, while aryl hydrocarbon receptor pathway and orexin receptor pathway were the most prominent altered pathways. Investigated mixture induced protein expression of interleukin-6 (IL-6), NAD(P)H quinone dehydrogenase 1 (NQO1) and prostaglandin-endoperoxide synthase 2 (COX2) but led to decreased protein activity of superoxide-dismutase 2 (SOD2). Aromatase (CYP19A1) mRNA levels decreased leading to a reduction in the biosynthesis of estrogen. This findings indicated that the proposed toxicogenomic data-mining approach may be effective for a better understanding of the molecular mechanisms underlying the cocktail effects of Pb and PCBs involved in ESDs. Nevertheless, this approach should be regarded as preliminary data for further *in vitro* and *in vivo* tests.

Keywords: Lead and PCBs mixture; Endocrine disrupters; Interleukin; COX2; SOD2; Comparative Toxicogenomics Database;

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PP-40. The Important Role of NLRP3 Inflammasome in Rheumatoid Arthritis

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Rheumatoid arthritis, a systemic autoimmune and inflammatory disease, causes pain, swelling, stiffness, and irreversible damage to the joints. Despite being the most common autoimmune disease, its mechanism remains unclear. The nucleotide-binding oligomerization domain-like receptor pyrin domain-containing-3 (NLRP3) inflammasome, plays an important role in the pathogenesis of autoimmune diseases by activating caspase-1 and releasing IL-1 β and IL-18 in response to inflammation and cellular damage. The large spectrum of inflammatory infectious and endogenous ligands, including damage- and pathogen-associated molecular patterns, react with NLRP3, which contributes to the pathogenesis of several inflammatory disorders. Therefore, we aimed to determine the possible role of NLRP3 in rheumatoid arthritis and also the effect on disease activity. The Enzyme-linked ImmunoSorbent Assay was performed to measure plasma NLRP3 levels. The mean NLRP3 level of the patient group was significantly higher than the control group ($p < 0.001$). The highest NLRP3 levels were observed in patients with active disease, while the lowest was in the remission group. Additionally, NLRP3 levels were determined using multivariate logistic regression, which adjusted for age, gender, and smoking history as confounding factors. NLRP3 levels in the patient group were statistically higher than those in the control group even after confounding factors were eliminated (95% CI: 1.024-1.058) ($p < 0.001$). AUC for NLRP3 according to ROC analysis was 0.926 ± 0.027 (95% CI: 0.872-0.979) ($p < 0.001$). The diagnostic value was found quite high in distinguishing between control and patient groups. This feature suggests that NLRP3 can be a crucial marker in the mechanism of the disease. Further studies are needed to support this approach.

Keywords: inflammasome, inflammation, NLRP3, rheumatoid arthritis



PP-41. The Effects of Melatonin and its Analogues on Steroidogenesis Pathway: a Possible Therapeutical or Odverse Effect

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Melatonin (MLT) is a hormone that is secreted from pineal gland and regulates the circadian rhythm. It is reported to have anticancer effects in hormone dependent breast cancer via endocrine modulation, namely antagonising the estrogen receptor (ER) or inhibiting aromatase enzyme. However, its use is restricted because of the short half-life and poor bioavailability. Therefore, new indole derived MLT analogues were synthesized previously, and they were shown to have potential antioxidant and anticancer effects. The present research is aimed to investigate the potential of MLT and its two newly synthesized analogues (M6 and M20) on steroidogenesis pathway by using an OECD validated in vitro method, H295R steroidogenesis assay (TG-456 test guideline). The compounds were incubated with H295R, human adrenocarcinoma cells for 48 hours and the hormone levels (testosterone; T and estradiol; E2) were detected by LC-MS/MS. The method partial validation was performed by using reference compounds forskolin, prochloraz, letrozole and ketoconazole. MLT decreased both E2 and T levels and its effect on E2 levels were dose dependent. On the other hand, M6 and M20 showed biphasic effects on both hormone levels. None of the compounds decreased H295R cell viability. These results demonstrate that depending on the use and the dose of these compounds, their potential effects on hormone production can result either in a therapeutical effect (such as anticancer agent) or a non-targeted endocrine related adverse effect when **used as a pharmaceutical**.

Keywords: Steroidogenesis, melatonin, melatonin analogues, therapeutical effect, endocrine related adverse effect, LC-MS/MS



PP-42. Cytotoxicity and Genotoxicity Evaluation of Certain Herbal Weight Loss Products on Endothelium, Liver, and Kidney Cell Lines

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Obesity is one of the chronic diseases that plays an important role in the global health burden in both developed and developing countries. Lately, weight-loss products have been widely used throughout the world. Many herbal products which are sold without any safety regulation, are available in the market. Although we benefit from a large number of plant-derived active pharmaceutical ingredients in modern medicine, these herbal weight-loss products that have not undergone adequate toxicity testing should be carefully evaluated as they may cause various problems. Several case reports showed that herbal weight loss products may cause hepatotoxic, nephrotoxic, and cardiovascular toxic effects in patients. This study is aimed to evaluate the possible cytotoxic and genotoxic effects on the endothelium, liver, and kidney of five different herbal products in the form of tea and capsules sold for weight loss on the Turkish market. HUVEC, HepG2, and Vero cell lines were treated for 48 h with these samples (coded as FI, MS, HS, TLS, CLJ), and MTT test and alkaline comet analyses were conducted. Dramatically decreasing cell viability % was observed in HS and CLJ samples (respectively, $38.99 \% \pm 4.95$ and $41.25 \% \pm 4.54$) on HUVEC at 1000 $\mu\text{g/ml}$ concentration ($p < 0.0001$). CLJ were also cytotoxic on Vero cells at 1000 $\mu\text{g/ml}$ concentration ($\% 66.74 \pm 4.80$). MS showed statistically significant genotoxic activity on HUVEC cells at the 1000 $\mu\text{g/ml}$ concentration ($p = 0.0227$). These results are important for public health in terms of drawing attention to the irrational use of herbal products.

Keywords: Cytotoxicity, genotoxicity, herbal weight loss products, obesity



PP-43. The Effect of a Single Overdose of Paracetamol on Mitochondrial and Nuclear DNA Oxidative Damage Level in Kidney and Liver of Mice

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Paracetamol (acetaminophen; PAR) is one of the widely used analgesic and antipyretic drugs, however, its overdose can cause severe hepatotoxicity. Although studies on its toxicity have focused on the mechanism of liver injury, in recent years, attention has gradually increased to other possible damages, such as oxidative damage to DNA. Therefore, the objective of the present study is to investigate whether the application of a single overdose of PAR caused oxidative base damage in both nuclear (nDNA) and mitochondrial DNA (mtDNA) of C57BL/6J mice. The effect of MitoTempo, which is an antioxidant specifically for mitochondria, on the potential base damage has also been studied. After the administration of the drug, kidney and liver tissue samples have been taken and nDNA and mtDNA isolations were carried out. DNA samples were amplified by PCR to test for cross-contamination with each other. 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels were determined with ELISA. The results have shown that the 8-OHdG levels were not significantly increased in the nDNA and mtDNA of both the liver and the kidneys in the PAR-treated group relative to the Control while they were significantly reduced in the renal nDNA of the PAR group. Paradoxically, 8-OHdG levels in the MitoTempo group were significantly increased compared to the PAR group. However, 8-OHdG levels in the liver nDNA were found to be significantly lower in the MitoTempo group as compared to both the Control and PAR groups. These results demonstrate that MitoTempo had a completely different effect on the liver and kidneys. However, this effect does not alter the oxidative base damage that PAR induces, because PAR has not induced base damage to either the liver or the kidney.

Keywords: Paracetamol, DNA base damage, hepatotoxicity, nephrotoxicity, in vivo animal model



PP-44. Evaluation of Toxicity of Some Antiretroviral Drugs and Their Combinations on Sertoli And Leydig Cells

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Antiretroviral drugs are used to treat and prevent HIV infection. They are divided into three groups: (1) nucleoside reverse-transcriptase inhibitors (NRTIs), (2) non-nucleoside reverse-transcriptase inhibitors (NNRTIs), and (3) protease inhibitors (PIs). It is recommended to use a PI or an NNRTI in addition to two NRTIs in primary therapy currently. The aim of this study is to investigate the potential testicular toxicity on TM3 (Mouse Leydig cell line) and TM4 (Mouse Sertoli cell line) cells of abacavir (A), ritonavir (R), nevirapine (N), zidovudine (Z), and their combinations (A+Z+R, A+Z+N). For this purpose, MTT and neutral red uptake (NRU) assays were chosen for determining cytotoxicity and 2',7'-dichlorofluorescein diacetate (DCFDA) assay for evaluating the production of reactive oxygen species (ROS). According to results of MTT and NRU assays, cytotoxicity on TM3 and TM4 cells at 24 h may be induced by the drugs and especially their combinations. It can be emphasized that cytotoxicity was detected in both techniques, with ritonavir and drug combinations generally inhibiting cell viability at more significant levels. Also, exposure to drugs and their combinations can lead to oxidative stress. The ROS generation caused by ritonavir on TM3 cells and drug combinations on TM4 cells was found to be remarkable.

Keywords: Antiretroviral drugs, Sertoli cells, Leydig cells, cytotoxicity, oxidative stress.



PP-45. Investigation of Cytotoxic Effects of Novel Hydrazone Derivatives as Cholinesterase Inhibitors

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Alzheimer's disease (AD) is among the most common neurodegenerative diseases in the world. The hydrazones are very effective compounds in terms of biological activity for this disease. Acetylcholinesterase inhibitory activities have also been proven by literature studies. For this purpose, novel hydrazone derivatives were synthesized within the scope of this study. The structures of the compounds to be obtained elucidated by ¹H and ¹³C NMR and HRMS spectroscopic methods. The inhibitory effects of the final compounds on AchE and BchE enzymes investigated using *in vitro* Ellmann methods. According to the results of the activity studies, compounds 4d, 4f and 4h were determined as the most active derivatives in the series with IC₅₀=0.116±0.004 µM, IC₅₀=0.042±0.001 µM and IC₅₀=0.059±0.002 µM, respectively. For a compound to be a drug candidate, it must be active as well as not showing cytotoxic properties. For this purpose, the cytotoxic effects of active compounds were evaluated by MTT method against NIH/3T3 (mouse embryonic fibroblast cells). MTT assays of active compounds were evaluated at 24 hours and 48 hours. As a result of cytotoxicity studies, compounds 4d, 4f, and 4h were found to be non-cytotoxic.

Keywords: Cytotoxic effects, MTT, Hydrazone, Cholinesterase inhibitors.



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PP-46. Special Issue in Toxics Journal: "Biological Risk Monitoring of Exposure to Chemical Pollutants and/or Physical Agents"

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Biological monitoring (BM) (used mostly in occupational, but also accidental, at home and environmental exposure) is an important, but still underrepresented and not efficiently used, tool in risk assessment. There are also different BM regulations in each country, mostly due to the lack of strong data and databases to be analyzed for final confirmation about specific chemical/physical agent risk. New data, improvements of already existing methods, use of newly developed sensitive methods, or the combination of analysis techniques for quantitative determinations have made it possible to establish new limits of exposure or even helped in classifying some substances as carcinogens or mutagens with limits of exposure, especially in the workplace. Still, the development of new materials, chemicals, co-exposures with mixed toxicokinetic pathways and similar/different modes of action, and new uses of already known/unknown chemical and physical agents are still demonstrating the need for new data collection for a strong database fulfilment in order to finally reveal whether a specific mixture of agents is dangerous and, if so, to what extent. At present, for exposure biomarkers and other types of biomarkers used in biomonitoring, such as effect and susceptibility biomarkers, data regarding possible databases and literature surveys are still insufficient. Therefore, the aim of this Special Issue is to contribute to further collection of data on the evidence of biological monitoring and risk assessment considering all types of biomarkers and exposure types and routes in living organisms or revealing their pathways *in vivo* and *in vitro* to obtain new insights into the old/new and novel use of chemicals and/or physical agents. This Special Issue will include original articles, as well as systematic/reviews on these topics including human, *in vivo*, *ex vivo*, and *in vitro* studies. Deadline for manuscript submissions is 28 March 2023.



PP-47. Determination of Postmortem Interval (PMI) in Frozen Rat Liver and Kidney Tissues by Alkaline Comet Assay

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Purpose: The postmortem interval (PMI), which is defined as the time elapsed after the death of the person, has significant role in forensic medicine. Accurate PMI estimate most importantly helps determine the time of the murder. The disadvantage of the current molecular techniques results in various difficulties in consequence of estimated and approximate times instead of a precise time. Therefore, the aim of the study is to contribute to the evaluation of PMI with better precision by alkaline comet assay (1-4).

Methods: Ethics committee approval was obtained from Marmara University Experimental Animals Ethical Committee. 45 healthy, albino, male adult Sprague Dawley rats were used in division of 5 different groups within 9 animals in per group. After sacrifice, tissue samples of liver and kidney were taken separated into 3 parts at different times (Group 1: Hour 0, Group 2: Hour 6, Group 3: Hour 12, Group 4: Hour 24, Group 5: Hour 48), stored at -80°C. Samples were prepared by frozen tissues in ice-cold mincing solution and Comet assay was applied with slight modifications to Sardas *et al.* and Lu *et al.* (5-7).

Results: All samples spread on precoated slides were observed by BAB Fluorescent Microscope with BAB Bs200ProP/BsComet DNA Comet Assay Image Analyzer program. However, instead of normal comet cell structure, the slides showed consistence of ghost cell which indicates a short stage in the process of cell death, aforesaid nuclear membrane rupture under microscope. For ghost cells, qualitative evaluation is suggested; therefore, cells are noted as C0, C1 and ghost cell according to Choucroun *et al.* (8). Intermediate damage shape cells in 6 hour PMI group and ghost cells in 24 hour group were observed as compared to 0 Hour PMI group. Finalized qualitative evaluation of 100 cell count will be displayed in detail in our poster.

Keywords: Comet assay, postmortem interval (PMI), frozen tissue, forensic toxicology



PP-48. Determination of The Antioxidant Activity of Black, Green and White Tea on Genotoxic Damage-Induced *Caenorhabditis Elegans* Model

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Purpose: *Caenorhabditis elegans* (*C. elegans*) having similar aspects to human metabolism, giving human-like innate immunity and immune response, having a short life span of one month, allowing short-term exposures to cover a larger percentage of lifetime, and being easily observed intracellularly under microscopes (1-3) are promising model organism in toxicology studies. The aim of this study is to determine the antioxidant effects of green, black and white tea on *C. elegans* nematodes exposed to genotoxic pollutant benzo(a)pyrene and in parallel the same procedure was applied *in vitro* to human healthy blood samples evaluated by using the alkaline comet assay.

Methods: The model organism *C. elegans* N2 strain and *E. coli* OP51 strain used in the study were obtained from the *Caenorhabditis* Genetics Center (CGC) of the University of Minnesota. Experimental groups included healthy human blood samples and *C. elegans* model classified as control, benzo(a)pyrene-induced and tea extracts-exposed. Comet assay was applied in *in vitro* blood samples according to Sardas *et al.* and to *C. elegans* with slight modifications Sobkowiak *et al.* after 48 hours exposure to benzo(a)pyrene (4, 5) evaluated by BAB Fluorescent Microscope with BAB Bs200ProP/BsComet DNA Comet Assay Image Analyzer program.

Results: Decreased comet tails were observed in benzo(a)pyrene-induced *in vitro* human lymphocytes exposed to white tea compared with black, and green tea. The study is continuing and hope to observe this antioxidant effect on genotoxic damage-induced *C. elegans* which will be a valuable model for studying toxicogenomic responses of chemicals, both at the molecular and organismal level. Finalized evaluations will be displayed in detail in our poster.

Keywords: *C. elegans*, Comet Assay, antioxidant, genotoxicity



PP-49. EpiDerm™ Phototoxicity test – a test to study the phototoxic potential and ability to form ROS of selected compounds

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An *in vitro* phototoxicity test using the human reconstructed epidermis model EpiDerm™ (EpiDerm™ H3D-PT) has been developed and pre-validated almost 20 years ago and can be used either as standalone method for the phototoxicity testing of topically applied materials, or in combination with the 3T3 NRU PT, to minimize the potentially false positive results from this assay. In June 2021, OECD Test Guideline (TG) 498: In vitro Phototoxicity: Reconstructed Human Epidermis Phototoxicity test method was adopted. OECD TG 498 is a stand-alone method for evaluating the phototoxic potential of a test chemical after topical application in reconstructed human epidermis (RhE) in the presence and absence of simulated sunlight. EpiDerm™ H3D-PT is currently the only tissue model accepted under this test guideline.

The aforementioned method is based on evaluating the viability of exposed and irradiated tissues. However, another key determinant of photoreactive changes in cells is presence of reactive oxygen species (ROS). Therefore we have selected a set of 13 compounds with known phototoxic potential and analyzed them using EpiDerm™ phototoxicity test. Moreover, we have studied the ability to form ROS using dichlorofluorescein diacetate assay (DCFH-DA) in combination with the UVA irradiation and the exposure to the test compound. Using this approach all 13 compounds were correctly identified as either having or not having phototoxic potential.

These preliminary results confirm that EpiDerm™ H3D-PT is a reliable test for the detection of phototoxicity and prediction of the phototoxic potential of selected substances and suggest that it can be also used to identify the formation of ROS after UVA irradiation. To confirm these results and to further examine potential of such combined method we will apply this approach to an expanded set of phototoxic and non-phototoxic compounds.



PP-50. Sub-categorization of ocular irritants using the EpiOcular tissue model and prediction models for liquids and solids

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Determination of serious eye damage/eye irritation originally involved the use of laboratory animals (OECD TG 405). In 2015, a new test guideline (OECD TG 492) was accepted which enables the use of an in vitro procedure based on RhCE to distinguish between chemicals not requiring classification and those that must be labeled for eye irritation or serious eye damage. Chemicals identified as requiring classification for eye irritation/serious eye damage must be further tested to distinguish between eye irritants and those causing serious eye damage. There have been several projects focused on the development of tiered testing strategies for eye irritation assessment which takes in account all drivers of classification. The goal of these projects has been to develop a testing strategy to sub-categorize chemicals which: a) do not require labeling for serious eye damage or eye irritancy (No Category), b) can cause serious eye damage (Category 1 or Cat 1), and c) are eye irritants (Category 2 or Cat 2). In the current project, a set of 13 chemicals (7 liquids and 6 solids) that are listed as proficiency chemicals in OECD TG 492B were tested using EpiOcular model. We used a testing strategy developed in CON4EI project and confirmed in ALT4EI project, which combines the most predictive time-points of EpiOcular time-to-toxicity neat and dilution protocols. The set of chemicals consisted of 4 Cat 1 chemicals, 5 Cat 2 chemicals and 4 No Cat chemicals. Using the proposed testing strategy, we were able to correctly identify 100% of Cat 1 chemicals (4/4), 100% of Cat 2 chemicals (5/5) and 100% of No Cat chemicals (4/4). The testing strategy proposed in CON4EI and verified in ALT4EI projects to achieve optimal prediction for all three categories – prediction models for liquids and solids seems to be a very promising tool in an integrated testing strategy (ITS) that can discriminate chemicals to No Cat, Cat 2 and Cat 1.



PP-51. Use of Reconstructed Human Small Intestine Tissue Model EpiIntestinal to Study Effects of Fruit Proteases on Intestinal Integrity and Permeability

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Intestinal absorption via paracellular route is an attractive concept for drug delivery, however, the efficiency of transport via this route may be insufficient for some substances. Certain fruit enzymes have been shown to enhance intestinal paracellular uptake of low molecular weight molecules as studied *in vivo*, *post-vivo* and Caco-2 cell lines. The present study aimed to validate effects of cysteine proteases such as bromelain from pineapple (*Ananas comosus*) or papain from papaya fruit (*Carica papaya*). To study the effects *in vitro* we chose the reconstructed 3D model of human small intestine epithelium EpiIntestinal (SMI-100), which is based on normal untransformed donor cells and thus should allow studying tissue integrity and function in more physiologically relevant setting than cell line-based models.

Purified bromelain and papain in powder form, both from BioChemica, were dissolved in HBSS buffer, pH 6.5, and applied on the apical surface of tissue at for 30 minutes followed by Lucifer yellow (LY) permeability test for another 30 min. Transepithelial electrical resistance (TEER) measurement was performed to assess the intestinal integrity before the enzyme application (0 min), after termination of treatment (at 30 min) and after 30 min washout period (at 60 min). The measurements of bromelain (BRM, n=9) or papain-treated (PAP, n=6) inserts were compared to the untreated control (Ctrl, n=9). Viability of the tissues was assessed using the MTT assay at the end of experiment (60 min).

Results, presented as mean \pm SEM, showed significant increase in the proportion of LY passage from apical to basolateral side for BRM (2.7 ± 1.0 , $p < 0.05$) and PAP (1.3 ± 0.3 , $p < 0.01$) compared to Ctrl (0.4 ± 0.07). This was confirmed by histology analysis of the tissues, which demonstrated presence of LY dye in the BRM-treated tissues, but not in Ctrl group. However, the TEER result showed significant decrease of tissue integrity at 60 min for BRM group compared to the control group ($67.8 \pm 17.05 \Omega \cdot \text{cm}^2$ vs $133 \pm 11 \Omega \cdot \text{cm}^2$, respectively, $p < 0.05$). It is worth mentioning that the tissues viability (determined by MTT assay) was not affected by either PAP or BRM during exposure period.

In conclusion, although both enzymes were administered at the same dose, the papain has shown slighter effects on intestinal paracellular permeability than bromelain. Present study has confirmed that EpiIntestinal tissue model is useful to investigate effects of bioactive food compounds such as fruit enzymes on intestinal integrity and permeability and can be further implemented in nutritional research to improve the modeling of the human intestinal function comparatively to existing animal models.



PP-52. Evaluating the inflammatory and genotoxic effects of smokeless tobacco using a human organotypic model of oral epithelium

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In addition to the well-known effects of tobacco use on the causation of lung and cardiovascular disease, tobacco use is also implicated as a major cause of oral cavity disease that leads to thousands of deaths per year. Snus, a smokeless tobacco applied to the oral cavity, has been proposed as a less harmful alternative to smoking although its safety has not been adequately evaluated. The objective of this study was to evaluate the cytotoxic, genotoxic and inflammatory effects of snus using an in vitro model of human oral mucosa (EpiOral™). EpiOral tissues were treated topically with 5 or 25 milligrams of snus for 24-48 hours and evaluated for cytotoxicity by MTT. Tissues treated with 5 mg of snus had comparable viability to vehicle treated controls while those treated with 25 mg displayed approximately a 20% decrease in viability after 24 and 48 hours of exposure. Histological analysis revealed hyperchromic staining in tissues treated with 5 mg of snus at 24 hours post-treatment whereas tissues treated with 25 mg of snus displayed a significant amount of sloughing of the apical layers. Following treatment, an inflammation-specific cytokine panel was used to analyze markers of inflammation at 24 and 48 hours post treatment. Of the cytokines analyzed, significant increases (1.5-2 fold) in IP-10, GM-CSF and RANTES were observed at both 24 and 48 hours post treatment in tissues treated with 25 mg of snus. As a measure of genotoxicity, the presence of γ -H2AX foci (specifically, phosphorylation at Serine 139) was evaluated in treated tissues. γ -H2AX is a phosphorylated derivative of the H2AX histone and is tightly bound to double strand DNA break sites, therefore serving as a biomarker of genotoxic insult. γ -H2AX foci were readily detected in the apical layer of tissues treated with 25 mg of snus at 24 and 48 hours post treatment. These results demonstrate the utility of this organotypic oral tissue model to evaluate the safety of smokeless tobacco products.



PP-53. Application of MelanoDerm, an epidermal model containing functional melanocytes, for skin lightening studies

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Considerable interest exists in evaluating cosmetic and/or skin care pharmaceutical formulations which cause lightening of the skin. These products are utilized to cosmetically alter one's natural skin color or to combat skin pigmentation disorders such as melasma, post-inflammatory hyperpigmentation, and other hyperpigmentation lesions. In order to aid in the development and testing of such products, we have developed a melanogenesis/whitening protocol using MelanoDerm™, a highly differentiated, three-dimensional tissue culture model of human epidermis that contains normal human melanocytes (NHM) and keratinocytes (NHK). The use of this 3D model along with the whitening protocol can provide invaluable in vitro data as an early screening tool for raw materials prior to the commencement of costly clinical trials. Skin lightening protocols have been produced containing NHM of varying skin phototypes which follow the expected pigmentation level of the donor tissue, i.e. black>Asian>Caucasian. Tissues are treated topically three times a week over a two to three week treatment period to mimic consumer application. Several over-the-counter skin lightening products were evaluated in MelanoDerm tissues containing NHM from black donors. Over the two-week period, control tissues became increasingly pigmented with retention of normal epithelial morphology. In contrast, tissues treated topically with cosmetic skin lightening agents containing Kojic acid or magnesium ascorbyl phosphate remained distinctly lighter than control cultures. The skin lightening effect on treated tissues was quantitatively evaluated for melanin content using a melanin assay. Treated tissues showed varied although significant changes in overall melanin content (reductions of 12-21%) compared to negative control (water-treated) tissues. The results described herein demonstrate the utility of this model for studying melanogenesis, skin lightening, and other pigmentation phenomena of skin in vitro.



PP-54. Physiologically Relevant in Vitro Model to Predict Acute Respiratory Toxicity of mists and Volatile Liquids

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Acute respiratory toxicity (ART) testing is required to assess the health effects of inhaled substances. OECD accepted methods utilize GHS categorization that is based on animal death. There is no validated in vitro ART assay, even though animal tests have been discredited as predictors of human responses and on ethical grounds. The goals of this work were to develop physiologically relevant ART tests using the EpiAirway™ tissue model, demonstrate interlaboratory transferability, and correlate the results to an established categorization system relevant to human respiratory irritation.

Test articles (TA, n=53) were applied to EpiAirway tissues (0.6 cm²) at MatTek (USA) and IVLSL (Slovakia) with ART protocols developed for exposure to mists/sprays (Direct Application Protocol, DAP) and vapors/volatile liquids (Vapor Cap Protocol, VCP). In both protocols, tissues were exposed for 4 hours to 4 fixed doses of the TA (0.5, 2, 10, 20 mg/tissue, diluted in corn oil or water). In the DAP, TAs were applied to the apical tissue surface and in the VCP - to an absorbent material in a specially designed cap that forms a tight seal above the tissue allowing exposure to TA vapor. The effects on tissue viability (MTT assay) and barrier properties (Transepithelial Electrical Resistance, TEER) were determined. The effective doses which reduced tissue viability by 25% (ED-25) or by 75% (ED-75) were mathematically interpolated for the DAP and VCP methods, respectively. The ED-25 and ED-75 were correlated to the acute irritation Health Effects (HE) Codes (HE14/15/16) listed by OSHA, which are relevant to human exposure. Using the MTT assay, the DAP discriminated between HE14/15/16&NH with a Sensitivity/Specificity/Accuracy (S/S/A) of 77.6/87.6/82.6% (MatTek) and 75.5/86.1/80.8% (IVLSL); correlation to GHS Cat.1&2/3&4/5&NC gave results of 63.5/76.1/69.8% (MatTek) and 63.8/76.1/70.0% (IVLSL) S/S/A. The VCP discriminated between HE codes with S/S/A of 80.9/90.5/85.7% (MatTek) and 77.6/90.0/83.8% (IVLSL); correlation to GHS was 70.1/82.9/76.5 S/S/A (MatTek) and 71.1/82.2/76.7% (IVLSL).

Both protocols demonstrated high predictivity of human HE Codes, which are more relevant to human respiratory toxicity than the GHS categories. Good inter-laboratory reproducibility was observed for the VCP. The VCP and DAP provide physiologically relevant, organ-specific in vitro tests that can improve the predictivity of human responses and significantly reduce the number of animals being used.



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PP-55. Evaluation of Serum Growth Factors in Wound Healing Using a Full-Thickness In Vitro Human Skin Model

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Timely and efficient epidermal wound healing is necessary to maintain normal skin barrier function. Cutaneous wound healing involves interactions between dermal fibroblasts and epidermal keratinocytes as well as cell-extracellular matrix interactions (1-4). The current study describes wound healing experiments conducted in a full-thickness in vitro human skin model (EpiDermFT™). This model exhibits stratified epidermal components and a fully developed basement membrane and resembles in vivo skin in regard to morphology and barrier function. Small epidermal-only wounds (3mm biopsy punch) or full-thickness wounds (cauterizer burns) were induced in the tissue model and cultures were processed for histological evaluation at various time points to observe the healing process under various conditions. Changes in gene expression during wound healing were also monitored using qPCR.



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PP-56. Assessment of heavy metals exposure in prenatal life

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In recent years due to the increase in industrial, agricultural and technical developments highly heavy metal exposures became inevitable. Heavy metals can show their toxic effects by replacing the elements that necessary for the organism's regular body functions or by creating oxidative stress with reactive oxygen species.

Prenatal life is considered the most important period for human development due to the high fetal cellular division and differentiation. Heavy metal exposure at this period is thought to be responsible for some problems in the pregnancy and birth process. Recent studies have shown that there is a relationship between heavy metal exposure and the occurrence of problems such as premature birth, low birth weight and others (Hu et al., 2015; Irwinda et al., 2019). For these reasons; detecting and measuring of these environmental pollutants in maternal and neonatal materials and the determination of the potential risks they are associated with are becoming increasingly important.

This review aims to present the studies conducted on heavy metal levels in biological samples of mothers and newborns in the last 20 years.



PP-57. Investigation of cytotoxic effects of novel hydrazone derivatives as cholinesterase inhibitors

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Alzheimer's disease (AD) is among the most common neurodegenerative diseases in the world. The hydrazones are very effective compounds in terms of biological activity for this disease. Acetylcholinesterase inhibitory activities have also been proven by literature studies. For this purpose, novel hydrazone derivatives were synthesized within the scope of this study. The structures of the compounds to be obtained elucidated by ¹H and ¹³C NMR and HRMS spectroscopic methods. The inhibitory effects of the final compounds on AchE and BchE enzymes investigated using *in vitro* Ellmann methods. According to the results of the activity studies, compounds 4d, 4f and 4h were determined as the most active derivatives in the series with IC₅₀=0.116±0.004 µM, IC₅₀=0.042±0.001 µM and IC₅₀=0.059±0.002 µM, respectively. For a compound to be a drug candidate, it must be active as well as not showing cytotoxic properties. For this purpose, the cytotoxic effects of active compounds were evaluated by MTT method against NIH/3T3 (mouse embryonic fibroblast cells). MTT assays of active compounds were evaluated at 24 hours and 48 hours. As a result of cytotoxicity studies, compounds 4d, 4f, and 4h were found to be non-cytotoxic.

Keywords: Cytotoxic effects, MTT, Hydrazone, Cholinesterase inhibitors.



PP-58 Chemical, Biologic, Radiologic and Nuclear Risk and Prevention Approaches

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Purpose: Human health and the environment are harmed as a result of deliberate or accidental release of Chemical, Biological, Radiological, Nuclear (CBRN) agents. Advances in technology in our age and chaos in the world will cause more CBRN agents to pose significant risks in the future. Therefore, in this review, it is aimed to summarize the current CBRN threats and the precautions that can be taken.

Methods: Research data is obtained by snowball search methods using the relevant keywords through the Pub Med, Researchgate and Web of Science databases, as well as the directories and websites of health and CBRN authorities.

Findings: Current findings indicate that CBRN terrorism has been widespread in recent years. Programs are being created to support primary research agents of chemical or biological terrorism, to develop model systems to evaluate new medical countermeasures, and to support early-stage drug discovery through grants to government and private research institutions. It is stated that the low level of knowledge of the society about CBRN threats, the diversity of CBRN agents and the inadequacy of the personnel in the ambulances in the CBRN management process can create uncertainty and problems in the response to CBRN threats. In addition, the absence of detailed content about CBRN in the hospital disaster plans, the insufficient protective clothing, equipment and the presence of decontamination area indicate that the preparations on this subject are not at the desired level.

Results: Studies show that the process of precautions against CBRN threats and the available information are still in their infancy in our country. For an effective and continuous CBRN management process, sanctions should be developed to raise awareness of the society about CBRN, to train the personnel who will cooperate, to organize exercises at regular intervals, to make evaluations to measure their competencies, and to strengthen inter-institutional communication.

Keywords: CBRN, threats, toxicology, public health, disaster education



PP-59. Quantity Determination and Toxicological Evaluation of Pyrrolizidine Alkaloids in Supplementary Foods and Herbal Tea

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Pyrrolizidine alkaloids (PA) are the most common toxic secondary plant metabolites found in more than 6000 plant species worldwide. These compounds especially those with 1,2 unsaturated bonds, pose a threat to human health with their genotoxic and carcinogenic properties. There is concern and growing awareness among regulators about the risks associated with consuming PA-contaminated products. For this purpose, a method based on quadrupole time-of-flight mass spectrometry-liquid chromatography (LC-MS-QTOF) was validated to determine the amount of PA in herbal supplements and teas. 28 PA were analyzed. Quantification of products such as green tea and green tea supplements, form teas and chamomile teas, mixed herbal teas, and products containing PA alkaloids such as comfrey and coltsfoot were performed. In our study, 11 different PAs, mostly intermidine and N-oxide, were found in 34 different products. At least one PA was detected in 44% (14) of 31 samples, excluding PA-containing samples. The highest total PA amounts belong to *Sympytm officinale* samples, as expected. In the analyzed samples, varying amounts of PAs were found in the range of 2.70 ng/g-9.37 µg/g in 17 of them. It was concluded that some of the amounts detected in the samples were above the allowable limit values. The first data on different types of samples found in our country were obtained.



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