TTD 2025

12TH INTERNATIONAL CONGRESS OF THE TURKISH SOCIETY OF TOXICOLOGY

November 6-9, 2025, İstanbul

Toxicology in Protecting Human and Environmental Health

ABSTRACT BOOK

12th International Congress of the
Turkish Society of Toxicology
06-09 November 2025 - İstanbul, Türkiye

ABSTRACT BOOK

WELCOME

It is my great pleasure to invite all of you to the **12th International Congress of the Turkish Society of Toxicology (TST)**, which will be held in Istanbul, Turkiye between 06-09 November, 2025.

Our congress, themed **"Toxicology in Protecting Human and Environmental Health"** will provide a distinguished platform for esteemed toxicology professors, researchers, and students to exchange knowledge and discuss the latest scientific advancements. This meeting aims to foster interdisciplinary collaboration and contribute to the development of innovative solutions to safeguard human and environmental health in the face of global challenges.

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, 2022) 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 explore Istanbul, a city where continents meet and history comes alive. As Turkiye's cultural and historical heart, Istanbul offers a unique blend of ancient landmarks and modern vibrancy. You can immerse yourself in its rich heritage, enjoy the breathtaking views of the Bosphorus, and experience the dynamic spirit of this timeless metropolis.

We invite you to attend TST2025 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 TST2025!
Prof. Dr. Yalçın Duydu, ERT
President of TST

COMMITTEES

Congress Chair
Dr. Nurşen BAŞARAN Türkiye

Organizing Commiittee - Executive Committee of TST Congress	
Dr. Yalçın DUYDU	Congress Co Chair
Dr. Özlem ATLI EKLİOĞLU	Congress Secreteriat
Dr. Bensu KARAHALİL	Member
Dr. Onur ERDEM	Member
Dr. Aylin ÜSTÜNDAĞ	Member
Dr. Suna SABUNCUOĞLU	Member
Dr. Sibel ÖZDEN	Member

Scientific Committee	
Dr. Ahmet AYDIN, Türkiye	Dr. Helena KANDORAVA, Slovakia
Dr. Ali Esat KARAKAYA, Türkiye	Dr. Marco RACCHI, Italy
Dr. Andrea HARTWIG, Germany	Dr. Merve GÜDÜL BACANLI, Türkiye
Dr. Anja HAVERIC, Bosnia & Herzegovina	Dr. Mirta MILIC, Croatia
Dr. Bensu KARAHALİL, Türkiye	Dr. Neslihan AYGÜN KOCABAŞ, Belgium
Dr. Biljana ANTONIJEVIC, Serbia	Dr. Nurşen BAŞARAN, Türkiye
Dr. Carina LEDEIRA, Portugal	Dr. Salmaan INAYAT-HUSSAIN, UK
Dr. Emanuela CORSINI, Italy	Dr. Sibel ÖZDEN, Türkiye
Dr. Hilmi ORHAN, Türkiye	Dr. Sinan Süzen, Türkiye
Dr. Jose MANATOU, USA	Dr. Yalçın Duydu, Türkiye

SUPPORTING ORGANIZATONS

SPONSORS

The Organising Committee of TTD2025 is thankful to the above organizations and sponsors for their contribution and support

SCIENTIFIC PROGRAMME

November 6, 2025 – Thursday	
Eğitim – Klinik Araştırmalarda Risk Değerlendirmesi ve Biyobenzer Ürünlerde İmmünojenisite Testleri	
Moderatorler: Uzm. Dr. Muhammed Emin Çelik, Prof. Dr. Nurşen Başaran	
13.00-13.30	Faz I klinik çalışmalarını desteklemek için kullanılan klinik öncesi verilerin risk değerlendirmesi **Prof. Dr. Yalçın Duydu**
13.30-14.00	Klinik araştırmalarda risk değerlendirme raporunda dikkat edilecek hususlar **Dr. Ecz. Hilal İlbars**
14.00-14.30	İlaç sektörü açısından klinik araştırmalarda risk değerlendirmesinde karşılaşılan sorunlar **Dr. Mahir Kurt (Roche Türkiye)**
14.30-15.00	Soru – Cevap
15.00-15.30	Kahve arası
Eğitim – Biyobenzer Ürünlerde İmmünojenisite Testlerinin Önemi	
Moderatorler: Uzm. Dr. Muhammed Emin Çelik, Prof. Dr. Nurşen Başaran	
15.30-16.00	Biyobenzer ürünlerde immünojenisite testleri **Prof. Dr. Bensu Karahalil**
16.00-16.30	Biyobenzer ürünlerde ruhsatlandırma **Prof. Dr. Alper İskit**
16.30-17.00	Biyobenzer ürünlerde klinik araştırmalardaki güncel durum **Dr. Kim. Müh. Ayla Özsar**
17.00-17.30	Biyobenzer ürünlerde karşılaşılan sorunlar **Dr. Hasan Ersin Zeytin (Yerlikaya Biopharma İlaç Sanayi ve Tic. A.Ş.)**
18.00-18.20	**Welcome** Congress Chair: Nurşen Başaran President of TST: Yalçın Duydu
Opening Lecture	
Chair: Ali Esat Karakaya	
18.20-18.50	One health in the perspective of a toxicologist **Félix Carvalho (Portugal)**
19.00-21.00	Opening Reception

SCIENTIFIC PROGRAMME

November 7, 2025 – Friday	
Plenary Lecture	
Chair: Yalçın Duydu	
09.00-09.30	Toxicological consideration and risk assessment of drug impurities **Biljana Antoijevic (Serbia)**
Session 1, Transporters in Toxicology – Unraveling Mechanisms of Xenobiotic Exposure, Metabolic Dysfunction, and Reproductive Health	
Chairs: José E. Manautou, Bensu Karahalil	
09.30-10.00	Fetoplacental toxicity and mycoestrogens at the placental barrier **Lauren M. Aleksunes (USA)**
10.00-10.30	Multidrug resistance protein 4 dysfunction drives hepatic steatosis and metabolic dysregulation: Integrated *in vivo* and *in vitro* insights **José E. Manautou (USA)**
10.30-11.00	Integrating drug development tools to predict per- and polyfluoroalkyl (PFAS)-transporter interactions and disposition to human liver **Angela L. Slitt (USA)**
11.00-11.15	Coffee break
Session 2, Epigenetic Mechanisms in Toxicology	
Chairs: Sibel Özden, Benay Can Eke	
11.15-11.45	Epigenetic biomarkers for chemical hazard assessment **Joëlle Rüegg (Sweden)**
11.45-12.15	AHR-mediated m6A RNA methylation contributes to PM2.5-induced cardiac defects **Tao Chen (China)**
12.15-12.45	Role of epigenetic modifications in the toxicity mechanisms of Fusarium-induced mycotoxins **Ecem Fatma Karaman (Türkiye)**
12.45-14.00	Lunch break
14.00-16.00	Parallel Session - Industry Sponsored Workshop / MatTek *In vitro* 3D reconstructed human skin tissue models and their use in toxicology and pharmacology

SCIENTIFIC PROGRAMME

November 7, 2025 – Friday	
Session 3, New Approaches to *In Vitro* Efficacy/Toxicity Assessment – Advantages and Limitations	
Chairs: Félix Carvalho, Merve Güdül Bacanlı	
14.00-14.30	New air-liquid interface (ALI) system approach for drug efficacy/toxicity testing **Merve Güdül Bacanlı (Türkiye)**
14.30-15.00	Challenges in implementing new approaches methodologies in the risk assessment of nanomaterials **Ivana Vinković Vrček (Croatia)**
15.00-15.30	Organoid models to test drug safety and efficacy **Esra Erdal (Türkiye)**
15.30-16.00	Advancements of in vitro methods for inhalation toxicity towards next generation risk assessment **Tommaso Serchi (Luxembourg)**
16.00-16.15	Coffee break
Session 4, Safety in Psychotropic Medications Treatment	
Chairs: Sinan Süzen, Christoph Hiemke	
16.15-16.45	Monitoring the safety and tolerability of antipsychotic drug treatment **Christoph Hiemke (Germany)**
16.45-17.15	Comprehensive overview on intoxications with psychotropic drugs **Fredrik C. Kugelberg (Sweden)**
17.15-17.45	Pharmacokinetic genes variations and response to treatment: the examples of risperidone, aripiprazole and clozapine **Eap-Bin Chin (Switzerland)**
17.45-18.15	The impact of pharmacodynamic gene variations on adverse drug reactions in antidepressant treatment **Sinan Süzen (Türkiye)**
Session 5, Advances in Food Safety and Risk Assessment: Emerging Challenges and Solutions	
Chair: Türkan Yurdun, Biljana Antoijevic	
18.15-18.45	Toxic metals in food and their emerging role as risk factors in hormone-related reproductive cancers **Aleksandra Buha (Serbia)**
18.45-19.15	Food safety risk assessment in Türkiye **Serap Hancı (Türkiye)**
19.15-19.45	Challenges of the risk assessment of dietary microplastics **María-Carmen Rubio-Armendáriz (Canary Islands, Spain)**

SCIENTIFIC PROGRAMME

November 8, 2025 – Saturday	
Plenary Lecture	
Chair: Onur Erdem	
09.00-09.30	Toxicology risk management through interdisciplinary approaches to meet ESG requirements and sustainability challenges **Salmaan Hussain B Inayat Hussain (UK)**
09.30-09.45	Coffee break
Session 6, The Importance of Genotoxicity Tests in the Biomonitoring of Chemical Hazards in Occupational Settings	
Chairs: Mirta Milic, Aylin Üstündağ	
09.45-10.10	The comet assay in human biomonitoring **Andrew Collins (Norway)**
10.10-10.35	Alkaline comet and micronucleus cytome assay – Methods and Perspectives through HCOMET, HUMNAP, EDIAQI, AND BIOMOLTOX Projects **Mirta Milic (Croatia)**
10.35-11.00	Steel industry in Bosnia and Herzegovina – Human biomonitoring in exposed population **Anja Haveric (Bosnia and Herzegovina)**
11.00-11.25	Genotoxicity biomarkers in occupational exposure to hazardous compounds in Portugal: Lessons on formaldehyde, antineoplastics and volatile organic compounds **Carina Ladeira (Portugal)**
11.25-11.50	Occupational exposure to mineral fibers – biomarkers of exposure, effect and susceptibility **Maria Dusinska (Slovakia/Norway)**
12.00-13.30	Lunch
Session 7, Cutting-edge Advances in NAMs – What's New and What's Next	
Chairs: Emanuela Corsini, Özlem Atlı Eklioğlu	
13.30-14.00	Advancing immunotoxicology: NAMs for immunosuppression and developmental immunotoxicity **Emanuela Corsini (Italy)**
14.00-14.30	Advancing ocular safety: insights into the new OECD guideline **Helena Kandarova (Slovakia)**
14.30-14.45	NAMs in cosmetics: the future of safety and innovation **Zehra Sarıgöl Kılıç (USA)**
14.45-15.00	Computational and predictive analysis in toxicology **Sanin Haveric (Bosnia and Herzegovina)**
15.00-15.15	Coffee break

SCIENTIFIC PROGRAMME

November 8, 2025 – Saturday	
Parallel Session - Introducing CAS (A division of the American Chemical Society)	
14.00-14.30	From Data to Discovery: Exploring the Role of CAS Solutions in Advancing Toxicological Research **Miriam Plana (Spain)**
Session 8, Microplastics – State of the Science and the Impacts on Human Health	
Chairs: Neslihan Aygün Kocabaş, Erik Rushton	
15.15-15.45	Understanding microplastics and human health concerns **Neslihan Aygun Kocabaş (Belgium)**
15.45-16.15	Challenges of microplastics research in risk assessment: Ensuring generation of quality data **Erik Rushton (Netherlands)**
16.15-16.45	Plastic chemical additives: determining human risk from microplastic exposure **John Norman (USA)**
16.45-17.15	Microplastics: unfolding consequences **Marijana Curcic (Serbia)**
Session 9, Advancements in Understanding the Mechanisms of Organ-Specific Toxicity Induced by Chemicals and Drugs	
Chairs: Hilmi Orhan, Sinem Ilgın	
17.15-17.45	New insight into molecular targets and early key events in genotoxicity and renal carcinogenicity of the mycotoxin ochratoxin A **Johannes Borchers (Germany)**
17.45-18.15	Towards understanding the mechanism of methanol-induced optic nerve damage by leveraging human data **Hilmi Orhan (Türkiye)**
18.15-18.45	Particle-induced acute phase response as a mechanism-of-action of particle-induced cardiovascular disease **Ulla Vogel (Denmark)**
18.45-19.15	Human in vitro models for the detection and understanding of chemical-induced nephrotoxicity **Paul Jennings (The Netherlands)**
20.00	Gala dinner

SCIENTIFIC PROGRAMME

November 9, 2025 – Sunday	
Plenary Lecture	
Chair: Ahmet Aydın	
09.00-09.30	Mode of action-based refined risk assessment for direct and indirect genotoxic carcinogens **Andrea Hartwig (Germany)**
Session 10, European and National Regulation on Medical Devices – Challenges and Opportunities for Toxicologists	
Chairs: Gül Özhan, Suna Sabuncuoğlu	
09.30-10.00	Medical devices made of substances: challenges on definitions for classification and biosafety assessment **Marco Racchi (Italy)**
10.00-10.30	Toxicological challenges for industry in the application of MDR 745/2017 **Nina Eriksen (Denmark)**
10.30-11.00	Methodological aspects for biosafety compliance in medical devices **Emanuela Testai (Italy)**
11.00-11.30	Toxicological risk assessment for extractables/leachables in pharmaceuticals and medical devices **Ahmet Aydın (Türkiye)**
11.30-12.00	Closing ceremony

November 7, 2025 – Friday	
Oral Presentations - Molecular Toxicology	
Chairs: Burcu Ünlü Endirlik, Ayşe Eken	
14.10-14.20	Mitochondrial Effects of PFOA and its Alternative HFPO-TA in Cardiac Cells **Tuğçe Boran**
14.20-14.30	Comparative *In Vitro* Toxicity of Tartrazine and Its Primary Metabolite Sulfanilic Acid in HT-29 Human Colon Cells **Merve Baysal**
14.30-14.40	Investigation of the Cytotoxic and Cell Migration Inhibitory Effects of Doramectin on Human Cervical Carcinoma and Liver Adenocarcinoma Cell Lines **Dilan Aşkın Özek**

SCIENTIFIC PROGRAMME

November 7, 2025 – Friday	
14.40-14.50	Investigation of Genetic/Oxidative Damage and Molecular Docking Potential of Dinotefuran Insecticide on Blood Lymphocytes **Mazlume Pırıl Coşkun**
14.50-15.00	Tilorone-Induced Autophagy-Dependent Apoptosis in Triple-Negative Breast Cancer **Şükran Ozdatlı Kurtuluş**
15.00-15.10	Epigenetic Modulation of DNMT1 and Estrogen Receptors (ESR1/ESR2) by SGI-1027 in Triple-Negative Breast Cancer **Seher Karslı**
Oral Presentations - Environmental and Organ-Specific Toxicology	
Chairs: Sevtap Aydın, Onur Kenan Ulutaş	
15.30-15.40	Targeting Amyloid Toxicity: Naringin Mitigates Phe-Phe-Induced Neurodegeneration in SH-SY5Y Cells **Emre Korkmaz**
15.40-15.50	Epigenetic Modulation of Circadian Genes PER1 and CHRONO by Histone Deacetylation in a Parkinson's Disease Model *In Vitro* **Zülfinaz Betül Çelik**
15.50-16.00	Doxylamine-Pyridoxine in Pregnancy: What is the Real Risk? **Onur Kenan Ulutaş**
16.00-16.10	Evaluation of Thymoquinone's Neuroprotective Role Against Olanzapine-Induced Gene Expression Alterations in Rat Brain **Miyase Yaylagül**
16.10-16.20	Determination of Organophosphate Flame Retardants in Children with Thyroid Dysfunctions **İrem İyigündoğdu**
16.20-16.30	Effects of Clothianidin on Global DNA Methylation in Hepg2 Cells **Metin Caner Çakır**
16.30-16.40	Environmental Toxicant-Induced Epigenetic Transgenerational Inheritance in Male Infertility **Merve Arıcı**
16.40-16.50	Evaluation of the Effect of Herpes Simplex Glycoprotein B on the Necroptosis Pathway in An *In Vitro* Model of Parkinson's Disease **Berfu Şura Güneş**
16.50-17.00	Liver Safety of Oral Antidiabetics: From the Perspective of MASLD **Sena Ergün**
17.00-17.10	A Study on the Awareness of University Students about the Healthy Use of Skin Care Products and Their Toxicological Effects **Hülya Gül**

SCIENTIFIC PROGRAMME

November 8, 2025 – Saturday	
Oral Presentations - Therapeutic Agents and Natural Products	
Chairs: Pınar Erkekoğlu	
14.00-14.10	*In Vitro* Antioxidant Activity of *Anthemis altissima* L. Flower Extract Against Acetamiprid in HepG2 Cell Line and Comparison with AI Predictions **Yağmur Emre Arıcan**
14.10-14.20	Evaluation of Cytotoxic and Genotoxic/Antigenotoxic Effects of *Rheum Ribes* L. Root and Stem Extracts on A549 Lung Cancer Cells **Halime Serinçay**
14.20-14.30	Investigation of the Effects of Royal Jelly Against UV-Induced Photoaging in Hacat Cell Cultures **Hilal Akar**
14.30-14.40	Cytotoxic, Genotoxic/Antigenotoxic and Epigenetic Effects of Bee Bread on Healthy and Cancer Lung Cell Lines **Gökçe Taner**
14.40-14.50	Cytotoxic and Anti-Migration Effects of Thymoquinone on Various Cancer Cell Lines **Hande Yüce**
14.50-15.00	Protective Effects of Green Tea Extract Against Titanium Dioxide Nanoparticle-Induced Toxicity in Lung Cells **Anıl Yirün**
15.00-15.10	From Waste to Cure: Anticancer Potential of Supercritical and Agro-Residual Extracts of Stinging Nettle **Jülide Secerli**
15.10-15.20	Rosmarinic Acid Restores Redox Balance and Limits Apoptosis in Testes of Type 2 Diabetic Rats **Cagatay Oltulu**
15.20-15.30	Evaluation of the Potential Anticancer Activity of Extracts from Endemic Species Origanum Acutidens (Hand.-Mazz.) Ietswaart **Ayşenur Bilgehan**
15.30-15.40	Coffee break

SCIENTIFIC PROGRAMME

November 8, 2025 – Saturday	
Oral Presentations - *In Silico* and Mechanistic Toxicology	
Chairs: Asuman Karakaya, Bülent Ergun	
15.40-15.50	Therapeutic Performance and Toxicological Profiles of Gold and Silver Nanoparticles in Photothermal Applications **Şevval Çelikten**
15.50-16.00	Reducing Toxicological Risks and Drug Waste: Stability and Efficacy Profiling of Monoclonal Antibodies in Healthcare Settings **Orhan Ziya**
16.00-16.10	The Effects of Azithromycin on Some Biological Parameters of *Galleria Mellonella* L. (Lepidoptera: Pyralidae) **Kübra Aslan**
16.10-16.20	Toxicity Profiling of Novel Tetrazolato Ligands: Bridging the Knowledge Gap with Multi-Tool *In Silico* Approaches **Fatma Okuş**
16.20-16.30	Integrated Evaluation of Mediterranean Herbs and Spices for Antiviral and Immunomodulatory Activities Against SARS-CoV-2 Using *In Silico*, Highthroughput, and *In Vitro* Methods **Yuksel Cetin**
16.30-16.40	Glyphosate and the Gut: An In Silico Toxicogenomic Approach to Healthy Human Microbiome-Related Disease Pathways **Mine Çağlayan**
16.40-16.50	Network Toxicology and Molecular Docking for Elucidating Mutagenicity Mechanisms of Imidacloprid **Tuğrul Mehtiyev**
16.50-17.00	Mycotoxin Exposure in Children: A Biomarker-Based Approach **Gizem Sena Elagoz**

CONTENTS

LECTURES

CONTENTS

CONTENTS

ORAL PRESENTATIONS

CONTENTS

CONTENTS

CONTENTS

CONTENTS

Lectures

LE-01. One Health in the Perspective of a Toxicologist

Félix Carvalho

UCIBIO/i4HB, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, Portugal.

The One Health concept acknowledges the fundamental interdependence among human, animal, and environmental health, promoting transdisciplinary collaboration to achieve sustainable protection of all three domains. While traditionally applied to infectious diseases and antimicrobial resistance, this integrative framework is equally critical in addressing chemical pollution and toxicological threats that increasingly compromise global health and ecosystem integrity. From historical cases such as Minamata, Itai-Itai, and Seveso to current global concerns, including pharmaceutical and plastic pollution, lithium accumulation, endocrine disruptors, and the effects of climate change, chemical stressors continue to challenge both ecosystems and public health. The One Health framework provides an essential context for toxicology to address these complex interactions through mechanistic research, mixture toxicity assessment, and advanced biomonitoring. The exposome concept further enhances this perspective by encompassing the totality of environmental exposures across the life course. Toxicology thus plays a pivotal role in evidence-based risk assessment, regulatory guidance, and the development of preventive policies. Integrating toxicological science into the One Health paradigm strengthens its capacity to safeguard planetary health and ensure the sustainability of future generations.

LE-02. Toxicological Consideration and Risk Assessment of Drug Impurities

Biljana Antonijević

University of Belgrade – Faculty of Pharmacy, Department of Toxicology, Belgrade, Serbia,

Qualification of non-mutagenic impurities (NMIs) in drug substances and products includes safety evaluation strategies and alternative methodologies for assessing these impurities. The qualification „is the process of acquiring and evaluating data that establishes the biological safety of an individual impurity or a given impurity profile at the level(s) specified“. The limitation of ICH Q3A/B guidelines is that only the biological safety of a drug substance or drug product with a given impurity profile has been established (i.e., qualified), which is not the same as characterising the safety profile of an impurity. Therefore, they offer limited guidance on novel or elevated impurity levels. In general, impurities are assessed based on exposure levels, route of administration, physicochemical properties, bioavailability, degradability, clinical conditions, and target population. Factors like treatment duration, clinical indication, and target population (e.g., children, pregnant individuals) influence impurity qualification, too. To overcome the gaps, in the proposed methodologies, the emphasis is put on the alternative strategies to *in vivo* animal studies, aligning with the 3Rs principles (replacement, reduction, refinement). Risk assessment is based on the calculation of the acceptable level (AL) for product-specific impurity limits. AL is derived using toxicological data (e.g., NOAEL, BMDL) and uncertainty factors (e.g., interspecies variability, bioavailability differences). When impurity-specific safety information is required, alternative strategies to gather this information may be followed, including the use of threshold of toxicological concern (TTC), (Q)SAR, read-across (RAX), and *in vitro* approaches. However, if the alternative options mentioned above have not provided the necessary information to qualify an impurity at a proposed specification limit, a preferred *in vivo* study can be used. A weight-of-evidence (WoE) approach that involves evaluating multiple sources of information to support a conclusion can characterize the safety of NMIs at specified levels, minimizing reliance on animal studies.

Keywords: API, impurities, risk evaluation, alternative methodologies, acceptable level

LE-03. Fetoplacental Toxicity and Mycoestrogens at the Placental Barrier

Lauren Aleksunes

Rutgers University, Pharmacology and Toxicology, USA

As global temperatures rise and environments become more humid, concerns are growing over hidden fungal toxins in our diet. Warm, damp conditions promote the overgrowth of *Fusarium* fungi and the release of mycotoxins, including zearalenone. Structurally similar to 17β-estradiol, zearalenone interacts with estrogen receptors and is classified as a *mycoestrogen*. Our research team of toxicologists and epidemiologists has investigated zearalenone exposure and its metabolites during human and rodent pregnancy, focusing on: 1) dietary sources of these toxins, 2) effects on sex steroid production, and 3) placental transport and toxicities. We have identified the Q141K variant in the *BCRP/ABCG2* gene as a risk factor for mycoestrogen-associated reductions in fetoplacental ratios and birth weight. As research progresses, identifying factors that compromise BCRP-mediated protection will be crucial to safeguarding vulnerable populations at higher risk of perinatal mycoestrogen toxicity.

LE-04. Multidrug Resistance Protein 4 Dysfunction Drives Hepatic Steatosis and Metabolic Dysregulation: Integrated *In Vivo* and *In Vitro* Insights

José E. Manautou

University of Connecticut, Storrs, CT, United States

Multidrug Resistance Protein 4 (MRP4) is an efflux transporter that plays a pivotal role in drug metabolism and endogenous substrate clearance. Our integrated *in vivo* and *in vitro* studies reveal that MRP4 deficiency disrupts hepatic lipid homeostasis and promotes metabolic syndrome. In mice, the absence of MRP4 prolonged post-hepatectomy steatosis and, under a high-fat, high-sucrose diet, exacerbated liver injury, dyslipidemia, and insulin resistance. Adipose tissue of knockout mice exhibited increased inflammatory and fibrotic markers with activation of the cyclic adenosine monophosphate (cAMP)–cAMP response element-binding protein (CREB)–CREB regulated transcription coactivator 2 (CRTC2) pathway. In parallel, *in vitro* MRP4 silencing in HepaRG cells impaired lipid metabolism, altering the expression of lipogenic and adipogenic genes.

These studies indicate that impaired efflux of key substrates, such as cAMP and prostaglandin E2 (PGE2), underlies hepatic steatosis and metabolic dysfunction, offering novel insights into metabolic dysfunction-associated steatotic liver disease (MASLD), opening avenues for in-depth mechanistic studies and identifying potential targets for precision therapies.

LE-05. Integrating Drug Development Tools to Predict Per- And Polyfluoroalkyl (PFAS)-Transporter Interactions and Disposition to Human Liver

Angela L. Slitt

University of Rhode Island, Kingston, RI, United States

Per- and polyfluoroalkyl substances (PFASs) are man-made chemicals found in food packaging, stain repellents, and non-stick products. PFOS and PFOA can persist for years in humans and are linked to dyslipidemia, hypothyroidism, liver injury, and diminished vaccine response. As thousands of novel PFAS continue to emerge, as well as other unwanted environmental chemicals, there is an urgent need for efficient toxicokinetic and toxicodynamic prediction tools. We collaborated with Pfizer Global Research to apply drug development approaches for 14 PFAS, and applied the Extended Clearance Classification System (ECCS) to predict transporter interactions. Nine PFAS had low-permeability, whereas five showed moderate or high permeability. Some PFAS were identified as substrates for human xenobiotic transporters, such as Organic Anion Transporters (OATs) 3 and 4, Organic Anion Transporting Polypeptides (OATPs), and ATP-Binding Cassette G2 (ABCG2). Mining matched human serum-liver specimens further exemplifies PFAS structures that the ECCS predicts for liver distribution. These findings illustrate the promise of ECCS in predicting PFAS transporter interactions and mechanisms of clearance.

LE-06. Epigenetic Biomarkers for Chemical Hazard Assessment

Joëlle Rüegg

Uppsala University, Department of Organismal Biology; Physiology and Environmental Toxicology, Sweden

Epigenetics refers to the stable propagation of processes across generations of cells or organisms, independent of changes in the underlying DNA sequence; such processes include DNA methylation, chromatin structure modification, and the activity of non-coding RNAs. Epigenetic processes regulate temporal and spatial patterns of transcription and play a critical role in cell differentiation and tissue organisation during development. The epigenome is responsive to environmental exposures such as chemicals, and evidence is increasing that early-life chemical exposure induces epigenetic changes that might underlie long-lasting effects in one or over several generations. Due to their nature as heritable marks, epigenetic changes are less transient than, e.g., transcriptional alterations. As such, they could be used as predictors of delayed toxicity in one and over several generations.

In my presentation, I will give three examples on identifying epigenetic biomarkers for chemical hazards. Firstly, in a targeted approach combining insights from experimental and epidemiological studies, we have identified DNA methylation patterns at the neurodevelopmental gene *GRIN2B* mediating parts of the association between prenatal bisphenol F (BPF) exposure and lower IQ in 7 year old children. Secondly, using a genome-wide approach, we have identified DNA methylation changes induced by developmental exposure to six chemicals (BPF, butyl benzyl phthalate, DINCH, PFOS, permethrin, and triphenyl phosphate) associated with learning and memory impairments in a rat model. Thirdly, in a transgenerational study using zebrafish developmentally exposed to PFOS or PFBS, we could identify DNA methylation alterations induced in the directly exposed generation (F0) associated with behavioural changes in the unexposed generation (F2). Such epigenetic changes could become biomarkers predicting adverse outcomes induced by developmental exposures, and thus be used as a complement to, or instead of, testing in adult animals in one or several generations.

LE-07. AHR-mediated m6A RNA methylation contributes to PM2.5-induced cardiac defects

Shoushuang Zhao[1], Yizhou Tao[1], Xiaoxiao Li[1], Yan Jiang[1], Tao Chen[1,2]

[1] School of public health, Suzhou medical college, Soochow University, Suzhou, China.

[2] Soochow University, Suzhou, China.

Recent studies have linked maternal exposure to fine particulate matter (PM2.5) with congenital heart diseases in offspring, yet the underlying mechanisms remain unclear. We hypothesized that m6A RNA methylation is a key mechanism in PM2.5-induced cardiac developmental toxicity. Our experiments showed that extractable organic matter (EOM) from PM2.5 caused cardiac defects in zebrafish embryos and impaired cardiac differentiation in rat H9c2 cardiomyocytes. Crucially, EOM exposure led to a reduction in global m6A RNA methylation, an effect that was prevented by inhibiting the aryl hydrocarbon receptor (AhR). Further experiments demonstrated that AhR activation by EOM directly repressed the transcription of methyltransferases *mettl14* and *mettl3*, which upregulated the expression of *traf4a* and *bbc3*, triggering apoptosis and leading to cardiac malformations in zebrafish embryos. However, in rat H9c2 cells, AHR activation directly promoted the transcription of demethylase FTO, which enhances Nox4 expression by reducing its m^6A RNA methylation. The resulting oxidative stress inhibited the Wnt/β-catenin signaling, thereby compromising cardiomyocyte differentiation. Furthermore, we found that AhR activation by EOM reduced the methyl donor S-adenosylmethionine (SAM) levels by suppressing Dhfr, leading to global RNA hypomethylation in both zebrafish embryos and H9c2 cells. In conclusion, our findings indicate that PM2.5 induces cardiac defects by causing m6A RNA hypomethylation through two synergistic mechanisms: disturbing the expression of m6A regulators, and reducing the availability of the essential methyl donor.

Keywords: PM2.5; AhR; m6A RNA methylation; heart development

LE-08. DNA Methylation, Histone Modifications, and Beyond: How Emerging *Fusarium* Mycotoxins and Their Derivatives Disrupt Epigenetic Regulation

Ecem Fatma Karaman

Biruni University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Istanbul, Turkiye

Emerging *Fusarium* mycotoxins are secondary metabolites produced by *Fusarium* fungi that are gaining attention due to their increasing presence in food and feed crops, potential health risks, and challenges in detection and regulation. While traditional *Fusarium* mycotoxins like deoxynivalenol (DON), zearalenone (ZEA), fumonisins (FBs), and T-2/HT-2 toxins are well-studied, emerging mycotoxins are less characterized but pose significant concerns. Emerging mycotoxins such as enniatins (ENNs), beauvericin (BEA), moniliformin (MON), fusaproliferin and alternariol, have gained increasing attention due to their widespread occurrence in food and feed and their potential health risks. Unlike traditional mycotoxins, the toxicological profiles of these compounds are not yet fully understood, particularly concerning their epigenetic effects. Recent studies suggest that emerging mycotoxins may influence epigenetic mechanisms, including DNA methylation, histone modifications, and non-coding RNA expression, potentially leading to altered gene regulation and disease susceptibility. These epigenetic modifications could play a role in long-term health effects, such as carcinogenesis, reproductive disorders, immune dysfunction, and metabolic disorders. Among these mycotoxins, FB1 disrupts sphingolipid metabolism-related gene regulation through histone modifications, and ZEA exerts estrogenic effects via epigenetic modulation of hormone-responsive genes. *In vitro* studies suggest ENNs and BEA may reduce global DNA methylation, potentially activating pro-cancer pathways, while MON could be linked to mitochondrial DNA methylation changes. However, most emerging *Fusarium* mycotoxins are not yet regulated, but the European Food Safety Authority (EFSA) and other agencies are evaluating their risks. Research is ongoing to establish safe limits and mitigation strategies (e.g., biocontrol, resistant crop varieties). Notably, recent studies suggest that these modified forms, including masked, conjugated, and bound mycotoxins, can also interfere with epigenetic regulation, though their specific mechanisms remain poorly understood. Understanding these mechanisms is crucial for accurate risk assessment and the development of effective mitigation strategies to safeguard human and animal health.

Keywords: Epigenetics, *Fusarium*, Gene regulation, Modified mycotoxins, Emerging mycotoxins, Toxicity mechanisms.

LE-09. New Air-Liquid Interface (ALI) System for Drug Efficacy/Toxicity Testing

Merve Güdül Bacanlı[1], Sevde Altuntaş[2]

[1] *University of Health Sciences Turkey, Gülhane Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Türkiye*

[2] *University of Health Sciences Turkey, Tissue Engineering Department, Experimental Medicine Research and Application Center, İstanbul, Türkiye*

Advancements in *in vitro* modeling are essential for improving the predictability of preclinical drug screening. Here, we present a novel air-liquid interface (ALI) system that integrates vascularized Gelatin Methacryloyl (GelMA) hydrogels to create a physiologically relevant 3D model of colorectal cancer. Also, another bioengineered platform is specifically designed for evaluating the oral efficacy and toxicity of drug candidates under biomimetic conditions.

The system features a compartmentalized ALI setup in which colorectal cancer cells are cultured on a vascularized GelMA matrix, simulating both tumor architecture and microvascular perfusion. The vascular structures are embedded within the hydrogel and support nutrient exchange and metabolic waste removal, closely replicating the *in vivo* tumor microenvironment. The air-exposed apical side allows for dynamic exposure to orally delivered drug formulations or toxins, mimicking intestinal drug absorption dynamics.

Real-time readouts such as cell viability, and pro-inflammatory markers can be continuously monitored. This integrated model enables high-content screening of drug efficacy, cytotoxicity, and off-target effects in a system that bridges the gap between conventional 2D cultures and animal models.

By incorporating vascularization and tumor-specific architecture into an ALI-based drug screening platform, this system represents a transformative approach in oral drug testing, cancer pharmacology, and organotypic toxicity assessment.

This study was supported by TÜBİTAK (project number 123S129, awarded to Dr. Merve GÜDÜL BACANLI) and TÜSEB (project number 33514, awarded to Dr. Merve Güdül Bacanlı).

Keywords: *in vitro* model, colon cancer, oral efficacy, oral toxicity, hydrogel, GelMA

LE-10. Challenges in Implementing New Approaches Methodologies (NAMs) in The Risk Assessment of Nanomaterials

Ivana Vinković Vrček, Lucija Božičević, Nikolina Peranić, Nikolina Kalčec, Mateo Celinić

Institute for Medical Research and Occupational Health, Croatia

Manufactured nanomaterials (NMs) are increasingly used in a wide range of industrial applications leading to a constant increase in the market size of nano-enabled products. The increased production and use of NMs are constantly raising concerns among different stakeholder groups with regard to their effects on human and environmental health. Given the limitations associated with animal testing, in recent years there has been interest in developing faster, less expensive, and more informative new approaches, often referred to as New Approaches Methodologies – thus, facilitating implementation of the Three R's principles (3R's - Replacement, Reduction and Refinement of animal testing). However, the development and regulatory uptake of NAMs face several critical challenges. Scientifically, NMs exhibit complex, size-dependent physicochemical behaviors - such as agglomeration, corona formation, and dynamic transformations - that complicate accurate hazard and exposure assessment. Conventional *in vitro* systems often fail to replicate key *in vivo* processes, limiting the reliability of extrapolations. Dosimetry, particularly regarding relevant metrics like surface area or particle number, remains unresolved, and reference materials for benchmarking are scarce. Regulatory acceptance is further hindered by a lack of standardized protocols and limited validation of NAMs for nanotoxicology. Many NAMs yield mechanistic or high-throughput data that, while informative, do not align with the apical endpoints traditionally required in regulatory frameworks. Infrastructure barriers—including poor data standardization, lack of FAIR data implementation, and variability in testing procedures—further limit reproducibility and cross-study comparability. While NAMs offer promise in reducing animal testing and enhancing mechanistic insights, broader adoption will require concerted efforts to establish harmonized guidelines, validate physiologically relevant models, and foster regulatory confidence. Initiatives such as the OECD WPMN and European NanoSafety Cluster play a pivotal role in addressing these gaps and supporting the transition toward a modern, science-based risk assessment paradigm for nanomaterials.

Keywords: New Approaches Methodologies (NAMs), nanomaterials, risk assessment

LE-11. Organoid Models to Test Drug Safety and Efficacy

Esra Erdal

Izmir Biomedicine and Genome Center, Izmir, Turkiye,

Dokuz Eylul University, Faculty of Medicine, Department of Medical Biology, Izmir, Turkiye

Conventional animal and two-dimensional (2D) cell models have long been the foundation of pharmacological and toxicological testing. However, these systems often fail to capture the complexity of human physiology, resulting in poor prediction of drug efficacy and adverse reactions such as idiosyncratic drug-induced liver injury (DILI). Increasing ethical and regulatory attention to the 3R principles (Replacement, Reduction, and Refinement) has driven the need for human-relevant new approach methodologies (NAMs) that better reproduce organ-level structure and function.

Organoid technology, derived from human pluripotent stem cells (hPSCs) or patient tissues, offers a transformative platform for studying drug safety and efficacy *in vitro*. In our work, we established iPSC-derived endodermal hepatic organoids (eHEPOs) that closely mimic key liver-specific functions, including cytochrome P450 enzyme activity and bile canaliculi formation, and exhibit clinically relevant hepatotoxicity responses. Beyond toxicity testing, eHEPOs have been successfully used for disease modeling, such as citrullinemia and liver fibrosis, demonstrating their versatility.

In parallel, we have developed patient-derived cancer organoids (PDCOs) from colorectal and pancreatic adenocarcinomas to evaluate personalized therapeutic responses and explore mechanisms of drug resistance.

By combining multi-omics profiling, AI-driven data integration, and organoid-based functional assays, this approach bridges the translational gap between preclinical testing and real-world patient outcomes. Altogether, organoids represent an ethical, predictive, and human-relevant platform that advances drug discovery, regulatory innovation, and the practice of precision medicine.

Keywords: Organoids, iPSC-derived hepatic organoid, PDCO, drug safety, DILI, NAMs, 3R principles, toxicology, personalized medicine

LE-12. Advancements of *In Vitro* Methods for Inhalation Toxicity Towards Next Generation Risk Assessment

Tommaso Serchi[1], Pamina Weber[1], Emma Arnesdotter[1], Nour Attar[1], Dario Greco[2], Angela Serra[2], Luca Mannino[2], Antonio Federico[2], Francesco Dondero[3], Steffi Friedrichs[4]

[1] *Luxembourg Institute of Science and Technology, SUSTAIN Unit - Environmental Health Group, Belvaux, Luxembourg*

[2] *Tampere University, FHAIVE, Faculty of Medicine and Health Technology, Tampere, Finland*

[3] *Università del Piemonte Orientale, Department of Science and Technological Innovation, Alessandria, Italy*

[4] *AcumenIST, Brussels, Belgium*

The development and implementation of *in vitro* New Approach Methodologies (NAMs) are critical for enabling the transition toward animal-free safety assessment. In the context of inhalation toxicity, there is a growing need for mechanistically informed, human-relevant models that can be integrated into Next Generation Risk Assessment (NGRA) frameworks. Particular challenges include modeling complex endpoints such as respiratory sensitization, irritation, and fibrotic remodeling under physiologically relevant exposure conditions.

Recent progress within the Horizon Europe projects CHIASMA, SCENARIOS and MACRAME has contributed to advancing the LIST's developed ALIsens model, an air–liquid interface (ALI) co-culture system designed to assess respiratory toxicity. Comparative testing under different exposure conditions has been conducted, including the use of VitroCell Cloud, VitroCell PowderX, semi-ALI configurations, and TECAN digital dispensing systems. These approaches were evaluated for their capacity to deliver aerosols, dry powders, or liquid formulations reproducibly and without inducing unspecific stress responses.

To assess the biological relevance and discriminatory capacity of the model, both bulk and single-cell transcriptomic analyses were performed following exposure to selected reference compounds, including known respiratory sensitizers, irritants, and pro-fibrotic agents. The resulting datasets provided insights into both global and cell-type–specific molecular responses and were used to explore mode-of-action differentiation and biomarker discovery.

The integration of these data into NGRA concepts demonstrates the potential for *in vitro* inhalation models to support hazard identification and read-across, and to contribute to risk assessment without the use of animals. These advancements represent important steps toward the broader adoption of NAMs in regulatory contexts, helping to align scientific innovation with evolving ethical and policy frameworks in chemical safety evaluation.

LE-13. Monitoring the safety and tolerability of antipsychotic drug treatment

Christoph Hiemke

University Medical Center Mainz, Department of Psychiatry, Mainz, Germany

The discovery of the antipsychotic efficacy of chlorpromazine in France 1952 was a milestone in psychopharmacotherapy. Before that time, most affected patients had to be hospitalized after the initial onset, usually at a young age, and often lifelong care was required. With the introduction of chlorpromazine, an effective medication was available for schizophrenic patients and lifelong hospitalization came to an end. After the release of chlorpromazine an intensive search started for other active ingredients, not just antipsychotics. Chlorpromazine was soon replaced by more selective compounds, especially the highly potent butyrophenone haloperidol, and by clozapine and its derivatives, atypical antipsychotics. The search for new antipsychotics was largely driven by the search for better tolerability and safety. Over 50 drugs with proven efficacy are now available. However, tolerability and safety continue to limit their use. Toxic effects can manifest as extrapyramidal symptoms, metabolic disturbances leading to obesity, hormonal changes, especially hyperprolactinemia, that may lead to breast cancer or QTc prolongation leading to arrhythmia. Therefore, it is necessary to monitor antipsychotic drug therapy. Measuring drug concentrations in the blood, i.e. the use of therapeutic drug monitoring (TDM), is important. TDM can not only improve efficacy but also safety. Imaging studies have shown that dopamine receptor occupancy exceeding 80% should be avoided because it can trigger motor disturbances. Dopamine D2 receptor occupancy correlates well with blood levels. The antipsychotic drug concentration in blood plasma or serum is a suitable biomarker of dopamine receptor occupancy. TDM is particularly important when using clozapine, which is the most potent antipsychotic and has a narrow therapeutic index. In patients, such as smokers, who are rapid metabolizers of clozapine and therefore require high doses but tolerate them poorly, it may be beneficial to combine treatment with fluvoxamine as a booster to slow clozapine metabolism. This should be monitored by measuring blood levels. This presentation will focus on toxic symptoms associated with antipsychotic drug use and show how to avoid them especially by monitoring drug levels in blood.

LE-14. Comprehensive Overview on Intoxications with Psychotropic Drugs

Fredrik C. Kugelberg

National Board of Forensic Medicine, Department of Forensic Genetics and Forensic Toxicology, Linköping, Sweden

Linköping University, Department of Biomedical and Clinical Sciences, Division of Clinical Chemistry and Pharmacology, Linköping, Sweden

Psychotropic drugs play a central role in the treatment of psychiatric disorders, but their use requires close monitoring due to the risk of both intentional and unintentional overdose. Fatal drug intoxications remain a major public health concern, particularly in the context of increasing polypharmacy among the elderly and the associated risk of adverse drug interactions. Medication errors and adverse drug reactions are relatively common in geriatric patients, who are frequently prescribed sedatives, antidepressants, and antipsychotics. In contrast, drug-related fatalities among adolescents and young adults often involve recreational use of psychoactive substances. The types of drugs implicated in poisoning deaths vary between countries depending on factors such as socioeconomic conditions, prescribing practices, and access to pharmaceuticals. In Sweden, approximately 800 individuals die annually from drug intoxications. Of these, 45% are classified as unintentional poisonings, 30% as suicides, and 25% as of undetermined intent. Among women, poisoning is the most common method of suicide (around 40%), while among men, it accounts for about 15% of suicides. To support preventive efforts, it is crucial to understand whether the substances involved were prescribed to the deceased. Cases of fatal drug intoxication were identified through the Swedish National Board of Forensic Medicine's database. Substances assessed as direct contributors to death were included and linked to data from the Swedish Prescribed Drug Register to determine if the deceased had been dispensed the drug during the year prior to death. Substances involved in unintentional poisonings were more often associated with the illicit drug market or opioid substitution therapy. A high prevalence of prescriptions was observed in suicide cases and among women. Improved knowledge and increased awareness in prescribing practices may help reduce the incidence of fatal drug intoxications.

Keywords: Drugs, Fatalities, Intoxication, Overdose, Poisoning, Prescription

LE-15. Pharmacokinetic Genes Variations and Response to Treatment: The Examples of Risperidone, Aripiprazole and Clozapine

Chin B Eap,

Unit of Pharmacogenetics and Clinical Psychopharmacology, University of Lausanne and Centre les Toises, Lausanne, Switzerland

The cytochrome P450 2D6 (CYP2D6) metabolizes around 20% of currently prescribed medications, including the antipsychotics risperidone and aripiprazole, while CYP1A2 metabolizes clozapine. Risperidone and aripiprazole treatment failure, defined as treatment duration, was analyzed among 515 and 466 psychiatric patients, respectively. After 1 year of treatment, the proportion of patients who switched from risperidone was 44%. The proportion was increased to 70% among poor metabolizers (PMs), vs. 42% among the other CYP2D6 phenotypes (P=0.026)1. CYP2D6 extreme metabolizers (ie, poor and ultrarapid metabolizers) had higher risk of treatment discontinuation from aripiprazole versus intermediate and normal metabolizers after 3, 6, and 12 months of treatment (HR: 2.08,1.75, 1.59, respectively; P=.013, P=.019, and P=.047, respectively)2. Cost-benefit ratios of CYP2D6 genotyping prior to treatment for personnalization of treatment with risperidone and aripiprazole will be presented.

Contrary to CYP2D6, whose activity can be estimated through genotyping and phenotyping methods, CYP1A2 activity is best measured by phenotyping, measuring the caffeine metabolic ratios (CMR) following monitored caffeine intake. Given caffeine's ubiquity, we tested whether random CMR from dietary caffeine were associated with clinical, genetic, and epigenetic factors linked to CYP1A2 activity; plasma concentrations of clozapine and olanzapine; and psychotropic treatment response. First, we analyzed two population-based studies (CoLaus|PsyCoLaus, N= 4898; SKIPOGH, N= 2054) to investigate random CMR associations with clinical, genome-wide, and epigenome-wide factors associated with CYP1A2 activity. Second, in psychiatric cohorts, we tested CMR associations with dose- normalized plasma concentrations (C/D) of clozapine (N= 164) and with psychotropic treatment response, including hospital admission risk (N= 1019) and prolonged stays (N= 1349). CMR were positively associated with age, CYP1A2 inducers including smoking, and negatively with female sex. CMR were negatively associated with clozapine C/D, explaining up to 14.9% of the variance; over six-fold the variance explained by genetic factors. A one-unit increase in CMR was associated with a 26% increased likelihood of hospital admission (p=0.002) and reduced short-stay chance by 11% (p< 10−3). Random CMR provides a useful method to probe CYP1A2 activity, contributing, alongside other variables, to personalizing clozapine doses and identifying psychiatric patients at risk of hospital admission and lengthy stays.

LE-16. The Impact of Pharmacodynamic Genes Variations on Adverse Drug Reactions in Antidepressants Treatment

Merve Demirbügen Öz, H. Sinan Süzen

Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Ankara University, Türkiye

Selective serotonin reuptake inhibitors (SSRIs) are commonly prescribed as first-line pharmacotherapy for depression. Poor clinical outcome (response rates range from 53% to 64%) and moderately high (at least %20) relapse rates are still prominent problems during pharmacotherapy of depression with SSRIs. A primary factor contributing to the inadequacy of depression treatment with SSRIs is the occurrence of adverse drug reactions (ADRs) during pharmacotherapy, which often results in patient discontinuation of these medications. SSRI use is frequently associated with sexual dysfunction (SD) and nausea/vomiting. Since their potential importance in the physiology of sexual functioning and differences of the occurrence of the SD; genetic variations in the neurotransmitters, receptors and proteins of serotonergic pathway, and brain derived neutrophic factor (BDNF) might be plausible targets in SSRI-induced SD. Within this context, we investigated to determine the relationship between serotonin-2A receptor (HTR2A) gene −1438A/G and 102T/C polymorphisms, serotonin transporter gene (SLC6A4) 5-HTT-linked polymorphic region (5-HTTLPR) insertion/deletion variant and BDNF gene Val66Met polymorphisms and the occurrence of SD adverse effect in major depressive disorder patients treated with citalopram (CIT) or sertraline (SERT). A total of 133 patients with major depressive disorder (MDD), were included in the study. SD were assessed with the use of the UKU Side-effects Rating Scale. GVs were identified using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) technique. The findings of this study demonstrated that the −1438A/G and 102T/C polymorphisms appear to be associated with the SD induced by CIT. It was also demonstrated that patients receiving SERT, carrying T allele of HTR2A or L allele of 5-HTTLPR more likely to experience SD. Most important overall finding of the study is the combined effects of −1438A/G, 102T/C, and 5-HTTLPR polymorphisms. In a logistic regression model, the occurrence of SD increased with the number of risky alleles.

Keywords: Citalopram, sertraline, sexual dysfunction, pharmacogenetics

LE-17. Toxic Metals in Food and Their Emerging Role as Risk Factors in HormoneRelated Reproductive Cancers

Aleksandra Buha Đorđević

Department of Toxicology, University of Belgrade-Faculty of Pharmacy, Belgrade, Serbia

Purpose: Toxic metals such as cadmium (Cd), lead (Pb), arsenic (As), and mercury (Hg) contaminate staple foods through natural occurrence and anthropogenic activities. These metals accumulate in rice, cereals, leafy vegetables, seafood, and beverages, and some can mimic estrogens by activating the estrogen receptor α. This abstract synthesizes recent evidence on how dietary metal exposure contributes to hormone-related reproductive cancers.

Methods: Articles describing exposure sources, mechanistic studies, and epidemiologic associations between toxic metals and breast, ovarian, endometrial, or prostate cancers were examined. Data were extracted on dietary sources, biological mechanisms, and risk estimates.

Results: Rice and vegetables contribute most to Cd intake; values above 31.5 µg/day were linked to increased risk of estrogenreceptor-positive breast cancer in Japanese women, while Swedish men consuming ~19 µg/day showed higher prostate cancer incidence. A meta-analysis of 17 studies found that high Cd exposure raised overall breastcancer risk (pooled OR = 1.13), although dietary Cd alone was not significant. Prospective data from NHANES 20032018 showed that urinary Cd (OR ≈ 1.745) and As (OR ≈ 1.005) were risk factors for ovarian cancer, and Cd increased endometrialcancer risk (OR ≈ 1.617). In women under 50 years, blood Pb levels above 9.39 µg/L doubled the risk of developing any cancer and breast cancer compared with lower levels. Advanced endometrialcancer stage correlated strongly with urinary Cd and Pb, whereas Hg showed little effect. Mechanistically, Cd, As, and Pb induce oxidative stress, DNA damage, and epigenetic alterations, and can act as metalloestrogens that enhance estrogen-dependent tumour growth.

Conclusions: Emerging epidemiologic evidence links dietary exposure to Cd, As, and Pb with higher risks of breast, ovarian, endometrial, and prostate cancers. Food contamination arises from industrial pollution, metal-containing pesticides, and phosphate fertilizers, highlighting the need for stringent environmental control and dietary riskreduction strategies. Further mechanistic studies and longitudinal cohorts are warranted to clarify dose-response relationships and evaluate whether reducing metal exposure can lower the hormone-related cancer burden.

LE-18. Food Safety Risk Assessment in Türkiye

Serap Hancı

Ministry of Agriculture and Forestry, General Directorate of Food and Control, Department of Risk Assessment, Türkiye

Ensuring food safety remains a critical public health priority worldwide, with growing complexity in food production and increasing global trade. In Türkiye, as in many countries, food safety authorities have taken significant steps to establish risk-based approaches for managing foodborne hazards. A central component of this effort is scientific food safety risk assessment, which provides an evidence-based foundation for effective decision-making.

Risk assessment is one of the three pillars of risk analysis, along with risk management and risk communication. It is defined as a structured, science-based process that includes hazard identification, hazard characterization, exposure assessment, and risk characterization. In Türkiye, this process is conducted by Scientific Commissions operating under the Department of Risk Assessment (DRA) within the Ministry of Agriculture and Forestry.

This presentation aims to provide an updated overview of food safety risk assessment practices in Türkiye. It will describe the organizational structure of the DRA, the role and selection process of Scientific Commission members, and the operational principles that guide risk assessment activities—such as independence, transparency, and scientific rigor. In addition, the presentation will cover how the risk assessment process interacts with other components of the food safety system, including regulatory decision-making and communication with stakeholders. Examples of current practices and selected case studies will also be presented to illustrate the functioning of the system in practice.

By sharing Türkiye's evolving experience in risk assessment, this presentation seeks to contribute to the broader dialogue on improving food safety systems through science-based approaches.

Keywords: Risk assessment, food safety, hazard

LE-19. Challenges of the Risk Assessment of Dietary Microplastics

María-Carmen Rubio-Armendáriz
Universidad de La Laguna (ULL), Canary Islands, Spain

Dietary exposure to microplastics represents an emerging and highly relevant area within risk assessment, management, and communication, attracting increasing attention from the scientific community, regulatory bodies, and consumers alike. Microplastics ingested through food contribute to the overall human exposure, complementing the inhalation route. The identification and characterization of different types of microplastics are essential steps to understanding and predicting their potential toxicity. Monitoring their presence in food and estimating dietary exposure across populations with diverse dietary patterns remain major challenges. Such data are crucial for food safety authorities—particularly national food safety agencies—to protect consumers and ensure safer food systems. However, comprehensive risk characterization is still not feasible due to significant knowledge gaps and methodological limitations in current analytical approaches.

LE-20. Toxicology Risk Management Through Interdisciplinary Approaches to Meet ESG Requirements and Sustainability Challenges

Salmaan H. Inayat-Hussain

Ipieca, London, United Kingdom,

The UN 2030 Agenda for Sustainable Development calls for global collaboration to promote peace and prosperity for people and the planet. Central to this vision is the integration of sustainability principles—balancing economic, social, and environmental dimensions for the well-being of current and future generations. Environmental, Social, and Governance (ESG) frameworks have emerged as essential tools for evaluating organizational performance and stakeholder impact, particularly in areas related to health, safety, and the environment (HSE).

Toxicological risk management plays a critical role within ESG, especially in environmental and occupational health domains. As regulatory and voluntary reporting expectations evolve, organizations must adopt interdisciplinary approaches to identify, assess, and mitigate toxicological risks. These approaches encompass HSE and product stewardship strategies that align with ESG metrics and sustainability goals.

This plenary will explore how toxicology intersects with ESG reporting, by examining frameworks such as the Global Reporting Initiative (GRI), Ipieca Sustainability Reporting guidance, and the EU Corporate Sustainability Reporting Directive (CSRD), emphasizing how toxicological data and risk assessments can be effectively integrated into ESG narratives. Cross-disciplinary collaboration among toxicologists and other subject-matter experts can enhance an organisation's ESG performance while safeguarding human health and the environment.

LE-21. The Comet Assay in Human Biomonitoring

Andrew Collins
University of Oslo, Norway
NorGenotech SA

The general aim of human biomonitoring is to establish the causes of disease – or to test ways of preventing disease. Observational studies compare a population exposed to some environmental, intrinsic or lifestyle factor with a control group. Intervention studies test the effect of a change, for instance in diet, on disease outcome. Such studies can be long-term, but a short-cut is provided by the use of biomarkers, i.e. metabolic factors measured, typically, in samples of serum, blood cells or urine. Biomarkers can represent exposure to a putative causative agent, protection against such an agent, or they can indicate risk of disease. Regarding cancer risk, the appropriate markers are related to DNA damage, mutations, and chromosome changes. The comet assay is a standard tool for detecting DNA damage – DNA strand breaks, or potentially mutagenic oxidised bases using a modified version of the assay. Versions of the assay are also used to measure the capacity of cells to repair DNA damage. The assay has been applied in many studies of environmental and occupational exposure to chemicals, and also in the field of nutrition, where it is possible to carry out intervention trials, looking at the effect of taking supplements of antioxidants, for example; or of changing the diet to one thought to be more healthy. The comet assay is simple, sensitive, and economical, and standard protocols exist to ensure reliability.

LE-22. Alkaline Comet and Micronucleus Cytome Assay – Methods and Perspectives Through The Results of The hCOMET, HUMNap, EDIAQI, Biomoltox and PROMETHEUS Projects

Mirta Milić, Goran Gajski, Marko Gerić, Vilena Kašuba, Nevenka Kopjar, Katarina Matković, Luka Kazensky, Bruno Bekić

Institute for Medical Research and Occupational Health, Division of Toxicology, Ksaverska cesta 2, 10000 Zagreb, Croatia

The alkaline comet assay, or single-cell gel electrophoresis, is one of the most popular methods for assessing DNA damage, and for further evaluation of genomic instability, the micronucleus assay (now evolved into cytome assay) has been mostly used. Although both assays are well known, with their OECD guideline protocols for some cell types, there are still open issues concerning possible new applications, the use of the assays, and even different types of samples, e.g. in human biomonitoring.

There are concerns about identifying factors that explain the considerable interindividual and inter-laboratory variation, along with standardisation and harmonisation among laboratories and diagnostics.

Here we will explain how various projects have addressed these issues through collaborative initiatives such as the hCOMET, in which many new standards and protocols have been made, with the establishment of a database of 19,320 subjects with pooled data from 105 studies run by 44 laboratories in 26 countries between 1999 and 2019 that helped to establish reference values and to examine the effects of different factors.

We will give an overview of using both assays in human biomonitoring studies, accounting for air pollution factors from HUMNap and EDIAQI projects, and future perspective use through the PROMETHEUS project (bus drivers). We will also mention the new OECD recommendations for the use of micronuclei and the problems addressed in the Guiding Principles for Mixture Threshold Derivation from Effect Biomarkers, effective as of September 2025.

LE-23. Steel Industry in Bosnia and Herzegovina – Human Biomonitoring in Exposed Population

Anja Haverić[1], Irma Durmišević[1], Tamara Ćetković Pećar[1], Maida Hadžić Omanović[1], Sanin Haverić[1]

[1]University of Sarajevo, Institute for Genetic Engineering and Biotechnology.

The use of raw materials and the energy-intensive nature of steel industry production processes release a variety of pollutants into the air. Emissions such as particulate matter (PM), sulfur dioxide (SO_2), nitrogen oxides (NO_x), and volatile organic compounds (VOCs) are well-documented for their adverse impacts on human health, including respiratory and cardiovascular diseases, neurological effects, and an increased risk of cancer. Environmentally, these pollutants contribute to smog, acid rain, reduced visibility, and the contamination of soil and water through the deposition of toxic substances. Human biomonitoring has emerged as a valuable approach for identifying the effects of exposure at the individual level and, together with air quality monitoring, has been regulated in some countries for specific hazardous substances. In Bosnia and Herzegovina (BiH), however, air pollution frequently exceeds legal limits, and environmental permits for steel production facilities are often violated. A human biomonitoring study was coordinated with the support of a non-governmental organization, focusing on genotoxicity biomarkers in populations residing near steel and coke production centers. We hypothesized that residents of BiH's main steel industry center and the country's coke production hub would exhibit higher levels of DNA damage compared to residents of nearby rural areas and the capital city, Sarajevo. Genotoxicity was assessed in salivary cells collected from inhabitants of industrial and rural areas, and results were compared with historical controls from 40 young adults in Sarajevo. A statistically significant increase in DNA damage among individuals exposed to industrial pollution was confirmed. Despite several obstacles, this citizen-led initiative provided crucial evidence on the health impacts of industrial pollution and contributed to public pressure that ultimately led to the closure of the coke production facility—an action followed by a marked improvement in local air quality.

Acknowledgment: Eko Forum, prof. dr. Samir Lemeš and Igda Lemeš

Keywords: air quality, DNA damage, science communication

LE-24. Genotoxicity Biomarkers in Occupational Exposure to Hazardous Compounds in Portugal: Lessons on Formaldehyde, Antineoplastics and Volatile Organic Compounds

Carina Ladeira

Polytechnic University of Lisbon, Portugal

Occupational exposure to hazardous compounds represents a critical concern for workers' health, particularly when it involves agents with proven or suspected genotoxic potential. In Portugal, several professional groups, such as healthcare workers, laboratory technicians, and industry employees, are at risk of chronic exposure to substances such as formaldehyde, antineoplastic drugs, and volatile organic compounds (VOCs). This work provides an overview of biomonitoring studies conducted in occupational settings, focusing on the use of genotoxicity biomarkers, particularly micronucleus frequency, and DNA strand breaks. Evidence indicates that workers exposed to these compounds may present higher levels of genetic damage when compared to non-exposed controls, reinforcing the importance of biological monitoring as a preventive tool and early detection disease. Lessons learned from the Portuguese experience highlight the need for continuous surveillance, the implementation of protective measures, and the adoption of standardized methodologies for biomarker assessment. These findings contribute to the global debate on occupational health and safety, while underlining the role of genotoxicity biomarkers in risk assessment and policy-making.

LE-25. Occupational Exposure to Mineral Fibers – Biomarkers of Exposure, Effect and Susceptibility

Maria Dusinska[1], Elise Rundén-Pran[1], Naouale El Yamani[1], Eleonora Martha Longhin[1], Sivakumar Murugadoss[1], Katarina Volkovova[2], Magdalena Barancokova[2], Marta Staruchova[2], Bozena Smolkova[3], Stefano Bonassi[4], Andrew Collins[5]

[1] Health Effects Laboratory, Departmnet of Environmental Chemistry and Health, The Climate and Environmental Research Institute-NILU, Kjeller, Norway

[2]Department of Biology, Faculty of Medicine, Slovak Medical University, Bratislava, Slovakia

[3]Cancer Research Institute, Biomedical Research Center, Slovak Academy of Sciences, Slovakia

[4] IRCCS San Raffaele Pisana, Unit of Clinical and molecular epidemiology, Rome, Italy

[5]Norgenotech AS, Oslo Cancer Cluster, Radiumhospitalet, Oslo, Norway

Human biomonitoring elucidates the role of genetic and occupational/environmental exposure factors important in the etiology of chronic diseases such as cancer via identification of markers associated with their origin. Biomonitoring provides an integrated chemical exposures assessment considering all routes and sources of exposure. The accurate interpretation and comparison of biomarkers depend on precise design of the study, harmonized, quality-assured sampling, processing, and analysis to generate high-quality data.

We conducted several human biomonitoring studies in Slovakia creating biobank with up to 2000 samples, with accompanying data from hundreds of biomarkers. One of the largest studies included occupational exposure monitoring of workers exposed to asbestos, stone wool, or glass fibres (together 387 subjects, 239 exposed, and 148 controls) to assess markers of of exposure, effect and individual susceptibility. A proportion of these fibres are likely to be in nanoform.

Asbestos-exposed workers had significantly higher levels of chromosomal aberrations, oxidised and alkylated DNA bases, all correlating with years of exposure. DNA strand breaks also increased with age in exposed workers, indicating persistent genotoxic risk. In 98 stone wool-exposed workers and 43 controls, all exposed subjects and exposed non-smokers showed more DNA strand breaks than their corresponding controls, but no increase in specific base damage or change in DNA repair capacity. Among glass fibre workers, even low-level exposure was linked to increased strand breaks and oxidised DNA bases. DNA damage was influenced by antioxidant enzyme activity, while repair capacity showed an inverse relationship with damage. These findings demonstrate that mineral fibre exposure, even at low levels, can lead to measurable DNA damage.

The results highlight the importance of incorporating DNA instability biomarkers such as comet assay endpoints into routine occupational health monitoring to identify and mitigate genotoxic risks in exposed populations.

Supported by the European Union (project FIBRETOX no. QLK4–1999-01629), and by the Horizon Europe project PARC (no. 101057014).

LE-26. Advancing Immunotoxicology: New Approach Methodologies for Immunosuppression and Developmental Immunotoxicity

Emanuela Corsini

Department of Pharmacological and Biomolecular Sciences 'Rodolfo Paoletti', Università degli Studi di Milano, Milano, Italy

The immune system is highly sensitive to xenobiotic exposures, with consequences ranging from immunosuppression to inappropriate immunostimulation, with in utero and early life exposure being very sensitive periods. Conventional testing strategies, primarily reliant on animal models, are limited by ethical concerns, cost, and translational relevance to human biology. In this context, New Approach Methodologies (NAMs) offer significant opportunities to modernize immunotoxicology by providing mechanistic, human-relevant insights into immune dysfunction. This presentation will highlight recent advances in NAMs for assessing immunosuppression and developmental immunotoxicity (DIT). Key developments include *in vitro* human primary cell systems, high-content imaging, and omics-based platforms, which enable the identification of immune cell perturbations at both molecular and functional levels. Computational models and adverse outcome pathways (AOPs) further integrate these data, supporting predictive risk assessment. Special emphasis will be placed on the potential of NAMs to capture critical windows of immune development, identify novel biomarkers of effect, and provide quantitative inputs for regulatory decision-making. By bridging mechanistic immunology with regulatory toxicology, NAMs are paving the way toward a more efficient, ethical, and predictive immunotoxicity assessment framework. Their implementation will be essential for protecting vulnerable populations, reducing reliance on animal testing, and aligning immunotoxicology with next-generation safety sciences.

LE-27. Advancing Ocular Safety: Insights into the New OECD Guidelines and Guidance Document

Helena Kandarova

Institute of Experimental Pharmacology and Toxicology, Centre of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovakia

Ocular safety remains a critical aspect of chemical, cosmetic, and pharmaceutical development due to the potential for severe eye damage or irritation by many substances. Recent updates in the OECD's testing strategies and guidance represent a paradigm shift toward more robust, integrated, and non-animal approaches. The newly released OECD Guidance Document on Integrated Approaches to Testing and Assessment (IATA) for Serious Eye Damage and Eye Irritation (3rd edition, 2024), together with the accompanying test guidelines, advances established strategies for hazard identification, classification, and regulatory decision-making, while incorporating multiple New Approach Methodologies (NAMs) to enhance predictive accuracy and reduce reliance on animal testing.

The updated Guidance Document expands the modular IATA framework by incorporating newer *in vitro* and defined-approach methodologies (e.g. OECD TG 492B, TG 467, TG 494, TG 496) alongside traditional data sources, facilitating more flexible top-down and bottom-up testing strategies.

Emphasis is placed on the weight-of-evidence (WoE) integration of human, *in vivo*, *in vitro*, and *in silico*/non-testing data, enabling more informed decisions on classification and the need for further testing. The guidance also details when and how to deploy new assays or defined approaches to reduce reliance on the rabbit *in vivo* eye test (TG 405), which is reserved as a last resort.

The presentation will also discuss the remaining challenges—such as gaps in mechanistic understanding, limited coverage of some chemical classes or mixtures, and harmonisation across regulatory jurisdictions—that may slow down the full uptake.

Keywords: Ocular safety, OECD, regulatory toxicology

LE-28. NAMs in Cosmetics: The Future of Safety and Innovation

Zehra Sarıgöl Kılıç
L'Oreal USA R&I, United States

The cosmetics industry is undergoing a transformative shift driven by the growing demand for ethical, sustainable, and scientifically advanced safety assessments. New Approach Methodologies (NAMs)—which include *in vitro* models, computational toxicology, high-throughput screening, and omics technologies—offer innovative alternatives to traditional animal testing. These methods promise enhanced human relevance, faster screening capabilities, and more mechanistic insights into toxicity pathways. Regulatory frameworks, such as the EU Cosmetics Regulation (EC) No 1223/2009 and OECD guidelines, increasingly support the integration of NAMs into safety evaluations. As the industry continues to embrace digital tools, artificial intelligence, and organ-on-chip technologies, NAMs are poised to become the cornerstone of next-generation cosmetic safety assessment.

This presentation explores the current state and future trajectory of NAMs in cosmetic safety assessment, emphasizing their integration into international regulatory frameworks. Ultimately, NAMs are not just a replacement for animal testing—they represent a paradigm shift toward a more intelligent, humane, and innovative future in cosmetic science.

Keywords: NAMs, cosmetics, toxicity, alternative methods

LE-29. Computational and Predictive Analysis in Toxicology

Sanin Haveric[1], Maida Hadzic Omanovic[1], Din Dolarevic[2]

[1]University of Sarajevo – Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina

[2]University of Sarajevo – Faculty of Science, Sarajevo, Bosnia and Herzegovina

Computational and predictive analysis in toxicology employs various methods and tools to assess the toxicity of chemicals, predict adverse effects, and understand how substances interact with biological systems. These methods are essential for reducing reliance on animal testing and offer faster, more cost-effective ways of evaluating chemical safety. Their application can follow several alternative approaches. In our studies, we used two distinct approaches. The first, within the field of toxicogenomics, involved functional analysis using the DAVID (Database for Annotation, Visualization, and Integrated Discovery) 6.8 software to identify relevant functional pathways and the mechanism of action of halogenated boroxine ($K_2B_3O_3F_4OH$) in the human leukemic UT-7 cell line. These findings contribute to a deeper understanding of its mode of action and toxicological potential, following years of our research on its effects in various cell lines. The second approach focused on identifying miRNAs with the potential to significantly influence the IC50 values of drugs used in cancer treatment, by applying linear models to cancer cell line data. This approach still requires experimental validation and confirmation.

Keywords: toxicogenomics; functional pathway analysis, cytotoxicity

LE-30. Understanding Microplastics and Human Health Concerns

Neslihan Aygun Kocabaş[1], Erik Rushton[2]

[1] *TotalEnergies Refinery &Chemicals, Seneffe, Belgium*

[2] *LyondellBasell Industries, Åstorp, Sweden*

Microplastics (MP) MPs, defined as plastic particles smaller than 5 mm, are heterogeneous mixture of particles and fibers of various shapes, sizes, polymer composition, surface chemistry and associated chemicals. They are found ubiquitously in the environment (air, water, soil, food, etc), leading to widespread human exposure primarily via oral and inhalation routes, raising concerns about potential health risks due to their detection in human tissues. Physicochemical and environmental factors such as polymer type, composition, particle size, shape, surface chemistry may influence their uptake, behavior and biological interactions, complicating exposure assessment and health effect studies.

There has been an increasing number of studies suggesting the potential human health impacts related to exposure to MPs in both the scientific literature and the media. Early clinical findings associate MPs with immune modulation, reproductive and cardiovascular effects via oxidative stress, and inflammation; however, studies are limited by small sample sizes and inadequate exposure characterization, preventing definitive risk conclusions. Although the limited data provide some evidence that MP have adverse effects in humans, this presentation will summarize the available data and prioritize research needs required for assessing human health implications to MPs.

LE-31. Challenges of Microplastics Research in Risk Assessment: Ensuring Generation of Quality Data

Erik K. Rushton[1], Neslihan Aygun Kocabaş[2]

[1] *LyondellBasell Industries, Åstorp, Sweden*

[2] *TotalEnergies Refinery &Chemicals, Seneffe, Belgium*

Research into the potential health effects of microplastics is expanding rapidly, generating a vast and diverse body of data across *in vivo* and *in vitro* studies. As the field matures, there is an increasing need to consolidate findings and identify trends related to polymer type, particle size, shape, surface charge, and other physicochemical properties. However, efforts to synthesize this information are hindered by inconsistent and incomplete reporting practices.

This presentation will explore the critical role of "Good Reporting Requirements" in ensuring that microplastics research is both scientifically robust and policy-relevant in risk assessments. Drawing on some anonymized examples from recent literature, the talk will highlight commonly overlooked areas in study design and data reporting, such as inadequate particle characterization, missing exposure metrics, and lack of standardized endpoints. These overlooked areas have the potential to limit the utility of findings for risk assessment, regulatory decision-making, and scientific verification and advancement.

This session will look at several frameworks that exist for reporting scientific research as well as those that have been developed specifically for microplastics. The aim is to present key criteria that should be considered when reporting on or evaluating microplastic research.

LE-32. Plastic Chemical Additives: Determining Human Risk From Microplastic Exposure

John Norman

American Chemistry Council

Micro- nanoplastic particles (MNPs) do not have a single chemical identity and therefore require a risk assessment framework that accounts for this variation. Size, shape, density, and polymeric composition are only some of the factors that must be considered when conducting a MNP risk assessment. In addition to these characteristics, recent attention has been given to plastic additives. Plastic additives are chemicals that are intentionally incorporated into a polymer to modify or improve its properties during manufacture, processing, or use. This presentation will introduce and discuss the International Council of Chemical Associations (ICCA)'s Plastic Additive Database. The database is an initiative to bring greater transparency to the use of chemicals in plastic and aggregates existing hazard, exposure, and risk information. The database can serve as a starting point to assess the potential risk of plastic additives from MNPs.

LE-33. Microplastics: Unfolding Consequences

Marijana Ćurčić

Centre for Toxicological Risk Assessment and Department of Toxicology, University of Belgrade - Faculty of Pharmacy, Serbia

Microplastics pose a threat through their intrinsic chemical composition, the release of adsorbed environmental pollutants, and the physical damage they can cause to biological systems. Plastics release additives, concentrate environmental contaminants, and serve as substrates for biofilms, including exotic and pathogenic species. Microplastics' porous structure allows them to adsorb existing environmental contaminants like heavy metals, pesticides, dioxins etc., acting as transport vectors for these harmful substances into organisms. Physical toxicity of microplastics is usually observed as cellular, tissue or barriers damage/disruption. Toxicity mechanisms attributed to microplastics physical properties are oxidative stress, inflammation, cell death (apoptosis), increased accumulation of other harmful substances in the body and metabolic imbalances. Exposure can trigger inflammatory reactions by stimulating the release of inflammatory cytokines, as well as DNA damage, and alter gene expression. Moreover, exposure can compromise feeding, metabolic processes, reproduction, and behavior. Microplastics can release plasticizers, such as phthalates and bisphenol A, or other additives like toxic metals or flame retardants. Uncertainties related to the microplastics issues are the size, shape, and chemical composition that vary significantly, making it difficult to standardize research and interpret results, as well as to isolate the toxicological effects of microplastics from those of the co-occurring environmental pollutants they carry. Human ingestion of contaminated food and water remains a concern. Indoor microplastics pose yet uncharacterized risks, magnified by the time we spend indoors and by the abundance of polymeric products therein. Key scientific challenges include improving microplastics sampling and characterization, understanding long-term behavior, assessing additive bioavailability, and evaluating health risks. Proposed solutions include globally coordinated pollution prevention through the concept of global health.

Keywords: Microplastics, health concerns, toxicology

LE-34. New Insights into Molecular Targets and Early Key Events in Genotoxicity of The Mycotoxin Ochratoxin A

Borchers, Johannes [1]; Schlosser, Andreas [2]; Lutz, Leonie [1]; Pedroni, Lorenzo [3]; Perugino, Florinda [3]; Dellafiora, Luca [3]; Mally, Angela [1]

[1]University of Würzburg, Department of Toxicology, Würzburg, Germany

[2]University of Würzburg, Rudolf Virchow Center, Würzburg, Germany [3]University of Parma, Department of Food and Drug, Parma, Italy

Ochratoxin A (OTA) is a mycotoxin and prevalent contaminant widely recognized for its nephrotoxic effects and renal tumor induction in rodents and is classified as a possible human carcinogen (IARC Group 2B). While OTA-induced replication stress has been recently suggested as cause for mitotic abnormalities and associated compensatory cell proliferation, the precise molecular initiating event (MIE) has not yet been elucidated [1]. To address this knowledge gap critical for evidence-based evaluation of health risks associated with human exposure to OTA, we implemented a chemoproteomics approach aimed to identify direct molecular targets of OTA to characterize initial early key events.

For this purpose, OTA was covalently immobilized via its carboxylic acid group to aminefunctionalized agarose beads, creating an affinity matrix. Kidney epithelial cell lysates were applied to this matrix to capture OTA-interacting proteins, which were then subsequently identified and quantified through tandem mass spectrometry. This study revealed specific interactions between OTA and members of the small GTPase superfamily, proteins known for their critical regulatory roles in processes such as protein and vesicular trafficking, mitosis and cytoskeleton dynamics. Coherent with these findings, small GTPases were shown to be transcriptionally dysregulated following OTA exposure [2, 3]. Complementary *in silico* docking experiments suggest that OTA binds within conserved GDP/GTP-binding pockets of these GTPases, with molecular dynamics simulations indicating stable interactions over toxicodynamically relevant timeframes.

Together these results suggest that interference of small GTPase functionality may represent an early molecular event linking OTA exposure to cyto- and genotoxic effects. Our findings highlight small GTPases as putative molecular targets in OTA-induced carcinogenicity and underscore their potential relevance in future mechanistic studies.

LE-35. Towards Understanding the Mechanism of Methanol-Induced Optic Nerve Damage by Leveraging Human Data

Hilmi Orhan[1], Kemal Atmaca[1], Şadiye Mıdık[1], Mehmet Tokdemir[2]

[1] Ege University, Türkiye

[2] İzmir Katip Çelebi Universiy, Türkiye

Methanol-induced visual impairment is a serious and often permanent toxic effect, yet its underlying mechanism remains poorly understood. Although methanol, being a small and lipid-soluble molecule, distributes widely throughout the body including whole brain, it is not clear why damage occurs predominantly in the optic nerve and retina. Moreover, the basis for the differential vulnerability between retinal tissue and the optic nerve has not been fully established. In this study, we investigated the mechanism of ocular toxicity using *in vitro* models, *in vivo* animal tissues, and human ocular samples. Cytotoxic and mitochondrial toxic effects of methanol and its major metabolites—formaldehyde and formic acid—were determined in *in vitro* ARPE-19 cells and HepG2 cells for comparison. Upon preliminary data showing significant alterations in mitochondria, mitochondrial toxicity was then examined separately in freshly isolated bovine retina and optic nerve tissues, as well as in post-mortem human tissues. Enzyme activities of alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH), and 10-formyltetrahydrofolate synthetase (MTHFD), all involved in methanol biotransformation, were measured in bovine and human ocular tissue fractions. Our results demonstrated similar patterns of enzyme activity across bovine and human retina and optic nerve. In human tissues, bioactivation enzyme activities were 2–3 fold higher in the optic nerve than in the retina, whereas detoxification activity of MTHFD was 3–4 fold higher in the retina. These findings provide a mechanistic explanation for why retinal damage may be partially reversible, while optic nerve injury is irreversible in methanol intoxication. Ongoing work with larger numbers of human tissues aims to confirm these findings and to investigate the potential contribution of genetic variation to interindividual differences in enzyme activity.

Keywords: Methanol, visual toxicity, optic nerve, retina, cell culture, bovine, human

LE-36. Particle-Induced Acute Phase Response as A Mechanism-of-Action of Particle-Induced Cardiovascular Disease

Ulla Birgitte Vogel

National Research Centre for the Working Environment, Denmark

Inhalation of particles induces inflammation and acute phase response. Small particles are more hazardous than larger particles of the same chemical composition for two reasons. First, small (ultrafine) particles have a higher alveolar deposition than larger (fine) particles, resulting in delayed pulmonary clearance of the particles. Secondly, particle-induced inflammatory and acute phase responses are predicted by the total surface area of the pulmonary deposited particles. The total surface area of insoluble particles is larger for smaller particles (inversely proportional to the diameter). Particle-induced inflammation and dose-dependent induction of acute phase response has been causally linked to increased risk of cardiovascular disease, and proposed as adverse outcome pathways (AOP) 237.

In the presentation, the biological mechanism of action will be described along with examples of epidemiological evidence and a proposed health-based occupational exposure limit for ZnO.

The causal relationship between inhalation of particles and dose-dependent induction of acute phase response and the causal link to atherosclerosis underscores cardiovascular disease as an occupational disease and calls for revision of particle-related occupational exposure limits.

Keywords: Particles; nanomaterials, acute phase response, cardiovascular disease

LE-37. Human *In Vitro* Models for The Detection and Understanding of Chemical-Induced Nephrotoxicity

Paul Jennings

VU Amsterdam, The Netherlands

There are many different options to be used as models for human proximal tubular renal cells including primary cells, cell lines such as RPTEC/TERT1 and HK-2, and now induced Pluripotent Stem Cells differentiated in to Proximal Tubule Like cells (PTL). In one example we compared the transcriptional profile of 3 primary donors, 3 iPSC donors, RPTEC/TERT1 and HK-2 cells treated with 3 concentrations of the aminoglycoside compounds gentamycin and tobramycin in a 7 day repeat dose exposure. The iPSC PTLs were much more comparable to the primary cells than the cell lines. This talk will present other examples to illustrate the unique advantages of iPSC derived cells for understanding chemical-induced nephrotoxicity.

LE-38. Mode of Action-Based Refined Risk Assessment for Direct and Indirect Genotoxic Carcinogens

Andrea Hartwig

Karlsruhe Institute of Technology (KIT), Institute of Applied Biosciences (IAB), Food Chemistry and Toxicology, Germany

One of the most demanding tasks in toxicology is related to risk assessment of chemical carcinogens. Although exposure has been effectively reduced in recent decades, low levels of carcinogens are still present in food and in the workplace and are often unavoidable. An important and widely accepted distinction concerns genotoxic and non-genotoxic carcinogens; for the latter group the existence of no-effect concentrations (thresholds) is assumed. In contrast, genotoxic carcinogens, their metabolic precursors and DNA-reactive metabolites are considered risk factors at any concentration, since even one or a few DNA lesions can in principle lead to mutations and thus increase the risk of tumors. However, in recent years, updated risk assessments for genotoxic carcinogens have been proposed. They consider mechanistic knowledge of the substance (group) under investigation, including the cellular response to DNA damage, but also endogenous exposure due to physiological metabolic processes. Together with significant improvements in analytical techniques for quantifying even background levels of DNA lesions and mutations, as well as the increasing application of "omics" approaches, refined dose-response assessments appear appropriate in case of well-investigated compounds. Specific examples of genotoxic carcinogens to which humans are exposed both exogenously and endogenously are formaldehyde and acetaldehyde. In other cases, such as benzo[a]pyrene diolexpoxide, the assumption of linear dose-response relationship appears to be more appropriate. Finally, special attention is given to some carcinogenic metal compounds that are considered indirect genotoxins, accelerating mutagenicity through interactions with the cellular response to DNA damage, but at particularly low exposure conditions. Thus, a refined strategy for assessing the carcinogenic risk associated with exposure to direct and indirect genotoxic compounds is proposed.

Keywords: Genotoxic carcinogens, risk assessment

LE-39. Medical Devices Made of Substances: Challenges on Definitions for Classification and Biosafety Assessment

Marco Racchi

Department of Drug Sciences - University of Pavia, Italy

Medical devices encompass a wide range of products used in disease prevention, diagnosis, monitoring, and treatment. Recent advances have introduced an increasing number of devices containing "substances," often resembling medicinal products in presentation and application. Regulation 2017/745/EC explicitly addresses "medical devices made of substances," including those that can be absorbed, many of which derive from natural sources.

These products often fall into the "borderline" category, requiring complex evaluation of their mechanism of action. The decisive factor distinguishing a medical device from a medicinal product lies in its non-pharmacological (immunological or metabolic) mechanism of action. However, this distinction is not always intuitive and depends on precise interpretation of regulatory terms, which is essential for proper classification.

Substances of natural origin pose additional challenges, as they may fall under different regulations ranging from food law to therapeutic use. Their complex composition complicates scientific, regulatory, and therapeutic assessments. While isolated molecules can be evaluated through established rules ensuring quality, efficacy, and safety, these approaches must be adapted for complex natural substances, requiring appropriate methodologies and regulatory frameworks.

Emerging scientific approaches, such as systems biology, may offer new tools for defining the mechanisms of complex substances. However, Directive 2001/83/EC on medicines is primarily designed for single molecules, and Regulation 2017/745/EC, while introducing the term "medical devices made of substances," does not yet provide clear methods for demonstrating a non-pharmacological mechanism of action. Pharmacologists play a central role in clarifying definitions and guiding innovation, opening opportunities for therapeutic advances with complex natural substances.

Toxicological challenges for industry in the application of MDR 745/2017

LE-40. One Health in the Perspective of a Toxicologist

Nina Eriksen
Coloplast A/S, Denmark

The implementation of the EU Medical Device Regulation (MDR) 2017/745 has introduced several toxicological challenges for industry, particularly in the context of chemical safety requirements, lifecycle considerations and substance-based medical devices. Here is a summary of the key toxicological challenges:

1. Chemical Safety and CMR/ED Substances

MDR mandates strict controls on substances that are: Carcinogenic, Mutagenic or Reprotoxic (CMR) or Endocrine Disruptors (ED). Devices that are invasive and come into contact with the body must justify the presence of such substances above 0.1% w/w, including:

- Risk-benefit analysis
- Justification of clinical necessity
- Availability of safer alternatives
- Labelling requirements

2. Biological Safety and Lifecycle Considerations

- MDR emphasizes total lifecycle safety, including during use and disposal.
- Nanoparticles, wear debris, and degradation products must be evaluated for toxicological impact.
- Challenge: Generating sufficient biological and toxicological data for long-term exposure scenarios and finding appropriate testing methods.

3. Substance-Based Medical Devices (MDR Rule 21)

Devices composed of substances that are introduced into the human body and absorbed or locally dispersed, face stricter classification and toxicological scrutiny:

- Re-classification to higher risk classes based on absorption and systemic effects.
- Requirement for Absorption, Distribution, Metabolism, Excretion (ADME) data to support safety and performance claims.
- Challenge: Demonstrating non-pharmacological mechanisms of action for complex mixtures like natural products is difficult due to their multifactorial interactions.

4. Scientific and Regulatory Paradigm Shift

- MDR demands new scientific approaches to assess complex substances and mixtures.
- Traditional toxicological models may not suffice for evaluating non-pharmacological mechanisms.
- Transition to *in-vitro methods*: MDR pushes to move away from animal testing towards *in-vitro* methods.
- Challenge: Developing validated methods and standards for toxicological testing of novel substances, as well as technical difficulties and regulatory skepticism towards alternative methods.

LE-41. Methodological Aspects for Biosafety Compliance in Medical Devices

Emanuela Testai

formerly at Istituto Superiore di Sanità – Rome Italy

Ensuring biosafety in medical devices is a fundamental requirement to protect patients, healthcare professionals, and the environment from potential biological hazards. The methodological aspects for achieving biosafety compliance involve a systematic approach that integrates risk assessment, regulatory adherence, material evaluation, biocompatibility testing, and post-market surveillance.

In Europe the MDR mandates that manufacturers demonstrate biological safety.

The methodological aspects are described in the EN ISO 10993 series – Biological evaluation of medical devices, providing harmonized standards for methods to be applied (depending on the type and classification of the medical device). Manufacturers must compile all relevant biosafety data in the Technical Documentation (Annex II and III MDR), which serves as the basis for conformity assessment by a Notified Body.

Under the MDR, risk management is a continuous process integrated throughout the design and manufacturing stages. Biological risk assessment must consider:

- Material composition and exposure (depending on duration, type, and site of contact with the body). Each material must be characterized for its chemical composition, surface properties, and degradation behavior. This step often includes extractables and leachables analysis, ensuring that no harmful substances are released under physiological conditions.
- Potential biological hazards. Comprehensive biological evaluation is performed according to ISO 10993 guidelines. Tests typically include cytotoxicity, sensitization, irritation, systemic toxicity, genotoxicity, and implantation studies (studying interactions between device components and biological tissues). For medical devices made of substances also toxicokinetics studies are requested. The testing strategy should be proportional to the device's intended use, duration of contact, and type of patient exposure.

Manufacturers are expected to document the rationale for test selection and provide justification when omitting specific biological evaluations.

Technical files should include biological risk assessments, test results, and validation reports. Biosafety does not end at product launch: post-market monitoring collects real-world performance data to identify emerging biosafety concerns.

LE-42. Toxicological Risk Assessment for Extractables/Leachables in Pharmaceuticals and Medical Devices

Ahmet Aydın

Yeditepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Türkiye

During the manufacturing and storage of pharmaceuticals, components such as packaging materials, delivery systems, or production equipment can release small amounts of chemicals into the final product. Similarly, medical devices or materials that come into direct or indirect contact with the human body may transfer certain substances to the patient or user.

Leachables are the substances that migrate into a drug or device product under normal manufacturing, storage, and use conditions. In contrast, extractables are compounds that can be intentionally drawn out under controlled laboratory conditions using solvents of varying polarity, such as acidic, alkaline, or nonpolar media. Generally, extractables represent potential leachables

Because these substances can pose health concerns, manufacturers and marketing authorization holders are required to evaluate them. Once extractables and leachables are identified, a toxicological risk assessment must be conducted to ensure that any potential exposure is within acceptable safe limits.

This process considers both biological and toxicological risks and should follow internationally recognized standards—such as ICH guidelines for pharmaceuticals and ISO standards for medical devices.

In this presentation, it will be outlined the fundamental principles of toxicological risk assessment for extractables and leachables and share practical examples of how these assessments are applied in real-world scenarios.

Keywords: Pharmaceuticals, Medical Devices, Toxicology, Risk Assessment

Oral Presentations

OP-01. A Study on The Awareness of University Students Regarding The Healthy Use of Skin Care Products and Their Toxicological Effects

Hülya Gül[1], Busenaz Doğan [2], Berdıl Dinç [2], Emine Ergin [2], Aylin Kıybar [2], Gülsün Çiçek [2], Rayan Kayari [2]

[1] Istanbul Aydin University, Faculty of Medicine, Department of Public Health, Istanbul, Türkiye

[2] Istanbul Aydin University, Faculty of Medicine, 4th Term Student, Istanbul, Türkiye

Introduction: Awareness of the toxicological effects of regularly used products is critical for protecting and sustaining public health. The increasing frequency of cosmetic and personal care product use, particularly among young people, prioritizes research on awareness levels regarding ingredient safety and toxicological risks. The purpose of this study is to assess university students' knowledge and awareness of skin care products and the toxic ingredients they contain.

Materials and Methods: This preliminary study was conducted using a 28-item structured survey form with 310 students studying at various faculties at various universities on the European side of Istanbul. Data were collected between May 1 and June 15, 2025. Data were collected through surveys prepared with Google Forms and links sent through social media. The data were analyzed using appropriate statistical methods using SPSS 25.0 (SPSS Inc., IL, USA).

Results: Of the students who participated in the study, 74.5% were female and 25.5% were male. While 68.4% of participants stated that they had little knowledge about toxic substances, only 21.9% stated that they had sufficient knowledge. The percentage of those who believed that excessive use of skin care products could lead to skin problems was quite high (92.3%). 81% of participants stated that their universities provided insufficient training on toxic substances. 40.6% of participants stated that they regularly read the ingredients of the products they use, and 48.4% stated that they occasionally review this information. The percentage of those who pay attention to "organic" and "natural" labels is 72.6%. The most common source of information is social media (42.6%), followed by dermatologists (31.6%). 68.7% of participants reported using products without consulting a doctor.

Conclusion: A significant portion of study participants were found to have only limited knowledge of the potential toxic substances in dermatological products and a low habit of examining product ingredients. There is a clear lack of knowledge about the side effects of cosmetic products, which can have serious health consequences. In this context, toxicology education should be expanded, individuals should be directed to reliable sources of information, product ingredients should be presented in an understandable and transparent manner, and conscious consumption behaviours should be encouraged through awareness-raising campaigns.

Keywords: Public health; Dermatology; Toxicology; Side effects; Türkiye

OP-02. *In Vitro* Antioxidant Activity of *Anthemis altissima* L. Flower Extract Against Acetamiprid in HepG2 Cell Line and Comparison with AI Predictions

Yağmur Emre Arıcan[1], Samet Atılgan[2], Mine Koçyiğit Avcı[3], Hikmet Can[2], Müberra Özcan[1], Gül Özhan[4], Özlem Batu[1], Mustafa Yıldız[5]

[1] *Süleyman Demirel University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Isparta, Türkiye*

[2] *Süleyman Demirel University, Faculty of Pharmacy, Isparta, Türkiye*

[3] *İstanbul University, Faculty of Pharmacy, Department of Pharmaceutical Botany, İstanbul, Türkiye*

[4] *İstanbul University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, İstanbul, Türkiye*

[5] *Meddem Hospital, Nuclear Medicine, Isparta, Türkiye*

In Turkish folk medicine, *Anthemis altissima* L. flowers are used externally for wounds and burns due to their antimicrobial, antiseptic, and antioxidant properties; internally for gastrointestinal disorders as a digestive aid, in gynecological issues to regulate the menstrual cycle and relieve menstrual pain, and for various internal conditions and infections. The antioxidant-rich *Anthemis altissima* L. flower extract (AAFE) is noteworthy as a potential protective herbal drug. Environmental exposure to oxidants presents a significant risk, leading researchers to explore novel and effective antioxidants. Our study examined the antioxidant potential of *Anthemis altissima* L. flower against acetamiprid (ACE) exposure, a neonicotinoid insecticide known for causing oxidative stress and damage.

To achieve this objective, 4.6 mM ACE-exposed HepG2 cell lines underwent *in vitro* treatment with 0.015-0.244 mg/ml AAFE. Glutathione (GSH) and malondialdehyde (MDA) levels were determined spectrophotometrically using ELISA kits, and cell viability was evaluated by the MTT assay. Furthermore, experimental results are juxtaposed with predictions produced by the artificial intelligence (AI) of ChatGPT-4 Mini.

Treatment with 0.015-0.061 mg/ml AAFE significantly reduced 4.6 mM ACE-induced cytotoxicity. 0.010-0.125 mg/ml AAFE treatment against 4.6 mM ACE exposure significantly reduced GSH and MDA levels. Although 4.6 mM ACE exposure enhanced GSH formation in HepG2 cells, AAFE mitigated lipid peroxidation and free radicals through its antioxidant properties rather than by boosting GSH production. AAFE may be employed as a food supplement due to its capacity to decrease lipid peroxidation, requiring more investigation on optimizing its dosage. Even while accuracy dropped for difficult inquiries, ChatGPT usually answered accurately. AI can reduce costs and labor with pre-simulation and support toxicological and pharmaceutical research, as confirmed by ChatGPT's qualitative assessment of AAFE's antioxidant properties.

The study was funded by Health Institutes of Türkiye (TÜSEB) (Call code: 2023-A102, Project number: 37694).

Keywords: *Anthemis altissima* L. flower extract, acetamiprid, oxidative stress, GSH, MDA, MTT assay, *in vitro*, HepG2, ChatGPT-4 mini, artificial intelligence

OP-03. Toxicity Profiling of Novel Tetrazolato Ligands: Bridging The Knowledge Gap with Multi-Tool *In Silico* Approaches

Fatma Okuş[1], Fatma Ünal[2], Deniz Yüzbaşıoğlu[2], Gonca Çakmak[3]

[1]*Gazi University, Graduate School of Natural and Applied Sciences, Teknikokullar, Ankara 06500, Turkiye*

[2]*Gazi University, Faculty of Science, Department of Biology, Teknikokullar, Ankara 06500, Turkiye*

[3]*Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Yenimahalle, Ankara 06330, Turkiye*

Computational predictions offer valuable preliminary insights in toxicological evaluations of newly synthesized molecules, complementing essential experimental studies for comprehensive risk assessment. *In silico* approaches for preclinical safety assessments in drug research are an evolving and supported area. This study aims to assess the preclinical safety parameters of newly synthesized tetrazolato ligands (TetSalCl, TetSalH), characterized by Schiff base structures incorporating a tetrazole ring (Okus et al., 2023), by integrating ACD Labs PERCEPTA (commercial) and Simulation Plus (academic access) *in silico* tools, which predict endpoints, with StopTox (public) tool, offering mechanistic analysis based on chemical structure. Several preclinical safety parameters: acute toxicity, Ether-à-go-go-Related Gene (hERG) interaction, blood-brain barrier (BBB) permeability, ADME properties, and eye irritation potential are determined. *In silico* estimations by Percepta and Simulation Plus indicate low acute oral toxicity (Globally Harmonized System, Category 4, 300-2000 mg/kg). Furthermore, neither ligand showed interaction with hERG channels nor permeability across the BBB. Both ligands are predicted to be non-inhibitory to cytochrome P450 enzymes, exhibit moderate permeability in Caco-2 cells, and show high predicted absorption in the human intestinal system, suggesting favorable ADME profiles. However, ligands are predicted to cause eye irritation. *In silico* structural analysis by StopTOX indicate that the acute toxicity of TetSalCl is primarily attributed to the aromatic ring, while TetSalH is linked to both aromatic and tetrazole rings. Eye sensitization is predicted to be caused by the -OH group attached to the aromatic ring. These findings emphasize the effectiveness of a multi-tool *in silico* approach, enabling quantitative data output comparison and prediction consistency evaluation. The integrative approach, supported by three distinct tools, provided crucial preliminary data for the preclinical safety assessment of tetrazolato ligands, highlighting their promising potential use in drug candidates. The complementarity of the tools offers valuable insights for future studies.

Acknowledgments: This study was financially supported by The Scientific and Technological Research Council of Türkiye (TÜBİTAK 1001) under project number 122Z762.

Keywords: *In silico* toxicology, preclinical safety assessments, tetrazolato ligands, multi-tool *in silico* approach

OP-04. Effects of Clothianidin on Global DNA Methylation In HepG2 Cells

Metin Caner Cakir[1,2], Ela Kurt[1,3], Sibel Ozden[1]

[1]*Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Istanbul, Turkiye*

[2]*Istanbul University, Institute of Graduate Studies in Health Sciences, Istanbul, Turkiye*

[3]*Institut auf dem Rosenberg, St. Gallen, Switzerland*

Clothianidin (CLO, 1-[(2-chloro-1,3-thiazol-5-yl)methyl]-3-methyl-2-nitroguanidine) is a neonicotinoid pesticide which was prohibited by the European Union in 2018. However, no regulations are present in many countries and farmers may continue using CLO due to their low cost and high efficacy despite the regulations. Previous studies have linked neonicotinoid exposure to adverse health outcomes such as hepatotoxicity and cancer. Epigenetic mechanisms are gaining attention as biomarkers for early detection of toxicity and for their possible use in regulatory toxicology. However, epigenetic alterations induced by CLO exposure are not well documented. Due to this, we aimed to investigate global DNA methylation alterations, one of the most studied epigenetic mechanisms, in human hepatocellular carcinoma (HepG2) cells exposed to CLO at 0, 5, 10 and 20 µM for 24h. Global DNA methylation assessment was done with a 5-methylcytosine (5-mC) ELISA kit which revealed dose dependent increases in 5-mC% levels. DNA methylation processes in mammals are mainly regulated by DNA methyltransferase (DNMT) and ten-eleven translocation (TET) enzymes. DNMT enzymes recruit methyl groups and facilitate DNA methylation while TET enzymes facilitate demethylation by hydroxylation of 5-mC. To further investigate the role of DNA methylation alterations in CLO toxicity, *DNMT-1*, *DNMT-3a*, *DNMT-3b*, *TET-1*, *TET-2* and *TET-3* relative gene expressions were analyzed by real-time polymerase chain reaction (RT-PCR). RT-PCR results revealed alterations in genes involved in DNA methylation which is in support of the ELISA results. Findings of this study indicate that DNA methylation may be a contributing factor to CLO-induced toxicity, which further demonstrates the importance of epigenetic mechanisms in toxicology.

OP-05. Therapeutic Performance and Toxicological Profiles of Gold and Silver Nanoparticles in Photothermal Applications

Şevval Çelikten[1], Jülide Secerli[1], Onur Erdem[1], Hakan Erdoğan[2], Merve Güdül Bacanlı[1]

[1]University of Health Sciences Turkiye, Gülhane Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Turkiye

[2]University of Health Sciences Turkiye, Gülhane Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Turkiye

Photothermal therapy (PTT), which destroys cancer cells by converting photon energy into heat, has emerged as a promising non-invasive treatment in cancer. Near-infrared (NIR) light, particularly at 808 nm, is favored in PTT owing to its deep tissue penetration and minimal absorption by biological components. Photothermal agents (PTAs) mediate cancer cell death via necrosis or apoptosis under laser exposure. Among metallic nanomaterials, gold (AuNPs) and silver nanoparticles (AgNPs) have attracted significant attention for their photothermal properties and biological effects.

This study aimed to comparatively evaluate the photothermal efficacy and toxicity of AuNPs and AgNPs in human lung carcinoma A549 cells, both with and without 808 nm laser irradiation. Nanoparticles were characterized in terms of morphology and thermal behavior. The cytotoxicity of increasing concentrations of AuNPs and AgNPs (0.0625–20 µg/mL) was assessed following 24- and 72-hour incubations using the MTT assay, with or without 5-minute 808 nm laser exposure. Reactive oxygen species (ROS) generation was quantified, while genotoxicity, pro- and anti-inflammatory cytokines (TNF-α, IL-6, IL-10), and apoptosis-related complement components (C1q, C3, C6, and C9) were also analyzed. Laser exposure alone did not induce significant toxicity. Both nanoparticles exhibited dose-dependent toxicity, which was further enhanced by laser irradiation. AgNPs were more toxic than AuNPs in the absence of laser, whereas AuNPs showed higher toxicity under laser exposure. ROS levels were elevated in AgNP-treated cells without laser, while laser exposure enhanced ROS generation in AuNP-treated cells. DNA damage was more pronounced in AgNP-treated groups. Inflammatory markers and caspase-related complement components (excluding C1q) were elevated, especially in groups receiving combined NP and laser treatments, suggesting apoptosis induction.

In conclusion, both AuNPs and AgNPs demonstrated potential as photothermal agents, with distinct toxicity mechanisms influenced by time and laser exposure.

This study was supported by TÜSEB (project number 31034, awarded to Dr. Merve Güdül Bacanlı).

Keywords: 808 nm laser, lung cancer, cytotoxicity, genotoxicity, ROS, apoptosis, inflammation

OP-06. Evaluation of Cytotoxic and Genotoxic/Antigenotoxic Effects of *Rheum Ribes L.* Root and Stem Extracts on A549 Lung Cancer Cells

Halime Serinçay[1], Ceren Su Ergin[1], Nisa Nur Deniz[1], Hilal Akar[2], Gökçe Taner[1]

[1]Bursa Technical University, Faculty of Engineering and Natural Sciences, Department of Bioengineering, Bursa, Turkiye

[2]Bursa Technical University, Graduate Education Institute, Department of Biotechnology, Bursa, Turkiye

Rheum ribes L., commonly used in traditional medicine, is considered a natural source with anticancer potential due to its rich content of bioactive compounds. In particular, anthraquinone derivatives such as emodin and aloe-emodin have demonstrated antiproliferative and apoptotic effects, which have increasingly attracted scientific interest toward this plant. A perennial species belonging to the Polygonaceae family, *Rheum ribes* is utilized both as a food source and in the treatment of various ailments. In this study, the *in vitro* cytotoxic, genotoxic, and antigenotoxic effects of root and stem extracts of *Rheum ribes L.* were evaluated on the A549 human lung cancer cell line. *Rheum ribes L.* were collected from Saso Mountain in Batman, Turkey. Root and stem parts were extracted separately using 80% methanol–water solution, followed by concentration and lyophilization. The dry extracts were stored at +4 °C for analyses. Cytotoxic effects of *Rheum ribes* L. extracts on the A549 cell line were evaluated by both Methyl Thiazolyl Tetrazolium (MTT) assay and Neutral Red Uptake (NRU) assay. Genotoxic effects of the extracts and also antigenotoxic effects against DNA damage caused by hydrogen peroxide were evaluated by comet assay. The results of this study demonstrate that the root and stem extracts of *Rheum ribes L.* exhibit distinct cytotoxic, genotoxic, and antigenotoxic effects on the A549 lung cancer cell line. The root extract significantly reduced cell viability at higher concentrations, increased DNA damage, and showed a marked antigenotoxic effect by mitigating H_2O_2-induced damage. In contrast, the stem extract displayed more limited effects. These results suggest that the root extract may possess a stronger and more consistent anticancer potential.

This study was supported by Bursa Technical University Scientific Research Projects Coordination Office within the scope of project number 230Y009.

Keywords: *Rheum ribes L.*, cytotoxicity, genotoxicity, antigenotoxicity, comet assay

OP-07. Targeting Amyloid Toxicity: Naringin Mitigates Phe-Phe-Induced Neurodegeneration in SH-SY5Y Cells

Emre Korkmaz[1], Jülide Secerli[1], Onur Erdem[1], Hakan Erdoğan[2]

[1] *University of Health Sciences, Gülhane Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Turkiye*

[2] *University of Health Sciences, Gülhane Faculty of Pharmacy, Department of Analytical Chemistry, Turkiye*

Alzheimer's disease (AD) is a progressive neurodegenerative disorder marked by amyloid-β (Aβ) accumulation, tau hyperphosphorylation, neuroinflammation, and neuronal apoptosis. Given the limited efficacy of current treatments, phytochemicals with multifunctional properties have emerged as promising neuroprotective agents. This study investigates the protective effects of naringin—a Citrus-derived flavonoid—using an *in vitro* AD model based on diphenylalanine (FF)-induced amyloid toxicity in human SH-SY5Y neuroblastoma cells. Naringin (NAR) was applied at various concentrations to determine its cytotoxic threshold via MTT assay. Amyloid-like aggregates were confirmed using high-resolution scanning electron microscopy (SEM) and light microscopy. Subsequent experiments employed non-toxic NAR doses to evaluate intracellular reactive oxygen species (ROS), DNA damage (Comet assay), and apoptosis (Annexin V-FITC/PI flow cytometry). ELISA quantified AD-relevant biomarkers—APP, tau, and Aβ—as well as pro- and anti-inflammatory cytokines (IL-1α, IL-6, IL-10, TNF-α, TGF-β) and complement proteins (C1q, C3, C6, C9).

When the results were examined, NAR markedly attenuated FF-induced ROS generation, DNA damage, and caspase-mediated apoptosis. It also suppressed the expression of APP, tau, and Aβ, while modulating inflammatory cytokines and complement activation.

These findings demonstrate that NAR exerts neuroprotective effects by simultaneously targeting oxidative stress, amyloidogenesis, inflammation, and apoptosis. This positions naringin as a compelling multi-target therapeutic candidate for AD, warranting further validation through *in vivo* and pharmacokinetic studies.

Keywords: Diphenylalanine, neuroblastoma, genotoxicity, apoptosis, Alzheimer's biomarkers

OP-08. Investigation of The Effects of Royal Jelly Against UV-induced Photoaging in HaCaT Cell Cultures

Hilal Akar[1], Gökçe Taner[2]

[1]*Bursa Technical University, Graduate Education Institute, Department of Biotechnology, Bursa, Turkiye*

[2]*Bursa Technical University, Faculty of Engineering and Natural Sciences, Department of Bioengineering, Bursa, Turkiye*

Ultraviolet (UV) radiation accelerates the photoaging process in the skin by causing serious cytotoxic effects through DNA damage, reactive oxygen species (ROS) production and inflammatory responses in epidermal cells. In particular UVB rays, target keratinocytes even at low doses, causing mutation, apoptosis and tissue deterioration; these effects are associated with toxicological processes that threaten cellular integrity as well as photoaging. Royal jelly, a natural bioactive substance, has the potential to reduce ROS-induced cellular stress thanks to its high antioxidant capacity and 10-hydroxy-2-decenoic acid (10-HDA) content. Royal jelly, with its collagen synthesis-supporting and matrix metalloproteinase activity-suppressing properties, can exhibit both protective and reparative effects against UV-induced biological damage. With these properties, royal jelly has been the focus of this study as a potent natural bioactive agent with the potential to reduce dermal toxicity, whose efficacy against UV-induced photoaging and cellular damage has been investigated. In this study the photoprotective effect of royal jelly at different concentrations (250-2000 µg/mL) against the UV mediated-cell and DNA damage in a human keratinocyte cell line (HaCaT) and for this purpose, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) test and comet assay were used. The HaCat cells were preteated with royal jelly concentrations and exposed to UVB irridation with Osram Ultra Vitalux 300W 230V lamp and then cultured 24h. Royal jelly significantly prevented UVB-induced cytotoxicity and reduced DNA damage expressed as DNA tail length, tail intensity and tail moment, depending on its concentration. Targeting the effects of photoaging at the cellular and molecular level, this study revealed that royal jelly not only prevents cellular damage by reducing UVB-induced oxidative stress and DNA damage, but also may act as a potential photoprotective agent in restructuring the biological integrity and youth of skin tissue.

Keywords: Royal jelly, UVB, Human keratinocytes, cytotoxicity, genotoxicity

OP-09. Cytotoxic, Genotoxic/Antigenotoxic and Epigenetic Effects of Bee Bread on Healthy and Cancer Lung Cell Lines

Hacer Ergün[1], Elife Kıldalı[2], Halime Serinçay[2], Hilal Akar[1], Gökçe Taner[2]

[1]*Bursa Technical University, Graduate Education Institute, Department of Biotechnology, Bursa, Turkiye*

[2]*Bursa Technical University, Faculty of Engineering and Natural Sciences, Department of Bioengineering, Bursa, Turkiye*

Past and present studies show that natural bee products can be used in the prevention and treatment of many diseases. Bee bread (perga) is one of the valuable honeybee products, which is a mixture of plant pollen and honey fermented with lactic acid and is used by bees as a most nutritious food source. Although many studies exist for other bee products, studies on bee bread are rare and limited. In this study the cytotoxic, genotoxic and antigenotoxic effects of bee bread on BEAS-2B (healthy epithelial lung cell) and A549 (cancer cell) cell lines were investigated. Bee bread obtained from Bursa was extracted in methanol:water (80:20) and cytotoxic effects were evaluated by neutral red uptake (NRU) and Methyl Thiazolyl Tetrazolium (MTT) tests, its genotoxic/antigenotoxic effects were evaluated by comet assay. In order to determine the epigenetic effects, Total 5mC analysis was performed using the Cayman Chemical DNA Methylation ELISA Kit. Bee bread (6.25-800 µg/mL) did not show cytotoxic effects on BEAS-2B and A549 cells by MTT and NRU cytotoxicity tests. According to the genotoxicity results evaluated with the comet assay, bee bread applied at concentrations of 100, 200, 400, 800 µg/mL did not show a genotoxic effect on BEAS-2B and A549 cells compared to the untreated negative control group, on the other hand, when its antigenotoxic effect was evaluated in cells subjected to DNA damage with H_2O_2, it was determined that it reduced the damage compared to the positive control applied with H_2O_2 alone. After application for 24 hours in BEAS-2B cells, a decrease in total genomic DNA methylation in all application groups compared to the control group was determined. The results obtained revealed that bee bread showed cell and DNA damage reducing activity due to its strong antioxidant activity.

Keywords: bee bread (perga), cytotoxicity, genotoxicity, antigenotoxicity, comet assay

OP-10. Epigenetic Modulation of Circadian Genes PER1 and CHRONO by Histone Deacetylation in A Parkinson's Disease Model *In Vitro*

Zülfinaz Betül Çelik, Rabia İleri

Samsun University, Faculty of Medicine, Department of Medical Biology, Samsun, Turkiye

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by dopaminergic neuron loss and alpha-synuclein aggregation. Recent studies suggest that circadian rhythm disruptions and epigenetic mechanisms, particularly histone modifications, contribute to PD pathogenesis. *PER1* and *CHRONO*, key circadian genes, may be epigenetically modulated in disease contexts. This study aimed to evaluate the expression levels of *PER1* and *CHRONO* genes and the effect of the class IIa histone deacetylase (HDAC) inhibitor TMP-195 in a human astrocyte-based *in vitro* PD model induced by alpha-synuclein. C8-D1A human astrocytes were treated with alpha-synuclein (500 nM), TMP-195 (100 nM), or a combination of both for 6 hours. Total RNA was isolated, cDNA synthesized, and gene expression levels were quantified by qRT-PCR using *GAPDH* as a reference. Statistical significance was determined by ANOVA and Student's t-test. Alpha-synuclein significantly reduced *PER1* expression, while TMP-195 partially restored it. In contrast, alpha-synuclein increased *CHRONO* expression, and TMP-195 further enhanced this upregulation. Our findings suggest that alpha-synuclein disrupts circadian gene expression, possibly contributing to PD pathology, and histone deacetylation plays a key role in this regulation. TMP-195 modulates *PER1* and *CHRONO* expression, indicating that class IIa HDAC inhibitors may offer therapeutic potential through epigenetic restoration of circadian gene dynamics in PD. This study was supported by TÜBİTAK 2209-A-Research Project Support Programme for Undergraduate Students (Project No: 1919B012322068).

Keywords: Circadian rhythm, Parkinson's disease, Epigenetic

OP-11. Doxylamine-Pyridoxine in Pregnancy: What is The Real Risk?

Cansel Çalışkan[1,2], Onur Kenan Ulutaş[1], Münire Funda Cevher Akdulum[3], Nesligül Özdemir Ayduran[4], Şeyma Osmanlıoğlu Coşkun[5], Figen Karadogan[6], İrem Budakoğlu[7]

[1] *Gazi University Faculty of Pharmacy, Department of Toxicology, Ankara, Türkiye*

[2] *Ankara Etlik City Hospital, Ankara, Türkiye*

[3] *Gazi University Faculty of Medicine, Department of Obstetrics and Gynecology, Ankara, Türkiye*

[4] *Gazi University Faculty of Pharmacy, Department of Clinical Pharmacy, Ankara, Türkiye*

[5] *Ankara Medipol University Faculty of Medicine, Ankara, Türkiye*

[6] *Governors State University, Division of Psyc and Counseling, Chicago, Illinois, United States of America*

[7] *Gazi University Faculty of Medicine, Department of Medical Education, Ankara, Türkiye*

Although extensive human studies have demonstrated the fetal safety of the doxylamine succinate and pyridoxine hydrochloride combination, and the drug has held FDA Category A status since 2013, lingering concerns regarding its potential teratogenicity continue to shape prescribing behaviors and medication adherence in the management of nausea and vomiting of pregnancy (NVP) and hyperemesis gravidarum (HG). This study aims to investigate the safety profile and real-world use of doxylamine-pyridoxine among pregnant women in Turkey, while also exploring potential discrepancies between scientific evidence and the risk perceptions of pharmacists and patients. A retrospective evaluation of prescription and dispensing records from Ankara Etlik City Hospital (2022–2024) was conducted to analyze prescribing trends, adherence rates, and pregnancy outcomes among women who did or did not fill their prescriptions. In parallel, a structured survey targeting community pharmacists assessed their knowledge, perceptions of risk, and counseling practices regarding doxylamine-pyridoxine use during pregnancy. This study generates the first comprehensive data from Turkey on both the clinical application of doxylamine-pyridoxine and pharmacist-driven factors affecting its utilization. The findings are expected to highlight existing gaps between clinical guidelines and real-world practice, particularly misconceptions regarding teratogenic risks, which may hinder optimal management of NVP/HG and contribute to avoidable maternal and fetal complications.

OP-12. Investigation of the Cytotoxic and Cell Migration Inhibitory Effects of Doramectin on Human Cervical Carcinoma and Liver Adenocarcinoma Cell Lines

Dilan Aşkın Özek[1], Hande Yüce[2], Songül Ünüvar[2]

[1]Fırat University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Elazığ, Türkiye

[2]İnönü University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Malatya, Türkiye

Purpose: Cervical (3.6%) and liver (7.8%) cancers account for a significant proportion of cancer-related deaths worldwide. The limitations and serious side effects of current treatments necessitate the development of new treatment strategies. Drug repositioning, defined as the identification of new therapeutic uses for approved drugs, presents considerable advantages in terms of time and cost compared to new compound discovery in cancer treatment. In this context, there is increasing interest in avermectin (AVM) compounds, which are employed in anthelmintic and antiparasitic therapies. This study aimed to elucidate the cytotoxic and migration inhibitory effects of doramectin, a third-generation derivative of the AVM class, on human cervical carcinoma (HeLa) and liver adenocarcinoma (Hep3B) cell lines.

Methods: HeLa, Hep3B, and healthy fibroblast L929 cells were incubated with increasing concentrations of doramectin (1, 2.5, 5, 10, 20, 25, 50, and 75 µM) for 24 and 48 h. Each assay was repeated four times, and cell viability was evaluated using a 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTS) assay. Half-maximal inhibitory concentration (IC_{50}) were calculated using GraphPad Prism 10 software. Cell migration inhibitory effects were analyzed using a wound healing assay. Wound areas were evaluated using ImageJ software.

Results: Doramectin exhibited time-dependent cytotoxicity in cancer cells. The IC_{50} in HeLa and Hep3B cells were 35.14 µM and 19.74 µM at 24 h, respectively, and decreased to 21.61 µM and 12.92 µM at 48 h. In contrast, the IC_{50} were higher in L929 cells, measuring 23.66 and 22.34 µM, indicating lower sensitivity. In the wound healing assay, doramectin significantly inhibited cell migration in HeLa cells at 48 h and in Hep3B cells at both 24 and 48 h. No significant change was observed in L929 cells. These results suggest that doramectin selectively impedes the migration and proliferation of cancer cells while exerting no significant effect on healthy fibroblasts.

Keywords: Doramectin, cytotoxicity, HeLa, Hep3B, cell migration

OP-13. Cytotoxic and Anti-Migration Effects of Thymoquinone on Various Cancer Cell Lines

Hande Yüce[1], Dilan Aşkın Özek[2], Songül Ünüvar[1]

[1]İnönü University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Malatya, Türkiye

[2]Fırat University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Elazığ, Türkiye

Purpose: Cancer-related mortality rates are increasing worldwide. Although current treatments prolong progression-free survival, they often have serious side effects. Therefore, new agents with selective cytotoxic effects on cancer cells are needed. In this context, the anticancer effects of naturally derived compounds are remarkable. Thymoquinone (TQ), the main bioactive component of black seed (*Nigella sativa*) and a potent antioxidant, has shown promising anticancer effects against various cancers. This study aimed to investigate the cytotoxic and anti-migration effects of TQ in colon (HCT-116), cervical (HeLa), and breast cancer (MCF-7) cell lines.

Method: Increasing concentrations of TQ (1, 5, 10, 25, 50, 100, and 200 µM) were applied to cancer (HCT-116, HeLa, and MCF-7) and healthy fibroblast (L929) cell lines, and cell viability at 24 and 48 h was evaluated using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTS) assay. Half-maximal inhibitory concentration (IC_{50}) were calculated using the GraphPad Prism 10 software. The anti-migration effect was analyzed using a wound-healing assay. The wound area was evaluated using the ImageJ software.

Results: The IC_{50} of TQ were determined to be 74.27 µM at 24 h and 24.27 µM at 48 h in L929 cells. In cancer cell lines, the IC_{50} (24–48 h, respectively) were 194.7–142 µM in HCT-116, 166.4–124.7 µM in HeLa, and 81.9–53.08 µM in MCF-7 cells. These results indicate that the cytotoxic effect of TQ increased time-dependent, with the MCF-7 cell line exhibiting the highest sensitivity to TQ. In the wound healing assay, TQ incubation significantly inhibited cell migration in HCT-116 and MCF-7 cells at 48 h and in HeLa cells at both 24 and 48 h; however, no significant difference was observed in L929 cells. In conclusion, TQ demonstrates substantial cytotoxic effects, particularly in MCF-7 cells, indicating the selective anticancer potential of this compound.

Keywords: Thymoquinone, cytotoxicity, breast cancer, cell migration, natural compound

OP-14. The Effects of Azithromycin on Some Biological Parameters of *Galleria Mellonella* L. (Lepidoptera: Pyralidae)

Kübra Aslan[1], Cihat Çelik[2], Kemal Büyükgüzel[3]

[1]Zonguldak Bülent Ecevit University, Science Faculty, Department of Biology, Zonguldak, Turkiye,

[2]Zonguldak Bülent Ecevit University, Çaycuma Food and Agriculture Vocational School, Department of Chemistry and Chemical Processing Technologies, Zonguldak, Turkiye

[3]Zonguldak Bülent Ecevit University, Science Faculty, Department of Biology, Zonguldak, Turkiye

Safer and more environmentally friendly alternative chemicals to combat agriculturally pest insects has become extremely important due to negative effects of currently used insecticides on the environment, human and animal health. The effects of Azithromycin (AZR), a macrolide antibiotic used in the treatment of bacterial infections, on the survival rate, developmental time and longevity of *Galleria mellonella* (Lepidoptera: Pyralidae), which is a model organism, were investigated. Different AZR concentrations were used as 0.0012, 0.0072, 0.0432, 0.2592, 0.7776 and 1.0368% (g/100 g diet) in the artificial diet, respectively. While 93.75 ± 2.07% of the larvae reached the 7th instar in the control group, this rate was reduced to 20.00 ± 2.5% at the highest AZR concentration. Similarly, adult emergence rate was decreased from 86.25 ± 4.8% to 6.66 ± 0.00%. Remarkable differences were also observed in terms of developmental duration. While the average time to reach the 7th instar was 21.01 ± 0.92 days in the control group, this period was extended to approximately 38.09 ± 0.87 days at 1.0368% of AZR. Similarly, the time to adult stage was 36.33 ± 1.15 days in the control group, while this period reached 48.66 ± 0.00 days in the highest concentration. These findings show that AZR significantly prolongs the developmental period of the insect. The adult lifespan, which was 10.64 ± 0.56 days in the control group, decreased to approximately 6.66 days at the highest concentration. The data show that AZR causes significant adverse effects on survivorship and developmental time on *G. mellonella* in a concentration-dependent manner and therefore can be evaluated as a potential bioinsecticide. This study demonstrates the effects of AZR, which is widely used in clinical drug, on insect physiology and provides important information in terms of both basic science and agricultural applications.

*This study was prepared from the Master's thesis.

Keywords: *Galleria mellonella*, Azithromycin, survival rate, developmental time, longevity

OP-15. Reducing Toxicological Risks and Drug Waste: Stability and Efficacy Profiling of Monoclonal Antibodies in Healthcare Settings

Orhan Ziya[1], Gamze Varan[2], Gözde Koşarsoy Ağçeli[3], Merve Güdül Bacanlı[1]

[1] *University of Health Sciences Turkey, Gülhane Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Türkiye*

[2] *Hacettepe University, Vaccine Institute, Department of Vaccine Technology, Türkiye*

[3] *Hacettepe University, Department of Biology/Biotechnology, Türkiye*

Due to the limited number of studies on physicochemical and microbiological stability, as well as the biological activity of monoclonal antibodies (mAbs) after dilution and preparation, and the lack of sufficient information in their product leaflets, healthcare professionals often face challenges in decision-making under exceptional conditions. This study aims to evaluate the physicochemical and microbiological stability and biological activity of five commonly used mAbs —Bevacizumab, Nivolumab, Rituximab, Cetuximab, and Trastuzumab— under various environmental stress conditions following dilution and preparation.

Each mAb was diluted and prepared according to the minimum and maximum therapeutic doses. Samples were stored at 0–4°C and room temperature (20–25°C) and analyzed on days 0, 1, 3, 5, and 7. The biological activities of the antibodies were assessed in their respective cancer cell lines: human breast cancer (MCF-7), colorectal cancer (HT-29), glioblastoma (U87), lung cancer (A549), melanoma (SK-MEL-30), and Burkitt lymphoma (Daudi and BJAB). Microbiological stability was also evaluated under the same conditions. Additionally, to simulate mechanical stress during transport, samples were subjected to vibration at 100 rpm and 500 rpm for 1, 2, and 3 hours, after which their biological activity was reassessed.

Overall, results showed that samples stored at 0–4°C retained their biological activity up to day 3, while those kept at room temperature exhibited a marked decrease in activity after day 1. Microbiological analyses indicated that the samples remained sterile up to day 5 when stored at 2–8°C, whereas microbial contamination was detected under room temperature storage. Furthermore, vibration-induced mechanical stress led to a time- and intensity-dependent reduction in activity.

In conclusion, the findings of this study provide critical data that may support clinical decision-making in non-standard storage and transport conditions of mAbs. These insights may help reduce unnecessary drug disposal, promote environmental sustainability, and provide pharmacoeconomic benefits.

Keywords: Monoclonal antibodies, cytotoxicity, microbial contamination, drug waste

OP-16. Protective Effects of Green Tea Extract Against Titanium Dioxide Nanoparticle-Induced Toxicity in Lung Cells

Anıl Yirun[1], Göksun Demirel[1], Berfu Şura Güneş[1,2], Selen Selvi[1,3], Pınar Erkekoğlu[2,4]

[1]Çukurova University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Adana, Turkiye

[2]Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Turkiye

[3]Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Turkiye

[4]Hacettepe University, Vaccine Institute, Department of Vaccine Technology, Ankara, Turkiye

This study investigated the potential protective effects of green tea extract against titanium dioxide (TiO_2) nanoparticle-induced toxicity in the human lung cell line A549. TiO_2 nanoparticles are widely used, raising concerns about their potential health effects, particularly on the respiratory system. While the mechanisms of their toxicity are still under investigation, oxidative stress and inflammation have been implicated. Green tea, known for its antioxidant and anti-inflammatory properties, was investigated as a potential countermeasure. A549 cells were exposed to TiO_2 nanoparticles and various parameters related to toxicity, inflammation, and apoptosis were assessed. Using the MTT assay, the IC20 for TiO_2 was determined to be 57.51 µg/ml. Based on this, the experimental groups included control, TiO_2-treated cells, green tea-treated cells, and cells co-treated with TiO2 and green tea extract. The results showed that TiO_2 treatment led to increased levels of C-reactive protein (CRP) and interleukin-18 (IL-18), indicating inflammation. In addition, TiO_2 exposure increased caspase-3 activity, suggesting the induction of apoptosis. Notably, co-treatment with green tea extract significantly reduced TiO_2-induced increases in CRP, IL-18, and caspase-3 activity. Morphological analysis also supported these findings, showing that green tea extract attenuated the cytotoxic effects of TiO_2 on cell structure. In conclusion, this *in vitro* study suggests that green tea extract has a protective effect against TiO_2 nanoparticle-induced inflammation and apoptosis in lung cells. These findings highlight the potential of green tea as a natural therapeutic agent to counteract the adverse effects of titanium exposure in the lung and warrant further *in vivo* investigation.

Keywords: Titanium dioxide, green tea extract, A549, inflammation, apoptosis

OP-17. Evaluation of Thymoquinone's Neuroprotective Role Against Olanzapine-Induced Gene Expression Alterations in Rat Brain

Miyase Yaylagül[1], Nevin Kocaman[2]

[1] *Vocational School of Health Services, Aksaray University, Aksaray, Turkiye*

[2] *Department of Histology, Faculty of Medicine, Fırat University, Elazığ, Turkiye*

Purpose: This research aimed to explore the potential neuroprotective properties of thymoquinone (TQ), a known antioxidant, against the detrimental effects of olanzapine (OLZ) on brain tissue.

Methods: Thirty-five female Sprague-Dawley rats were allocated into five groups (n = 7 per group): group 1 as Control, group 2 receiving OLZ, groups 3, 4, and 5 receiving TQ at doses of 25, 50, and 100 mg/kg, respectively. OLZ was administered orally at 4 mg/kg once daily during the first week, increased to 8 mg/kg daily in the second week, concurrently with daily oral TQ treatments for 14 days.

Results: Administration of 25 mg/kg TQ significantly elevated Neuropeptide Y1 (NPY1) expression levels ($p=0.01$). Additionally, expressions of dopamine D1 receptor (D1R) and serotonin 5-HT2c receptor were notably reduced with 25 mg/kg TQ treatment ($p=0.001$). Treatment with 50 mg/kg TQ caused a significant increase in hypothalamic AMP-activated protein kinase α (AMPKα) expression ($p=0.046$). No significant changes were observed in dopamine D4 receptor (D4R), serotonin 5-HT1a receptor, or histamine H1 receptor (H1R) expressions following OLZ and TQ administration. The OLZ group showed a marked rise in apoptotic cell counts compared to controls ($p < 0.05$), which was significantly attenuated by 25 mg/kg TQ treatment ($p < 0.05$). Findings indicate that TQ administration may confer protective effects on brain tissue against OLZ-induced side effects, suggesting its potential as an adjunctive therapeutic agent to improve treatment outcomes.

Keywords: Olanzapine, Thymoquinone, D1R, D4R, H1R, 5-HT1a, 5-HT2c, NPY1, AMPKα, Apoptosis

OP-18. From Waste to Cure: Anticancer Potential of Supercritical and Agro-Residual Extracts of Stinging Nettle

Jülide Secerli[1], Duygu Sevim-Tatar[2], Merve Güdül Bacanlı[1], Abdullah Levent Alparslan[3]

[1] *University of Health Sciences Turkey, Gülhane Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Türkiye*

[2] *University of Health Sciences Turkey, Gülhane Faculty of Pharmacy, Department of Pharmacognosy, Ankara, Türkiye*

[3] *Istinye University, Faculty of Pharmacy, Department of Pharmaceutical Technology, İstanbul, Türkiye*

In recent years, supercritical fluid extraction (SFE) has gained significant attention as a green extraction technique due to its use of non-toxic, eco-friendly solvents, operation at relatively low temperatures—thereby minimizing thermal degradation of sensitive bioactive compounds—and its efficiency in rapid and selective extraction. Notably, while the primary focus of SFE has been on the target compounds, the residual plant material, often regarded as waste, may still contain substantial amounts of valuable phytochemicals. However, studies exploring the bioactive potential of spent plant material following SFE are limited. In this context, we focused on *Urtica dioica* (commonly known as Stinging Nettle), an edible, perennial plant from the Urticaceae family, widely recognized for its traditional medicinal uses. *U. dioica* has demonstrated a broad spectrum of pharmacological activities, including anti-inflammatory, astringent, depurative, galactagogue, diuretic, and stimulant properties. More importantly, a growing body of evidence suggests its anticancer potential against various cancer types, including breast, prostate, cervical, epidermoid, colon, gastric, and lung cancer. In our study, we investigated the anticancer effects of both SFE-derived extracts and extracts obtained from the residual plant material (using ethyl acetate and ethanol) of *U. dioica*. The bioactivities were evaluated on human cervical cancer (HeLa), aiming to assess both primary extracts and post-extraction residues for their cytotoxic potential via MTT method. Genotoxicity was assessed using the Comet assay, while the potential for reactive oxygen species (ROS) generation and apoptotic activity were analyzed via flow cytometry. When all results are considered collectively, it can be concluded that the extracts of *Urtica dioica* may exhibit promising therapeutic effects against female-specific cancer types.

Keywords: Supercritical fluid extraction, Urtica dioica, HeLa, cytotoxicity, genotoxicity, oxidative stress, apoptosis

OP-19. Determination of Organophosphate Flame Retardants in Children with Thyroid Dysfunctions

İrem İyigündoğdu[1], İsmet Çok[1], Zeynep Şıklar[2], Betül Ulukol[3], Mustafa Çelebier[4], Ozan Kaplan[4], Fatih Günay[3], Emine Koç[4]

[1] Gazi University, Faculty of Pharmacy, Department of Toxicology, Ankara, Turkiye

[2]Ankara University, Faculty of Medicine, Department of Pediatric Endocrinology, Ankara, Turkiye

[3]Ankara University, Faculty of Medicine, Department of Pediatrics, Division of Social Pediatrics, Ankara, Turkiye

[4] Hacettepe University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Turkiye

Purpose: Flame retardants (FRs) are chemicals applied to lower flammability and prevent fires from starting and spreading. Organophosphate flame retardants (OPFRs) are among of these chemicals which are determined in many environmental samples and emerging studies pointed out many possible health effects. The aim of this study is to investigate the possible impact of exposure to OPFRs on development of thyroid dysfunction in children who are a susceptible population to chemical exposures such as FRs.

Method: In the scope of this study, 86 samples were collected from 5-12-year-old children who have thyroid dysfunctions while control group consisted of 100 samples from healthy children of same age. To determine diphenylphosphate (DPHP) and bis(2-butoxyethyl) phosphate (BBOEP) compounds, samples were analyzed via ultra performance liquid chromatography quadrupole time of flight mass spectrometer (LC-Q-TOF-MS) consequent to solid phase extraction. Urinary levels of DPHP and BBOEP were corrected with creatinine.

Results: DPHP was determined in 10 samples in control group and 31 samples in thyroid dysfunction group while BBOEP were determined in 48 samples in control group and 47 samples in thyroid dysfunction group. Median levels of DPHP and BBOEP were quantified as 1,66 and 6,84 ng/ml creatinine in control group; 3,11 and 7,21 ng/ml creatinine in thyroid dysfunction group respectively. Levels of DPHP and BBOEP were not found to be statistically correlated with different types of thyroid dysfunctions or thyroid dysfunction biomarkers. However urinary levels of DPHP and BBOEP were found to be positively and significantly correlated in thyroid dysfunction group ($r=0,483$; $p=0,012$). Among thyroid dysfunction group, the ratio of samples in which DPHP was determined, was significantly higher($p<0,001$). Childhood is an important period of time regarding exposures to chemicals and thyroid has a critical role in growth and development of children thus investigating chemicals with potential thyroid disrupting effects holds significance.

OP-20. Glyphosate and the Gut: An *In Silico* Toxicogenomic Approach to Healthy Human Microbiome-Related Disease Pathways

Mine Çağlayan

Biruni Üniversitesi, Eczacılık Fakültesi, Farmasötik Toksikoloji Anabilim Dalı, İstanbul, Türkiye

Glyphosate is one of the most widely used agricultural chemicals in the world and has been declared safe because humans and other animals do not possess the target enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). However, a growing number of studies have shown potential risks to humans and animals due to the presence of the shikimate metabolic pathway in many microorganisms. In this study, we evaluated the potential effects of glyphosate on the healthy human microbiome using toxicogenomic data and *in silico* methods. We collected a total of 215 human gut microbiome genes from the GutMgene database and 3,144 glyphosate-related genes from the CTD database. Forty-six final targets were identified as key genes and used to determine key signaling pathways via PPI network and KEGG pathway analysis. Overall, we identified *IL6*, *TNF*, *IL1B* and *NFKB1* as common genes, and the IL-17 signaling pathway as a common pathway. Additionally, the top ten transcription factors and miRNAs regulating these genes were identified. Phenotypes and diseases with increased incidence upon alteration of these genes were also determined. Furthermore, we found that reperfusion injury is the primary disease potentially associated with increased incidence due to glyphosate exposure. Studies have shown that the gut microbiota is associated with cardiovascular diseases. It has been proposed that low cardiac output and systemic circulatory obstruction may cause inadequate intestinal perfusion, leading to ischemia and intestinal barrier dysfunction. The resulting bacterial translocation may contribute to inflammation. However, further empirical studies are needed to determine the effect of glyphosate on the healthy human microbiome.

Keywords: Glyphosate; Gut microbiome; *In silico* toxicogenomics; IL-17 signaling pathway; Reperfusion injury

OP-21. Rosmarinic Acid Restores Redox Balance and Limits Apoptosis in Testes of Type 2 Diabetic Rats

Çağatay Oltulu[1], Onur Ersoy[2], Melek Akıncı[3], Ayça Çevikelli-Yakut[3], Mustafa Dasman[1], Elvan Bakar[4], Eray Özgün[5]

[1]Trakya University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Edirne, Turkiye

[2]Trakya University, Vocational School of Health Services, Department of Pathology Laboratory Techniques, Edirne, Turkiye

[3]Trakya University, Faculty of Pharmacy, Department of Pharmacology, Edirne, Turkiye

[4]Trakya University, Faculty of Pharmacy, Department of Basic Pharmaceutical Sciences, Edirne, Turkiye

[5]Trakya University, School of Medicine, Department of Medical Biochemistry, Edirne, Turkiye

Background: Oxidative stress plays a major role in diabetes-induced testicular dysfunction. Rosmarinic acid, a potent natural antioxidant, was evaluated for its protective effects on testicular tissue in a type 2 diabetic rat model.

Objective: To investigate the protective role of rosmarinic acid in preserving testicular redox homeostasis, histological integrity, and endocrine function in high-fat diet and streptozotocin (HFD-STZ)-induced diabetic rats

Methods: Sprague-Dawley rats were assigned to three groups: Control, Diabetes (HFD-STZ), and Diabetes+Rosmarinic acid (50 mg/kg per day orally, 4 weeks). Fasting blood glucose, insulin, HOMA-IR, testicular GPx, catalase, MDA, and SHBG were quantified. Histopathological (H&E), immunohistochemical (SHBG), and apoptotic (TUNEL assay) assessments were performed.

Results: Rosmarinic acid reduced hyperglycemia and HOMA-IR in diabetic rats. Diabetic testes showed elevated GPx, CAT, and MDA, all of which were reversed by rosmarinic acid. Histopathological evaluation revealed reduced Johnsen scores and increased apoptosis in diabetics; rosmarinic acid significantly improved spermatogenesis and reduced apoptotic indices. SHBG expression was restored in the Diabetes+Rosmarinic acid group, indicating endocrine protection.

Conclusion: Rosmarinic acid ameliorates oxidative stress and apoptosis in diabetic testes, supporting its therapeutic potential in male subfertility associated with diabetes.

OP-22. Network Toxicology and Molecular Docking for Elucidating Mutagenicity Mechanisms of Imidacloprid

Tuğrul Mehtiyev

Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Fenerbahce University, Istanbul, Turkiye

The widespread use of imidacloprid, a neonicotinoid insecticide, has raised concern about its potential genotoxic effects, with mutagenicity recognized as one of the key underlying mechanisms. To explore its mutagenic effects, this study adopted a strategy integrating network toxicology and molecular docking. Computational analyses revealed evidence supporting imidacloprid's mutagenic potential. Imidacloprid was found to interact with 655 potential protein targets. In our constructed mutagenicity gene universe (6470 genes), 253 were identified as overlapping with imidacloprid putative targets. Enrichment results indicated that the implicated processes include chemical stress responses and protein kinase activity, mainly localized in the cytoplasm and plasma membrane regions. KEGG pathway analysis identified key cancer and stress related signaling pathways, including PI3K-Akt signaling, Pathways in Cancer, resistance to EGFR inhibitors, and Prostate Cancer. Analysis of the PPI network revealed 38 potential core targets, of which six (AKT1, TNF, BCL2, CASP3, EGFR, HSP90AA1) were selected for molecular docking. Molecular docking results indicated strong binding affinities (binding energies < –7.0 kcal/mol), suggesting stable interactions between imidacloprid and the selected proteins. Collectively, these data imply that imidacloprid may influence mutagenic pathways by targeting apoptosis and DNA repair, thereby supporting the need for future in-depth toxicological studies.

Keywords: Imidacloprid, Mutagenicity, Network Toxicology, Molecular Docking, DNA Damage and Repair

OP-23. Liver Safety of Oral Antidiabetics: From the Perspective of MASLD

Sena Ergün, Merve Hıdıroğlu, Tuğçe Yeşil
Marmara University, Faculty of Pharmacy, İstanbul, Türkiye

Objective: Type 2 Diabetes Mellitus (T2DM) is affecting a large portion of the population. T2DM is an important risk factor for metabolic dysfunction-associated steatotic liver disease (MASLD). MASLD can progress to metabolic dysfunction-associated steatohepatitis (MASH), cirrhosis, and hepatocellular carcinoma. Since hepatotoxicity is the main reason for drug withdrawal, drug-induced liver injury (DILI) must be carefully evaluated at risk of MASLD. Therefore, it is significant to select an antidiabetic medication with favorable hepatic safety.

Methods: The hepatotoxic and hepatoprotective effects of widely used oral antidiabetics were assessed by reviewing clinical trials, cohort studies, meta-analyses, and case reports from the previous 20 years.

Findings: A study found that diabetic patients receiving combination therapy with sulfonylureas and metformin had more than twice the risk of developing MASLD compared to those treated with only metformin. Troglitazone was withdrawn because of the hepatotoxicity. While pioglitazone in the same class is recommended for glycemic control in MASLD and MASH due to hepatoprotective effects. Additionally, semaglutide, the only oral GLP-1 receptor agonist, has shown positive effects in MASLD and MASH patients. There are various studies showing the positive effects of sodium-glucose cotransporter 2 inhibitors (SGLT-2is) on the liver, although some case reports have noted hepatotoxicity. A population-based study indicated that the risk of acute liver injury is higher with dipeptidyl-peptidase-4 inhibitors, especially linagliptin, compared to SGLT2 is while some studies suggest their hepatoprotective properties. Some Alpha-glucosidase inhibitors have been linked to hepatotoxicity.

Conclusion: While some drugs are considered to have a favorable hepatic safety profile, adverse effects can still occur in certain individuals. Therefore, it is crucial to evaluate each case based on individual factors, and liver function tests should be performed when needed.

Keywords: Type 2 diabetes mellitus, MASLD, oral antidiabetics, liver safety

12TH INTERNATIONAL CONGRESS OF THE TURKISH SOCIETY OF TOXICOLOGY
November 6-9, 2025, İstanbul
Toxicology in Protecting Human and Environmental Health

OP-24. Evaluation of The Potential Anticancer Activity of Extracts from Endemic Species *Origanum Acutidens* (Hand.-Mazz.) Ietswaart

Ayşenur Bilgehan[1], Tuğba Danış[2], Gizem Gülsoy Toplan[3], Gül Özhan[4]

[1] *University of Health Sciences, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Istanbul, Turkiye*

[2] *University of Health Sciences, Faculty of Pharmacy, Istanbul, Turkiye*

[3] *University of Health Sciences, Faculty of Pharmacy, Department of Pharmacognosy, Istanbul, Turkiye*

[4] *Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Istanbul, Turkiye*

Origanum acutidens (Hand. -Mazz.) Ietswaart, known locally in Eastern Anatolia as "Onıx," "Anıx," or "Kekik," is a plant species endemic to Turkey belonging to the *Origanum* genus. Traditionally, its leaves and flowers have been used as herbal tea and culinary spice. Numerous hytochemical analyses have identified the presence of bioactive constituents, including carvacrol, p-cymene, β-caryophyllene, and rosmarinic acid, which are attributed to its significant antimicrobial, antioxidant, and antispasmodic properties. While previous research is limited, available evidence suggests that *O. acutidens* exerts cytotoxic and apoptotic effects on various cancer cell lines, such as those derived from lung, breast, cervical, and epidermoid cancers. However, the limited scope of previous research has prevented a thorough evaluation of its anticancer potential. This study aims to fill this gap by examining the anticancer effects of *Origanum acutidens* extracts on the SH-SY5Y human neuroblastoma cell line. Plant specimens collected from the Bingöl province were subjected to drying and extraction using *n*-hexane, ethyl acetate, methanol, respectively. Also, the infusion method was used to prepare water extract. The antioxidant capabilities were measured using DPPH and CUPRAC assays, whereas antiproliferative activity was determined via the MTT assay. Notably, the ethyl acetate extract exhibited the most potent antioxidant activity, followed by the methanol extract. Furthermore, the MTT analysis revealed that the ethyl acetate extract significantly diminished cell viability in a dose-dependent manner. These findings indicate that *O. acutidens*, particularly its ethyl acetate extract, possesses considerable antioxidant and cytotoxic potential. This research contributes to the existing knowledge and supports further exploration of its utility as a phytotherapeutic agent for cancer treatment. Our results suggest that further studies are needed to identify the bioactive constituents of extract. In addition, we are planning to perform LDH release and caspase-3/7 apoptosis assays to further elucidate the mechanisms involved.

Keywords: *Origanum acutidens*, anticancer effect, antioxidant activity.

OP-25. Investigation of Genetic/Oxidative Damage and Molecular Docking Potential of Dinotefuran Insecticide on Blood Lymphocytes

Mazlume Pırıl Coşkun[1], Ayla Çelik[2], Hakan Arslan[3]

[1] *Department of Biology, Graduate School of Natural and Applied Science, Mersin University, Türkiye*

[2] *Department of Biology, Faculty of Science, Mersin University, Türkiye*

[3] *Department of Chemistry, Faculty of Science, Mersin University, Türkiye*

Purpose: Neonicotinoids, a subgroup of these pesticides, offer an effective solution in combating insect pests. This study aims evaluating the oxidative DNA damage potential and molecular docking of the dinotefuran insecticide

Methods: Oxidative DNA damage potential was analyzed in human peripheral blood lymphocyte cultures using the single-cell gel electrophoresis (comet assay) method at three different concentrations (0.05 µg/ml, 0.15 µg/ml, and 0.30 µg/ml). The potential of dinotefuran to interact with DNA structure was also evaluated through molecular docking analysis.

Results A statistically significant increase ($p<0.001$) was observed in the GDI and DCP parameters at all three concentrations compared to the negative control. For the molecular docking analysis, the optimized molecular structure of dinotefuran, total energies, molecular orbital energy values, molecular electrostatic potential (MEP) maps, and global reactivity parameters were obtained using the DFT/B3LYP/6-311G(d,p) method. The geometric parameters of the fully optimized structure were compared with single-crystal structural data. Computational studies, including density functional theory (DFT) and molecular electrostatic potential (MEP) analysis, provided detailed insights into the geometric structure and electronic properties of dinotefuran. The findings indicate that the ligand possesses significant characteristics in terms of chemical reactivity and potential biological activity upon interaction with DNA. The molecular interactions of dinotefuran with DNA (PDB ID: 1BNA) were investigated through molecular docking studies. The docking results revealed a minimum binding energy of -6.24 kcal/mol for dinotefuran. It was determined that dinotefuran forms four conventional hydrogen bonds, two carbon-hydrogen bonds, and one π-donor hydrogen bond with DNA. These interactions suggest a strong binding affinity particularly with guanine, cytosine, and thymine bases. The docking results are highly consistent with the experimental data on genetic and oxidative damage.

Acknowledgements: This study was supported by the Scientific Research Unit under project number 2024-TP2-5159. The studies were conducted with permission from the Mersin University Rectorate Scientific Research Ethics Committee, dated October 18, 2023, and numbered 2023/683.

OP-26. Targeting the Tumor Microenvironment: Tilorone-Induced Autophagy-Dependent Apoptosis in Triple-Negative Breast Cancer

Sukran Ozdatlı Kurtulus[1], Fulya Alkan[2], Hilmi Kaan Alkan[2], Hasan Korkaya[2]

[1] University of Health Sciences, Faculty of Pharmacy, Department of Toxicology, Istanbul, Turkiye
[2] Wayne State University, School of Medicine, Department of Oncology, Detroit, MI, USA

The tumor microenvironment (TME) plays a pivotal role in cancer progression and therapeutic resistance through dynamic interactions between tumor and immune cells. Tilorone, a synthetic interferon inducer, has established antiviral and antitumor activities; however, its effects in breast cancer from a microenvironmental perspective remain insufficiently defined. This study aimed to evaluate the effects of tilorone on autophagy, apoptosis, and immune responses using murine 4T1 breast cancer cells and RAW264.7 macrophages.

Murine 4T1 breast cancer cells and RAW264.7 macrophages were treated with tilorone for 48 hours. Effective concentrations were determined by MTT-based dose–response assays. Gene expression was analyzed by RT-PCR, targeting immune- and stress-related markers (IL-6, IL-1α, IL-1β, TMEM173, STAT1, IFN-γ, HSPA1A/B in 4T1; CEPBD, TLR2/4, NRF2, IRF3, TMEM173, IL-1β, IFN-A1R in RAW264.7). Protein-level modulation of Gasdermin E, LC3-I/II, PARP, and cleaved PARP was validated by Western blot. Flow cytometry was performed to assess immune phenotypes, apoptotic cell death, and cancer stem cell populations.

Tilorone reduced 4T1 cell viability in a dose-dependent manner and induced apoptosis, as confirmed by Annexin V positivity and PARP cleavage. Increased LC3-II and cleaved Gasdermin E expression indicated the involvement of autophagy and pyroptosis. RT-PCR revealed upregulation of pro-inflammatory and interferon-related genes, including IL-6, IL-1β, STAT1, IFN-γ in 4T1 cells, and NRF2, IRF3, TMEM173 in RAW264.7 macrophages. Flow cytometry demonstrated expansion of antigen-presenting cell subsets CD11c+MHCII+CD11b+ and a reduction in immunosuppressive monocytes CD115+Cx3CR1+ CD11b+, whereas cancer stem cell populations remained largely unaffected.

Tilorone induces autophagy-dependent apoptosis and enhances immunogenic signaling in 4T1 breast cancer cells while reshaping immune responses in RAW264.7 macrophages. These findings suggest that tilorone holds promise as a potential therapeutic candidate in breast cancer immunotherapy.

This study was supported by the Scientific and Technological Research Council of TURKEY (TUBİTAK) Project no: 1059B192301041

12TH INTERNATIONAL CONGRESS OF THE TURKISH SOCIETY OF TOXICOLOGY
November 6-9, 2025, İstanbul
Toxicology in Protecting Human and Environmental Health

OP-27. Epigenetic Modulation of *DNMT1* and Estrogen Receptors (*ESR1/ESR2*) By SGI-1027 in Triple-Negative Breast Cancer

Tuçe Kibar[1,2], Tuğçe Yeşil[1], Seher Karslı[1]

[1]Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Istanbul, Türkiye
[2]Marmara University, Institute of Health Sciences, Istanbul, Türkiye

Purpose: Triple-negative breast cancer (TNBC) exhibits a more aggressive clinical course compared to other breast cancer subtypes. DNA methylation, an epigenetic modification, contributes to the etiology of various diseases, including cancer. Toward this aim, DNA methyltransferase (DNMT) inhibitors have drawn increased attention due to their therapeutic potential. This study aimed to evaluate the therapeutic value of the DNMT inhibitor SGI-1027 for TNBC and to investigate its effects on the DNA methylation profiles of the *ESR1* and *ESR2* promoter regions.

Methods: TNBC model MDA-MB-231 (ER-, PR-, HER2-) and estrogen receptor-positive MCF-7 cell lines were used in this study. The cytotoxic effect of SGI-1027, as well as the levels of *DNMT1*, *ESR1* and *ESR2* mRNA and the CpG methylation status of the CpG *ESR1,* and *ESR2* genes, were investigated. Following determination of IC_{50} concentrations, cells were treated with 5 µM of SGI-1027, and gene expressions of *DNMT1, ESR1,* and *ESR2* were analyzed by quantitative real-time polymerase chain reaction (qPCR). DNA samples underwent bisulfite conversion, and CpG islands in the promoter regions of *ESR1* and *ESR2* were amplified and analyzed by PCR and sequencing.

Results: SGI-1027 exhibited dose-dependent cytotoxicity. Treatment with SGI-1027 reduced the level of DNMT1 expression by 0.4-fold, and this was statistically significant (p=0.001). Bisulfite sequencing revealed that SGI-1027 treatment reduced *ESR2* promoter CpG methylation levels from 83.3% to 42.9% in MDA-MB-231 cells and from 58.3% to 50.0% in MCF-7 cells, indicating a cell type–dependent epigenetic demethylating effect.

Conclusion: Our findings demonstrate that SGI-1027 exerts cytotoxic and epigenetic effects in breast cancer cells by suppressing *DNMT*1 expression and modifying *ESR2* promoter methylation in a cell type–specific manner. These results suggest that SGI-1027 can be a potential therapeutic candidate for targeting epigenetic regulation in triple-negative breast cancer.

This study was supported by The Scientific and Technology Research Council of Turkey (grant no: 424S086).

Keywords: SGI-1027, triple-negative breast cancer, DNA methylation, DNMT inhibitors, *ESR1*, *ESR2*

OP-28. Comparative *In Vitro* Toxicity of Tartrazine and Its Primary Metabolite Sulfanilic Acid in HT-29 Human Colon Cells

Merve Baysal, Abdullah Burak Karaduman, Büşra Korkut Çelikateş, Merve Güven, Özlem Atlı Eklioğlu[1], Sinem Ilgın[1]

Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Eskisehir, Turkiye

Tartrazine is a synthetic azo dye widely used in food and consumer products. In humans, it is metabolized primarily into sulfanilic acid by gut microbiota. Although generally considered safe within acceptable daily intake limits, tartrazine has been associated with potential toxic effects on various organs. Data on sulfanilic acid — a key metabolite potentially relevant to tartrazine-induced toxicity — are limited. This study aimed to assess the toxic effects of tartrazine and sulfanilic acid in human colon adenocarcinoma cells (HT-29), chosen as a model for intestinal epithelial cells, which are primary targets due to the compounds' metabolism in the gut. Cell viability was evaluated using MTT and neutral red uptake (NRU) assays. DNA damage was assessed via the comet assay, and apoptosis was measured through annexin V binding, caspase-3/7 activity, and mitochondrial membrane potential (MMP) analysis. Tartrazine (1–5000 µM) significantly reduced HT-29 cell viability after 24, 48, and 72 h in both MTT and NRU assays, with greater inhibition observed in MTT. In contrast, sulfanilic acid showed no cytotoxicity at any tested concentration or time point. Following 24 h exposure, both compounds increased apoptotic cell percentages in a concentration-dependent manner, slightly higher for tartrazine. A concentration-dependent decrease in MMP was noted for tartrazine, while sulfanilic acid caused no significant changes. For both compounds, no significant differences in caspase 3/7 levels were recorded compared to the control. Preliminary comet assay results suggested slight DNA damage induced by both tartrazine and sulfanilic acid at certain concentrations compared to controls. In conclusion, under the present experimental conditions, tartrazine induced mild cytotoxicity, apoptosis, and DNA damage in HT-29 cells, whereas its primary metabolite, sulfanilic acid, did not exhibit any significant toxic effects.

Keywords: Tartrazine, sulfanilic acid, HT-29 cells, cytotoxicity, genotoxicity, apoptosis.

OP-29. Mitochondrial Effects of PFOA and its Alternative HFPO-TA in Cardiac Cells

Tugce Boran[1], Özce Esma Pala Çamlı[2,3], Gül Özhan[4]

[1]*İstanbul University-Cerrahpaşa, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, İstanbul, Türkiye*

[2]*Institute of Health Sciences, Istanbul University, İstanbul, Türkiye*

[3]*İstanbul Medipol University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Türkiye*

[4]*İstanbul University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Istanbul, Türkiye*

Perfluoroalkyl acids (PFAAs) are surfactants frequently used in many different fields, such as food, cosmetics, and textiles, due to their resistance to degradation. Perfluorooctanoic acid (PFOA) has been one of the most extensively studied and used PFAAs. However, PFOA has been phased out in many regions due to its negative health and environmental impacts, and alternative compounds such as HFPO-TA have been developed. However, emerging evidence suggests that these substitutes may exhibit similar persistence and toxicity profiles. Studies have demonstrated that exposure to HFPO-TA can also be toxic and can cause the inhibition of cellular development and morphological abnormalities.

Cardiovascular diseases are one of the most common causes of death worldwide. The increasing prevalence and high mortality rates associated with cardiovascular diseases indicate significant gaps in the understanding of the underlying mechanisms that impair cardiovascular health. Recent studies suggest that cardiovascular tissues may have greater sensitivity to environmental chemicals compared to other tissues. Due to its high energy demand, the heart contains a large number of mitochondria. Functional defects in mitochondria have been shown to play a significant role in the pathogenesis of cardiovascular diseases. Therefore, in this study, the mitochondrial toxicity of PFOA and its alternative, HFPO-TA, was investigated in cardiac cells. Intracellular ATP amount, mitochondrial membrane potential change, mitochondrial mass, and mitochondrial ROS amount parameters were used in the evaluation of mitochondrial toxicity.

In cytotoxicity studies, IC_{20} values were calculated to be nearly 150 µM for both the chemicals following 48 h exposure. The mitochondrial effects were evaluated at the 37.5-150 µM concentrations. Intracellular ATP level decreased significantly at the highest concentration after HFPO-TA exposure (40%); the decrease was not found significant in PFOA-exposed cells. Mitochondrial membrane potential depolarization was detected in a dose-dependent manner (>11%), and the decrease was only significant at the highest concentration. A significant change in mitochondrial mass was also observed in HFPO-TA-exposed cells at the highest concentration. Besides, the mitochondrial ROS production was induced following HFPO-TA exposure (>30%). The preliminary research showed underlying cardiotoxicity of HFPO-TA, a PFOA alternative, may result from mitochondrial dysfunction.

This study was supported by TUBITAK with project number 124S490.

Keywords: PFOA, HFPO-TA, mitochondrial toxicity, environmental toxicity, cardiotoxicity

OP-30. Environmental Toxicant-Induced Epigenetic Transgenerational Inheritance in Male Infertility

Merve Arici

Istinye University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Istanbul, Türkiye

Infertility rates have increased in developing and industrialized countries in recent years. It is considered as a serious health issue affecting 15% of couples throughout the world. Male infertility factors contribute to approximately half of all infertile cases. Anatomical defects, endocrine dysfunction, immunological problems, molecular defects, impaired spermatogenesis, lifestyle factors, idiopathic factors and chromosomal abnormalities are most common reasons for male infertility. Besides, the male reproductive system seems to be particularly vulnerable to environmental exposures. Early development exposures linked to impaired male reproductive functions. Epigenetic modifications control all steps of fertilization from testicular cells development to embryo development and regulate spermatogenesis and male fertility. Epigenetics regulates gene regulation without any changes in the DNA sequence. These modifications consist of methylation of DNA, post-translational histone modifications and chromatin rearrangement. Normal spermatogenesis requires the regulation of gene expression through epigenetic modifications. Current findings indicate that numerous genes in testicular cells are subject to epigenetic control, emphasizing their essential role in sperm formation and count, maturation, fertilization, general testis health and male reproductive capacity. In recent times, environmental toxicant-induced epigenetic transgenerational inheritance is a phenomenon to be considered in male infertility etiology. It is stated as early developmental exposures that cause altered epigenetic programming in the germline, which is then passed on to subsequent generations even in the absence of continued environmental exposure. A lot of environmental toxicants, pesticides, phthalates, industrial compounds, dioxins and plasticizers, might trigger transgenerational abnormalities and induce exposure specific alteration in sperm DNA methylation. Environmental exposures and associated epigenetic changes, as well as transgenerational inheritance, are important factors to consider as they may affect the susceptibility of future generations to male infertility.

OP-31. Mycotoxin Exposure In Children: A Biomarker-Based Approach

Gizem Sena Elagoz

Istanbul Aydin University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Turkiye

Mycotoxins, toxic fungal metabolites, present significant health risks to children due to their developmental susceptibility. It is important to evaluate mycotoxin exposure in children using a biomarker-based approach, particularly in high-risk developing regions.

Recent investigations reveal that 50-85% of children exhibit detectable biomarkers, including aflatoxin-lysine adducts (5-40 pg/mg albumin) and urinary fumonisins (0.1-0.8 ng/mL), reflecting dietary exposure to contaminated grains. Co-exposure to deoxynivalenol and ochratoxin A occurs in 20-50% of cases, predominantly in cereal-consuming populations. Elevated biomarker levels are associated with growth impairment (height Z-score reduction), immune suppression, and elevated liver enzymes (ALT).

Biomarkers provide a superior measure of internal exposure compared to food contamination assessments. It is crucial to underscore the role of biomarker monitoring in the identification of at-risk groups, while also advocating interventions including dietary diversification and improved storage practices. Future investigations should focus on multi-biomarker panels and longitudinal studies to evaluate chronic effects.

Keywords: Biomarkers, Child exposure, Developmental toxicity, Health outcomes, Mycotoxins.

OP-32. Evaluation of The Effect of *Herpes Simplex* Glycoprotein B on The Necroptosis Pathway in an *In Vitro* Model of Parkinson's Disease

Berfu Şura Güneş[1,2], Anıl Yirün[1], Göksun Demirel[1], Deniz Arca Çakır[3], Selen Selvi[1], Pınar Erkekoğlu[2,2]

[1]*Department of Pharmaceutical Toxicology, Çukurova University Faculty of Pharmacy, Adana, Türkiye*

[2]*Department of Pharmaceutical Toxicology, Hacettepe University Faculty of Pharmacy, Ankara, Türkiye*

[3]*Department of Vaccine Technology, Hacettepe University Vaccine Institute, Ankara, Türkiye*

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the *substantia nigra*, resulting in motor and non-motor symptoms. Multiple molecular mechanisms, including α-synuclein misfolding, mitochondrial dysfunction, oxidative stress, neuroinflammation, and programmed cell death, contribute to PD pathology. Recent evidence implicates necroptosis, a caspase-independent cell death pathway mediated by receptor interacting serine/threonine protein kinases (RIPK1, RIPK3) and mixed lineage kinase domain-like protein (MLKL), in neuronal loss. Furthermore, viral infections, particularly *Herpes simplex* virus type 1 (HSV-1), have been suggested to contribute to the progression of neurodegenerative diseases by triggering neuroinflammation and activating various cell death pathways. In this study, an *in vitro* PD model was established by treating the SH-SY5Y cell line with 1-methyl-4-phenylpyridinium (MPP^+), and the effects of *Herpes simplex* glycoprotein B (HSV-gB) on cell death through the necroptosis pathway were investigated. To determine whether HSV-gB–induced cytotoxicity was mediated through the necroptosis pathway, cells were treated with necrostatin-1 (Nec-1), a specific inhibitor of RIPK1. Necroptosis activation was assessed by measuring tumor necrosis factor alpha (TNF-α), dopamine, RIPK1, RIPK3, and MLKL protein levels using ELISA kits, and cytotoxicity was assessed using the MTT assay (IC_{20}: MPP^+ 70.22 µM, IC_{20}: HSV-gB 193.04 pg/ml).The experimental groups were designed as follows: (1) control; (2) MPP^+; (3) HSV-gB (HSV); (4) necrostatin-1 (NEC); (5) HSV-gB + MPP^+ (HM); (6) MPP^+ + Nec-1 (MN); (7) HSV-gB + Nec-1 (HN); and (8) MPP^+ + HSV-gB + Nec-1 (HMN). The results demonstrated that HSV-gB could activate necroptotic pathways by enhancing TNF-α production, and that this effect was partially suppressed by Nec-1. Moreover, co-treatment with MPP^+ and HSV-gB elicited a stronger inflammatory response, suggesting that necroptosis plays a critical role in both cell death and inflammation in the pathogenesis of PD. These findings support the potential of RIPK1 inhibitors as therapeutic strategies against neurotoxicity and virus-associated effects.

Keywords: Parkinson's disease, necroptosis, *herpes simplex* glycoprotein b, necrostatin-1, *in vitro* Parkinson's model

12TH INTERNATIONAL CONGRESS OF THE TURKISH SOCIETY OF TOXICOLOGY
November 6-9, 2025, İstanbul
Toxicology in Protecting Human and Environmental Health

OP-33. Integrated Evaluation of Mediterranean Herbs and Spices for Antiviral and Immunomodulatory Activities Against SARS-CoV-2 Using *In Silico*, Highthroughput, and *In Vitro* Methods

Yüksel Çetin[1], Gülşah Akbaş[2], Jenya Dursun[1], Şeyma Aydınlık Şanlı[1]

[1] *TUBITAK Marmara Research Center, Life Sciences*

[2] *Yildiz Technical University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics*

The key challenges in antiviral drug development have increased attention in phytochemicals as promising, nature-derived alternative or complementary antiviral agents against SARS-CoV-2 infection. The main goal of this study is to conduct an integrated evaluation of the antiviral and immunomodulatory potential of 40 extracts from Mediterranean herbs and spices against SARS-CoV-2, utilizing *in silico* modeling, high-throughput screening, and *in vitro* assays. A range of *in vitro* cellular assays included MTT assays for cell viability; Hematoxylin and Eosin (H&E) staining for cellular morphology; Annexin V/PI/Hoechst staining for apoptosis detection; and the DCFDA assay for measuring intracellular oxidative stress were conducted using HEK293T, Vero E6, Caco-2, and Calu-3 cell lines. The antiviral activities of the extracts were investigated using high-throughput techniques included an ELISA assay to assess the inhibition between the SARS-CoV-2 receptor-binding domain (RBD) and ACE2, and a FRET assay to evaluate the inhibition of the viral main protease (Mpro). In addition, the effecacy of the extracts to block viral entry was examined using a SARS-CoV-2 pseudovirus S1 neutralization assay in ACE2-transfected HEK293T cells. 26 of the extracts exhibited very strong inhibition of RBD-ACE2 binding (30 - 100%) as determined by ELISA, while 24 extracts showed greater than 30% inhibition of SARS-CoV-2 pseudovirus S1 entry into HEK293T cells. These 24 extracts demonstrated potent inhibitory activity (30 - 100%) against the viral Mpro. Immunomodulatory profiling reveled that the extracts exerted significant immunosuppressive and/or immunostimulatory effects on the production of IL-1β, TNF-α, IFN-γ, IL-6, and IL-8 as measured by ELISA in a SARS-CoV-2 pseudovirus S1-infected co-culture system comprising THP-1 and HEK293T cells in a transwell insert system. Collectively, the findings demonstrate that the extracts possess notable antioxidant capacity, antiviral efficacy, and diverse immunomodulatory effects, highlighting their potential as phytochemical-based therapeutic agents against SARS-CoV-2, potentially offering valuable alternatives or complements to existing treatment strategies.

Keywords: SARS-CoV-2, Mediterranean herbs and spices, Antiviral activity, İmmunomodulatory effect, MTT assay, DCFDA assay

12TH INTERNATIONAL CONGRESS
OF THE TURKISH SOCIETY OF TOXICOLOGY
November 6-9, 2025, İstanbul
Toxicology in Protecting Human and Environmental Health

Poster Presentations

PP-01. Method Development for the Rapid Detection of Avermectin Drug Residues in Cow, Sheep and Goat Milk by Liquid Chromatography-Tandem Mass Spectrometry

Esma Söylemez-Yeşilçimen[1,2], Gülşen Yıldırım[2], Caner Sayın[2], Eren Ozcagli[3], Gülden Zehra Omurtag[1]

[1] *Istanbul Medipol University, School of Pharmacy, Department of Pharmaceutical Toxicology, İstanbul, Türkiye*

[2] *Pendik Veterinary Control Institute, Department of Pharmacology, Veterinary Drug Residue Laboratory, İstanbul, Türkiye*

[3] *Istanbul University, Department of Pharmaceutical Toxicology, Faculty of Pharmacy, İstanbul, Türkiye*

The detection of veterinary drug residues in various edible animal products such as meat, honey, eggs and milk is essential for ensure safe food consumption. The aim of this study was to develop and validate a new rapid, simple and sensitive method performed by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method with fewer extraction steps for the detection of avermectin veterinary drugs in cow, sheep and goat milk according to the criteria and recommendations of the Commission Implementing Regulation (EU) 2021/808. The veterinary anthelmintic drugs abamectin (ABA), doramectin (DOR), ivermectin (IVR), eprinomectin (EPR) and moxidectin (MOX) were extracted by liquid-liquid extraction with acetonitrile followed by evaporation. Selamectin was chosen as the internal standard. Chromatographic separation was performed by an accucore C-18 column (100 mm x 2.1 mm x 2.6 µm) using a suitable gradient of 2 mM ammonium formate-0.1% formic acid in water (mobile phase A) and 2 mM ammonium formate-0.1% formic acid in methanol (mobile phase B). Calibration curves were obtained by least squares linear regression analysis of peak area versus concentration and the response was linear over the range tested ($r^2 \geq 0.99$). For confirmatory quantitative validation trueness, precision, selectivity, specificity, stability and ruggedness were determined. For all analytes inter and intra-day reproducibility CV% was not exceed 10.3%. CCα of the analytes were calculated for IVR; DOR; ABA, EPR, MOX respectively;1.18; 1.20;1.25; 22.11; 42.2 µg/kg.

In conclusion, a new, rapid and practical method for ABA, DOR, IVR, MOX, EPR in cow, sheep and goat milk samples were developed and validated.

Keywords: veterinary drug residues, avermectin, LC MS/MS

PP-02. Familial Multiple Sclerosis: Assessment of Heavy Metal Levels in Blood Sample from Two Affected Siblings and the One Healthy Sibling

Esma Söylemez-Yeşilçimen[1], Fulya Kürekçi[2], Eylem Funda Göktaş[3], Oya Yeter[4], Eren Ozcagli[5], Edibe Pembegül Yıldız[2], Gülden Zehra Omurtag[1]

[1] *Istanbul Medipol University, School of Pharmacy, Department of Pharmaceutical Toxicology, Istanbul, Türkiye*

[2] *Istanbul University, Faculty of Medical, Department of Pediatric Neurology, Istanbul, Türkiye*

[3] *Pendik Veterinary Control Institute, Department of Pharmacology, Istanbul, Türkiye*

[4] *Council of Forensic Medicine, Department of Chemistry, Istanbul, Türkiye*

[5] *Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Istanbul, Türkiye*

Multiple Sclerosis (MS) is a chronic autoimmune disease. There is evidence that genetic and environmental factors interact to influence the development and progression of MS, although the exact cause of MS is not fully understood. The aim of this study was to compare the levels of selected heavy metals (Ni, As, Se, Zn, Cd, Co, Mo, Hg, and Al) in the blood samples of two siblings diagnosed with MS and one healthy sibling. The levels of heavy metals in the blood of these children were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). In the blood of siblings with MS and one healthy sibling: Ni (2.38; 2.41 and 1.81 µg/L), As (3.37; 13.27 and 2.5 µg/L), Se (122.14; 135.16 and 97.39 µg/L), Zn (5304; 6327 and 4590 µg /L), Cd (0.41; 0.39 and 0.22 µg/L), Co (0.46; 0.21 and 0.03 µg/L), Mo (1.74; 0.58 and 0.85 µg/L), were detected respectively. The Al and Hg level were 567 and 0.61 µg/L in one MS sibling. There was no difference between the other sibling and the healthy one in the levels of both Al and Hg. (< 0.1 µg/L).

In conclusion, our study demonstrated that blood levels of Ni, As, Se, Zn, Cd, Co were increased in siblings with MS compared to healthy sibling.

Keywords: Multiple Sclerosis, heavy metals, ICP-MS

PP-03. Effect of Recombinant Enzymes on Glucuronide-Bound Components

Eylem Funda Göktaş[1], Esma Söylemez-Yeşilçimen[2]

[1] *Pendik Veterinary Control Institute, Department of Pharmacology, Istanbul, Türkiye*

[2] *Istanbul Medipol University, School of Pharmacy, Department of Pharmaceutical Toxicology, Istanbul, Türkiye*

Urine samples play an important role in toxicological analyses for detecting a wide range of pharmacological agents and their metabolites. Enzymatic hydrolysis is commonly used to determine the concentrations of free forms of compounds metabolized through conjugation, such as glucuronides and sulfates in urine samples. An efficient hydrolysis step is essential for the accurate determination of the free forms of glucuronide-bound active substances, which are particularly challenging to hydrolyze. In this study, the efficiency of 3rd generation recombinant enzymes, which have been increasingly used in recent years, on compounds that are difficult to hydrolyze in low volume urine samples (codeine-6-glucuronide (C6G), morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G)) was evaluated using direct injection method and the results were compared with β-glucuronidase enzyme. For this purpose, 25, 50 and 100 µL of B-one recombinant enzyme were added to 100 µL urine samples containing C6G, M3G and M6G at a concentration of 200 ng/mL, and the samples were incubated at room temperature for 15 minutes. The other comparison samples to which β-glucuronidase was added were incubated at 55°C for 1 hour. The hydrolysis reaction in both enzyme groups was stopped by adding 100 µL acetonitrile and then the sample volume was made up to 500 µL with water. Finally, the samples were centrifuged at 18000 rpm for 10 minutes. After centrifugation, 200 µL of the supernatant was transferred to a vial and analyzed by HPLC-HRMS. The results demonstrated that the recombinant β-glucuronidase B-One® enzyme provided almost complete cleavage of glucuronide bonds when 100 µL was added. It was particularly effective in converting C6G to its free form, outperforming the abalone-derived β-glucuronidase enzyme.

In conclusion, it was demonstrated that recombinant enzymes provide a user-friendly, practical, and effective for converting glucuronide-bound compounds to their free forms in toxicological analyses.

Keywords: glucuronides; deconjugation; β-glucuronidase; recombinant enzyme

PP-04. *In Vitro* Assessment of The Cytotoxic Effects of Bovine-Derived Collagen Hydrolysates on Various Mammalian Cell Lines

Simge Kara Ertekin[1], Muhammet Enes Yurteri[2]

[1]Istanbul Yeni Yuzyil University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Istanbul, Turkiye

[2]Istanbul Yeni Yuzyil University, Graduated from the Faculty of Pharmacy, İstanbul, Turkiye

Aim: Collagen is a major structural protein in the extracellular matrix and plays a fundamental role in maintaining tissue structure and function. Hydrolyzed collagen, particularly from bovine sources, has become a widely used ingredient in cosmetic and pharmaceutical products due to its improved solubility and bioavailability. However, its safety profile remains inadequately characterized. This study aimed to evaluate the *in vitro* cytotoxic effects of a commercially available bovine collagen hydrolysate blend (Type I and III) on different mammalian cell lines.

Methods: Four cell lines—L929 (mouse fibroblast), HOB (human osteoblast), HEK293 (human embryonic kidney), and MCF-7 (human breast adenocarcinoma)—were cultured and treated with increasing concentrations (1, 2, 3, 5, 10, and 20 mM) of collagen hydrolysate for 24 hours. The MTT assay was used to assess cell viability. Dose–response curves were generated, and IC_{50} values were calculated using non-linear regression via GraphPad Prism v9.5.1.

Results: A concentration-dependent decrease in cell viability was observed in all tested cell lines. Statistically significant cytotoxicity ($p < 0.05$) occurred primarily at 10 and 20 mM concentrations. IC_{50} values were found to be 21.4 mM (HEK293), 22.3 mM (HOB), 34.21 mM (MCF-7), and 54.9 mM (L929), suggesting differential sensitivity.

Conclusion: The results demonstrate that collagen hydrolysates can exert varying levels of cytotoxicity across cell types and concentrations. These findings underscore the importance of conducting detailed *in vitro* toxicological assessments of collagen-based formulations prior to their therapeutic or cosmetic application. Moreover, this study addresses a gap in the current toxicological literature by evaluating a commercially relevant collagen hydrolysate blend across multiple mammalian cell types. The observed differences in cell line sensitivity contribute to a more nuanced understanding of its safety profile, which may support more targeted and safe application in formulation science."

Keywords: Collagen Hydrolysate, Cytotoxicity, Type I collagen, Type III collagen, MTT assay

PP-05. *In Vitro* Evaluation of The Cytotoxic Effects of The Endemic Arenaria Kotschyana Subsp. Kotschyana Extracts

Simge Kara Ertekin[1], Hüseyin Servi[2], Nursena İlhan[3]

[1]Istanbul Yeni Yüzyil University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Istanbul, Turkiye

[2]İstanbul Yeni Yüzyıl University, Faculty of Pharmacy, Department of Pharmacognosy, Istanbul, Türkiye

[3] Istanbul Yeni Yuzyil University, Student of the Faculty of Pharmacy, İstanbul, Turkiye

Purpose: Arenaria kotschyana subsp. kotschyana is an endemic species of Türkiye. While some Arenaria species are traditionally used as diuretics and anti-inflammatory agents, their toxicological properties have not been sufficiently characterized. This study aimed to evaluate the *in vitro* cytotoxic potential of extracts from this species to assess its safety for possible traditional or pharmacological use.

Methods: Aerial parts of the plant were extracted using petroleum ether, ethyl acetate, methanol, and methanol:water (70:30). Extracts were applied to L929 (normal fibroblast), A549 (lung carcinoma), MCF-7 (breast carcinoma), and U87MG (glioblastoma) cell lines for 48 hours. Cell viability was assessed via the MTT assay. IC_{50} values were calculated for each extract in cell lines.

Results: The methanol extract exhibited the strongest cytotoxic activity, with an IC_{50} of 37.26 µg/mL on U87MG cells. Moderate cytotoxicity was observed on MCF-7 and A549 lines, while minimal toxicity was found in L929 cells. This selective cytotoxic profile suggests potential biological activity associated with the methanol-soluble secondary metabolites. Literature indicates that Arenaria species are rich in flavonoids, phenolic acids, and triterpenes, which may contribute to these effects via ROS modulation and apoptotic mechanisms.

Conclusion: The results demonstrate that A. kotschyana subsp. kotschyana contains bioactive compounds capable of inducing cytotoxic effects in tumor cells. These findings highlight the necessity of toxicological evaluation for endemic plants with potential traditional usage, particularly to determine safe exposure levels and identify active constituents for further study.

Keywords: Arenaria kotschyana, cytotoxicity, MTT assay, endemic plant, secondary metabolites

PP-06. Assessing the Genotoxicity of Botanicals Using Standard Assays – an Update from the Botanical Safety Consortium

Zehra Sarıgöl Kılıç[1], Kristine Witt[2], Jan Ven Benthem[3], James MacGregor[4]

[1] *L'Oreal USA R&I, United States*

[2] *NIEHS/DNTP, United States*

[3] *RIVM, United States*

[4] *Toxicology Consulting Services, United States*

Thirteen botanicals selected by the Botanical Safety Consortium as data-rich case studies were tested in four standard genotoxicity assays covering mutation and chromosome damage endpoints. The goal of this exercise was to determine whether the results from the four selected assays provided an assessment of genotoxicity consistent with the existing data for each case study, thereby supporting their use in generating genotoxicity profiles of botanicals. The four assays included the bacterial reverse mutation assay (Ames test), the *in vitro* micronucleus (MN) assay in TK6 cells and in HepaRG cells, and the ToxTracker® assay. The Ames and MN tests were conducted in accord with their respective OECD Test Guidelines.

Based on existing information for the 13 botanicals tested, 4 were expected to be positive in the Ames test and/or were expected to induce MN. Our results were consistent with the existing literature. 4 Ames-positive botanicals were identified. The MN test in TK6 cells, conducted +/- S9, identified 1 botanical as positive while the MN test conducted using metabolically competent HepaRG cells identified 4 botanicals as positive or equivocal. ToxTracker® assays identified four positives, consistent with previously published data.

Our results suggest that currently available *in vitro* genotoxicity assays are suitable for testing botanicals. Currently, we are evaluating all the data to determine a recommended testing scheme. In addition, our test data will be compared with *in silico* predictions of genotoxicity that were made for each botanical based on their identified constituents to determine how to combine *in silico* data, *in vitro* test data, and human exposure data to produce a comprehensive assessment of genotoxicity.

Keywords: Dietary supplements; Mutagenicity testing; micronucleus; *in vitro* assays; *in silico* tools; mixtures

PP-07. Effects of Short-Term Storage Conditions on DNA Damage in Isolated Human Lymphocytes

Seda İpek Tekneci[1,2], Tuğçe Bilgen[1,2], Seniha Kerimoğlu[2], Aylin Üstündağ[1], Yalçın Duydu[1]

[1] Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Türkiye

[2]Graduate School of Health Sciences, Ankara University, Ankara, Türkiye

Introduction: The comet assay is a prevalent method for evaluating DNA damage. One application of comet is in human biomonitoring investigations, where peripheral lymphocytes readily extracted from human venous blood are the most commonly utilized cell type. The evaluation of DNA damage in lymphocytes is crucial for indicating the human body's response to genotoxic agents and the extent of oxidative stress (1,2,3). Lymphocyte samples obtained from fresh venous blood can be preserved at low temperatures for extended durations prior to examination (2,3). Nonetheless, storage settings that induce further DNA damage in cells may result in erroneous interpretations of human biomonitoring research outcomes. Consequently, examining the impact of storage conditions on human peripheral blood lymphocytes is a significant concern.

Methods: Our study sought to assess DNA damage in human isolated peripheral blood cells at room temperature and 4°C over a duration of 7 days. Lymphocyte isolation was conducted using fresh blood from a healthy 34-year-old female volunteer, and the alkaline comet assay was employed to assess DNA damage at the designated time and storage conditions.

Results: The statistical analysis of the study involving duplicate samples ($p<0.05$ significance threshold) revealed that DNA damage in lymphocytes preserved at +4°C on days 4, 5, 6, and 7, as well as on days 3 and 4 at room temperature, was greater than that in lymphocytes derived from fresh blood. Analysis could not be conducted on lymphocytes maintained at room temperature on days 5, 6, and 7 due to a cloudy appearance resulting from significant DNA damage. The results indicate that lymphocytes isolated from fresh blood can be stored at +4°C for an extended duration more effectively than at room temperature. This scenario indicates that +4°C storage temperatures are better appropriate for short-term analyses involving lymphocytes.

Conclusion: The findings are crucial for the accurate interpretation of results derived from DNA damage tests conducted with lymphocytes. Further extensive research is required in this context.

Keywords: isolated lymphocyte, storage, DNA integrity, comet

PP-08. Antioxidant Potential of Common Walnut Leaf Extract Against Acetamiprid: *In vitro* Analysis in HepG2 Cell Line and Comparison with AI Predictions

Yağmur Emre Arıcan[1], Hikmet Can[2], Gülsen Kendir[3], Samet Atılgan[2], Müberra Özcan[1], Gül Özhan[4], Özlem Batu[1], Mustafa Yıldız[5]

[1] *Süleyman Demirel University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Isparta,*

[2] *Süleyman Demirel University, Faculty of Pharmacy, Isparta*

[3] *Süleyman Demirel University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Isparta*

[4] *İstanbul University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, İstanbul*

[5] *Meddem Hospital, Nuclear Medicine, Isparta*

In Turkish folk medicine, the leaves of the common walnut are utilized internally as an appetite stimulant, antidiarrheic, antidiabetic, and tonic, while externally they are employed for their analgesic properties. Common walnut (*Juglans regia* L.) leaf extract (JRLE), demonstrated to possess antioxidant properties, is of scientific interest as a potential safeguard against oxidative stress. Environmental exposure to oxidants poses a considerable risk, prompting researchers to investigate novel and effective antioxidants. In our study, we aimed to evaluate the antioxidant potential of common walnut leaf in response to acetamiprid (ACE) exposure, which has widespread use as a neonicotinoid pesticide and is recognized for inducing oxidative stress and damage.

To achieve this goal, 4.6 mM ACE-exposed HepG2 cell lines were treated *in vitro* with 0.008-0.128 mg/ml JRLE. The levels of glutathione (GSH) and malondialdehyde (MDA) were quantified spectrophotometrically using the ELISA kit, while cell viability was assessed via the MTT assay. Additionally, experimental findings are compared with predictions generated by the artificial intelligence (AI) of ChatGPT-4 Mini.

Depending on dosage, 0.008-0.128 mg/ml JRLE treatment against 4.6 mM ACE reduced MDA levels significantly. JRLE reduced lipid peroxidation in HepG2 cells and scavenged free radicals rather than producing GSH thanks to its antioxidants. Cytotoxicity results show that 0.008-0.128 mg/ml JRLE with 4.6 mM ACE did not affect cell viability (67.38-71.13%). Due to its capacity to reduce lipid peroxidation, JRLE may be employed as a food supplement following more research to optimize the dosage of JRLE. Additionally, while accuracy declined for difficult inquiries, ChatGPT usually answered correctly. AI pre-simulation before studies will reduce expenses and labor, and AI in toxicology and pharmacology research will have several benefits.

The study was funded by Health Institutes of Türkiye (TÜSEB) (call code: 2023-A102, project number: 37694).

Keywords: common walnut, *Juglans regia* L., acetamiprid, oxidative stress, GSH, MDA, MTT assay, *in vitro*, HepG2, ChatGPT-4 mini, artificial intelligence

PP-09. *In Silico* Toxicity Assessment of Long- And Short-Chain Per- and Polyfluoroalkyl Substances Based on Human Health Effect Probabilities

Rumeysa Çetin Türker[1,2], Fatma Okuş[3], Metin Yangın[4], Ayça Çakmak Pehlivanlı[4], Gonca Çakmak[2]

[1] *Afyonkarahisar Health Sciences University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Afyonkarahisar, Turkiye*

[2] *Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Turkiye*

[3] *Gazi University, Graduate School of Natural and Applied Sciences, Department of Biology, Teknikokullar, Ankara, Turkiye*

[4] *Mimar Sinan Fine Arts University, Faculty of Science and Letters, Statistics Department, İstanbul, Türkiye*

Per- and polyfluoroalkyl substances (PFAS) are synthetic, persistent, and bioaccumulative chemicals widely used since the 1940s in industrial and consumer products, and they are known as 'forever chemicals' with health and environmental concerns. PFAS are classified into two groups based on carbon chain length: long-chain (LC)- and short-chain (SC)-PFAS, the latter generally considered to have lower toxicity. Our aim is to perform an *in silico* prediction of structure-related human health effect probabilities for LC- and SC-PFAS species using the ACD/Labs Percepta *in silico* tool. 55 LC- and 24 SC-PFAS present in human biological sample species, determined in a Web of Science search, are included for prediction. SMILES codes of the mentioned PFAS species are used for *the in silico* evaluation of health effect probabilities on a number of organs (blood, cardiovascular system (CVS), gastrointestinal system (GI), kidneys, liver, and lungs). Statistically significant differences were found between LC- and SC-PFAS across all organ systems using the non-parametric Mann-Whitney U test ($p<0.05$). PFAS are found to be non-toxic to the GI but highly toxic to the CVS. Toxicity ranking for LC-PFAS was: CVS (0.90) > liver (0.81) > lungs (0.65) > kidney (0.56) > blood (0.41) > GI (0.03), and for SC-PFAS, the ranking was: CVS (0.73) > lungs (0.31) > liver (0.30) > blood (0.22) > kidney (0.14) > GI (0.04). The most significant finding is that LC-PFAS present higher toxicity than SC-PFAS, with approximately 2-fold higher toxicity for lungs and blood, 3-fold for liver, and 4-fold for kidneys. These findings not only underscore the widespread cardiotoxicity of PFAS but also critically highlight a pronounced chain-length-dependent toxicity, with LC-PFAS exhibiting substantially higher toxic potentials for vital organs like the liver, lungs, kidneys, and blood than SC-PFAS. These results offer critical directions for guiding subsequent toxicological investigations.

PP-10. Toxicity and Safety Profile of Eugenol: A Comprehensive Review

Serhat Bayram[1], Mahmoud Abudayyak[2]

[1] *Institute of Graduate Studies in Health Sciences, Istanbul University, Istanbul, Turkiye*

[2] *Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Istanbul University, Beyazit, 34116, Istanbul, Turkiye*

Eugenol is a natural phenolic compound primarily derived from cloves (*Syzygium aromaticum*), basil (*Ocimum gratissimum* L.), and other herbs. Due to its significant antioxidant, anti-inflammatory, antibacterial, and cytoprotective effects, it has recently received considerable attention in biological and medical studies. Researchers have demonstrated that eugenol and its derivatives can effectively neutralise reactive oxygen species, reduce inflammatory pathways, and mitigate cellular damage in various biological models. Beyond its pharmacological effects, eugenol-based substances are widely used in everyday products thanks to their antimicrobial and aromatic properties, being important components of food, dental preparations, cosmetics, and natural flavouring agents. However, excessive exposure to eugenol can lead to genotoxicity, cytotoxicity, reproductive toxicity, and hepatotoxicity. The safety profile and bioavailability of eugenol depend on metabolic factors, the hepatic cycle, and interindividual differences in absorption, which may pose a greater risk to sensitive individuals. This study critically reviews the toxicological data and presents realistic, evidence-based insights into the use of eugenol and its derivatives. Additionally, ADMETlab3 and SwissADME *in silico* models were employed to clarify the pharmacological and toxicological findings further.

Keywords: Eugenol, Toxicity, Safety Assessment, *In Silico*

PP-11. Long-Term Low-Level Exposure to Perfluorooctanoic Acid Induces Oxidative Stress Without Overt Cytotoxicity in Human Hepatocytes *In Vitro*

Katarina Borkovic, Dunja Kokai, Bojana Stanic, Nebojsa Andric

University of Novi Sad, Faculty of Sciences, Serbia

Perfluorooctanoic acid (PFOA), a synthetic chemical belonging to per- and polyfluoroalkyl substances (PFAS), is known for its extreme environmental persistence and potential to bioaccumulate in human tissues. PFOA has been associated with various adverse health outcomes, including hepatotoxicity, but the effects of long-term low-level exposure on liver cells remain insufficiently explored, particularly under exposure conditions relevant to the general population.

In this study, we investigated the impact of prolonged exposure to low concentrations of PFOA on human hepatocytes *in vitro*, using the HepG2 cell line as a model. Cells from four independent cryopreserved vials (biological replicates) were cultured under control conditions (0.05% DMSO) or exposed to 1, 10, or 100 nM PFOA for 12 weeks. The following endpoints were assessed after 6 and 12 weeks: metabolic activity (alamarBlue™ assay), cell viability (Trypan blue exclusion), proliferation (cell counting), cell death pathways (annexin V/propidium iodide flow cytometry), cell cycle distribution (propidium iodide staining), intracellular reactive oxygen species (ROS; dichlorofluorescein fluorescence), and lipid peroxidation (thiobarbituric acid reactive substances assay). No significant changes in cell viability, proliferation, apoptosis/necrosis, or cell cycle progression were observed at any PFOA concentration or time point. However, metabolic activity was significantly increased after 12 weeks of exposure to 10 nM and 100 nM PFOA. Notably, all concentrations of PFOA led to elevated ROS levels after 12 weeks, accompanied to a certain degree by an increase in lipid peroxidation. These findings suggest that while long-term low-level PFOA exposure does not overtly impair hepatocyte survival or proliferation, it induces oxidative stress, which may represent a molecular event preceding more overt cytotoxic effects.

Further studies are warranted to determine whether prolonged oxidative stress may compromise liver function over extended periods or in more physiologically relevant models.

This study was supported by Science Fund of the Republic of Serbia, #7010, Integration of Biological Responses and PBTK Modeling in Chemical Toxicity Assessment: A Case Study of Perfluorooctanoic Acid (PFOA) – ToxIN.

Keywords: Perfluorooctanoic acid, HepG2 cells, long-term exposure, ROS

PP-12. Is GenX Really Safer Than PFOA?

Burcu Ünlü Endirlik[1], Aylin Gürbay[2]

[1] *Erciyes University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Kayseri, Turkiye*

[2] *Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Turkiye*

Hexafluoropropylene oxide dimer acid (HFPO-DA), also known as GenX, is a perfluorinated chemical introduced as replacement for perfluorooctanoic acid (PFOA) in the industry. PFOA is a member of per- and polyfluoroalkyl substances (PFASs) which are called “forever chemicals” and had a wide range of industrial applications until its worldwide restriction or prohibition due to its environmental persistence and negative health effects. GenX, a short-chain PFAS, was adopted as substitute for PFOA in recent years. Consequently, environmental discharge of GenX is increasing rapidly with a wide detection range, including water, soils, grass and animals, as well as human plasma. It is known to be primarily distributed to the liver and excreted via urine almost unchanged. It has also the ability to cross the placenta. Despite its significantly shorter half-life relative to PFOA, emerging studies found GenX might pose the same human health and environmental risks as PFOA. *In vitro* and *in vivo* studies show that GenX has hepatotoxic, neurotoxic and thyroid disrupting effects, as well as toxicity on reproductive and developmental, immune, gastrointestinal, hematological and renal systems. It was also reported to have potential carcinogenic and genotoxic effects. In epidemiological studies, GenX exposure was found to be associated with reproductive dysfunctions. These findings necessitate addressing safety concerns about GenX. Despite the attention GenX has gained in recent years, several characteristics of these chemicals are yet to be illuminated. Further investigation is required to better understand the underlying toxicity mechanisms and adverse health effects of GenX.

Keywords: GenX, PFOA, HFPO-DA, PFASs, toxicity.

PP-13. Toxicity Predictions of Alternariol and Alternariol Monomethyl Ether Using *In Silico* Models

Furkan Berk Oğuzer[1,2], Sezen Yılmaz Sarıaltın[3]

[1]*Başkent University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Türkiye*

[2] *Ankara University, Graduate School of Health Science, Ankara, Türkiye*

[3]*Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Türkiye*

Alternaria toxins are secondary metabolites produced by fungi of the *Alternaria* genus, capable of contaminating a broad spectrum of agricultural commodities, including fruits, vegetables, cereals, and animal feed. These toxins, detected in both raw and processed food products, are associated with substantial reductions in crop yield and quality, as well as a significant health risk for humans. The European Food Safety Authority 2022 report stated that exposure levels to *Alternaria* toxins alternariol, alternariol monomethyl ether (AME), and tenuazonic acid exceeded toxicologically relevant thresholds. Consequently, the authorities emphasized the need for additional compound-specific toxicity data, and these substances are therefore regarded as emerging mycotoxins. In line with this call, this study aims to investigate the mutagenicity, genotoxicity, carcinogenicity, and developmental toxicity potential of alternariol and AME using *in silico* models available on the VEGA (v.1.2.4), US EPA TEST (v.5.1.2), and OECD QSAR (v.4.7) platforms. Both VEGA and EPA TEST mutagenicity (Ames test) consensus models assessed alternariol and AME as non-mutagenic. Six carcinogenicity models implemented in VEGA, including CAESAR (v.2.1.10), ISS (v.1.0.3), IRFMN-ISSCAN-CGX (v.1.0.2), IRFMN-Antares (v.1.0.2), and carcinogenicity oral and inhalation classification models (IRFMN) (v.1.0.1) predicted both compounds to be carcinogenic. These models range from low to good reliability scores. Chromosomal aberration model (CORAL) (v.1.0.1), *in vitro* (IRFMN-VERMEER) (v.1.0.1), and *in vivo* micronucleus activity (IRFMN) (v.1.0.2) models predicted both compounds as active/genotoxic with good reliability. The EPA TEST and VEGA developmental toxicity models (CAESAR, v.2.1.8) estimated both compounds as toxic with good reliability. Cramer classification by Toxtree (v.1.0.1) classified alternariol and AME in Group III, indicating high toxicity. Our results suggest that both alternariol and AME may have high genotoxicity and carcinogenicity potential. Considering the potential for chronic exposure through the food chain and the substantial health risks involved, these toxins warrant further toxicological investigation.

Keywords: Alternariol, alternariol monomethyl ether, carcinogenicity, genotoxicity, *in silico*

PP-14. Life Cycle Impact and Ecotoxicological Evaluation of Antibiotics Using *in silico* Tools

Zübeyde Merve Kala, Melek Aşlamacı, Esmanur Kuzucu, Semra Sardaş

İstinye University, Turkiye

Antibiotic usage represents a major global threat, as it significantly contributes to the acceleration of antimicrobial resistance. Previous reports demonstrates that Turkiye ranks as the highest antibiotic-consuming country among OECD members, with a resistance rate reaching 38.8%. Improper pharmaceutical waste disposal and discharge into wastewater systems contribute to the release of active pharmaceutical ingredients into aquatic environments, raising serious environmental concerns. Even at low concentrations, prolonged exposure to these substances may adversely affect aquatic and terrestrial organisms.

This study aims to evaluate the comparative ecotoxicological impacts of commonly used antibiotics based on their physicochemical properties (EPI Suite and ChemBL) and toxicological profiles (ECOSAR) on freshwater environment. Using the USEtox model within the Life Cycle Impact Assessment (LCIA) framework, nine antibiotics were assessed for their potential ecotoxicity. These antibiotics were selected based on two main criteria: (i) their inclusion in the 2025 European Union (EU) Watch List, developed under the EU Water Framework Directive to identify substances that pose a potential risk to aquatic environments, and (ii) their high usage frequency according to global and national (Türkiye) pharmaceutical consumption data.

The results, expressed as Comparative Toxic Units for ecosystems (CTUe), provide a scientific basis for identifying antibiotics with higher ecological risk potential and support the development of environmental prioritization strategies. The highest CTUe values, indicates ecotoxicity potential for clarithromycin, oxytetracycline, and sulfamethoxazole.

In conclusion, these findings support the consideration of prescription frequency in prioritizing antibiotics with higher ecotoxicological impacts, as reflected by their CTUe values.

Keywords: Antibiotic, ecological risk potential, computational approaches, life cycle impact assessment

PP-15. An Adverse Reaction Case Report of Hepatic Encephalopathy and Lactic Acidosis Induced by Eltrombopag

Candan İslamoğlu[1], Şule Haskoloğlu[1], Nesrin Yüzüak Okatan[2], Gökçe Demiray[2], Gökcan Öztürk[1], Fatih Alpkıray[1], Merve Havan[3], Tanıl Kendirli[3], Hakan Ergün[4], Onursal Sağlam[2], Figen Doğu[1], Aydan İkincioğulları[1]

[1] Ankara University Faculty of Medicine, Department of Pediatric Immunology and Allergy, Türkiye

[2] Novogenix Bioanalytical Drug R&D Center, Ankara, Türkiye.

[3] Ankara University Faculty of Medicine, Department of Pediatric Intensive Care, Ankara, Türkiye

[4] Ankara University Faculty of Medicine, Department of Medical Pharmacology, Ankara, Türkiye

A pediatric patient diagnosed with Wiskott-Aldrich Syndrome underwent a 10/10 HLA-matched unrelated donor allogeneic hematopoietic stem cell transplantation at the age of two. Due to graft failure and persistent thrombocytopenia, a boost stem cell infusion was administered followed by initiation of eltrombopag titrated up to 75 mg/day according to platelet counts.

During follow-up, the patient developed acute renal failure associated with calcineurin inhibitor use and intestinal graft-versus-host disease. Tacrolimus was replaced with sirolimus and anti-IL6 therapy (tocilizumab) was added due to persistent bloody diarrhea. Despite treatment platelet count remained ~25,000/mm³.

The patient presented to our emergency department with two days history of nausea, vomiting, and diarrhea and tachypnea, tachycardia, signs of dehydration, and lethargy were observed with laboratory investigations revealed lactic acidosis, increased liver enzymes, elevated INR and ammonia levels. Despite fluid resuscitation and supportive therapies, lactic acidosis persisted. Altered mental status progressively worsened, hepatic encephalopathy developed. Hepatic encephalopathy treatment protocol was initiated in the pediatric intensive care unit.

Although thiamine deficiency could not be definitively excluded, thiamine supplementation was initiated but showed no clinical response. During evaluation of the etiology, eltrombopag was identified as a potential cause in the literatüre and discontinued.

Forty hours post-discontinuation, plasma eltrombopag levels were measured using ethyl acetate-based liquid-liquid extraction followed by LC-MSMS analysis. The eltrombopag level was found to be 20 times higher than the therapeutic range. Subsequent measurements showed a gradual decline coinciding with clinical improvement, lactic acidosis resolution, and spontaneous platelet recovery above 50,000/mm³.

Eltrombopag as a thrombopoietin receptor agonist for thrombocytopenia requires monitoring of liver and renal function parameters and lactate levels. In cases of transaminitis, elevated creatinine, and/or lactic acidosis, immediate drug discontinuation and plasma level assesment are crucial steps to prevent potentially life-threatening complications.

Keywords: Advers reaction, eltrombopag, hepatic encephalopathy, bioanalsis, LC/MS-MS

PP-16. Dual Assault: Lead and PCB Mixture Targets Acetylcholinesterase and Compromises Sperm Quality in Rats

Nikola Stojilković[1], Miodrag Stojković[2], Aleksandra Stojanović[2], Aleksandra Zdravković[2], Tina Stojadinović[2], Biljana Radović[1], Katarina Baralić[1], Đurđica Marić[1], Jovana Živanović[1], Evica Antonijević Miljaković[1], Marijana Ćurčić[1], Aleksandra Buha Đorđević[1], Danijela Đukić-Ćosić[1], Zorica Bulat[1], Biljana Antonijević[1]

[1] *Department of Toxicology "Akademik Danilo Soldatović", University of Belgrade -Faculty of Pharmacy, Belgrade, Serbia*

[2] *SPEBO-Fertility Hospital, 16000 Leskovac, Serbia*

Infertility affects over 186 million individuals globally, with declining semen quality recognized as a key contributor to male infertility. Meta-analyses of semen parameters worldwide have revealed a downward trend in semen quality over recent decades. To better understand the role of complex environmental chemical mixtures in this decline, it is essential to evaluate semen parameters alongside other biomarkers, such as erythrocyte acetylcholinesterase (AChE) activity. In this study, adult male Wistar rats were subacutely exposed to environmentally relevant low doses of a lead (Pb) and polychlorinated biphenyls (PCBs) mixture. The objectives were to: (i) assess the impact of Pb–PCB mixtures on sperm parameters; (ii) examine the potential disruption of erythrocyte AChE activity; and (iii) explore dose–response relationships. Rats were randomly assigned to ten experimental groups, including a control, and treated with combinations of 0.1, 0.5, or 1 mg Pb/kg/day and 0.25, 0.5, or 1 mg PCBs/kg/day in a 3×3 dose design. The animals were anesthetized using a ketamine/xylazine mixture, and cardiac puncture was performed for blood collection. Sperm samples were obtained from the left cauda epididymis by diffusion in 37°C saline, and sperm motility, concentration, and morphology were evaluated under light microscopy. Erythrocytes were isolated to measure AChE activity. Statistical analyses included one-way ANOVA (SPSS v18.0, IBM, Armonk, NY, USA) and dose–response modeling using PROAST 71.1 (RIVM, Bilthoven, The Netherlands) within R v4.3.1. Our results demonstrated a significant decline in sperm quality, with sperm motility identified as the most sensitive parameter to mixture exposure. Additionally, elevated AChE activity was detected at higher mixture doses, suggesting a disruption of cholinergic transmission. The results, supported by existing literature, indicate that combined exposure to Pb and PCBs can adversely affect sperm quality, potentially via mechanisms involving cholinergic dysregulation.

Acknowledgments: This research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia through Grant Agreement with University of Belgrade-Faculty of Pharmacy No: 451-03-47/2023-01/ 200161.

Keywords: metal; pops; male fertility; cholinergic transmission; sperm; PROAST

PP-17. Predicting The Health Risks of Neonicotinoids: An *In Silico* ADMET And Endocrine Disruption Study

Sezen Yılmaz Sarıaltın[1], Can Özgür Yalçın[2]

[1] *Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Türkiye*

[2] *Karadeniz Technical University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Trabzon, Türkiye*

Background: Despite their widespread agricultural use, neonicotinoid insecticides raise environmental concerns, particularly regarding pollinator decline and potential human health effects. This study computationally assessed the absorption, distribution, metabolism, elimination, toxicity (ADMET), and endocrine-disrupting potential of fourteen neonicotinoid insecticides.

Methods: Computational approaches, including ADMETlab (v.3.0) and the VEGA-HUB platform's VEGA NRMEA (v.1.1.1) and VEGA QSAR (v.1.2.4) tools, were employed. Molecular docking simulations and binding affinities were assessed using Endocrine Disruptome and Panscreen.

Results: Neonicotinoids generally showed good absorption, with cycloxaprid, flupyradifurone, guadipyr, imidacloprid, and nitenpyram particularly having high rates. Distribution profiles indicated significant toxicity from transporter protein (BSEP, OATP1B1/3) inhibition, including acetamiprid, clothianidin, flupyradifurone, imidacloprid, imidaclothiz, nitenpyram, sulfoxaflor, and thiacloprid. The high blood-brain barrier penetration of thiacloprid is an additional risk factor. Regarding metabolism, acetamiprid, cycloxaprid, flupyradifurone, guadipyr, paichongding, and thiacloprid pose high risks due to their interactions with CYP enzymes. Excretion profiles did not suggest significant accumulation-related toxicity, as no compounds exhibited low clearance or prolonged half-lives. Cycloxaprid, flupyradifurone, imidaclothiz, and nithiazine showed weak interaction potential in the Tox21 Nuclear Receptor (NR) pathways (especially NR-ER and SR-ARE). These compounds also yielded positive results in multiple toxicity parameters, including liver injury, genotoxicity, cytotoxicity, hERG inhibition, nervous system, and respiratory toxicity. The developmental toxicity model (CAESAR, v.2.1.8) predicted all compounds as toxicants except nithiazine and thiacloprid, though these predictions had low reliability. The developmental/reproductive toxicity library (PG, v.1.1.2) estimated all compounds as non-toxicants. VEGA models predicted no activity in estrogen, androgen, thyroid, or glucocorticoid receptor-mediated effects, and IRFMN (v.1.0.0) screening found them inactive for endocrine disruption. However, the steroidogenesis (OBERON) (v.1.0.0) model determined clothianidin, imidacloprid, and thiacloprid as active based on experimental data, while cycloxaprid, dinotefuran, imidaclothiz, and paichongding were predicted to be active, with varying reliability. Docking results show cycloxaprid, flupyradifurone, guadipyr, imidacloprid, and paichongding are most concerning for human endocrine disruption, as they bind strongly to many endocrine receptors.

Conclusion: Overall, these findings underscore the potential health risks associated with neonicotinoid exposure, particularly concerning their interactions with crucial biological pathways and their broad toxicological profiles.

Keywords: ADMET, *in silico*, neonicotinoids, endocrine disruption

PP-18. Analysis of Glucocorticoid Withdrawal Syndrome Based on Patient Demographics

Lutfi Mangal[1], Burcu Eda Arda[1], Hande Sipahi[1]

[1]Yeditepe University Faculty of Pharmacy, Department of Toxicology, Istanbul, Turkiye

This study investigated the relationship between different glucocorticoids and withdrawal syndrome in relevance to patient demographics. We extracted and analyzed 11 years of patient data (1,713 cases) from glucocorticoid withdrawal syndrome (GWS)-related VigiBase reports. Prednisone accounted for 28% of all GWS cases, followed by hydrocortisone (17%), betamethasone (14%), triamcinolone (9%), and cortisone (< 1%). In general, the number of cases associated with these glucocorticoids showed an increasing trend. The highest GWS incidence was observed for the age range of 18–44, with a rate of 36%, followed by the 45–64 age group with 20%, the 65–74 group with 7%, and the 2–11 group with 7%. Of all cases, 44% were reported in the Americas, 40% in Europe, 10% in Oceania, and 6% in Asia. Moreover, regional variations were observed for different glucocorticoids: the majority of reports for hydrocortisone and betamethasone originated from Europe, while those for triamcinolone, prednisone, and cortisone came from the Americas. Among the 1,713 reported GWS cases, a higher prevalence was found for females (1,032 cases, 60%) as compared to 591 cases (34%) for males. Regarding different glucocorticoid types, 60% of the patients treated with hydrocortisone, 64% treated with triamcinolone, 75% treated with betamethasone, 55% treated with prednisone, and 61% treated with cortisone were female. Most cases (77%) were classified as serious, and 18% were non-serious, with 18% of the cases requiring prolonged hospitalization, 13% resulting in disabling capacity, 4% being life-threatening, and 2% leading to death. Comparing different glucocorticoids, methylprednisolone (5%), dexamethasone (4%), prednisolone (2%), and prednisone (2%) were more frequently associated with mortality. Healthcare providers should inform patients about the risk of serious adverse drug reactions related to GWS, taking into account patient demographics, particularly in female patients.

PP-19. Genotoxic and Cytotoxic Potential of Bis(2,4,6-Tribromophenoxy) Ethane (BTBPE) in HEK-293 Cells

Şuheda Taştan, Mine Çağlayan

Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Istanbul, Turkiye

Istanbul University, Institute of Health Sciences, Istanbul, Turkiye

Biruni University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Zeytinburnu, İstanbul.

1,2-bis(2,4,6-tribromophenoxy) (BTBPE) ethane is one of the new-generation brominated flame retardants (NBFRs) developed to replace banned decabromodiphenyl ethers (deca-BDE). It is widely used in plastics, electronics, furniture, and textiles, with production reaching 16,710 tons in the US and Europe in 2001. Due to its high hydrophobicity and resistance to degradation from polymers, BTBPE is persistent in the environment and is mainly released from electronic and industrial waste. According to NBFR toxicity research, BTBPE can have negative health effects to human via several mechanisms of action, including genotoxicity, hormone and endocrine disruption, and behavioral change. BTBPE exhibits bioaccumulation and long-range transport potential comparable to that of polybrominated diphenyl ethers (PBDEs), highlighting the need for further research on its risks to human health and ecosystems. BTBPE demonstrates poor gastrointestinal absorption, with more than 99% excreted unchanged in feces and minimal tissue accumulation; however, trace levels have been detected in fat, kidney, skin, and thymus following repeated dietary exposure in rats. In this study, the genotoxic and cytotoxic potential of BTBPE was investigated in human embryonic kidney (HEK-293) cells. To assess cytotoxicity, an MTT assay was performed following 24 h exposure to BTBPE at concentrations ranging from 0 to 100 µM, and the IC50 value was determined > 100 µM compared to the control group. For genotoxicity assessment, the alkaline comet assay was employed, and cells were exposed to BTBPE at concentrations of 5, 10, and 20 µM for 24 h. No statistically significant differences were observed in DNA damage at these concentrations. Further studies, including oxidative stress, apoptosis, and endoplasmic reticulum (ER) stress, are ongoing to better understand the underlying toxicity mechanisms of BTBPE in kidney cells. The results are expected to provide valuable contributions to the existing literature, especially in the context of environmental toxicology and potential risks to human health.

PP-20. Targeting The Powerhouse: The Mitochondrial Perspective on Gentamicin-Induced Kidney Injury

Sinem Ilgin, Busra Korkut Celikates, Melis Umay Yilmaz, Ozlem Atli Eklioglu

[1]*Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Eskisehir, Turkiye*

Gentamicin, an aminoglycoside antibiotic widely used for treating severe bacterial infections, is associated with significant nephrotoxicity, limiting its clinical utility. Mitochondrial dysfunction has emerged as a crucial factor in the pathogenesis of gentamicin-induced nephrotoxicity. Mitochondria play a vital role in cellular energy metabolism, redox balance, and apoptotic regulation, making them highly susceptible to toxic insults. Gentamicin accumulates in renal proximal tubular cells, where it induces excessive reactive oxygen species production, mitochondrial DNA damage, oxidative phosphorylation impairment, and the opening of the mitochondrial permeability transition pore. These mitochondrial alterations lead to ATP depletion, cytochrome c release, and apoptosis, contributing to renal injury. Furthermore, high concentrations of gentamicin can trigger necrotic cell death via ATP depletion and lysosomal proteolysis. In addition to mitochondrial dysfunction, endoplasmic reticulum stress and inflammatory cascades further exacerbate nephrotoxicity. Given the critical role of mitochondria in gentamicin-induced renal damage, mitochondria-targeted therapeutic strategies, including antioxidants, permeability transition pore inhibitors, and mitochondrial biogenesis stimulators, have been explored for nephroprotection. Understanding the intricate mechanisms underlying gentamicin-induced mitochondrial toxicity is essential for developing effective nephroprotective interventions. Future studies should focus on refining these therapeutic approaches to mitigate renal complications while preserving the antibiotic's clinical efficacy.

Keywords: Gentamicin, nephrotoxicity, mitochondrial dysfunction

PP-21. Stem Cells: A Promising Tool for Enchanging Toxicological Testing

Özlem Atlı Eklioğlu[1], Achmet Ali Tsaous[2], Merve Güven[1], Sinem Ilgın[1]

[1] Anadolu University Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Türkiye

[2] University of Helsinki Faculty of Pharmacy, Türkiye

Stem cells are pluripotent cells capable of differentiating into multiple cell lineages within an organism. They also have the remarkable ability to self-renew and proliferate. Owing to these properties, stem-cell-based technologies are now used in many areas, including regenerative medicine, drug screening, diagnostic testing, investigation of disease mechanisms, developmental toxicity assessment, and studies of target-organ toxicity. Numerous toxicological studies have sought to improve the precision of risk assessments for drugs and chemicals. Yet conventional *in vivo* animal models and *in vitro* cell cultures remain limited in their ability to predict adverse effects in humans because of fundamental physiological differences. As a result, stem cells have emerged as a promising alternative tool in toxicological research.

Historically, stem cell research in Asia was thought to lag behind, largely due to ethical debates and economic constraints. Recent analyses, however, show that the region has made significant strides and now recognizes the value of stem cells in toxicology. Among stem cells, pluripotent embryonic stem cells (ESCs or ES cells) are particularly advantageous because of their broad differentiation capacity. Furthermore, they are widely considered key to enabling more personalized evaluations of both therapeutic benefits and potential toxicities, as they can reflect an individual's unique genetic makeup and help address ethical concerns about traditional ESC use.

This review highlights how stem cells contribute to toxicological investigations, with a focus on their application in assessing developmental toxicity, cardiotoxicity, neurotoxicity, hepatotoxicity, and nephrotoxicity. It also provides an in-depth overview of the current state of stem cell–based toxicity testing across Asia, emphasizing both the progress achieved and the future opportunities in this rapidly advancing field.

Keywords: Alternatives to animal testing, embryonic stem cells, *in vitro* toxicity screening, ınduced pluripotent stem cells, stem cells, asia

PP-22. Sulfur Mustard Disrupts Epithelial Adhesion and Cell-Cell Communication in Göttingen Minipig Skin

Kevin Ozkuyumcu[1], Peihong Zhou[1], Claire R. Croutch[2], Debra L. Laskin[1], Diane E. Heck[1], Jeffrey D. Laskin[1], Laurie B. Joseph[1]

[1]Rutgers University, Piscataway NJ, USA

[2]MRIGlobal, Kansas City, MO, USA

Sulfur mustard (SM; bis(2-chloroethyl) sulfide) is a highly reactive bifunctional alkylating agent and a potent skin vesicant. In human skin, SM causes inflammation and blistering. During skin injury, cell-cell adhesion and communication are disrupted. E-cadherin and β-catenin form a cadherin/catenin complex that controls adhesion, migration, and proliferation of keratinocytes. Also important is connexin43, a gap junction cell-cell communication protein involved in organizing adherens junctions. We have been using the Göttingen minipig model to investigate mechanisms of skin injury and wound repair following SM exposure. Herein, the role of cell adhesion and communication proteins in SM toxicity was investigated. Using an IACUC approved protocol, air control or saturated SM vapor caps were placed on the dorsal flanks of male Göttingen minipigs (3-months-old) for 30min (MRIGlobal). After 48h, SM wounded sites were debrided daily for 7d with wet-to-wet saline gauze. Animals were euthanized 9, 28, and 60d post-SM and full thickness skin biopsies prepared for immunohistochemical analysis. Primary antibodies against E-cadherin, β-catenin, connexin43, and phospho-connexin43(S368) and appropriate IgG controls were used. In control animals, E-cadherin and β-catenin were expressed throughout all viable layers of the epidermis. SM reduced E-cadherin and β-catenin expression 9-28d post-exposure; a marked reduction was noted in the stratum basale. In contrast, connexin43 was contiguously expressed within the stratum granulosum of control skin. Phosphorylation of connexin43 is a dynamic process that modulates gap junction permeability. A marked upregulation of connexin43 and phospho-connexin43(S368) was evident in the stratum spinosum and stratum granulosum 9-28d post-SM. These proteins returned to control levels by 60d post-SM. These data indicate that there are dynamic changes within the skin that contribute to epidermal injury and repair. Further studies evaluating mechanisms controlling epithelial cell adhesion and cellular communication will assist in the development of therapeutics that promote wound repair following exposure to SM.

PP-23. Establishment of Fast and Simple LC-MS/MS Method for Quantitative Analysis N, N-Diethyl-Meta-Toluamide (DEET) in Serum

Göksel Koç Morgil[1], N. Neslihan Bozkurt[1], Nurgül Özcan[1], İsmet Çok[2]

[1] *Minister of Health, General Directorate of Public Health, Department of Public Health Reference Laboratories, Türkiye*

[2] *Gazi University, Faculty of Pharmacy, Department of Toxicology, Türkiye*

Introduction and Aim: N, N-Diethyl-meta-toluamide (DEET) is the active ingredient commonly used in repellent products. Because consumer products containing DEET are typically applied to the skin or clothing, exposure is expected to occur predominantly dermally. Exposure to DEET occurs seasonally, with the majority of use occurring during the spring and summer months when biting insect season peaks. Exposure to DEET is widespread but highly variable in human populations through the use of DEET-containing products. Various rapidly developing toxic effects and deaths have been reported due to DEET exposure. The primary objective of this study is to develop a rapid and reliable analytical method for determining blood exposure in individuals to inform precautions against potential DEET-related health risks.

Materials and Methods: Several analytical methods have been utilized for the identification and quantification of DEET in human plasma and urine. In this study, we utilize a simplified, reliable method to analyze the DEET content in serum samples using fast and simple LC-MS/MS method. The levels of the DEET in bovine serum samples were determined quantitatively with LC-MS/MS (Sciex 5500) by using positive electrospray ionization (ESI) in the multiple reaction monitoring (MRM) mode. Five microliters (µL) of the extract were injected onto an Phenomenex Luna Omega Polar C18 100 × 3.0 mm, 3.5 µm particle size Polar column. The Gradient program was used in the analyses.

Results: The linear ranges for DEET were 0.5– 50 ng/mL. The correlation coefficient was 0.999. The limit of detection and limit of quantitation for the compound were 0.1 ng/ml, and 0.5 ng/ml, respectively. Full-scan spectra were obtained then DEET ions was selected from fragmentations occurring in the collision cell to obtain daughter scan spectra. Compound identities were confirmed by comparison of fragmentation with that of standards. Our validated method appears to be successfully applicable to the analysis of serum samples collected from randomly selected healthy individuals.

Conclusion: DEET has consistently been one of the top ten active ingredients in the home use category of pest control products. Therefore, products containing DEET can often play a role in the emergence of various health problems, especially poisoning. This method, which we developed to determine DEET levels in the blood to prevent unwanted health problems that may be related to DEET exposure in cases of intentional or accidental overexposure or in sensitive populations such as children, can be used in emergency situations to provide highly sensitive results quickly and reliably.

Keywords: DEET, Serum, LC-MSMS

12TH INTERNATIONAL CONGRESS OF THE TURKISH SOCIETY OF TOXICOLOGY
November 6-9, 2025, İstanbul
Toxicology in Protecting Human and Environmental Health

PP-24. 2-Mercaptobenzothiazole Triggers Oxidative Stress, DNA Damage and Hormonal Imbalance in Mouse Testicular Cells

Mustafa Daşman[1,2], Mahmoud Abudayyak[3]

[1]*Institute of Graduate Studies in Health Sciences, Istanbul University, Istanbul, Turkiye*

[2]*Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Trakya University, Edirne, Turkiye*

[3]*Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Istanbul University, Beyazit, Istanbul, Turkiye*

Background: 2-Mercaptobenzothiazole (MBT) is an industrial compound commonly used as a vulcanization accelerator and corrosion inhibitor. Evidence suggests its endocrine-disruptive and genotoxic potential, yet its direct impact on testicular cell populations remains poorly defined. This study investigated the oxidative, genotoxic, and endocrine-modulating effects of MBT in mouse Leydig (TM3) and Sertoli (TM4) cells using in vitro and in silico approaches.

Methods: TM3 and TM4 cells were treated with MBT (1.5625–100 µg/mL) for 24 h. Cytotoxicity (MTT, NRU), genotoxicity (comet assay), oxidative stress markers (ROS, MDA, GSH, SOD, 8-OHdG), apoptosis/necrosis (Annexin V/PI), and testosterone secretion (ELISA) were evaluated. Computational predictions were performed with ADMETLab 3.0 and SwissADME to estimate pharmacokinetic and toxicodynamic properties.

Results: At 100 µg/mL, MBT reduced viability to 29.03% (NRU) and 34.68% (MTT) in TM3 cells, and to 42.55% (NRU) and 30.27% (MTT) in TM4 cells. In TM4 cells, 40 µg/mL MBT caused significant ROS generation (1.45-fold, $p \leq 0.05$), MDA increase (1.40-fold, $p \leq 0.05$), and 8-OHdG elevation (2.54-fold, $p \leq 0.05$), accompanied by enhanced apoptosis (1.95-fold, $p \leq 0.05$) and necrosis (1.83-fold, $p \leq 0.05$). TM3 cells exhibited increased testosterone secretion (2.43-fold at 20 µg/mL, $p \leq 0.05$). In silico predictions indicated high genotoxicity probability (93.9%), oxidative stress pathway activation (78.8%), and aryl hydrocarbon receptor activation (99.6%), consistent with experimental findings.

Conclusions: MBT induces oxidative stress–mediated genotoxicity in Sertoli cells and stimulates testosterone production in Leydig cells, reflecting both cytotoxic and endocrine-disruptive potential. These results highlight MBT's relevance to male reproductive toxicity and underscore the need for further mechanistic in vitro and in vivo studies.

Keywords: Reactive oxygen species (ROS), Endocrine disruptor, Benzothiazole-2-thiol, Antioxidant depletion, Reproductive toxicity

PP-25. Effects of Enniatin B Exposure on Global DNA Methylation in HepG2 Cells

Zeynep Rana Guler[1, 2], Beyza Nur Ceylan[2], Sibel Özden[2]

[1] *Istanbul University, Institute of Graduate Studies in Health Sciences, İstanbul, Turkiye*

[2] *Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, İstanbul, Turkiye*

Emerging mycotoxins are toxic compounds produced by certain fungi that have recently gained attention due to improved detection methods or increased prevalence, but they are not yet regulated or routinely monitored in food and feed safety systems. Unlike well-known mycotoxins such as aflatoxins or ochratoxins, emerging mycotoxins were previously overlooked or considered unimportant, often due to limited toxicological data or detection challenges. Enniatin B (ENN-B) is one of the common emerging mycotoxins belongs to the enniatin group. ENN-B act as ionophores, disrupting cellular ion homeostasis and pH by facilitating the transport of monovalent cations like potassium, sodium, and calcium across cell membranes. This disturbance can lead to cytotoxicity, oxidative stress, genototoxicty, impairment of cell cycle distribution, modulation on estrogenic avtivity, apoptosis and necrosis and epigenetic alterations. Environmental toxins such as enniatins may induce global hypomethylation or site-specific hypermethylation, both of which can interfere with normal gene regulation. The aim of this study was to investigate the effects of ENN-B on DNA methylation in human hepatocellular carcinoma cells (HepG2). For this purpose, HepG2 cell line was exposed to 0-25 µM ENN-B for 24 hours. Global DNA methylation was found to increase significantly at 10 µM (1.96-fold, $p<0.05$) and 25 µM (1.57-fold, $p<0.05$) for 24 h after ENN-B exposure. There was no change were observed on the *DNMT1* gene expression in HepG2 cells exposed to ENN-B at 1, 5, 10 and 25 µM. Exposure to ENN-B significantly increased *DNMT3A* (2.41-, 1.61-, and 3.29-fold) and *DNMT3B* (2.72-, 4.98-, and 3.49-fold) genes expression in HepG2 cells at 5, 10, and 25 µM, respectively ($p<0.05$ for all). In conclusion, global DNA methylation may be involved in ENN-B toxicity in HepG2 cells. Further studies might provide more detailed information for the evaluation of epigenetic alterations in ENN-B toxicity in cell cultures. It is expected that the obtained data from study will make contribution to literature and the toxicity mechanisms of the ENN-B in the risk assessment process.

Acknowledgement: This work was supported by the 2209-A TÜBİTAK University Students Research Projects Support Program (Project No: 1919B012220043)

Keywords: Enniatin B, Epigenetic Mechanism, DNA Methylation, HepG2 Cell

12TH INTERNATIONAL CONGRESS OF THE TURKISH SOCIETY OF TOXICOLOGY
November 6-9, 2025, İstanbul
Toxicology in Protecting Human and Environmental Health

PP-26. Research Productivity and Trends in Individualized Therapies in European OECD Countries: A Bibliometric Analysis

Nergis Ayyıldız[1], Gül Özhan[2], **Mahmoud A**budayyak[2]*

[1] *Institute of Graduate Studies in Health Sciences, Istanbul University, İstanbul, Türkiye*

[2] *Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Istanbul University, İstanbul, Türkiye*

Introduction: Pharmacogenomics and genetic polymorphism have gained increasing attention in pharmacology, toxicology, and medical research due to their relevance in personalized medicine and individualized therapy. This study evaluates the research productivity and trends in this field within OECD European countries from a bibliometric perspective.

Methods: Keywords such as *individualized therapy* and *polymorphism* were used to collect data. Of 4,889 global articles published between 2000 and 2020, 1,502 originated from OECD European countries. Key indicators such as institutional contributions, publication trends, preferred journals, and most-cited articles were analyzed and interpreted in relation to demographic and economic data to assess relative productivity.

Results: Germany accounted for over 21% of total publications, followed by Italy (17%) and the United Kingdom (14%). *Journal of Antimicrobial Chemotherapy* and *Clinical Cancer Research* were the most common outlets. Publication output rose significantly after 2005, peaking in 2013 with 103 articles. The ten most-cited papers, published in journals such as *Nature* and *The Lancet Oncology*, received ~500–1,200 citations, demonstrating strong academic impact. When adjusted for Gross Domestic Expenditure on R&D (GERD), however, smaller countries such as Greece, Slovenia, and Lithuania ranked higher, emphasizing the need to consider economic factors when evaluating productivity.

Conclusion: This bibliometric analysis shows that most pharmacogenomics and polymorphism research originates from OECD European countries, reflecting national research priorities. Yet, significant disparities exist across countries in their support for such studies. Research in pharmacogenomics-based toxicology and medical fields continues to rise, but strategies are needed to strengthen capacity in underrepresented regions to promote a more inclusive scientific environment.

Keywords: Pharmacogenomics, genetic polymorphism, toxicology,bibliometric analysis

PP-27. Hematology, Oxidative Stress and Biochemical Responses in *Clarias Gariepinus* Post Fingerlings Exposed to Sublethal Doses of Emamectin Benzoate

Emmanuel Ogueji[1], Joshua Ekpenyong [1], Christopher Ezike[2], Elizabeth Tabi[1], Michael Olaolu[3], Abubakar Yaji[4], Gabriel Amana[5], Adewale Adeolu[3], Roseline Nwose[3], Joseph Nwakpa[6]

[1] *Department of Fisheries and Aquaculture, Alex Ekwueme Federal University, Ndufu Alike.Abakaliki, Nigeria*

[2] *Department of Animal/Fisheries Science and Management, Enugu State University of Science and Technology, Enugu State, Nigeria*

[3] *Department of Agriculture, Alex Ekwueme Federal University, Ndufu Alike, Abakaliki, Nigeria*

[4] *Department of Fisheries, Moddibo Adama University of Technology, Yola, Nigeria*

[5] *Department of Animal and Environmental Biology, Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria*

[6] *Department of Fisheries and Aquaculture, Ebonyi State University, Abakaliki, Nigeria*

Emamectin benzoate (EMB) is a highly neurotoxic insecticide used in agronomy, animal husbandry, and farming of aquatic organisms to control pests. Runoff from these sources can harm fish. In this work, experimental groups were subjected to four doses of 17 µg L-1, 34µgL-1, and 68 µg L-1, and control without EMB for 14 days, after which fish was monitored for one week (7 days) depuration period to estimate recovery potential of the fish. In days 1, 7 and 14 durations of exposure, stress biomarkers including aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and blood Urea and Creatinine levels increased with dose and duration, and after 7 days depuration period, they maintained elevated values. The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) decreased significantly ($p < 0.05$), as did packed cell volume (PCV), mean corpuscular volume (MCV), hemoglobin concentration (Hb), and red blood cell count (RBCC). The trend continued after 7 days of depuration. There were significant ($p<0.05$) leukocytosis, lymphopenia and neutrophilia in treated fish. In contrast, lipid peroxidation and glutathione reductase (GR) levels increased but returned to normal after 7 days of depuration. The altered hematological parameters were indicative of immune suppression and anemia in the exposed fish, while the altered antioxidant enzymes implied that reactive oxygen species (ROS) may have played a role in the toxic action of EMB. This study showed that7 day depuration time was insufficient to repair the harm caused by EMB stress to the fish.

Keywords: Biochemistry; Depuration; Fish health; Pesticide; Oxidative stress; Pollution

12TH INTERNATIONAL CONGRESS OF THE TURKISH SOCIETY OF TOXICOLOGY
November 6-9, 2025, İstanbul
Toxicology in Protecting Human and Environmental Health

PP-28. Evaluation of Cytotoxic, Genotoxic, and Cellular Internalization Properties of Green-Synthesized Palladium Nanoparticles in Human Peripheral Blood Lymphocytes

Zöhre Akdoğan[1], Ersan Turunç[2], Rıza Binzet[3], Hatice Oruç Demirbağ[4], Ayla Çelik[3]

[1] *Department of Biology, Graduate School of Natural and Applied Science, Mersin University, 33343 Mersin, Turkiye*

[2] *Department of Chemistry and Chemical Processing Technologies, Technical Science Vocational School, Mersin University, 33343 Mersin, Türkiye*

[3] *Department of Biology, Faculty of Science, Mersin University,33343 Mersin, Turkiye*

[4] *Department of Histology and Embriyology, Faculty of Medicine, Mersin University,Mersin, Turkiye*

Purpose: This study aims to evaluate the cytotoxic and genotoxic effects of palladium (Pd) nanoparticles synthesized via a green chemistry approach using Paliurus spina-christi fruit extract on human peripheral blood lymphocytes under *in vitro* conditions.

Methods: The biosynthesized Pd nanoparticles were characterized using Scanning Electron Microscopy Dynamic Light Scattering (DLS), which revealed an average particle size of 20 nm and a zeta potential of –35.8 mV. Genotoxic effects were evaluated using the Comet assay, while chromosomal damage was assessed through the Micronucleus (MN) assay. Additionally, the intracellular localization of Pd nanoparticles was confirmed by Transmission Electron Microscopy (TEM).

Results: Comet assay results indicated a significant increase in DNA damage in lymphocytes exposed to Pd nanoparticles, as evidenced by elevated Genetic Damage Index (GDI) and Damaged Cell Percentage (DCP) values ($p<0.001$). Furthermore, the micronucleus assay revealed a statistically significant increase in micronucleus frequency in Pd-exposed cells compared to the control group ($p<0.001$), indicating chromosomal instability. A marked decrease in the Cytokinesis-Block Proliferation Index (CBPI) was also observed, suggesting impaired cellular proliferation and potential cytostatic effects of Pd nanoparticles.

In conclusion, Pd nanoparticles synthesized via green methods demonstrate significant genotoxic and cytotoxic effects on human lymphocytes, including DNA fragmentation and chromosomal abnormalities. These findings underscore the importance of comprehensive safety evaluations in the biomedical application of Pd-based nanomaterials and highlight the necessity of incorporating genotoxicity assessments into the design and development of nanoparticle-based therapeutic and diagnostic platforms. With the increasing use of nanomaterials in biomedical applications, thorough assessment of their potential biological effects, particularly at the genetic level, is essential.

Aknowledgements: This thesis was supported by the Scientific Research Unit under project number 2023-2-TP2-4983. The studies were conducted with permission from the Mersin University Rectorate Scientific Research Ethics Committee, dated October 18, 2023, and numbered 2023/683.

PP-29. Environmental Endocrine Disruptors and Their Relevance to Hashimoto's Thyroiditis

N.Merve Güven Aksu, Ayşegül Koçyiğit, Benay Can Eke

Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Ankara University, Türkiye

Background: Hashimoto's thyroiditis (HT) is the most common cause of hypothyroidism in iodine-sufficient regions, characterized by lymphocytic infiltration, progressive thyroid destruction, and elevated thyroid autoantibodies. While genetic predisposition is substantial, environmental exposures play an important role in disease onset and progression.

Objective: The study addresses the relationship of selected endocrine-disrupting chemical (EDC) groups—bisphenol A (BPA), per- and polyfluoroalkyl substances (PFASs), and phthalates—with thyroid dysfunction and Hashimoto's thyroiditis.

Summary of evidence: Human studies on BPA demonstrate inconsistent associations with thyroid autoantibodies and hormone levels. Experimental data suggest that BPA can increase oxidative stress, modulate immune responses, and interact with estrogen receptors, providing biological plausibility; however, epidemiological findings remain variable depending on study design and exposure assessment. PFASs are persistent pollutants capable of impairing iodide transport and thyroid hormone homeostasis. High-throughput screening has shown sodium-iodide symporter inhibition, and a recent prospective cohort linked PFAS mixtures with altered thyroid outcomes. Phthalate exposure has been associated with changes in circulating thyroid hormone concentrations in adults; nevertheless, their associations with thyroid autoantibodies remain inconclusive in human studies.

Limitations: Most available studies are cross-sectional or limited in size, often rely on single spot measurements, use heterogeneous analytical platforms, and fail to adequately control for confounders such as iodine status, medications, and co-exposures. These limitations hinder causal inference.

Conclusions: BPA, PFASs, and phthalates have biologically plausible mechanisms to disrupt thyroid physiology and potentially modulate autoimmunity, yet current human evidence is insufficient to establish a causal role in HT. Large-scale, longitudinal studies with repeated exposure assessment and standardized immunological endpoints are required. Clinically, improved exposure assessment and patient education regarding modifiable EDC sources may be beneficial.

Keywords: Hashimoto's thyroiditis; endocrine-disrupting chemicals; bisphenol A; PFAS; phthalates; thyroid autoimmunity

PP-30. Curcumin Regulates Irinotecan- Induced Inflammatory Cytokines IL-1 Beta, TNF-Alpha, and Nfkappab in SW620 Colon Carcinoma Cells

Ebru Korkmaz[1], Merve Hazar[1,2], Berivan Ezgi Kaya[1], Sevtap Aydın Dilsiz[1]

[1] *Hacettepe University, Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Ankara,Türkiye*

[2] *Agri Ibrahim Cecen University, Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Agri, Türkiye*

The promising effects of plant phenolic substances in cancer treatments have attracted attention in research. Resistance mechanisms against chemotherapeutic drugs in colorectal cancer increase the need for the development of new treatment approaches. Curcumin, with its wide biological effects, has been shown to enhance the anti-cancer effects of chemotherapeutic agents and radiation in many *in vivo* and *in vitro* studies. In this study, we aimed to determine the effects of curcumin on ROS levels and inflammatory cytokines IL-1 beta, TNF-alpha, and NFkappaB in SW620 cells treated with irinotecan (IR). ROS levels were determined by fluorometric DC-FDA assay and colorimetric ELISA assay. It was found that compared to single IR treatment, curcumin reduced inflammatory cytokines at noncytotoxic high doses via reducing ROS levels. As a result, the combinations of curcumin with irinotecan can affect cellular inflammatory pathways. These results show that curcumin in combination with irinotecan may have an important role in the development of new treatment strategies for cancer.

This work was supported by Hacettepe University Scientific Research Projects Coordination Unit in Turkiye (Project number: THD-2022-19839).

Keywords: Colon carcinoma, curcumin, IL-1 beta, TNF-alpha, NFkappaB,

PP-31. E-Cigarettes: Friend or Foe? A Cross-Sectional Survey on E-Cigarette Use Habits and Perceptions Among Turkish Volunteers

Goksun Demirel, Mehmet Yılmaz

Çukurova University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Adana, Turkiye

Purpose: The electronic cigarette (e-cigarette), which has become very popular in the last decade, is widely used worldwide. Today it is very common, especially among young people and people who want to quit traditional cigarettes. This study aims to measure the participants' e-cigarette use habits, the use of e-cigarettes with traditional cigarettes, the e-cigarette users' level of knowledge about e-cigarettes, and their experiences of the harms and side effects of e-cigarette using an online survey study.

Materials and Methods: This cross-sectional survey study was conducted with volunteer participants in Türkiye between 10 January 2024 and 30 April 2024 using an electronic survey. The study was conducted with a questionnaire consisting of questions measuring participants' demographic information and participants' use and experience with electronic cigarettes.

Results: The survey included 419 people aged under 18 and over 65, 52.5% female and 47.5% male. The proportion of young people aged 18-25 who use or have used electronic cigarettes is 21.2%. The majority of e-cigarette users were young people aged 18-25 years ($p=0.002$). In our study, 7.6% of e-cigarette users reported using e-cigarettes to quit smoking, 17.4% reported using e-cigarettes for discretionary reasons, specifically because they liked the e-cigarette flavours, and 3.1% reported using e-cigarettes because they thought they could smoke indoors ($p < 0.05$).

Conclusion: This study presented data on the use of electronic cigarettes and the experiences of volunteers participating in the survey, and was designed to raise awareness of misconceptions about e-cigarettes among the volunteers surveyed.

Keywords: E-cigarette, survey, nicotine addiction, liquid, tobacco

PP-32. *In Silico* Study of *Ferula Latisecta*-Derived Compounds Molecular Interactions with A-Glucosidase

Sultan Mehtap Büyüker, Meryem Nur Baş, Özlem Esma Pala Çamlı, Gülden Zehra Omurtag

Istanbul Medipol University, Türkiye

Purpose: Our study aimed to investigate the sulfur compounds' antidiabetic effect in Ferula latisecta.

Methods: The molecular docking method investigated the α-glucosidase inhibitory effect of the components. In our study, the pharmacokinetic properties of Ferula latisecta compounds were also investigated with the SwissADME method, and the toxicity risk analyzes were investigated with Protox II tools. Ferula latisecta compounds were drawn from the literature in Chemdraw and and α-glucosidase enzyme structure was obtained from Protein Data Bank. Finally, the molecular interaction analysis between α-glucosidase and compounds from Ferula latisecta was performed by AutoDock 1.5.7. Molecular interactions were investigated using Discovery Studio Visualizer and Ligplot 2.1 program.

Results: All the selected sulfur compounds from Ferula latisecta followed Lipinski's rules, had sufficient binding energy, and lacked toxicity; therefore, they were appropriate candidates for α-glucosidase inhibition. Among these compounds, 2-(4-hydroxyphenyl) ethyl lignocerate and isosco-poletin showed the lowest binding energy and the highest inhibitory effect on α-glucosidase enzyme with −9.1 and −7.7 kcal/mol, respectively. These compounds also indicated a lower binding energy than the standard inhibitor (miglitol). Among the sulfur compounds in Ferula latisecta 2-(4-hydroxyphenyl) ethyl lignocerate and isoscopoletin were predicted to be the potent inhibitors due to having more hydrogen bonds and hydrophobic interactions with the active site of α-glucosidase.

Keywords: In Silico, α-Glucosidase Inhibition, Molecular, Ferula latisecta, Diabetes

PP-33. Emerging Mycotoxins: Potential Toxicity

Zeynep Ciftci

[1]*Trakya University, Arda Vocational College, Edirne, Türkiye*

Mycotoxins are secondary metabolites produced by certain filamentous fungi that can contaminate food and feed, posing serious health risks to both humans and animals. Maximum permissible levels for regulated mycotoxins such as aflatoxins, ochratoxins, fumonisins are prescribed in food and feed legislation. Emerging mycotoxins were defined as "mycotoxins, which are neither routinely determined, nor legislatively regulated; however, the evidence of their incidence is rapidly increasing". Emerging mycotoxins include metabolites from *Fusarium* (e.g., enniatins (ENNs), beauvericin (BEA), moniliformin (MON)), *Aspergillus* (e.g., emodin (EMO)), *Penicillium* (e.g., mycophenolic acid (MPA)), and *Alternaria* (e.g., alternariol (AOH)). BEA has been shown to exert strong cytotoxic effects in various cell lines, including liver, colon, germ, and pulmonary cells, by inducing cell cycle arrest, triggering apoptosis, and promoting the production of reactive oxygen species (ROS). Similarly, for ENNs, cytotoxicity has been observed in liver cells, colon cells, blood-brain barrier cells, and immune cells, with ENNA and ENNA1 exhibiting particularly high toxicity. Their cytotoxic mechanisms include reduced cell viability, cell cycle arrest, apoptosis induction, and elevated ROS production. MON exposure has frequently been associated with heart damage, including myocardial lesions and increased relative heart weights, as well as muscle weakness, respiratory distress, decreased feed intake and body weight, and impaired immune function. AOH induces DNA damage through single and double-strand breaks and oxidative stress mediated by ROS generated from their metabolites, including catechols and quinones. They also inhibit topoisomerase activity, stabilizing the enzyme–DNA complex and promoting DNA strand breaks. This study aims to compile and review the existing literature on the toxicity risks of emerging mycotoxins, with a particular focus on BEA, ENN, MON, and AOH, thereby contributing to their risk assessment.

Keywords: Emerging mycotoxins, enniatins, beauvericin, moniliformin, alternariol, toxicity

PP-34. Pediatric Inhalation Risk Assessment of Homosalate-Containing Spray Sunscreens

Selma Yazar[1] Zehra Kılıç[2]

[1]*Istanbul Yeni Yuzyil University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Istanbul, Turkiye*

[2]*Okan University, Faculty ofPharmacy, Department of Pharmaceutical Toxicology, İstanbul, Turkiye*

Organic UV filters have become an integral part of human lifestyle due to their ability to block harmful UV radiation through topical application. In addition to their widespread use in cosmetics and personal care products such as anti-aging creams, lipsticks, hair conditioners, body lotions, hair dyes, and shampoos, these heterogeneous organic chemicals are also employed in textiles, plastic coatings, and paint products to prevent photodegradation and discoloration.

Homosalate (HMS) is one of the most commonly used UV-B filters in sunscreens marketed for the pediatric population. Although some studies have reported endocrine-related effects, evidence regarding potential health risks in humans remains limited. Spray formulations represent the primary route of HMS exposure via inhalation among consumers. However, data on this exposure pathway in pediatric populations are insufficient, and a comprehensive assessment of inhalation risks in children has not yet been conducted.

In this study, the effects of daily usage frequency of sunscreen spray formulations containing HMS were evaluated in children aged 6–11 years. For risk assessment, systemic exposure calculations were performed under different usage scenarios (once, twice, and six times per day), and Margin of Safety (MOS) values for HMS were determined using the ConsExpo 1.2.0 program.

In conclusion, it was determined that an increase in the daily usage frequency of sunscreen spray products containing Homosalate leads to higher systemic exposure, and that long-term use of these products may pose potential health risks. The risk may be further elevated in individuals with allergic respiratory diseases. These findings indicate that caution should be exercised in the long-term use of such products in children, as safety thresholds may be exceeded.

Keywords: UV filters; homosalate; risk assessment; children; inhalation; sunscreen

12TH INTERNATIONAL CONGRESS OF THE TURKISH SOCIETY OF TOXICOLOGY
November 6-9, 2025, İstanbul
Toxicology in Protecting Human and Environmental Health

PP-35. Endocrine Disrupting Effects of Bisphenol A and Bisphenol F on 3t3-L1 Cells

İzem Bilinmiş[1], Deniz Arca Çakır[2], Hülya Tezel Yalçın[3], Funda Nuray Yalçın[4], Nurşen Başaran[1], Pınar Erkekoğlu[3]

[1]Başkent University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Turkiye

[2]Department of Vaccine Technology, Vaccine Institute, Hacettepe University, Ankara, Turkiye,

[3]Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Turkiye

[4]Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, Turkiye,

Obesity, one of the most persistent global health challenges, is characterized at the cellular level by increased fat mass, largely resulting from adipocyte hypertrophy, hyperplasia, and excessive energy storage within adipose tissue. To better elucidate the molecular mechanisms underlying adipogenesis and adipose tissue dysfunction, which contribute to obesity-associated metabolic disorders, extensive research has been conducted in recent years. Endocrine-disrupting chemicals (EDCs) are exogenous compounds that interfere with the synthesis, storage, release, transport, metabolism, binding, action, or elimination of endogenous hormones. Among these, bisphenols are widely used chemicals, most commonly employed in the production of hardened plastics and various industrial applications. Due to the significant health concerns associated with bisphenol A (BPA), many countries have restricted its use, resulting in the increasing use of analogs such as bisphenol F (BPF). The aim of this study was to investigate the effects of BPA and BPF on adipogenesis and their endocrine-disrupting potential in 3T3-L1 cells. An adipogenesis model was established using a differentiation protocol of 3T3-L1 cells. The MTT assay was performed to evaluate the effects of BPA and BPF on cell viability. At the highest non-cytotoxic concentrations, changes in the expression of aromatase, peroxisome proliferator-activated receptor alpha (PPARα), peroxisome proliferator-activated receptor gamma (PPARγ), fatty acid binding protein 4 (FABP4), and CCAAT/enhancer-binding protein beta (CEBPβ) were measured. We found that both BPA and BPF significantly increased aromatase (30.22% and 55.21%) and PPARα (12.98% and 48.16%) levels compared to control ($p<0.05$). A significant elevation in PPARγ expression was observed only with BPF (53.50%, $p<0.05$). FABP4 expression increased by 24.46% with BPA and 63.42% with BPF ($p < 0.05$). Similarly, C/EBPβ levels increased 39.44% and 69.90% after BPA and BPF application ($p<0.05$, both). In conclusion, both BPA and BPF exhibited adipogenesis-enhancing and endocrine-disrupting effects in 3T3-L1 cells, with BPF exerting a stronger impact on key adipogenic markers. These findings suggest that BPA analogs may pose comparable or even greater metabolic risks than BPA.

Keywords: Endocrine Disruptors, Obesity, Bisphenol A, Bisphenol F

PP-36. Evaluation of Cytotoxic and Proliferative Profiles of Novel Plant-Based Topical Formulations in Fibroblast Cell Lines

Aylin Balcı Özyurt[1], Tilbe Çevikelli[2], Gülşah Selin Akyüz Çetin[3], Umay Merve Guven Bolgen[4]

[1]Department of Pharmaceutical Toxicology, School of Pharmacy, Bahçeşehir University İstanbul, Turkiye

[2]Department of Pharmaceutical Technology, School of Pharmacy, Bahçeşehir University İstanbul, Turkiye

[3]Department of Pharmacognosy, School of Pharmacy, Bahçeşehir University İstanbul, Turkiye

[4]Department of Pharmaceutical Technology, Faculty of Pharmacy, Çukurova University, Adana, Turkiye

Purpose: Medicinal plants are widely used in wound care for their phytochemicals, which provide antimicrobial protection, regulate inflammation, and promote fibroblast proliferation and tissue regeneration. *Lavandula angustifolia* L. essential oil (LEO), and *Pinus brutia* L. extract (PE) have demonstrated wound-healing benefits. Microemulsions are needed to enhance the stability of plant-derived oils and to eliminate the toxic effects (irritation, sensitization) associated with their direct application to the skin. This study aimed to assess the cytotoxic and proliferative effects of LEO and PE on fibroblast cell lines using novel microemulsion formulations for wound healing.

Methods: Barks of *P. brutia* and flowering parts of L. *angustifolia* were collected from (respectively Çanakkale and Tekirdağ) Turkiye. Essential oils from L. *angustifolia* flowers were obtained via hydrodistillation, and proanthocyanidins were extracted from *P. brutia* bark using a standardized Masquelier method. Microemulsions were prepared using the water titration method with oleic acid (oil phase), Cremophor EL (surfactant), and Carbitol/Capryol PGMC (cosurfactants); 1% LEO and 2% Pinus extract were incorporated. The cytotoxic and proliferative effects of four groups (n=6); blank microemulsion (B), PE microemulsion (P), LEO microemulsion (L), and combined microemulsion (PL) at concentrations ranging from 0-25 µg/mL were evaluated in 3T3 and L929 cell lines using the MTT assay at 24 hours of exposure.

Results: Cell viability remained above 90% in all groups for both cell lines. Although a slight decrease was observed at higher doses in the P group compared to the control, a dose-dependent proliferation was noted in the L and PL groups. These results indicate that none of the formulations were cytotoxic within this dose range and suggest that the L and PL groups may exhibit significant wound healing potential. Further *in vitro* and *in vivo* wound healing studies are planned to evaluate this activity in greater detail.

PP-37. Evaluation of The Cytotoxic Effects of Dexketoprofen Trometamol in V9 Cell Line

Merve Kapı, Berivan Ezgi Kaya, Sevtap Aydın Dilsiz, Ülkü Ündeğer Bucurgat

[1]*Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Turkiye*

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly prescribed medications for the treatment of inflammation, fever, and pain.

Their primary mechanism of action is the inhibition of cyclooxygenase (COX), an enzyme responsible for the synthesis of prostanoids. Dexketoprofen trometamol is one of the most commonly used NSAIDs today. In addition to anti-inflammatory properties, NSAIDs have antitumor effects, including the ability to inhibit cell proliferation and angiogenesis, and to induce and increase apoptosis.

Dexketoprofen trometamol is the S-enantiomer of ketoprofen and has a higher potency compared to the racemic compound. It is also rapidly absorbed and reaches peak plasma distribution within a short time. This offers a potential benefit in the treatment of patients experiencing moderate to severe pain and, clinically, provides effective analgesic properties with the rapid onset of action of dexketoprofen trometamol.

In our study, the cytotoxic effect of dexketoprofen trometamol on V79 cells was evaluated by determining IC_{50} values at 24, 48, and 72 hours and over a wide concentration range using the MTT method.

It was observed that dexketoprofen trometamol significantly reduced the cell viability at the concentrations above 512 µM, 512 µM and 1024 µM at 24, 48, and 72 hours, respectively. The IC_{50} values of dexketoprofen trometamol were 7,763 mM, 1,898 mM, 1,047 mM at 24, 48, and 72 hours, respectively.

Additionally, based on the results obtained from this study, it is planned to investigate the antigenotoxic or potentiating effects of dexketotofen trometamol on DNA damage caused by hydrogen peroxide, an oxidative damaging agent, at non-cytotoxic doses using the Comet method and to contribute to the literature.

Keywords: Dexketoprofen trometamol, V79 cell lines, Cytotoxicity, Genotoxicity, Comet assay

PP-38. Investigating the Reproductive Toxicity of Ponatinib in TM3 Leydig Cells: Focus on Tyrosine Kinase Pathways and Epigenetic Regulation

Uluç Göktuğ Çiftçi[1,2], Seher Karslı[2], Gül Özhan[3]

[1] *Department of Pharmaceutical Toxicology, Istanbul University-Cerrahpaşa, Istanbul, Turkiye*

[2] *Faculty of Pharmacy, Department of Toxicology, Marmara University, Istanbul, Turkiye*

[3] *Department of Pharmaceutical Toxicology, Istanbul University, Istanbul, Turkiye*

Ponatinib is a multi-targeted tyrosine kinase inhibitor (TKI) primarily used in the treatment of Philadelphia chromosome-positive leukemias, including chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (Ph+ ALL). Its antitumor efficacy is well established through extensive clinical and preclinical studies. However, its toxicity profile which involves the cardiovascular, hepatic, and pancreatic systems has raised concerns about potential off-target effects in non-cancerous tissues.

To date, no comprehensive studies have specifically evaluated its toxicity in the male reproductive system. In particular, the possible impact of Ponatinib on Leydig cells, which play a crucial role in testosterone production and androgen-dependent reproductive functions, remains largely unexplored. This represents a significant gap in the current understanding of Ponatinib's safety profile.

Mechanistically, Ponatinib inhibits a broad spectrum of tyrosine kinases, including ABL (and its T315I mutant form) as well as kinases involved in key signaling pathways such as VEGFR, PDGFR, FLT3, and c-KIT. Notably, these pathways are also functionally active in the male reproductive system, particularly in Leydig cells. Given this wide inhibition spectrum, Ponatinib may potentially alter the expression or function of these receptors in testicular cells, suggesting a plausible molecular basis for reproductive toxicity. Initially, an MTT assay was performed to determine the appropriate dose range for subsequent mechanistic studies. Gene expression levels of VEGFR, PDGFR, FLT3, and c-KIT were analyzed using real-time quantitative PCR. Testosterone levels were quantified via a commercially available ELISA kit. Apoptosis was assessed by flow cytometry using Annexin V/PI staining, while mitochondrial membrane potential was evaluated with the JC-1 dye. Intracellular ATP levels were measured using a ready-to-use commercial assay kit and a spectrophotometer. These assays provide insight into the potential cellular mechanisms involved in Ponatinib-induced toxicity in leydig cells.

Keywords: Gene expression regulation, Ponatinib, Leydig cells, Apoptosis,Tyrosine kinase signaling

PP-39. Evaluation of Methylisothiazolinone Neurotoxicity in SH-SY5Y Cells via Cytotoxicity, Apoptosis, and Gene Expression Analyses

Gül Küçükkahraman[1], Özge Sultan Zengin[2], Gül Özhan[2]

[1] *Institute of Health Sciences, Istanbul University, Istanbul, Turkiye*

[2] *Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Istanbul University, Istanbul, Turkiye*

Methylisothiazolinone (MIT) is a widely used biocide and preservative found in numerous cosmetic and household products. In recent years, its potential neurotoxic effects have attracted increasing scientific attention. The aim of this study was to investigate the dose-dependent neurotoxic effects of MIT in human neuroblastoma SH-SY5Y cells and to evaluate its impact on key proteins involved in apoptosis, cell survival, and inflammation.

SH-SY5Y cells were treated with 60, 90, and 120 µM concentrations of MIT for 24 hours. Cell viability was assessed by the MTT assay, and the IC_{50} value was determined to be approximately 115 µM. Apoptotic cell death was quantified by flow cytometry following Annexin V/PI staining. To explore the molecular basis of MIT-induced responses, the expression levels of p53, BAX, BCL-2, APAF1, SRC, VEGFR2, COX, and COX-2 were measured using ELISA. Protein expression values were normalized to total protein content.

MIT exposure resulted in a clear dose-dependent increase in apoptotic cell death in SH-SY5Y cells. Protein analysis revealed a significant upregulation of multiple markers, including p53 (14.3-fold), BAX (8.3-fold), BCL-2 (8.2-fold), SRC (6.3-fold), and COX-2 (5.5-fold), indicating concurrent activation of apoptotic, survival, and inflammatory pathways.

Overall, MIT demonstrated pronounced neurotoxicity in SH-SY5Y cells by simultaneously activating apoptosis and inflammation-related mechanisms. This study provides a comprehensive molecular insight into the neurotoxic potential of MIT and contributes valuable evidence for re-evaluating its safety in consumer and industrial formulations.

PP-40. Investigation of The Apoptotic Effect of Cupferron in SH-SY5Y Neuroblastoma Cells

Özge Sultan Zengin[1,2], Esma Özce Pala Çamlı[1,2], Mahmoud Abudayyak[2], Gül Özhan[2]

[1] Department of Pharmaceutical Toxicology, Istanbul University, Istanbul, Turkiye

[2, 3] Department of Pharmaceutical Toxicology, Istanbul University, Istanbul, Turkiye

Cupferron is a chelating agent chemically known as ammonium nitrosophenylhydroxylamine. Cupferron is particularly preferred in the industry for the separation and analysis of iron, copper, and nickel complexes.In agriculture, cupferron is recognized for its antifungal properties and is used to control fungal pathogens.The chelating ability of cupferron, which enables it to inhibit fungal growth, can lead to toxicity in non-target living organisms if it contaminates the food chain and water, particularly as its solubility in water enhances its toxicity potential. In recent years, there have been increasing concerns about the potential health risks of Cupferron, and it was added to the IARC 2B (possible) carcinogens list in 2021. It has been observed that Cupferron causes DNA damage; it is clastogenic in Chinese hamster ovary cells, induces chromosomal abnormalities and sister chromatid exchanges in bacteria in the presence of S9, and gives a positive response in the SOS/umu test.Studies conducted on rats have proven its toxic effects on the reproductive system. While being used for its antifungal properties, cupferron, which can enter the bodies of living organisms through contamination of food and groundwater, is a compound that has not yet been sufficiently researched and has a high potential for toxicity.It is not yet known whether it has neurotoxic effects. In this study, the toxicity potential of Cupferron in SH-SY5Y neuroblastoma cells was investigated. First, an MTT test was performed and the IC50 value was determined to be 483.81 µg/mL, and the concentrations to be used in the experiment were established.Apoptosis determination with Annexin V-FITC, intracellular calcium determination, and expression levels of the p53, BAX, cytochrome c (Cyt-c), and APAF-1 genes were determined.In these experiments, where the apoptotic effect of Cupferron on neuroblastoma cells was investigated, a dose-dependent significant increase was observed, establishing the way for further experiments.

PP-41. Identification Of Compounds with Weak Skin Irritation Potential Using Human 3D Reconstructed Epidermis Model

Silvia Letasiova[1], Jan Markus[1], Jana Halajova[1], Lenka Hudecova[1], Christian Pellevoisin[2], Mitchell Klausner[2]

[1] MatTek In Vitro Life Science Laboratories, s.r.o., Bratislava, Slovakia

[2] MatTek Corporation, Ashland, Massachusetts, USA

This work was focused on utilization of an *in vitro* reconstituted human skin model to identify substances with a weak irritation potential. The *in vitro* method for determining skin irritation according to OECD test guideline 439 and the method for determining skin irritation for extracts from medical devices according to ISO standard - ISO 10993-23:2021, were used to detect the irritation of 15 test articles (TAs) in 5 concentrations in non-polar solvent (sesame oil) and in polar solvent (saline). If no effects were observed at these concentrations, TAs were also tested neat. The EC-50 was calculated, and release of pro-inflammatory cytokines was assessed. Allyl heptanoate, heptyl salicylate, linalyl acetate, methyl laurate, hexyl salicylate had viability comparable with negative control tissues in all concentrations in both solvents as well as in undiluted form. On the other hand, 10-undecenoic acid, lactic acid, 2-ethoxyehtyl methacrylate, 1-decanol, methyl methacrylate, 2-bromobutane and 50% sodium carbonate did not cause the irritation effect in all tested concentrations in both solvents, but irritation effect was observed using the TAs. Heptanoic acid, SDS and 10% sodium hypochlorite decreased tissue viability below 50%, so the EC-50s could be calculated. When using the skin irritation test for medical device extracts according to ISO 10993-23:2021 we found that heptyl butyrate, hexyl salicylate, methyl laurate did not decrease viability at any concentration in both solvents, and the viability of the undiluted form was 100%. On the other hand, methyl methacrylate and allyl heptanoate caused decreased tissue viability only in the undiluted form. All other compounds caused decreases in viability in at least one solvent as well as in the undiluted form. These results were confirmed by release of 1α, IL-6, and IL-8 and by the release of IL-18 when looking for skin sensitization potential.

PP-42. Development of An *In Vitro* Method Used for Assessment of Gastrointestinal Toxicity of Nanoparticles Contained in Food

Marek Puskar[1], Seyoum Ayehunie[2], Christian Pellevoisin[2], Jan Markus[1], Mitchell Klausner[2], Amanda Helfrick[2], Alex Armento[2]

[1] *MatTek In Vitro Life Science Laboratories, s.r.o., Bratislava, Slovakia*

[2] *MatTek Corporation, Ashland, Massachusetts, USA*

To evaluate acute and chronic effect of various NPs commonly used in food industry we have developed an *in vitro* method utilizing 3D reconstructed human tissue model of small intestine – EpiIntestinal. Barrier integrity, tissue viability, oxidative stress and inflammatory response were used for the assessment of toxicity of the NPs. In addition, a permeation study was conducted to assess applicability of the EpiIntestinal to study systemic exposure to NPs from the food industry. EpiIntestinal tissues were exposed at 4 doses of sonicated and dispersed NPs under rocking conditions for 4 hr and the tissues were further cultured for 20 hr under static conditions. Following acute exposure, CuO and ZnO, showed significant dose-dependent reduction in barrier integrity and tissue viability. These NPs also induced histological damage and increased release of the pro-inflammatory cytokine, IL-8. Analysis of culture supernatants showed a dose dependent increase of 8-isoprostane following exposure to CuO and a slight increase by ZnO, and Fe_2O_3 but not to Ag nanoparticles. For TiO_2, Ag, Al, and Fe_2O_3, minimal / no acute toxicity was observed at the tested concentrations. In additional experiments, the maximum tolerated dose, corresponding to concentrations with no acute toxicity signs, was used to model chronic exposure (dosing every 48 hrs for 18 days). Interestingly, signs of barrier impairment were noted with CuO and to a lesser extent for TiO_2, Au, Fe_2O_3 and, only to the longer exposure time, with Al. No significant changes were observed with ZnO, Ag and SiO_2. Overall, TEER measurements were a more sensitive endpoint compared to the MTT tissue viability assay. Assessment of genotoxic potential by the comet assay after repeated exposure showed a slight increase in tail length in CuO exposed tissues. To investigate permeation of nanoparticles through the EpiIntestinal model, polystyrene (PS) latex nanoparticles were used.

PP-43. Epiocular Time-To-Toxicity – A Test Method for Subcategorization of Eye Irritants

Silvia Letasiova[1], Lenka Hudecova[1], Jan Markus[1], Yulia Kaluzhny[2], Els Adriaens[3], Mitch Klausner[2]

[1] MatTek In Vitro Life Science Laboratories, s.r.o., Bratislava, Slovakia

[2] MatTek Corporation, Ashland, Massachusetts, USA

[3] Adriaens Consulting BVBA, Aalter, Belgium

In 2015 an OECD TG 492 was accepted and validated for the use of *in vitro* ocular tissue models. Initially, this only allowed distinguishing between substances and mixtures not requiring classification. More recently an OECD TG 492B was accepted, allowing for distinguishing between chemicals that: a) do not require labeling for serious eye damage or eye irritancy (No Cat), b) cause serious eye damage (Cat 1), and c) are eye irritants (Cat 2) according to the UN GHS ocular hazard categories. A new testing strategy was developed based on the results from 2 studies, CON4EI and ALT4EI projects. A robust final set of 144 reference chemicals– 78 liquids and 66 solids, was obtained and the results provided confirmation of the new testing strategy. The performance criteria, established by the OECD expert group overseeing OECD TG 492B, were met for all 144 chemicals mentioned above. Using this data set we developed the EpiOcular™ time-to-toxicity test method for eye hazard identification of liquid and solid chemicals according to UN GHS. The new testing strategy for liquids correctly predicted 78.7% of Cat 1 (N=27), 63.5% of Cat 2 (N=26) and 82.0% of No Cat (N=25). The testing strategy for solids correctly predicted 75.0% of Cat 1 (N=28), 59.4% of Cat 2 (N=16) and 80.3% of No Cat (N=22) materials. Overall, the new test method correctly predicted 76.8% of Cat 1 (N=55), 61.9% of Cat 2 (N=42), and 81.2% of No Cat (N=47) test articles. The EpiOcular™ time-to-toxicity test method is a novel approach for subcategorizing of both liquid and solid compounds. The prediction models that were developed for liquids and solids are capable of distinguishing substances and mixtures into the 3 UN GHS ocular hazard categories: No Cat, Cat 2, and Cat 1.

PP-44. Development of An *In Vitro* Test Method for Irritation of Medical Devices Used in The Oral Cavity

Marek Puskar[1], Jennifer Molignano[2], Christian Pellevoisin[2], Silvia Letasiova[1] and Mitchell Klausner[2]

[1] MatTek In Vitro Life Science Laboratories, s.r.o., Bratislava, Slovakia

[2] MatTek Corporation, Ashland, Massachusetts, USA

The irritation of any medical device (MD) contacting oral tissues (gingival, buccal, lingual, etc) needs to be evaluated. The objective of this project is to develop and validate *in vitro* assay to assess the oral irritation of MDs. This assay is intended to replace historical *in vivo* assay performed on Syrian hamsters.

The ISO 10993-23 standard requires that MDs be evaluated using an *in vitro* irritation test based on reconstructed human epidermis (RhE) prior to animal or human patch testing is performed. However, RhE models are not appropriate for MDs designed for use in oral cavity, therefore ISO recommends use of other *in vitro* models with relevant cells or tissues. The EpiOral tissue model consists of normal, human-derived oral epithelial cells cultured to form multilayered, highly differentiated model of the human buccal tissue. To assess the feasibility of an *in vitro* method, initial experiments tested solutions of irritant chemicals contained in MDs used in oral cavity. Increasing concentrations of ethanol, lactic acid, methyl methacrylate, sodium dodecyl sulfate, phosphoric acid, sodium hypochlorite, hydrogen peroxide, and chlorhexidine digluconate in NaCl or sesame oil were applied to the EpiOral model. The time required to reduce tissue viability by 50% (ET-50), was determined.

The results showed a clear relationship between tissue viability and exposure time and between ET-50 and concentration of the irritant chemical. Compared to historical *in vivo* data, the *in vitro* method classified the samples containing an irritant at the expected concentration. In addition, the ET-50s allowed differentiation between strong and mild irritants. The data demonstrate that this *in vitro* assay has equivalent or superior performance to *in vivo* method.

PP-45. Molecular Docking Analysis of Acetamiprid, Cypermethrin and Emamectin Benzoate with Steroidogenesis Enzyme CYP11A1

Yasemin Altun Ali[1,3], Gül Özhan[2]

[1] *Institute of Health Sciences Graduate Education, İstanbul University, 34126, İstanbul, Türkiye.*

[2] *Department of Pharmaceutical Toxicology, Faculty of Pharmacy, İstanbul University, İstanbul, Türkiye*

[3] *Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Karadeniz Technical University, Trabzon, Türkiye*

Background: The combined use of different groups of pesticides is a common practice both to reduce pest resistance to pesticides and to increase crop yields. Acetamiprid, a neonicotinoid insecticide, is a relatively new compound frequently used to kill Leipidoptera and Hemiptera species, such as aphids, on crops such as fruit, vegetables, and tea. Acetamiprid is similarly used alone or in combination with other pesticides such as cypermethrin and emamectin benzoate. Emamectin benzoate disrupts the insects' neurotransmitters, causing irreversible paralysis. It acts by activating chloride channels. Cypermethrin is a synthetic pyrethrin insecticide frequently used to control aphids, beetles, and caterpillars that damage a wide range of plants. It disrupts cell membrane potential by affecting Na^+ channel permeability, leading to cell death.

Pesticide-induced reproductive dysfunction is a current topic of research. Several enzymes are involved in testosterone biosynthesis, the first of them is CYP11A1. In this study, the affinity of two commonly used pesticides, acetamiprid and its combination, for CYP11A1, the primary enzyme involved in the testosterone biosynthesis pathway, was investigated using molecular docking.

Methods: The potential of 3 compounds to be metabolized by the CYP11A1 enzyme was investigated using molecular docking approaches. The molecular docking procedure was applied with AutoDock 4.2

Results: Cypermethrin demonstrated the highest binding affinity compared to other pesticides (-4.57 kcal/mol). Acetamiprid demonstrated moderate binding affinity to CYP11A1 with a binding energy of -4.20 kcal/mol. Emamectin benzoate demonstrated the lowest binding affinity to CYP11A1 with a binding energy of +71.92 kcal/mol.

Conclusion: Emamectin benzoate does not potentially affect steroidogenesis, but acetamiprid and cypermethrin potentially do. Further molecular docking studies are needed for acetamiprid and cypermethrin with other enzymes involved in testosterone biosynthesis.

PP-46. Selective Uptake of Novel Polyamine Transporter-Targeted Compounds by Wild-Type CHO Cells

Hilmi Orhan, Nil Bita Entezari Ökeer, Catalina Cheaburu Yılmaz, Evren Gündoğdu

Ege University, Faculty of Pharmacy, İzmir, Türkiye

Although gemcitabine, docetaxel, and cisplatin — the most commonly used drugs in non-small cell lung cancer — are highly effective, they cause severe systemic toxic effects such as bone marrow depression and neuropathies. One of the effective strategies to reduce these adverse effects is to target the drug specifically to tumor cells and limit its off-target distribution. For this purpose, conjugation of drug molecules with polyamine compounds to enable targeting to the polyamine transport protein (PAT), which is abundantly present on the membranes of lung tumor cells, has been an area of research for some time. In our study, docetaxel and cisplatin were conjugated with a synthetic polyamine compound, and their chemical structures were characterized using chromatographic and spectroscopic techniques. The cytotoxicity of the polyamine (PA) conjugate was then tested by incubating it with wild-type and PAT-deficient mutant CHO cells. The IC_{50} value in wild-type CHO cells, which are expected to uptake the compound, was found to be three times lower than that in mutant CHO cells, which are not expected to uptake it. Consistent with the cytotoxicity results, the compound labeled with Technetium-99 showed 60% higher uptake in wild-type CHO cells. These findings suggest that conjugation of polyamine compounds to anticancer drugs could enhance their efficacy in lung tumors compared to the unconjugated forms, offering a promising strategy for targeted chemotherapy.

Funding: This work was supported by the Research Fund of the Ege University (Project Number: 25269).

Keywords: non-small cell lung cancer, polyamine transporter, drug targeting, mutant CHO cells

PP-47. *In Vitro* Evaluation of Obesogenic Potentials of Indomethacin in 3T3-L1 Cells

Nil Bita Entezari Ökeer[1], Hande Gürer Orhan[2]

[1] *Istanbul Kent University, Faculty of Pharmacy, İstanbul, Türkiye*

[2] *Ege University Faculty of Pharmacy, İzmir, Türkiye*

Endocrine disruptors are suggested to act as potential "obesogens" by interfering with metabolic processes in adipose tissue. While industrial chemicals are commonly implicated in these effects, certain pharmaceuticals may also exert obesogenic side effects. However, research on the obesogenic potential of pharmaceuticals remains limited.

This study aimed to evaluate the possible *in vitro* adipogenic/lipogenic effects of indomethacin in the range of Cmax values in 3T3-L1. Its effects on lipid accumulation, adiponectin levels, glycerol-3-phosphate dehydrogenase (G3PDH) activity, and the expression of adipogenic/ lipogenic genes and proteins were assessed. Indomethacin increased lipid accumulation in a dose-dependent manner.

Moreover, adiponectin levels and G3PDH activity were elevated, suggesting its potential role in activating adipogenic pathways. Additionally, indomethacin altered the gene and/or protein expression of key adipogenic and lipogenic transcription factors, potentially disrupting metabolic programming in human relevant concentrations.

In conclusion, indomethacin may exhibit obesogenic effects through distinct mechanisms. Further *in vivo* and epidemiological studies are needed to assess its potential contribution to obesity risk.

Keywords: pharmaceuticals, indomethacin, adipogenesis, lipogenesis, *in vitro*

PP-48. *In Vitro* Assessment of The Cytotoxic Effects of Tritan Monomers in MCF-7 Cells

Polen Akkaya Dakmancı[1], Aylin Üstündağ[2]

*[1]*Ankara Üniversitesi, Sağlık Bilimleri Enstitüsü, 06110, Ankara,Türkiye,*

[2]Ankara Üniversitesi Eczacılık Fakültesi, Farmasötik Toksikoloji Anabilim Dalı, 06560, Ankara,Türkiye,

Bisphenol A (BPA) has been extensively employed in the plastics industry, yet its release into the environment results in continuous human exposure. Even at low concentrations, BPA is known to exert estrogen-mimicking activity by interacting with endocrine receptors, thereby impairing cellular physiological processes. Due to these documented health risks, regulatory measures have restricted its use, and BPA has been phased out from a range of consumer products, including infant feeding bottles and food container lids. To retain the durability and transparency of plastics without BPA, alternative compounds such as bisphenol analogs and Tritan-derived monomers have been introduced. Tritan consists of three principal monomers: dimethyl terephthalate (DMT), 1,4-cyclohexanedimethanol (CHDM), and 2,2,4,4-tetramethyl-1,3-cyclobutanediol (TMCD). Recent toxicological investigations, however, have raised concerns that these Tritan monomers may induce adverse effects similar to those of BPA, suggesting they might not represent a safer replacement. In this study, the cytotoxic potential of Tritan monomers was examined through the application of the MTT assay in MCF-7 cells. The results demonstrated that IC_{50} values were not reached for Tritan monomers even at the highest tested concentrations. Notably, only the CHDM monomer caused a reduction of approximately 20% in cell viability at concentrations of 5 µM and above.

Acknowledgment: This study, conducted as the thesis of Polen Akkaya Dakmancı, was supported by Ankara University Scientific Research Projects under grant number TDK-2024-3391.”

TTD 2025

12TH INTERNATIONAL CONGRESS OF THE TURKISH SOCIETY OF TOXICOLOGY

November 6-9, 2025, İstanbul

Toxicology in Protecting Human and Environmental Health