Arhiv za higijenu rada i toksikologiju

Archives of Industrial Hygiene and Toxicology

Arh Hig Rada Toksikol
Vol. 76/Suppl.1
Zagreb, October 2025

Institute for Medical Research
and Occupational Health,
Zagreb, Croatia

ISSN 1848-6312
UDC 613–6
CODEN: AHRTAN

Abstracts of the 7th CROTOX meeting

Rab, Croatia, 5 – 8 October 2025

Supplement 1
Vol. 76 / 2025

Arhiv za higijenu rada i toksikologiju

Archives of Industrial Hygiene and Toxicology

Covered by:

Web of Science - Science Citation Index Expanded
Medline
PubMed
PubMed Central
SCOPUS
AGRICOLA (National Agricultural Library)
Baidu Scholar
Cabell's Journalytics
CABI (over 50 subsections)
Chemical Abstracts Service (CAS) - CAplus
Chemical Abstracts Service (CAS) - SciFinder
CNKI Scholar (China National Knowledge Infrastructure)
Dimensions
DOAJ (Directory of Open Access Journals)
EBSCO
Elsevier - Nursing
Embase
Ergonomics Abstracts
ExLibris
FSTA - Food Science & Technology Abstracts
Google Scholar
Hrcak
International Nuclear Information System (INIS)
Japan Science and Technology Agency (JST)
J-Gate
Jisc - Open Policy Finder
Journal Citation Reports/Science Edition
JournalGuide
JournalTOCs
KESLI-NDSL (Korean National Discovery for Science Leaders)
MyScienceWork
Naver Academic
Naviga (Softweco)
ReadCube
Reaxys
ScienceON/AccessON
SCILIT
SCImago (SJR)
Scite_
Semantic Scholar
TDNet
TEMA Technik und Management
Ulrich's Periodicals Directory/ulrichsweb
WanFang Data
Web of Science - Biological Abstracts
Web of Science - BIOSIS Previews
Web of Science - Essential Science Indicators
WorldCat (OCLC)
X-MOL

Cover page: *Rab bell towers*
Photographed by Issa Bratinčević (Issa Arts), Croatia.
Disclaimer: This photo is intended to evoke the content of this issue of the journal. It is not intended for instructional or scientific purposes.
Cover design by Andrijana Marković

Free access to full-text articles:
Portal of the Scientific Journals of Croatia – HRČAK (http://hrcak.srce.hr) and e-publishing services by Sciendo (https://content.sciendo.com/view/journals/aiht/aiht-overview.xml).
Follow us:
https://hr.linkedin.com/showcase/archives-of-industrial-hygiene-and-toxicology/
https://www.facebook.com/arh.hig.rada.toksikol/
This journal is supported by the Ministry of Science, Education and Youth of the Republic of Croatia.

PUBLISHER: Institute for Medical Research and Occupational Health, Zagreb, Republic of Croatia

Arhiv za higijenu rada i toksikologiju
Archives of Industrial Hygiene and Toxicology

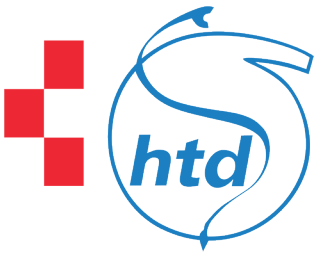
2025;76(Suppl. 1)

Abstracts of the 7th International Congress of the Croatian Society of Toxicology
CROTOX 2025

Expanding Horizons in Toxicology

Rab, Croatia
5–8 October 2025

Organised by



Croatian Society of Toxicology

Co-organiser

Institute for Medical Research and Occupational Health

Under the auspices of

REPUBLIKA HRVATSKA
Ministarstvo znanosti,
obrazovanja i mladih

Ministry of Science, Education and Youth of the Republic of Croatia

Sponsors

Gold sponsor

INEL MEDICINSKA TEHNIKA Zagreb

Silver sponsor

Altium

Bronze sponsors

avantor™

jasika®

SHIMADZU Excellence in Science

Other sponsors

KEFO

biovit

MEDIC

VITA LAB NOVA

Rab The Happy Island.

Ru-Ve tvoj lab partner

OKTAL PHARMA

toxics

ZAŠTITA SECURITY REPORT

SCUBAlife

Issa ARTS

INEL MEDICINSKA TEHNIKA Zagreb

Uvoz ▪ Distribucija ▪ Aplikacijska podrška ▪ Servis

Laboratorijska i medicinska oprema,

potrošni materijal i reagensi

SCIEX The power of precision

eppendorf

QIAGEN

SARSTEDT

Leica BIOSYSTEMS

BECKMAN COULTER

TECAN.

IMPLEN

THISTLE SCIENTIFIC

DOMEL

INDICAL BIOSCIENCE

fisher scientific part of Thermo Fisher Scientific

GeneProof part of NuvinkaDx

credo DIAGNOSTICS

info@inel-mt.hr

www.inel-mt.hr

Altium

Ultra-osjetljivo mjerenje PFAS spojeva iz materijala u dodiru s hranom

Korištenjem Agilent 6495D Triple Quadrupole LC/MS sustava, uspješno su kvantificirana **73 koncentrirana PFAS spoja** u papirnatim slamkama s granicom detekcije ispod 0,2 µg/kg. Metoda osigurava **izvrsnu linearnost (R^2 > 0,99)** i visoku preciznost s oporavkom u 90 % analita između 65 % i 120 %, uz RSD ≤ 20 %.

Ovaj sustav pruža pouzdano, osjetljivo i brzo praćenje PFAS kontaminacije materijala u dodiru s hranom što je ključno za sigurnost potrošača i usklađenost s regulativama.

Agilent | Authorized Distributor

Altium International d.o.o.
Karlovačka cesta 24, Blato
HR-10000 Zagreb, Croatia
info.hr@altium.net
prodaja.hr@altium.net
www.altium.net | www.alphachrom.hr

avantor™

LC-MS Made Easier

Accurate LC-MS analysis is the cornerstone of analytical chemistry in many of today's rapidly advancing fields of research. Because of the sensitivity and sophistication of LC-MS, results can depend heavily on the components and solvents chosen. Having access to technical support and a dependable product supply can also dramatically impact LC-MS results.

Considering how complicated it can be to use LC-MS. Avantor® manufactures a wide range of products, including:

- Columns
- Solvents and mobile phases
- Sample preparation products

VWR® Avantor® ACE®

J.T.Baker® Avantor® Hichrom

To access the **Avantor portfolio of products and services,** please visit www.avantorsciences.com/si/en/applications/chromatography/lc-ms-workflow

MOLEKULSKA SPEKTROSKOPIJA

PerkinElmer
For the Better

REAKTORI

PROČIŠĆAVANJE KISELINA

ANALIZA TEŠKIH METALA

TERMALNA ANALIZA

MIKROVALNA DIGESTIJA I EKSTRAKCIJA

KROMATOGRAFIJA

jasika®

info@jasika.hr
www.jasika.hr

revvity

STANIČNI BROJAČI

AUTOMATSKA IZOLACIJA DNA/RNA

ČITAČI MIKROTITARSKIH PLOČICA

Cole-Parmer

KRIOGENI MLINOVI

HOMOGENIZATORI

FLUKSERI

xylem

UREĐAJ ZA PROČIŠĆAVANJE VODE

SHIMADZU
Excellence in Science

Doprinos društvu kroz znanost i tehnologiju

Shimadzu je vodeći svjetski proizvođač analitičkih i mjernih instrumenata. Nudimo sofisticirana i optimalna rješenja neophodna za istraživanje, razvoj i kontrolu kvalitete.

Naša rješenja!

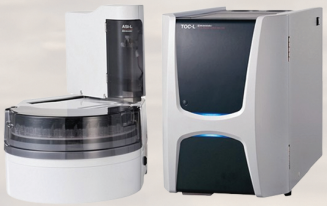
Sustavi za plinsku kromatografiju (GC) osiguravaju pouzdanu i osjetljivu analizu hlapljivih spojeva sadržanih u uzorku.

Masena spektrometrija s induktivno spregnutom plazmom (ICP-MS) je tehnika u kojoj se induktivno spregnuta plazma koristi kao ionizacijski izvor, a detekcija se vrši masenom spektrometrijom.

Spektrofotometri mjere intenzitet svjetlosti koju apsorbiraju ili emitiraju kemijske tvari.

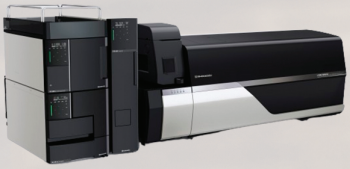
Analizatori ukupnog organskog ugljika (TOC) mjere ukupnu količinu organskog ugljika u vodi, plinovima i čvrstim tvarima. Zadovoljavaju raznolik raspon potreba u područjima kao što su istraživanje okoliša, kontrola kvalitete i upravljanje procesima.

Sustavi za tekućinsku kromatografiju (LC) uspješno razdvaja tvari na osnovi razmještanja između čvrste stacionarne faze i tekuće mobilne faze.



Sustavi za plinsku kromatografiju - masenu spektrometriju (GC/MS) omogućuju brzu identifikaciju nepoznatih komponenti i preciznu kvantitativnu analizu, čak i za komponente u tragovima.

Sustavi za visoko učinkovitu tekućinsku kromatografiju (HPLC) podržavaju širok raspon aplikacija, uključujući analizu hrane, lijekova te uzoraka iz okoliša.



KONTAKTIRAJTE NAS!

Shimadzu d.o.o.
Zavrtnica 17, Zagreb, Hrvatska
tel. +385 1 6185 777
shimadzu@shimadzu.hr

Ru-Ve
tvoj lab partner

Ru-Ve d.o.o. je privatna tvrtka osnovana 1990. godine u Zagrebu, specijalizirana za opskrbljivanje svih vrsta kemijskih, mikrobioloških i analitičkih laboratorija.

Djelatnosti tvrtke vezane su isključivo uz laboratorije:

- Distribucija:
  - Potrošni laboratorijski materijal,
  - Laboratorijski mjerni i pomoćni uređaji,
  - Kemikalije,
  - Laboratorijski namještaj.
- Servis laboratorijske opreme,
- Kvalifikacija mjerne opreme.

Godinama smo autorizirani distributer najrenomiranijih europskih proizvođača, a uz to surađujemo i s nekoliko specijaliziranih veletrgovaca u Europi.

Na vlastitom skladištu kraj Zagreba kontinuirano držimo više od 3.000 artikala, spremnih za isporuku u Vaš laboratorij unutar 24h.

Naših 25 zaposlenika stoji Vam, sa svojim stručnim znanjem i dugogodišnjim iskustvom, na usluzi savjetom, ljubaznošću, korektnom i brzom uslugom te razumnim cijenama i kvalitetnim proizvodom.

Vrlo rado ćemo Vam pomoći kod:

- Odabira laboratorijskog namještaja,
- Odabira laboratorijskih uređaja i pribora,
- Odabira analitičkih metoda,
- Odabira i traženja specijalnih roba ili proizvođača.

Od ožujka 2005 godine potvrdili smo svoju kvalitetu ISO 9001 certifikatom.

DURAN GROUP
magic of precision

HIRSCHMANN®

IKA®

memmert

sartorius

SI Analytics
a xylem brand

ATAGO®

HAMILTON
THE MEASURE OF EXCELLENCE™

Julabo

MACHEREY-NAGEL MN

VWR PROLABO

Ako nas do sada niste upoznali, pokušajte ..isplatit će se!

Ru-Ve
tvoj lab partner

Prosinačka 13, 10431 Sv. Nedelja – Kerestinec
Tel: 01/333-52-50
Fax: 01/333-52-59
info@ru-ve.hr www.ru-ve.hr

MEDIC *Znanstvena i laboratorijska rješenja!*

OTAPALA, REFERENTNI MATERIJALI, PRIPREMA UZORAKA

MERCK

UREĐAJI ZA ČISTU I ULTRAČISTU VODU, SUSTAVI ZA FILTRACIJU

MERCK MILLIPORE

MEDIJI, ANTITIJELA, DNA/RNA KITOVI, PCR, qPCR RJEŠENJA

Sigma-Aldrich
Lab Materials & Supplies

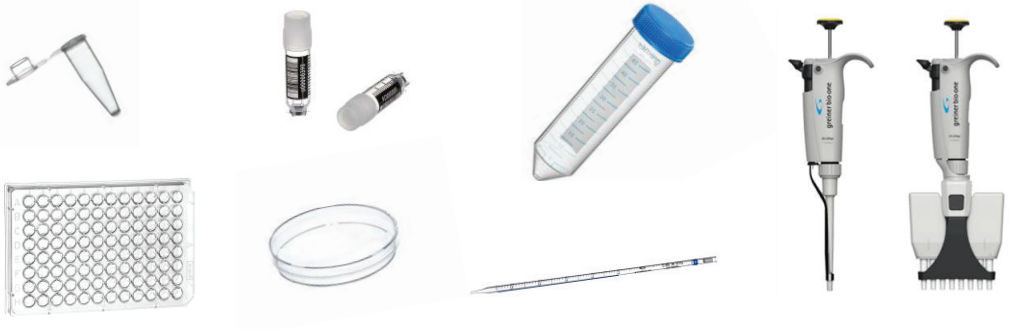
PROTOČNA CITOMETRIJA

CYTEK®
TRANSCEND THE CONVENTIONAL

Cytek Muse® Micro
Cytek® Guava® Muse®
Cytek® Guava® easyCyte™
Cytek® Amnis®ImageStream®X

LABORATORIJSKI POTROŠNI MATERIJAL

greiner
BIO-ONE



INFO: e: kemikalije@medic.hr w: www.medic.hr

biovit

Sve za Vaš laboratorij na jednom mjestu!

BIOVIT d.o.o. distribuira visokokvalitetne proizvode renomiranih proizvođača namijenjenih za upotrebu u medicini, laboratorijskoj dijagnostici, industriji i znanstveno-istraživačkim ustanovama.

- Kemikalije i reagensi za različite laboratorijske primjene
- Laboratorijski potrošni pribor
- Laboratorijska oprema
- Osobna zaštitna oprema

MERCK

Honeywell

fisher scientific
part of Thermo Fisher Scientific

BLD pharm

CARL ROTH

LGC

KEFO

Vita Lab Nova d.o.o.
Ilica 37, 10000 Zagreb

Phenomenex Vodeći svjetski proizvođač (U)HPLC, GC, SPE kolona i ostalog pribora za kromatografiju.

C.Gerhardt Analitički uređaji za analizu hrane, vode, stočne hrane i tla. Hidroliza, ekstrakcija, digestija...

Denovix mikrovolumen spektrofotometri i fluorimetri te brojači stanica.

BMG Labtech čitači mikrotitarskih ploča.

Zymo Research— epigenetika, DNA/RNA, protein izolacija i pročišavanje, NGS Library

highQu Kitovi za PCR, qPCR, RT-PCR, RT-qPCR, elektroforezu i enzime.

Tel: +385-1-4667-513; Fax: +385-1-4667-514; E-mail: info@vitalab.hr

TABLE OF CONTENTS

CROTOX 2025
Rab Croatia October 5-8

Organising Committee

Dubravka Rašić, *Congress President*
Maja Lazarus, *Chair*
Ana Huđek Turković, *Secretary*
Tena Čadež
Marko Gerić
Karla Jagić
Andreja Jurič
Anja Katić
Ena Oster
Blanka Tariba Lovaković
Antonio Zandona

Scientific Committee

Irena Brčić Karačonji, Institute for Medical Research and Occupational Health (Zagreb, Croatia), *Chair*
Biljana Antonijević, Faculty of Pharmacy, University of Belgrade (Belgrade, Serbia)
Vesna Benković, Faculty of Science, University of Zagreb (Zagreb, Croatia)
Petar Bulat, School of Public Health, University of Belgrade (Belgrade, Serbia)
Zrinka Dragun, Ruđer Bošković Institute (Zagreb, Croatia)
Ksenija Durgo, Faculty of Food Technology and Biotechnology, University of Zagreb (Zagreb, Croatia)
Goran Gajski, Institute for Medical Research and Occupational Health (Zagreb, Croatia)
Jasna Jurasović, Institute for Medical Research and Occupational Health (Zagreb, Croatia)
Nevenka Kopjar, Institute for Medical Research and Occupational Health (Zagreb, Croatia)
Daniel Mark Lyons, Ruđer Bošković Institute (Rovinj, Croatia)
Jelena Macan, Institute for Medical Research and Occupational Health (Zagreb, Croatia)
Mirta Milić, Institute for Medical Research and Occupational Health (Zagreb, Croatia)
Smiljana Milošev Tuševljak, Slovenian Society of Toxicology (Ljubljana, Slovenia)
Maja Peraica, Institute for Medical Research and Occupational Health (Zagreb, Croatia)
Alica Pizent, Institute for Medical Research and Occupational Health (Zagreb, Croatia)
Andreja Prevendar Crnić, Faculty of Veterinary Medicine, University of Zagreb (Zagreb, Croatia)
Arnes Rešić, Children's Hospital Zagreb (Zagreb, Croatia)
Sanja Stipičević, Institute for Medical Research and Occupational Health (Zagreb, Croatia)
Davorka Sutlović, School of Medicine, University of Split (Split, Croatia)
Bojan Šarkanj, University North (Koprivnica, Croatia)
Maja Šegvić Klarić, Faculty of Pharmacy and Biochemistry, University of Zagreb (Zagreb, Croatia)
Blanka Tariba Lovaković, Institute for Medical Research and Occupational Health (Zagreb, Croatia)
Ivona Vidić Štrac, Croatian Institute of Public Health (Zagreb, Croatia)
Bojana Žegura, National Institute of Biology (Ljubljana, Slovenia)
Suzana Žunec, Institute for Medical Research and Occupational Health (Zagreb, Croatia)
Irena Žuntar, Faculty of Pharmacy and Biochemistry, University of Zagreb (Zagreb, Croatia)

EDITORIAL

Dear Reader,

This Supplement to the *Archives of Industrial Hygiene and Toxicology* presents the abstracts of invited lectures, oral communications, and poster presentations of the 7th International Congress of the Croatian Society of Toxicology. This year marks a significant milestone in the history of our Congress. For the first time, it carries the designation *international* rather than *with international participation*. This change reflects not only the growth of our community but also the fulfilment of all criteria required by Croatian regulations for recognition as an international scientific congress. We are proud that CROTOX has reached this level of recognition, underscoring its expanding scope, global relevance, and enduring commitment to scientific excellence.

The subtitle *Expanding Horizons in Toxicology* has been chosen with care. It reflects not only the broadening thematic range of the Congress but also its growing international character. Toxicology is a field that is inherently global: environmental pollutants do not respect borders, industrial and agricultural practices have worldwide consequences, and human health is increasingly shaped by globalized exposure patterns. In this sense, our scientific discourse must transcend national perspectives and foster international collaboration.

The scientific programme of CROTOX 2025 reflects the diversity of toxicology today. It brings together invited lectures, oral presentations, and poster sessions covering a wide spectrum of subfields, incorporating clinical toxicology, risk assessment and regulatory science, food and environmental toxicology, occupational health, forensic toxicology, and the rapidly advancing areas of *in silico* modelling and alternative testing methods. The programme also includes nine *Young Scientist* lectures that give young researches the possibility to present their latest research results. Contributions addressing natural and foodborne toxins highlight the continuing importance of monitoring well-known contaminants such as mycotoxins, while research into bacterial toxins illustrates their significance as agents of potential misuse. At the same time, rapid advances in analytical techniques enable more precise identification of novel psychoactive substances, early detection of toxicants in biological samples, and improved pharmacovigilance, thus linking laboratory progress with direct clinical benefits. The environmental dimension of programme is equally prominent, with studies addressing the pervasive presence of micro- and nanoplastics, pesticide residues, toxic metal(oid)s, and emerging contaminants. Their implications extend from ecological integrity and aquatic biodiversity to subtle but lasting effects on child development and human reproductive health. Recent results regarding early biomarkers of genotoxicity and innovative *in vitro* approaches exemplify the role of toxicology in anticipating risks before they fully manifest. Finally, occupational exposures remain a pressing concern and the issue has been addressed by authors of several abstracts. Risk assessment is being refined, with more detailed exposure assessments, highlighting gender-specific vulnerabilities, consideration of mixtures, long-term low-dose exposures, vulnerable populations, and the use of new data types. Together, these contributions highlight the dual role of toxicology: to identify risks while also guiding safer practices, regulatory policies, and public health interventions.

The organization of a congress of this scope requires dedicated effort. I extend my sincere gratitude to the members of the Organizing and Scientific Committees for their invaluable work in shaping the programme, as well as to the journal *Archives of Industrial Hygiene and Toxicology* for publishing this Book of Abstracts. Above all, I acknowledge the contributions of all participants, whose research and engagement form the core of this Congress and ensure its continuing vitality.

Whether you are presenting your latest research, engaging in discussions, or simply drawing inspiration from the work of others, I hope this Congress provides you with valuable insights and new collaborations. Together, as a growing international community of toxicologists, we are indeed *expanding horizons* – advancing our science, strengthening our networks, and reinforcing our shared commitment to safeguarding health and the environment.

Guest Editor
Blanka Tariba Lovaković, PhD

WELCOME ADDRESS

Dear Friends and Colleagues,

It is my great pleasure and honour to welcome you, on behalf of the Croatian Society of Toxicology (CST), to the 7th International Congress of the Croatian Society of Toxicology, co-organized with the Institute for Medical Research and Occupational Health, and held under the auspices of the Ministry of Science, Education and the Youth of the Republic of Croatia.

This year's Congress takes place on the beautiful and happy island of Rab, a truly inspiring setting that blends natural charm with rich cultural heritage. We are confident that the tranquil surroundings and warm atmosphere will contribute to meaningful scientific exchange and memorable moments for all of the participants.

We are especially pleased that EUROTOX has recognized the value of this Congress and supported us, confirming the high scientific standard and international relevance of our gathering.

This year, we are joined by 130 participants, including eminent invited lecturers, scientists, and young scientists from Croatia and abroad, who will share their latest research through a dynamic program of oral presentations, discussions, and networking opportunities. As always, the Congress provides a platform for both experienced professionals and young scientists to exchange knowledge and strengthen collaboration.

We are proud to continue the tradition of supporting young scientists, as the winners of the Croatian Society of Toxicology Young Scientist Award will deliver oral presentations alongside leading experts in the field. This highlights our Society's strong commitment to supporting future leaders in toxicology.

The abstracts of all oral and poster presentations will be published in this Book of Abstracts as a supplement of the journal *Archives of Industrial Hygiene and Toxicology*, the official journal of the Croatian Society of Toxicology. I would like to express my sincere thanks to the Editor-in-Chief and the Editorial Board of the *Archives* for once again supporting CROTOX by accepting this publication. A special thank-you goes to the Guest Editor, who did an outstanding job in coordinating and preparing the abstracts for publication, contributing greatly to the scientific value of our Congress.

The success of this Congress is a result of the dedicated work of the Scientific and Organizing Committees, whose commitment to excellence is reflected in every aspect of the event. I am deeply grateful for their time, expertise, and enthusiasm. I would also like to extend my heartfelt thanks to our sponsors for their generous support.

On behalf of the Croatian Society of Toxicology and all members of the Organizing and Scientific Committees, I wish you a warm welcome and a stimulating and enjoyable congress experience.

President of the Congress
Dubravka Rašić, PhD

CONGRESS PROGRAMME

SUNDAY, 5 October 2025			
15:00–18:00	*Registration of participants*		
18:00–19:00	**Opening ceremony** Chairs: *Irena Brčić Karačonji, Maja Lazarus, Dubravka Rašić*		
19:00–19:45	IL 1	**Michael Burnet** *Tübingen, Germany*	The role of preliminary toxicology observations in early drug discovery – the need for predictive assays for new drug modalities
20:00	**Welcome reception** *(dinner&music)*		
MONDAY, 6 October 2025			
8:00–9:00	*Posters put up by presenters*		
SESSION 1 – BIOTHREATS & FOOD TOXINS Chairs: *Goran Gajski, Ana Hudek Turković*			
9:35–10:10	IL 3	**Dušan Mišić** *Wrocław, Poland*	Bacterial toxins as biological weapons
10:10–10:25	OP 1	**Aleksandra Daria Rajewska** *Wrocław, Poland*	Natural extracts as inhibitors of bacterial toxin production and the mechanisms of their inactivation
SESSION 2 – FROM ANALYTICS TO THE CLINIC *part 1* Chairs: *Željka Babić, Smiljana Milošev-Tuševljak*			
10:25–10:50	Gold Sponsor Presentation	**Jianru Stahl-Zeng** *SCIEX, Germany* *INEL – medicinska tehnika d. o. o.*	Simultaneous identification and quantitation of novel psychoactive substances (NPS) in human whole blood using liquid chromatography-tandem mass spectrometry (LC-MS/MS) technology
10:50–11:05	OP 2	**Tanja Živković Semren** Neuchâtel, Switzerland	Real-time exhaled breath analysis: investigating the dynamic nature of breath constituents
11:05–11:20	OP 3	**Mojca Dobaja Borak** Ljubljana, Slovenia	Snake venom snaclec *Vaa*-snaclec-3/2 induces reversible thrombocytopenia and prevents arterial thrombosis
11:20–12:00	*Poster viewing and coffee break sponsored by* ***Toxics***		

SESSION 2 – FROM ANALYTICS TO THE CLINIC *part 2* Chairs: *Arnes Rešić, Irena Žuntar*			
12:00–12:35	IL 4	**Elisabeth Varga** *Vienna, Austria*	Ichthyotoxins – from screening to exploring the mode of action
12:35–12:50	Silver Sponsor Presentation	**Borna Ferčec** *Altium International d. o. o.*	Altium per- and polyfluoroalkyl substance (PFAS) workflow solutions
12:50–13:05	OP 4	**Zrinka Franić** *Zagreb, Croatia*	Carbon monoxide poisonings reported in the Croatian Poison Control Centre in a ten year period
13:05–13:20	OP 5	**Ines Potočnjak** *Zagreb, Croatia*	Reported adverse drug reactions (ADRs) of semaglutide: assessing safety signals amid increasing off-label use – a pharmacovigilance analysis of the EudraVigilance database
13:20–14:45	*Lunch break*		
SESSION 3 – ECO HAZARDS Chairs: *Daniel Mark Lyons, Andreja Prevendar Crnić*			
14:45–15:20	IL 5	**Davor Šakić** *Zagreb, Croatia*	Chlorinated nucleobases: an unexpected (eco)toxicological profile
15:20–15:55	IL 6	**Marta Sendra** *Seville, Spain*	Using zebrafish (*Danio rerio*) to uncover the mechanisms of nanoplastics interaction and toxicity
15:55–16:45	*Poster viewing and coffee break sponsored by* ***Toxics***		
SESSION 4 – YOUNG SCIENTIST AWARDS Chairs: *Vesna Benković, Nevenka Kopjar*			
16:45–17:00	YSL 1	**Tim Ravnjak** *Ljubljana, Slovenia*	Cellular responses to nuclear receptor-activating chemicals in HepG2 spheroids: effects on viability, proliferation, and genotoxicity
17:00–17:15	YSL 2	**Iza Rozman** *Ljubljana, Slovenia*	Toward the safer use of nanomaterials: (geno)toxicity evaluation of ferrite-based nanoparticles in HepG2 spheroids
17:15–17:30	YSL 3	**Lucija Marcelić** *Zagreb, Croatia*	Effects of *N*-alkyl quaternary quinuclidines on oxidative stress biomarkers in SH-SY5Y cells
17:30–17:45	YSL 4	**Petra Tuksar** *Zagreb, Croatia*	Novel cholesterol- and quinoline-based oximes as potential antidotes for organophosphate nerve agent poisoning
17:45–18:00	YSL 5	**Jovana Živanović** *Belgrade, Serbia*	Disruption of ovarian redox balance by bisphenol mixtures: a comparison with individual exposures
18:00–18:15	YSL 6	**Mateo Jakac** *Pula, Croatia*	Cyto/genoprotective effects of propolis *in vitro*
18:15–18:30	YSL 7	**Jovan Baljak** *Novi Sad, Serbia*	Toxicological and clinical aspects of paediatric drug poisoning in Vojvodina, Serbia
18:30–18:45	YSL 8	**Zoran Kiralj** *Zagreb, Croatia*	The impact of abandoned coal mines on water and sediment pollution in the Mura River
18:45–19:00	YSL 9	**Anita Tarandek** *Zagreb, Croatia*	Effects of pharmaceuticals and personal care products on the fitness of freshwater fauna: a systematic review and meta-analysis
19:00–20:00	*Posters taken down by presenters*		

TUESDAY, 7 October 2025			
SESSION 5 – ENVIRONMENTAL MONITORING & CONTAMINANTS Chairs: *Sanja Stipičević, Blanka Tariba Lovaković, Suzana Žunec*			
9:00–9:35	IL 7	**Ivan Senta** *Zagreb, Croatia*	Municipal wastewater – a valuable source of epidemiological information
9:35–10:10	IL 8	**Darija Klinčić** *Zagreb, Croatia*	Brominated flame retardants – insights into environmental distribution and risks
10:10–10:25	OP 6	**Vlatka Buzjak Služek** *Osijek, Croatia*	Exposure assessment of children to lead from different types of food in Croatia
10:25–10:40	OP 7	**Antonija Sulimanec** *Zagreb, Croatia*	From market to table: fungicide residues in total diet samples in the Zagreb region
10:40–10:55	OP 8	**Marija Macan** *Zagreb, Croatia*	Association of dietary exposure to pyrethroid and organophosphate insecticides with gonadotropins, testosterone, and dehydroepiandrosterone sulphate in primary school boys
10:55–11:10	OP 9	**Maria de la Cruz Gomez Pellin** *Alicante, Spain*	Adipose tissue cadmium levels and reduced PON1 activity in serum: clinical implications from the GraMo cohort
11:10–11:25	OP 10	**Patricia Tomac** *Zagreb, Croatia*	Neuropsychology, toxicology, and human development: neurobehavioral effects of insecticide exposure during puberty and adolescence
11:25–12:00	*Coffee break*		
SESSION 6 – GENOTOXIC SIGNALS Chairs: *Mirta Milić, Bojana Žegura*			
12:00–12:35	IL 9	**Nancy B. Hopf** *Lausanne, Switzerland*	Micronuclei as early warning signals: strengthening exposure science for protecting human populations
12:35–13:10	IL 10	**Goran Gajski** *Zagreb, Croatia*	Spotlight on radiation: tracking its effects in children with blood and buccal micronucleus assays
13:10–13:25	OP 11	**Martina Štampar** *Ljubljana, Slovenia*	Automated quantitative fluorescence microscopy and image feature analysis for high content *in vitro* toxicity screening
13:25–13:40	OP 12	**Metka Filipič** *Ljubljana, Slovenia*	Genotoxicity of nicotine and tobacco products – a review of the available evidence
13:40–15:00	*Lunch break*		
15:30	**City tour**		
20:00	*Congress dinner (&music)*		
WEDNESDAY, 8 October 2025			
SESSION 7 – AQUATIC HAZARDS Chairs: *Zrinka Dragun, Maja Lazarus*			
9:00–9:35	IL 11	**Petra Burić** *Pula, Croatia*	Anthropogenic pollutants and the modern environmental burden: insights from sea urchin toxicity studies

9:35–10:10	IL 12	**Natalija Topić Popović** *Zagreb, Croatia*	Vulnerability of fish to treated sewage effluents
10:10–10:25	OP 13	**Marijana Erk** *Zagreb, Croatia*	Seasonal and environmental modulation of anaerobic strategies in *Synurella ambulans* from the hyporheic zone of the Sava River
10:25–11:00	*Coffee break*		
SESSION 8 – OCCUPATIONAL TOXICOLOGY Chairs: *Biljana Antonijević, Jelena Macan*			
11:00–11:35	IL 13	**Francesca Larese Filon** *Trieste, Italy*	Reprotoxics in workplaces: focus on gender differences
11:35–11:50	OP 14	**Jelena Macan** *Zagreb, Croatia*	Guidelines on workplace risk assessments for pregnant workers exposed to chemical hazards
11:50–12:05	OP 15	**Željka Babić** *Zagreb, Croatia*	Reprotoxic risks in the hairdressing profession: efforts to protect worker health
12:05–12:40	IL 14	**Dražen Lušić** *Rijeka, Croatia*	Unveiling honey origin and authenticity: a comprehensive perspective
12:40–13:00	**Closing ceremony**		

CONGRESS ABSTRACTS

Opening lecture

IL 1

The role of preliminary toxicology observations in early drug discovery – the need for predictive assays for new drug modalities

Michael Burnet and Julian Gotthard

Synovo GmbH, Tübingen, Germany
michael.burnet@synovo.com

Current drug discovery is characterised by the proliferation of agents with diverse modalities: nucleic acids being amongst the fastest growing [mRNA, short interfering RNA (siRNA), microRNAs (miRNAs), antisense oligonucleotides (ASOs), conjugated ASOs, lipid nanoparticles (LNPs), replicating RNAs, *in vivo* chimeric antigen receptor (CAR) constructs], but closely followed by multi-targeted glucagon-like peptide-1 (GLP-1) ligands, cellular therapies like CAR-T cells with bivalent constructs, various antibody formats, and combinations of the above. The pre-clinic is likewise changed in that much discovery is via under-funded biotechs and ideas of toxicology, translation, pharmacokinetics, and disease modelling are quite different *vs* small molecule dominated pipelines. Many originators are screening in engineered cells and transitioning relatively late to highly engineered humanised mouse strains where-almost all aspects of a drug's effect should be observed in parallel with toxicity considered unlikely and a rapid transition to non-human primates (NHPs) expected by investors. This leaves little opportunity to gain experience with constructs and thus, the preclinic has to make the most of any data that emerges from any model. Organ distribution in small animals is considered translatable to human settings and this may be of particular interest in early information requirements, especially off-target expression. Inflammatory immune responses are the most relevant of the possible toxicological end-points and this means that in any exposure in the *in vivo* setting, even minor immune excursions can be considered in the overall assessment of the materials in test. Typical is gene expression in small animal models and multi-donor human peripheral blood mononuclear cells (PBMCs), paired with multiplex data on the actual level of signalling molecules and cytokines. Clinical chemistry and behaviour complete all studies.

This study was funded by Synovo in the normal course of its business operations.

KEY WORDS: ASOs behaviour; diet; immune responses; metabolic profiling; nucleic acid drugs

Biothreats & Food Toxins

IL 2

Deoxynivalenol: a new effect of an old toxin

Isabelle P. Oswald

University of Toulouse, Toxalim Research Centre in Food Toxicology, INRAE, ENVT, INP-Purpan, UPS, Toulouse, France
isabelle.oswald@inrae.fr

Mycotoxins are the most prevalent natural dietary toxins that contaminate up to 70 % of global crop production. They represent a major issue for food safety. These secondary metabolites, produced by microscopic fungi, resist industrial processes and cooking, and contaminate finished processed food. Among mycotoxins, deoxynivalenol (DON) is a trichothecene mycotoxin produced by *Fusarium* species, commonly contaminating cereals and animal feeds. Its main effects are both acute and chronic, impacting multiple physiological systems. The main effect of DON is to cause gastrointestinal distress, suppress feed intake and growth, and disrupt immune and intestinal function through mechanisms involving oxidative stress, inflammation, and neuroendocrine signalling. The primary molecular target of DON is the ribosome; leading to ribotoxic stress. DON is not genotoxic on its own, but we recently described that this toxin is capable of increasing the genotoxicity induced by multiple compounds. Indeed, we observed that such an effect is present with several drugs with different modes of action, but also by captan, a food-contaminating pesticide, by colibactin, a genotoxin produced by the *Escherichia coli* bacteria in the gut, and by acrylamide, a compound formed during cooking processes and commonly found in fried foods and cereal products. The genotoxicity exacerbation is not only an effect caused by DON, but also caused by T-2, DAS, NIV, FX, and NX. This effect is mediated by the ability of TCT to bind to the ribosome as (i) it is observed with other ribosome inhibitors but (ii) not observed with de-epoxy-deoxynivalenol (DOM-1), a modified form of DON that does not induce ribotoxic stress. The association between dietary exposure to DON and hepatocellular cancers was further investigated in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. EPIC questionnaire data were matched to mycotoxin food occurrence data compiled by the European Food Safety Authority to assess long-term dietary mycotoxin exposure and then relate them to the risk of hepatocellular carcinoma (HCC). Analyses were conducted using multivariable Cox proportional hazards regression models, indicating that intake of DON and its derivatives was positively associated with HCC risk. Taken together, these data question the current classification of DON as "not classifiable as to its carcinogenicity to humans" and indicate that more data is needed to fully understand the toxicity of DON.

KEY WORDS: food safety; *Fusarium* species; genotoxicity; mycotoxins; ribotoxic stress

Arh Hig Rada Toksikol 2025;76(Suppl. 1):27–29

IL 3

Bacterial toxins as biological weapons

Dušan Mišić

Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland
dusan.misic@upwr.edu.pl

Bioterrorism implies the use of living agents – bacteria, viruses, fungi, or bacterial toxins – in order to cause illness or death in humans, animals, or plants. Despite the international laws and conventions prohibiting the use and development of biological weapons, it has been proven that all major global military powers continue to experiment with biological agents, and that there exists a constant risk of a bioterrorist attack wherever one may live. This is why various organizations such as the European Food Safety Authority (EFSA), Centers for Disease Control and Prevention (CDC), World Health Organization (WHO), European Centre for Disease Prevention and Control (ECDC), Food and Agriculture Organization (FAO), *Office International des Epizooties* (OIE) [now World Organisation for Animal Health (WOAH)], and Food and Drug Administration (FDA), provided detailed strategies as well as laboratory protocols to recognize and quickly respond to any biological attack, to prevent it, and to mitigate the consequences. The botulinum toxin, produced by *Clostridium botulinum*, is among the most researched toxins for bioterrorism purposes. It is a neurotoxin, causing flaccid paralysis, enlisted by both the Biological and Chemical Weapons Conventions (BWC, 1972; CWC, 1993) and it is on the CDC's "A" list of potential bioterrorist agents. Also, the Shiga toxin and Shiga-like toxins produced by *Shigella dysenteriae* and STEC *Escherichia coli*, which cause haemorrhagic colitis with potentially fatal hepato-uremic syndrome, as well as *Staphylococcus aureus* enterotoxin B (superantigen causing cytokine storm and toxic shock), are listed as potential biological weapons. Tetanospasmin, produced by *Clostridium tetani*, has also been studied as a possible aerosol toxin potentially causing respiratory paralysis in affected people. The use of all of the above listed toxins as biological weapons has been proven during recent history.

KEY WORDS: bacterial toxins; biological terrorism; botulinum toxin; *S. aureus* enterotoxin B; Shiga toxin

OP 1

Natural extracts as inhibitors of bacterial toxin production and the mechanisms of their inactivation

Aleksandra Daria Rajewska and Dušan Mišić

University of Environmental and Life Sciences, Faculty of Biotechnology and Food Sciences,
Department of Functional Food Products Development, Wroclaw, Poland
dusan.misic@upwr.edu.pl

Pathogenic bacteria are capable of producing harmful toxins, which play a crucial role in foodborne diseases. These toxins are not always effectively inactivated by conventional food processing methods such as cooking or pasteurization. Although antibiotics have traditionally been used to treat microbial diseases, antibiotics are limited in their action on already secreted toxins in the body of infected individuals. In addition, antibiotics and biocides are not allowed to be added to food in order to preserve food from the possible appearance of toxins. Therefore, alternative and safe strategies that limit bacterial toxin synthesis are urgently needed. Since microbial toxins play an important role in pathogenicity, they are appealing targets for prevention strategies. Natural molecules or plant extracts that have been proven safe for humans and are approved for use in the food industry are very interesting subjects for studying the possibility of neutralizing bacterial toxins. This review summarizes recent studies investigating natural compounds as inhibitors of bacterial toxins, with a focus on staphylococcal enterotoxins and Shiga toxins produced by *Escherichia coli* O157:H7. It discusses the associated illnesses, their prevalence and current treatment challenges, as well as the effectiveness of natural inhibitors. Representative molecules such as luteolin, eugenol, and cinnamaldehyde exhibit diverse mechanisms of action, including blocking toxin internalization and disrupting regulatory networks that govern virulence. Studies unraveling these anti-toxigenic mechanisms highlight the potential of natural compounds as innovative tools for reducing the pathogenic impact of bacterial toxins and contributing to the development of alternative strategies in food safety and clinical therapy.

KEY WORDS: antivirulence; cinnamaldehyde; eugenol; foodborne diseases; luteolin

From Analytics to the Clinic

IL 4

Ichthyotoxins – from screening to exploring the mode of action

Hélène-Christine Prause[1,2], Magdalena Pöchhacker[1,2,3], Zsuzsanna Neer[3,4], and Elisabeth Varga[3]

[1] University of Vienna, Faculty of Chemistry, Department of Food Chemistry and Toxicology, Vienna, Austria
[2] University of Vienna, Faculty of Chemistry, Vienna Doctoral School in Chemistry (DoSChem), Vienna, Austria
[3] University of Veterinary Medicine Vienna, Clinical Department for Farm Animals and Food System Science, Centre for Food Science and Veterinary Public Health, Unit Food Hygiene and Technology, Vienna, Austria
[4] Eötvös Loránd University, Faculty of Science, Department of Microbiology, Budapest, Hungary
elisabeth.varga@vetmeduni.ac.at

Ichthyotoxins within the context of this study are defined as compounds produced by microalgae that have a negative impact on fish. A general overview is provided, introducing the audience to harmful algal bloom forming species in different water bodies (fresh, brackish, and marine). Upcoming analytical challenges as well as an insight into their mode of action are presented based on our previous work. Finally, future challenges and perspectives are pointed out. The focus is placed on the haptophyte-microalga *Prymnesium parvum*, one of the better studied organisms. Its causative agents have previously been identified to be prymnesins, a group of over 50 different polyketide compounds. Currently, the strains are divided into A-, B-, and C-type producers based on the length of the carbon-backbone. Since no pure compounds are currently available, toxicological evaluations are based on investigating partly purified extracts. Using an *in vitro* test system of epithelium rainbow trout (*Oncorynchus mykiss*) gill cells (RTgill-W1), different end point assays were performed herein. These included assessing the metabolic activity, as well as cytotoxic and lytic properties. Potency ranked A>C>B-types with effective concentrations in the low to middle nmol/L range after just 3 h of incubation. The haemolytic potency in red blood cells from the Atlantic salmon (*Salmo salar*) reflected their cytotoxic potential. Further investigations showed the induction of membrane integrity changes, as well as chromatin condensation and nuclear fragmentation by *P. parvum* extracts. Moreover, the cellular redox status was affected.

This study was funded in part by the Austrian Science Fund (grant DOI:10.55776/I5707). Hélène-Christine Prause was funded by the University of Vienna, Faculty of Chemistry, Zsuzsanna Neer received a stipend from the Eötvös Loránd University. The project on which this presentation is based was partly funded by the German Federal Ministry of Education and Research (BMBF) within the Research Initiative for the Conservation of Biodiversity (FEdA) under the funding code 16LW0561k.

KEY WORDS: *Alexandrium* spp.; *Karlodinium armiger*; mass spectrometry; *Prymnesium parvum*; RTgill-W1

OP 2

Real-time exhaled breath analysis: investigating the dynamic nature of breath constituents

Tanja Živković Semren, Iris Legbre, Paul-Alain Singh Kalra, Philippe Alexandre Guy, and Diego Marescotti

PMI R&D, Philip Morris Products S.A., Neuchâtel, Switzerland
tanja.zivkovicsemren@pmi.com

Exhaled breath is a valuable biological matrix due to the presence of volatile organic compounds (VOCs), which can provide insights into biochemical processes occurring in the body. The non-invasive collection and unlimited availability of breath make it an attractive medium for analysis. However, some human activities performed prior to breath analysis can significantly alter the VOC composition in exhaled breath, necessitating a standardised protocol for breath sample collection. Secondary electrospray ionization coupled with high-resolution mass spectrometry (SESI-HRMS) is a powerful technology that enables real-time detection of VOCs and kinetic change monitoring. Our study focuses on the kinetics of VOCs in exhaled breath following brushing teeth with toothpaste. For this purpose, we recruited five volunteers and analysed their exhaled breath using SESI-HRMS to evaluate the influence of brushing teeth on the VOCs present in exhaled breath. Results showed significant changes in the chemical composition of exhaled breath, confirming the presence of distinct compounds in exhaled breath post-brushing. The monitoring of distinct compounds during the 30 minutes post-brushing and their subsequent decrease showcased the dynamic nature of breath constituents and washout patterns in the respiratory tract. These findings underscore the importance of considering pre-analytical variables in breath analysis protocols. In conclusion, SESI-HRMS technology offers a robust approach for real-time exhaled breath analysis. By emphasizing the kinetic aspects of VOCs, we aim to improve the accuracy and applicability of breath analysis in research settings.

This study was funded and sponsored by Philip Morris Products S.A.

KEY WORDS: breath kinetics; exhaled breath; SESI-HRMS; teeth brushing; volatile organic compounds

Arh Hig Rada Toksikol 2025;76(Suppl. 1):30–34

OP 3

Snake venom snaclec *Vaa*-snaclec-3/2 induces reversible thrombocytopenia and prevents arterial thrombosis

Mojca Dobaja Borak[1,2], Adrijana Leonardi[3], Kity Požek[2,3], Damjan Grenc[1], Tihana Kurtović[4], Beata Halassy[4], Katarina Reberšek[5], Helena Podgornik[5,6], Alenka Trampuš Bakija[7], Monika C. Žužek[8], Robert Frangež[8], Igor Križaj[3], and Miran Brvar[1,9]

[1] University Medical Centre Ljubljana, Centre for Clinical Toxicology and Pharmacology, Ljubljana, Slovenia
[2] University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia
[3] Jožef Stefan Institute, Department of Molecular and Biomedical Sciences, Ljubljana, Slovenia
[4] University of Zagreb, Centre for Research and Knowledge Transfer in Biotechnology, Zagreb, Croatia
[5] University Medical Centre Ljubljana, Department of Haematology, Ljubljana, Slovenia
[6] University of Ljubljana, Faculty of Pharmacy, Department of Clinical Biochemistry, Ljubljana, Slovenia
[7] University Medical Centre Ljubljana, Division of Pediatrics, Ljubljana, Slovenia
[8] University of Ljubljana, Veterinary Faculty, Institute of Preclinical Sciences, Ljubljana, Slovenia
[9] University of Ljubljana, Faculty of Medicine, Center for Clinical Physiology, Ljubljana, Slovenia
mojca.dobaja@kclj.si

In Slovenia, the nose-horned viper, *Vipera a. ammodytes* (*Vaa*), is the most medically important snake species. Envenomation may lead to severe thrombocytopenia, but no platelet aggregation agonists have yet been identified in *Vaa* venom. We hypothesized that snake C-type lectin-like proteins (snaclecs) contribute to this pathological effect. The aim of this study was therefore to isolate and characterize *Vaa* snaclecs and investigate their role in thrombocytopenia and thrombosis. We isolated snaclecs from crude *Vaa* venom using a combination of liquid chromatography techniques. Protein purity was confirmed by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). The effects of isolated snaclecs on platelets were assessed using turbidometry. Platelet receptor binding and activation were evaluated by flow cytometry. Platelet functionality in patients with *Vaa* envenomation and thrombocytopenia was analysed by thromboelastometry before and after treatment with $F(ab')_2$ antivenom fragments. *In vivo* effects were tested in a mouse model, where *Vaa*-snaclec-3/2 was administered and $FeCl_3$-induced carotid artery occlusion was used to evaluate antithrombotic activity. *Ex viv*o analyses revealed that *Vaa*-snaclec-3/2 binds to the GPIb platelet receptor and induces platelet agglutination, leading to a reduction in platelet count. The process was reversible, and platelets remained functionally competent. *Vaa*-snaclec-3/2 was found to be an acidic, non-glycosylated 30 kDa protein, composed of two subunits: *Vaa*-snaclec-3 (α) and *Vaa*-snaclec-2 (β). In patients with *Vaa* envenomation, thrombocytopenia resolved within one hour of $F(ab')_2$ antivenom therapy. In mice, administration of *Vaa*-snaclec-3/2 induced marked thrombocytopenia and effectively prevented $FeCl_3$-induced carotid artery occlusion. Our findings highlight the potential of *Vaa*-snaclec-3/2 as a safe and effective antithrombotic agent.

This study was funded by the following grants: UIP-2020-02-1317 (T.K.); ARRS-J3-2534 (M.B., I.K.); ARRS-P3-0019 (M.B.), ARRS-P1-0207 (I.K.), ARRS P4-0053 (MCŽ, FR).

KEY WORDS: antithrombotic; arterial occlusion; interventional cardiology; mice; nose-horned viper

OP 4

Carbon monoxide poisonings reported in the Croatian Poison Control Centre in a ten-year period

Zrinka Franić, Željka Babić, and Jelena Macan

Institute for Medical Research and Occupational Health, Division of Occupational and Environmental Health, Croatian Poison Control Centre, Zagreb, Croatia
zfranic@imi.hr

Carbon monoxide (CO) is an odourless, colourless gas that can cause sudden illness and death if inhaled. Every year, a part of the calls in the Croatian Poison Control Centre (CPCC) refers to CO poisonings, which can be life-threatening. A retrospective review of CO poisonings during a ten-year period (2015–2024) reported by telephone consultations with the CPCC was performed with the aim of understanding the characteristics of CO poisonings better. A total of 117 cases were recorded (56.8 % involved males). In 89.7% of cases, callers were healthcare providers. Half of the patients (51.2 %) were children under 18 years (median age 9.8 years) and 20 % of all cases involved children under 5 years. The majority of exposures (75.2 %) occurred in autumn and winter (from October to February). The majority of the poisonings (84.6 %) were unintentional, 11.9 % were occupational, and four cases were suicide attempts. Only 9.4 % of the patients were without symptoms, 70 % had minor or moderate symptoms, and 17.9 % severe symptoms at the time of the call. Occupational poisonings were mostly with mild symptoms and 64.2 % involved male workers. During the analysed period, the number of calls more than doubled per year (8 calls in 2015 *vs* 19 calls in 2024). It is alarming that half of the cases involved children, that 90 % of the cases were symptomatic, with the raising time-trend of CO poisonings. This highlights the need for warning the population more frequently, especially during winter months, and education regarding the dangers and prevention of CO poisoning.

KEY WORDS: child poisoning; occupational exposure; prevention; unintentional poisoning

OP 5

Reported adverse drug reactions (ADRs) of semaglutide: assessing safety signals amid increasing off-label use – a pharmacovigilance analysis of the EudraVigilance database

Ines Potočnjak[1,2] and Andrej Belančić[3]

[1] Sestre Milosrdnice University Hospital Center, Zagreb, Croatia
[2] Catholic University of Croatia, School of Medicine, Zagreb, Croatia
[3] University of Rijeka, Faculty of Medicine, Department of Basic and Clinical Pharmacology with Toxicology, Rijeka, Croatia
ines.potocnjak@yahoo.com

Semaglutide is a glucagon-like peptide-1 (GLP-1) receptor agonist indicated for the treatment of patients with inadequately controlled diabetes mellitus type 2, as well as for obesity or overweight in the presence of weight-related comorbidities. As recently reported, the annual number of serious adverse drug reactions (ADRs) reports for semaglutide (subcutaneous injection) has continued to rise, with a 67.1 % year-on-year increase, underscoring the scale of exposure and raising concern over safety in real-world use, particularly given the growing prevalence of off-label prescribing. This analysis aimed to evaluate the safety profile of semaglutide using data available in the European database of suspected ADR reports (EudraVigilance database). A total of 38,829 individual cases were identified. Most cases involved female patients (60.2 %). Healthcare professionals were the reporters in 53 % of cases. The most frequently reported ADRs involved gastrointestinal disorders, followed by injury, poisoning and procedural complications, general disorders, and administration site conditions. There was a notably high number of cases (9,436) reporting injury, poisoning, and procedural complications. Out of these, 6,407 were female, 21 fatal cases, and 797 not recovered/not resolved. The use of semaglutide is not without risk, especially if off-label. This is evidenced by the substantial number of ADRs reported after marketing authorisation. It is crucial to emphasise the need for rigorous ADR monitoring, responsible prescribing, and heightened awareness of the potential harms associated with widespread off-label use, particularly in populations seeking weight loss for non-medical or cosmetic reasons.

KEY WORDS: gastrointestinal disorders; glucagon-like peptide-1; patient safety; subcutaneous injection

Eco Hazards

IL 5

Chlorinated nucleobases: an unexpected (eco)toxicological profile

Davor Šakić[1], Antonio Ljulj[1], Lea Ulm[2], and Valerije Vrček[1]

[1] University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia
[2] Andrija Štampar Teaching Institute of Public Health, Zagreb, Croatia
davor.sakic@pharma.unizg.hr

Chlorinated derivatives of nucleobases are raising new concerns due to their unexpected genotoxic and ecotoxic effects. While halogenated analogues such as 5-fluorouracil (5-FU) are established antineoplastic agents, the impact of chlorination on the degradation pathways and toxicity of nucleobases remains poorly understood. In this study, we combined *in situ* chlorination, nuclear magnetic resonance spectroscopy (NMR), density functional theory (DFT) calculations, and *Daphnia magna* bioassays to investigate chlorination sites, rearrangement reactions, and (eco)toxicological profiles of uracil, thymine, 5-FU, 5-chlorouracil (5-ClU), 6-FU, and 6-ClU. Our findings revealed significant structural and biological differences introduced by chlorination, underscoring the need to reassess the environmental impact of these compounds and monitor their occurrence and transformation in aquatic systems.

This study was funded by the Croatian Science Foundation (grant HRZZ-IP-2022-10-2634).

KEY WORDS: bioassay; chlorination; density functional theory; NMR; pharmaceuticals

IL 6

Using zebrafish (*Danio rerio*) to uncover the mechanisms of nanoplastics interaction and toxicity

Marta Sendra[1], Patricia Pereiro[2], Beatriz Novoa[2], Antonio Figueras[2], and Bojana Žegura[1]

[1] National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, Ljubljana, Slovenia
[2] Institute of Marine Research IIM-CSIC, Vigo, Spain
marta.sendra@nib.si

Human exposure to nanoplastics (NPs) is an emerging health concern, yet the biological effects of NPs sizes and exposure routes remain poorly understood. To address this gap, we used zebrafish (*Danio rerio*) embryos, a 3Rs-compliant vertebrate model aligned with European regulatory directives. Zebrafish embryos are ethically acceptable for experimentation with high translational value. Their optical transparency and rapid development enable real-time *in vivo* visualization of NP biodistribution and cellular responses at single-cell resolution. This study evaluated the biodistribution of 50 nm and 1 μm polystyrene nanoplastics (PS-NPs) at environmentally and human-relevant concentrations administered through four distinct exposure routes: bath immersion dermal deposition, nutrient uptake, and gametal transmission. Using the embryo stage, we observed that particle accumulation was strongly dependent on exposure route: The 50 nm nanoplastics preferentially localized in yolk-intestines and vasculature, whereas the 1 μm particles were predominantly retained in dermal and pericardial regions. Gametal exposure resulted in widespread distribution during early development, highlighting a potential transgenerational transfer. Functional analyses with transgenic fish lines revealed that macrophages were preferentially recruited to NP-laden tissues (below 6 %), while neutrophil activation was modest with minimal colocalization. Vascular transgenics demonstrated that the PS-NPs tested induced subtle alterations in vessel branching and intersegmental vessels. Altogether, these findings provide evidence that both NPs and exposure route determine biodistribution, immune cell responses, and vascular development. Zebrafish embryos provide a powerful, 3Rs-aligned system to dissect NPs toxicology *in vivo*, offering mechanistic insights into immune and vascular interactions to advance the development of functional new approach methodologies (NAMs) for European risk assessment frameworks.

This study was funded by the HORIZON-WIDERA-2024-TALENTS-02-01 (ENLIVEN project; 101244206) and the Slovenian Research and Innovation Agency (ARIS) through the Strategic Project (NanoBreak; STR-0001) and program P1-0245.

KEY WORDS: 3Rs; animal model; health; immunotoxicity; uptake routes

Environmental Monitoring & Contaminants

IL 7

Municipal wastewater – a valuable source of epidemiological information

Ivan Senta, Karlo Jambrošić, Tin Županović, Ivona Krizman Matasić, Marijan Ahel, and Senka Terzić

Ruđer Bošković Institute, Zagreb, Croatia
isenta@irb.hr

Municipal wastewater is not only a waste stream and one of the most important sources of environmental pollution, but also a unique source of valuable information on the epidemiological status of the population. Raw municipal wastewater can be considered as a diluted urine (and faeces) sample containing biomarkers of almost everything we consume or are exposed to. The approach by which one obtains epidemiological information based on the analysis of specific human biomarkers in wastewater is called wastewater-based epidemiology (WBE) and is increasingly used to assess the consumption of illicit drugs, but also certain legal substances, such as alcohol, nicotine, and pharmaceuticals. On the other hand, few studies have investigated the potential of WBE to assess the dietary habits of the population. The project "Sewage chemical information mining to assess lifestyle and dietary habits in Croatian regions" focuses on the development and application of analytical methods, based on liquid chromatography-tandem mass spectrometry (LC-MS/MS), to determine a larger number of WBE biomarkers from different categories. These include alcohol, nicotine, caffeine, artificial sweeteners, various nutrients, and several groups of commonly abused pharmaceuticals, such as anti-anxiolytics, hypnotics, and sedatives, as well as erectile dysfunction drugs. The methods will be applied to study the spatial and temporal trends of lifestyle and dietary habits in six Croatian regions using the WBE approach. The presentation will introduce the general concept of WBE, as well as the analytical challenges and recent applications of the WBE approach in the assessment of lifestyle and dietary habits in Croatia.

This work was supported by the Croatian Science Foundation under projects number IP-2022-10-5332 and DOK-NPOO-2023-10-7882.

KEY WORDS: biomarkers; dietary habits; drugs; lifestyle; wastewater-based epidemiology

Arh Hig Rada Toksikol 2025;76(Suppl. 1):37–43

IL 8

Brominated flame retardants – insights into environmental distribution and risks

Darija Klinčić

Institute for Medical Research and Occupational Health, Zagreb, Croatia
dklincic@imi.hr

Brominated flame retardants (BFRs) are synthetic additives extensively used in consumer products such as furniture, electronics, textiles, and building materials to reduce flammability. Because they are not chemically bound to products, BFRs can easily migrate into the environment through volatilization, abrasion, and leaching. This has led to their widespread presence in environmental media – air, dust, soil, sediment, and biota – and ultimately in humans. Human exposure primarily occurs through contaminated food and indoor dust. Among BFRs, polybrominated diphenyl ethers (PBDEs) have been of particular concern due to their persistence, lipophilicity, bioaccumulative properties, and adverse health effects, including endocrine disruption and neurodevelopmental toxicity. As a result, three commercial PBDE mixtures have been listed under the Stockholm Convention on Persistent Organic Pollutants, and their use has been banned in the EU since 2004 (penta-, octa-BDE) and 2008 (deca-BDE). In Croatia, no systematic data on human PBDE exposure was available. To address this, we applied highly sensitive analytical methods to assess PBDE concentrations in both environmental and human matrices. Indoor dust was analysed as a key reservoir and exposure source. Paired dust and breast milk samples were collected to assess exposure in nursing mothers and their infants, allowing investigation of internal exposure in vulnerable populations. We extended this work to include PBDE levels in indoor air, human blood, and selected wildlife species of ecological and conservation interest. These findings provide the first national baseline for PBDE exposure in Croatia and support future environmental health surveillance of both legacy and emerging flame retardants.

KEY WORDS: BFRs; health risk assessment; human milk; indoor dust; polybrominated diphenyl ethers

OP 6

Exposure assessment of children to lead from different types of food in Croatia

Vlatka Buzjak Služek[1], Sanja Miloš[1], Jasna Bošnir[2], Ivana Kmetič[3], Andreja Prevendar Crnić[4], and Dražen Knežević[1]

[1] Croatian Agency for Agriculture and Food, Osijek, Croatia
[2] Teaching Institute for Public Health "Dr. Andrija Štampar", Zagreb, Croatia
[3] University of Zagreb, Faculty of Food Technology and Biotechnology, Zagreb, Croatia
[4] University of Zagreb, Faculty of Veterinary Medicine, Zagreb, Croatia
vlatka.buzjak.sluzek@hapih.hr

Exposure assessment to lead for three different age groups of children [infants (3 months to <1 year), toddlers (1 to <3 years), other children (3–10 years)] was conducted based on individual food consumption data and lead concentrations in food. Food consumption data were obtained from the Croatian National Food Consumption Survey 2017–2021. Lead concentrations in food were derived from results of the national monitoring program 2020–2023. For both data sets, food was divided into groups according to EFSA's FoodEx2 classification. Mean exposure of infants based on middle bound approach was 0.1633 μg/kg b.w./day, for toddlers 0.2860 μg/kg b.w./day, and for other children 0.2027 μg/kg b.w./day. The margin of exposure approach (MOE) was applied to assess the risk of lead to children's health. MOE was calculated for a benchmark dose ($BMDL_{10}$) of 0.5 μg/kg b.w./day for developmental neurotoxicity in young children. The MOE for infants was 3.06, for toddlers 1.75, and for other children 2.47, indicating very low risk. "Fruit and fruit products" and "milk and dairy products" contributed the most to dietary lead exposure of toddlers and other children, while "food products for young population" and "fruit and fruit products" were the highest contributors for infants. Statistically significant differences in exposure to lead between infants, toddlers, other children were determined using Kruskal-Wallis test and two-sample Mann-Whitney U test ($p<0.0001$). The Mann-Whitney U test showed no statistically significant difference in exposure between genders for all age groups of children (infants $p=0.5170$, toddlers $p=0.8270$, other children $p=0.2349$).

KEY WORDS: dietary exposure; food consumption; lead occurrence; MOE; risk assessment

Arh Hig Rada Toksikol 2025;76(Suppl. 1):37–43

OP 7

From market to table: fungicide residues in total diet samples in the Zagreb region

Antonija Sulimanec[1], Marija Macan[1], Andreja Jurič[1], Adriana Gustovarac[1], Jim Garvey[2], Jelena Kovačić[1], and Veda Marija Varnai[1]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] Department of Agriculture, Food and the Marine, Food Chemistry Division, Celbridge, Ireland
asulimanec@imi.hr

This work aimed to report on fungicide residues in food samples collected within the Total Diet Study conducted in Zagreb, Croatia between 2021 and 2023. After purchase in shops and local markets, food was prepared in ready-to-eat form (i.e., washed, peeled, and/or cooked as typical for household consumption), pooled in composite samples, homogenised, and stored at -20 °C before analysis. Samples were extracted using the standard miniLuke method and analysed by gas chromatography-high resolution accurate mass spectrometry (GC-HRAM) and liquid chromatography-mass spectrometry (LC-QQQ-MS). A total of 816 samples from 18 different food groups were analysed for pesticide residues. Fungicides were predominantly detected in fruits and vegetables (including their products) (145 out of 440 samples, 33 % positivity rate). The only exception was a sample of bacon. Among the thirty-six different fungicides, the most prevalent was boscalid (n=61), followed by fludioxonil (n=38), cyprodinil (n=27), and azoxystrobin (n=26). Two-thirds of positive samples contained a single residue. Five to seven residues were found in blackberries, black grapes, cucumbers, different types of lettuce (i.e., crisp, butterhead), lemons, peppers, strawberries (raw and jam), and tomatoes (corresponding to 10 % of positive samples). Only four samples contained fungicides above the EU maximum residue level (MRL): boscalid and fluopyram in nectarines, iprodione in strawberry jam, and biphenyl in processed and unprocessed bacon. These findings highlight the frequent co-occurrence of fungicides in commonly consumed fruits and vegetables, providing valuable data for further exposure assessments and consumer risk characterisation.

This study was funded by the Croatian Science Foundation (grant codes: HrZZ IP-2019-04-7193, DOK-2020-01-1312), aided by collaborative efforts with the Department of Agriculture, Food and the Marine, Ireland, and supported by the European Union – Next Generation EU funding Program Contract 533-03-23-0006 (HumEnHealth).

KEY WORDS: food analysis; fruits; pesticides; total diet study; vegetables

OP 8

Association of dietary exposure to pyrethroid and organophosphate insecticides with gonadotropins, testosterone, and dehydroepiandrosterone sulphate in primary school boys

Marija Macan[1], Jelena Kovačić[1], Andreja Jurič[1], Adriana Gustovarac[1], Saša Kralik Oguić[2], Antonija Sulimanec[1], Martina Pavlić[3], Darja Sokolić[3], Jim Garvey[4], and Veda Marija Varnai[1]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] University Hospital Centre Zagreb, Department of Laboratory Endocrinology, Zagreb, Croatia
[3] Croatian Agency for Agriculture and Food, Zagreb, Croatia
[4] Department of Agriculture, Food and the Marine, Celbridge, Ireland
mmacan@imi.hr

This study aimed to assess the thus far inadequately explored effects of dietary exposure to pyrethroid and organophosphate insecticides on the pubertal development of 445 boys, aged 10–13, from the Zagreb region, Croatia (first wave of the PyrOPECh project). The dietary exposure assessment was based on the participants' 24-hour dietary recall data and pesticide residue analysis in food samples (prepared as table-ready) collected in a total diet study. Pesticide residues were analysed at the Backweston Laboratory Complex, Department of Agriculture, Food and the Marine by gas chromatography-high resolution accurate mass spectrometry and liquid chromatography-mass spectrometry. Urinary gonadotropins [follicle-stimulating hormone (FSH) and luteinizing hormone (LH)] were measured by chemiluminescent microparticle immunoassay, and salivary testosterone and dehydroepiandrosterone sulphate (DHEA-S) by enzyme-linked immunosorbent assay. A statistically significant negative association was observed between pyrethroid exposure and log-transformed salivary testosterone levels (β=-0.019; 95 % CI=[-0.035, -0.003]; p=0.023) in a regression model adjusted for the boys' age, body mass index (BMI) z-score and usual physical activity, sampling season, parental education, residential area, maternal pubertal timing and weight gain during pregnancy, residence or temporary stay near an agricultural area treated with pesticides, and domestic use of pest control products for pets (for pyrethroids). No statistically significant associations were found between pyrethroids and organophosphates estimated dietary intakes and gonadotropins, or DHEA-S. This study suggests that dietary exposure to pyrethroids may be associated with lower testosterone levels in prepubertal boys, highlighting potential endocrine-disrupting effects.

Funding: Croatian Science Foundation (HrZZ IP-2019-04-7193; HrZZ DOK-2020-01-1312), with the support of the Department of Agriculture, Food and the Marine, Ireland, and EU – Next Generation EU funding (Contract 533-03-23-0006, HumEnHealth).

KEY WORDS: 24-hour dietary recall; hormones; puberty; total diet study; Zagreb Region

OP 9

Adipose tissue cadmium levels and reduced PON1 activity in serum: clinical implications from the GraMo cohort

Maria de la Cruz Gomez Pellin[1,2], Javier Esteban[1,3], Gonca Çakmak[4], Eduardo Linares-Ruiz[5], Inmaculada Salcedo-Bellido[5,6,7], María Eugenia Velasco García[8], Jose Barril[1,3], Suylen Galbán-Velázquez[1], Celia Pérez-Díaz[5,6], Francisco M. Pérez-Carrascosa[5,6], Željka Fiket[9], Petra Vrhovnik[10], Maria Cruz Pellín[1], and Juan P. Arrebola[5,6,7]

[1] Universidad Miguel Hernández de Elche, Instituto de Bioingeniería, Alicante, Spain
[2] Medical University of Vienna, Centre for Public Health, Department of Social and Preventive Medicine, Vienna, Austria
[3] Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL), Alicante, Spain
[4] Gazi University, Faculty of Pharmacy, Department of Toxicology, Ankara, Türkiye
[5] University of Granada, Department of Preventive Medicine and Public Health, Granada, Spain
[6] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain
[7] CIBER Epidemiology and Public Health (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain
[8] University of Granada, Department of Statistics and Operational Research, Granada, Spain
[9] Ruđer Bošković Institute, Division for Marine and Environmental Research, Zagreb, Croatia
[10] Slovenian National Building and Civil Engineering Institute (ZAG), Ljubljana, Slovenia
jesteban@umh.es

Environmental pollutants such as cadmium (Cd) are known inhibitors of Paraoxonase 1 (PON1), involved in the preventing atherosclerosis. The aims of this study were to assess the association between adipose tissue Cd concentrations and serum PON1 activity in serum and to evaluate the contribution of the $PON1_{Q192R}$ phenotype on the PON1 susceptibility to Cd. We measured three PON1 activities – PALSase, PAHSase, and CMPAase – in serum samples of participants from the GraMo cohort in Granada, Spain, allowing us to determine their $PON1_{Q192R}$ phenotypes (QQ, QR, or RR). Cd concentrations in adipose tissue were also available, alongside comprehensive demographic, lifestyle, dietary, and health information collected via interviews. The associations between PON1 activities in serum and Cd concentrations in adipose tissue (subset of n=138) were explored by multivariable regression models which were adjusted for sex, alcohol consumption, and $PON1_{Q192R}$ phenotype. Furthermore, benchmark dose (BMD) modelling was applied to assess dose-response relationships. The degree of susceptibility to Cd was found to increase in the following order: QQ<QR<RR. The Cd BMD ranges for an averaged subject were 0.002–0.016 mg $Cd/kg_{adipose\ tissue}$ for RR and 0.046–0.115 mg $Cd/kg_{adipose\ tissue}$ for QR, respectively. The calculated Cd BMD required to reduce PON1 activity in QQ cases was higher than the Cd concentrations found in the participants' adipose tissue. The present findings indicate that the elevated risk of cardiovascular disease in individuals with the RR genotype may be partly attributable to their greater sensitivity to chronic Cd exposure.

This study was funded by the Scientific and Technological Research Council of Türkiye (TUBITAK-2219, Gonca Çakmak) and the Instituto de Salud Carlos III, Spain (PFIS FI21/00269, Celia Pérez-Díaz; PI20/01568, Juan P. Arrebola).

KEY WORDS: atherosclerosis; benchmark dose modelling; dose-response; multivariable regression model

OP 10

Neuropsychology, toxicology, and human development: neurobehavioral effects of insecticide exposure during puberty and adolescence

Patricia Tomac[1], Adrijana Košćec Bjelajac[1], Ivana Hromatko[2], and Veda Marija Varnai[1]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] University of Zagreb, Faculty of Humanities and Social Sciences, Zagreb, Croatia
ptomac@imi.hr

Adolescence is marked by profound hormonal changes, brain maturation, and shifts in sleep and neurobehavioral functioning. Developmentally, this is the second window of heightened vulnerability, during which sleep and the neural circuits involved in cognition, emotion regulation, and social functioning are particularly sensitive to environmental influences. Recent findings indicated that even low-level, chronic exposure to common insecticides adversely affects the nervous and endocrine systems. Despite the intensive changes in these systems during adolescence, this period of human life remains underexplored, particularly with respect to neurodevelopmental outcomes. To bridge this gap, we will first provide a brief overview of the relevance of neuropsychological perspectives in toxicological research, particularly in detecting subtle impacts of chemical exposures on brain function and behaviour. We will then present findings from a literature review synthesising current evidence on the effects of postnatal exposure to organophosphate, carbamate, pyrethroid, and neonicotinoid insecticides on neurobehavioral functioning in individuals aged 8–20. Forty-eight studies, identified through Web of Science, PubMed, Scopus, and PsycINFO, met the inclusion criteria. Most employed cross-sectional designs and biomonitoring to estimate exposure. While cognitive outcomes were most commonly assessed neurobehavioral domain, sleep was evaluated in only one study. Nine studies focused on occupational exposure. Overall, our findings suggested that both occupational and residential insecticide exposure, primarily to organophosphates, may negatively impact adolescent neurobehavioral functioning, particularly executive functions and frontal-lobe-dependent processes. The results also highlight the importance of integrating developmental and psychological perspectives in toxicological studies, and call for longitudinal, multidisciplinary research to better characterise exposure-outcome relationships during sensitive periods.

This study was funded by the Croatian Science Foundation (HRZZ-IP-2019-04-7193, DOK-2021-02-7823) and European Union – Next Generation EU (Program Contract of 8 December 2023, Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006).

KEY WORDS: behaviour; cognition; emotions; neurotoxic insecticide exposure; pubertal development

Genotoxic Signals

IL 9

Micronuclei as early warning signals: strengthening exposure science for protecting human populations

Nancy B. Hopf

University of Lausanne, Center for Primary Care and Public Health (Unisanté), Lausanne, Switzerland
nancy.hopf@unisante.ch

Micronucleus (MN) frequency in buccal epithelial cells is gaining recognition as a sensitive, non-invasive biomarker of genomic instability and genotoxic exposure. Originally developed within cytogenetics and cell biology, MN assays are increasingly applied in environmental and occupational health to identify populations at risk from genotoxic exposures. This biomonitoring approach exemplifies how cellular-level methods are becoming integral to preventive medicine. Recent studies illustrate its utility across diverse exposure scenarios. Age-related increases in MN frequency were quantified in a general Swiss population, enabling the development of reference values for interpreting individual scores. In a smoking cessation trial, MN frequencies in buccal cells significantly declined only after 12–24 months of abstinence, demonstrating the biomarker's responsiveness to long-term genomic recovery. In an occupational study involving subway workers, MN frequency in buccal cells was found to be significantly associated with airborne titanium exposure, a known genotoxic agent, even at very low concentrations. Interestingly, MN frequency showed greater sensitivity to titanium than to polycyclic aromatic hydrocarbons (PAHs), highlighting its potential specificity for certain genotoxic agents. These findings support the integration of MN frequency analysis into exposure biomonitoring. However, further work is needed to standardize protocols, validate exposure-response relationships, and combine MN data with biological exposure markers. Despite these challenges, MN frequency in buccal cells offers a promising tool for early detection of genotoxic effects, helping to identify vulnerable populations and guide preventive interventions.

KEY WORDS: buccal cells; biomonitoring; effect biomarker; genotoxic exposure; particulate matter

IL 10

Spotlight on radiation: tracking its effects in children with blood and buccal micronucleus assays

Goran Gajski[1], Mirta Milić[1], Marko Gerić[1], Marijana Nodilo[2], Željka Knežević[2], Natko Beck[3], Vera Garaj-Vrhovac[1], Saveta Miljanić[2], Mária Ranogajec-Komor[2], and Đurđica Milković[3]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] Ruđer Bošković Institute, Zagreb, Croatia
[3] Srebrnjak Children's Hospital, Zagreb, Croatia
ggajski@imi.hr

Cytogenetic methods play a key role in human biomonitoring by assessing chromosomal damage induced by genotoxic agents, including diagnostic radiation. Among these, the lymphocyte micronucleus cytome (L-MN Cyt) assay is a well-established method for detecting chromosomal abnormalities. However, the buccal micronucleus cytome (B-MN Cyt) assay is gaining attention due to its non-invasive nature, making it particularly suitable for use in paediatric populations. Given that children may be more vulnerable to radiation, ongoing monitoring of their exposure to diagnostic X-rays is essential. This study aimed to investigate the effects of diagnostic chest and sinus X-ray exposure on lymphocytes and buccal epithelial cells in children (n=40, age range 4–17) using L-MN Cyt and B-MN Cyt assays before and after exposure. Results from the L-MN Cyt assay revealed a significant increase in micronuclei, nucleoplasmic bridges, and nuclear buds following X-ray exposure. In contrast, the B-MN Cyt assay showed a significant rise only in cells with condensed chromatin, indicative of early apoptotic processes. Notable interindividual variability was observed among the children in both assays. Both assays demonstrated potential for monitoring acute genotoxic exposure from physical agents in children. In particular, the B-MN Cyt assay offers a promising, minimally invasive approach for assessing genetic damage in paediatric patients frequently subjected to diagnostic imaging. Moving forward, it is important to minimise cumulative radiation doses and further explore age-related sensitivity to radiation exposure.

KEY WORDS: buccal cells; dosimetry; human biomonitoring; lymphocytes; paediatric X-ray diagnostics

OP 11

Automated quantitative fluorescence microscopy and image feature analysis for high-content *in vitro* toxicity screening

Martina Štampar[1], Špela Rozman[2], Alja Štern[1], Blaž Režonja[2], Karolina Berlingar[1], and Bojana Žegura[1]

[1] National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, Ljubljana, Slovenia
[2] University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia
martina.stampar@nib.si

With the growing number of chemicals requiring a safety evaluation, there is increasing demand for reliable, physiologically relevant *in vitro* models for genotoxicity testing. Three-dimensional (3D) hepatic spheroids offer improved structural and metabolic relevance compared to traditional 2D cultures and present a promising alternative to animal models. Automated quantitative fluorescence microscopy, combined with advanced image analysis, enables rapid, cost-effective, and high-content toxicological screening, particularly suited for multiplexed and time-dependent studies. This study aimed to optimise an automated fluorescence imaging and analysis workflow using the Cytation 5 Microplate Reader (Agilent BioTek), validated with an EVOS M7000 Imaging System. *In vitro* 2D and 3D HepG2 liver models were used for (geno)toxicity screening through automated fluorescence microscopy combined with quantitative image analysis. Two-dimensional cultures were seeded in 96-well plates and cultured for 24 hours. Three-dimensional spheroids were formed in AggreWell plates and maintained for 21 days in the ClinoStar rotating bioreactor system (CelVivo) under dynamic conditions. Spheroids were embedded in glycerol and transferred into black 96-well plates or sectioned into 5 mm paraffin slices. Both 2D and 3D models were exposed to two airborne polycyclic aromatic hydrocarbons (PAHs)–benzo[b]fluoranthene (BBF) and benzo[g,h,i]perylene (BGP)–individually and as a binary mixture. Method optimisation employed reference genotoxic agents: benzo[a]pyrene (BaP), etoposide (ET), and tert-butyl hydroperoxide (TBHP). Following treatment, cells were fixed and immunostained for key biomarkers of DNA damage (γH2AX, p-H3, PARP1), and oxidative stress (HMOX1, SRXN1). Imaging data were analysed using Gen5, Celleste, and ImageJ software with semi-automated segmentation and quantification. Our study revealed clear differences in the responses between 2D and 3D across multiple endpoints. These findings support the use of automated fluorescence microscopy as a robust approach for high-throughput toxicity screening. Future work will expand the biomarker panels and advance automated workflows for the confocal imaging.

This study was supported by the Slovenian Research and Innovation Agency (Research Core Funding no. P1-0245) and the HE projects Twinning for Excellence to Strategically Advance Research in Carcinogenesis and Cancer (CutCancer; g.a. no. 101079113) and HE project Evidence Driven Indoor Air Quality Improvement (EDIAQI; g.a. no. 101057497).

KEY WORDS: 2D/3D HepG2 liver models; genotoxicity; oxidative stress; polycyclic aromatic hydrocarbons; spheroids

OP 12

Genotoxicity of nicotine and tobacco products – a review of the available evidence

Metka Filipič

metkaf@gmail.com

The genotoxicity of nicotine and tobacco products has been extensively studied due to the significant health risks associated with smoking and tobacco use. While nicotine is primarily recognized for its addictive properties, some studies have suggested its weak genotoxic potential. Tobacco smoke contains harmful chemicals that mainly arise from the combustion of tobacco. The primary genotoxic carcinogens in tobacco smoke include polycyclic aromatic hydrocarbons, nicotine-derived nitrosamines, and carbonyl compounds. *In vitro* genotoxicity studies have shown that tobacco smoke induces mutations in bacterial and cultured mammalian cells and causes chromosomal damage, such as sister chromatid exchange, chromosomal aberrations, and micronucleus formation. *In vivo* studies have demonstrated that rodents exposed to tobacco smoke experience DNA damage in lung and bone marrow cells, along with an increased frequency of micronuclei and chromosomal aberrations. Human studies have indicated that smokers exhibit elevated levels of DNA adducts, markers of oxidative damage, and chromosomal instability in lymphocytes, buccal cells, and urothelial cells. Heated tobacco products (HTPs) have been developed as an alternative to cigarette smoking for harm reduction. The technology behind HTPs involves heating tobacco to a temperature that releases nicotine and aromas from the tobacco leaf without causing combustion. As a result, the levels of harmful chemicals in the aerosol are significantly reduced. From that perspective, HTPs are possibly a less hazardous alternative to conventional cigarettes. Evidence is presented here to support the assumption that transitioning from conventional cigarettes to HTPs reduces exposure to harmful chemicals and consequently reduces harm compared to combustible tobacco products.

KEY WORDS: carcinogenicity; harm reduction; heated tobacco products (HTPs); oxidative stress; tobacco smoke

Aquatic Hazards

IL 11

Anthropogenic pollutants and the modern environmental burden: insights from sea urchin toxicity studies

Petra Burić[1], Ines Kovačić[1], and Daniel Mark Lyons[2]

[1] Juraj Dobrila University of Pula, Faculty of Natural Sciences, Pula, Croatia
[2] Ruđer Bošković Institute, Center for Marine Research, Rovinj, Croatia
petra.buric@unipu.hr

With the global population growing, humanity is increasingly confronted with rising levels of environmental pollutants. Many of these pollutants ultimately reach aquatic ecosystems and eventually accumulate in marine waters. Coastal waters are inhabited by marine invertebrates such as sea urchins, which are commonly used in toxicological studies. One of the frequently employed methods in environmental toxicity studies is the Sea Urchin Embryo Development Test (SUEDT), which enables assessment of the effects of pollutants on early embryonic development as well as on sea urchin gametes and subsequent offspring. SUEDT offers several advantages: (i) easy collection of sea urchins from the shore; (ii) large quantities of gametes and embryos; (iii) similarity of early embryonic development to that of humans; (iv) a simple and cost-effective setup; and (v) a short experimental duration (48–72 hours). Various endpoints can be evaluated, including fertilization success, developmental progression from fertilization to larval stage, cytogenotoxicity and overall larval development after 2–3 days. Despite its advantages, SUEDT is susceptible to sample contamination, improper egg-to-sperm ratios, and require experienced microscopy for accurate identification of embryonic and larval malformations. Nevertheless, SUEDT provides valuable insights into both legacy pollutants and those of emerging concern. According to the literature, the most commonly studied pollutants in SUEDT over the past decade have been metals, followed by environmental sediment and water samples, plastics, nanoparticles, pharmaceuticals, marine toxins, hydrocarbons, and pesticides. Among these, metals, particularly heavy metals such as cadmium, arsenic, cobalt, copper, mercury, nickel and zinc, have emerged as pollutants of major concern.

KEY WORDS: metals; pesticide; plastics; sea urchin embryo development test

IL 12

Vulnerability of fish to treated sewage effluents

Natalija Topić Popović

Ruđer Bošković Institute, Zagreb, Croatia
ntopic@irb.hr

Treated wastewater often contains a variety of complex pollutants that can impact freshwater aquatic ecosystems and organisms. Fish are particularly sensitive to environmental pollutants, rapidly accumulating certain toxic substances even at low concentrations. Due to this sensitivity, fish are commonly used as model organisms to assess the effects of these pollutants, as they can influence key physiological functions and overall health. These biological effects can occur at multiple levels of biological organization. Wastewater treatment plant (WWTP) effluents impacted free-living Prussian carp (*Carassius gibelio*) and exposed zebrafish (*Danio rerio*) tissue's structural and functional characteristics. The histopathological analysis of effluent-caught carp (n=88) revealed significant alterations in gill tissues, primarily affecting the secondary lamellae. The gills exhibited hyperplasia, hypertrophy, and lamellar fusion. Kidney lesions were characterized by nephron and tubular system damage, glomerular atrophy, and necrosis. Liver abnormalities included karyolysis, binuclear hepatocytes, and sinusoidal distension. Additionally, spleen alterations were marked by the presence of intracellular vacuoles and granulomatous lesions. Effluent-impacted Prussian carp demonstrated elevated plasma albumin, total proteins, urea, triglyceride, alanine aminotransferase, alkaline phosphatase, and decreased activity of superoxide dismutase. Laboratory exposure of zebrafish embryos [embryos were exposed in five groups (one control and four treatments), n=20 per group, as one embryo per well, at 24 and 48 hours post fertilization (hpf)] to treated wastewater led to increased mortality and development abnormality rates. Additionally, heart rate, spontaneous movements, and pigmentation development were significantly impacted. The observed effects on fish provided valuable insights into the biological impact of pollutant mixtures under real-life and exposure conditions, contributing to the evaluation of the extent to which WWTP-treated water affects fish populations.

KEY WORDS: histopathology; plasma biochemistry; Prussian carp; treated wastewater; zebrafish

Abstracts of the 7th International Congress of the Croatian Society of Toxicology – Aquatic Hazards
Arh Hig Rada Toksikol 2025;76(Suppl. 1):48–50

OP 13

Seasonal and environmental modulation of anaerobic strategies in *Synurella ambulans* from the hyporheic zone of the Sava River

Marijana Erk[1], Zuzana Redžović[2], Sanja Gottstein[2], Inna M. Sokolova[3,4], Eugene Sokolov[5], Mirela Sertić Perić[2], and Mario Cindrić[1]

[1] Ruđer Bošković Institute, Zagreb, Croatia
[2] University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia
[3] University of Rostock, Institute for Biological Sciences, Department of Marine Biology, Rostock, Germany
[4] University of Rostock, Interdisciplinary Faculty, Department of Maritime Systems, Rostock, Germany
[5] Leibniz Institute for Baltic Sea Research Warnemünde, Rostock, Germany
erk@irb.hr

The hyporheic zone (HZ), a transitional area between the surface and groundwater with distinct ecological conditions, is highly susceptible to pollution and hypoxia, but the physiological responses of its aquatic inhabitants to these stressors remain poorly understood. The aim of this study was to investigate the effects of changes in the physicochemical properties of interstitial water from the HZ and the possible influence of contamination in the HZ on the activities of enzymes involved in aerobic/anaerobic energy metabolism (pyruvate kinase, PK; phosphoenolpyruvate carboxykinase, PEPCK; lactate dehydrogenase, LDH) in a stygophilous freshwater amphipod *Synurella ambulans* from the Sava River HZ. The study was conducted in two seasons (early autumn 2020 and spring 2021) with contrasting hydrological regimes at one site (Medsave) upstream of the wastewater treatment plant (WWTP) discharge and at two sites downstream (Podsused and Jarun). The populations of *S. ambulans* at sites downstream of the WWTP showed higher LDH activity than populations at the upstream site. PK/PEPCK ratio as an indicator of an organism's anaerobic potential was significantly lower in the *S. ambulans* populations at Podsused and Jarun compared to Medsave in early autumn, indicating a lower aerobic capacity and a greater reliance on anaerobic metabolic pathways. Overall, the relatively low PK/PEPCK ratio and high LDH activity indicated increased anaerobic potential of *S. ambulans* and its ability to tolerate low oxygen concentrations in the HZ. This study improves the understanding of the physiological responses of crustaceans exposed to contamination and environmental stressors, thus contributing to the ecological assessment of groundwater-connected ecosystems.

This study was supported by the European Union – NextGenerationEU, National Recovery and Resilience Plan NPOO.C3.2.R3-I1.04.0122. Support and funding are gratefully acknowledged.

KEY WORDS: anthropogenic contamination; biochemical biomarkers; Crustacea; hypoxia; spectrophotometry

Occupational Toxicology

IL 13

Reprotoxics in workplaces: focus on gender differences

Francesca Larese Filon, Francesca Rui, and Marcella Mauro

University of Trieste, Unit of Occupational Medicine, Trieste, Italy
larese@units.it

Reprotoxics are agents that can cause adverse effects on libido, sexual behaviour, spermatogenesis/oogenesis, interference with hormonal activity or physiological parameters that affects the ability to fertilise, as well as adverse effects on fertilization itself and the development of the fertilised ovum for and including implantation. We review the literature on this topic with regard to the EU legislation on reprotoxics exposure in workplaces. Agents with potential teratogenic effects are forbidden during pregnancy in EU countries (ionizing radiation, lead, mercury, solvents, antiblastic drugs, anaesthetic gases, etc.) and others are not allowed in Italy (noise over 87 dBa, night work, standing position for more than half of working hours, use of ladders, etc.). However, other effects have been reported, such as a decrease in sperm quality for men exposed to lead, solvents, pesticides/herbicides, and heavy metal in general (lead, cadmium, mercury). Studies on a maternal cohort demonstrated that early exposure during pregnancy to passive smoking or to heat were associated with lower birthweight, while exposure to dust was associated with higher caesarean section frequency. A large study on 13 EU cohorts with more than 200.000 mother-child pairs found that employment during pregnancy was associated with a reduction in preterm delivery with the exception of food workers. Moreover, mothers exposed to the higher quartile of polycyclic aromatic hydrocarbon were more likely to have offspring with heart defects. On the contrary, exposure to soldering fumes or nickel were not associated with reprotoxics effects. This review highlights the complexity of reprotoxic effects and the need for more comprehensive evaluations in occupational exposures.

KEY WORDS: occupational exposure; sexual behaviour; spermatogenesis; teratogenic effects

OP 14

Guidelines on workplace risk assessments for pregnant workers exposed to chemical hazards

Jelena Macan[1], Željka Babić[1], Tea Samardžić[2], and Roko Žaja[3]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] PROTEGO Healthcare Institution, Koprivnica, Croatia
[3] University of Zagreb, School of Medicine, Andrija Štampar School of Public Health, Zagreb, Croatia
jmacan@imi.hr

According to the current Croatian Maternity and Parental Benefits Act, which adopts the 1992 Pregnant Workers Council Directive, a pregnant worker exposed to reproductive chemical hazards at the workplace is entitled to health and pregnancy protection. In accordance with the employer's obligation, an authorised occupational health physician (OHP) assesses occupational pregnancy risk and recommends safety measures to the employer. Recently, a guideline on the occupational pregnancy risk assessment was published and shared with Croatian OHPs, along with the risk acceptance criteria based on medical literature, legislation, an OHP expert's opinion, and with the support of the Croatian Society on Occupational Health. Our aim here is to present a part of the guideline referring to the risk arising from chemical exposure at the workplace. The risk of exposure was stratified according to the European Commission Regulation on Classification, Labelling and Packaging of substances and mixtures (no. 1272/2008), which defines chemicals that "may damage the unborn child" (H360D), and those that are "suspected of damaging the unborn child" (H361d). According to the guidelines, the risk for pregnant worker is considered unacceptable if there is an inhalational exposure to H360D chemicals regardless of the implemented safety measures, or if there is an inhalational exposure to H361d chemicals with no or insufficient implementation of safety measures. The guideline additionally provides a list of documents and data needed for a proper workplace risk assessment, as well as information on sources of data about reprotoxic substances.

Supported by the project HumEnHealth funded by the European Union – Next Generation EU (Programme Contract of 8 December 2023, Class: 643-02/23-01/00016, Reg.no. 533-03-23-0006).

KEY WORDS: CLP Regulation; damage to unborn child; health protection; occupational health physicians; reprotoxic chemicals

OP 15

Reprotoxic risks in the hairdressing profession: efforts to protect worker health

Željka Babić and Jelena Macan

Institute for Medical Research and Occupational Health, Zagreb, Croatia
zbabic@imi.hr

Hairdressers are considered as being at risk of adverse reprotoxic effects, which has not yet been adequately addressed in the relevant legislation. Specifically, a chemical classified as reprotoxic, Category 2, can be used in cosmetic products in the European Union if the Scientific Committee on Consumer Safety (SCCS) has found it safe considering the frequency of use by consumers. However, occupational exposure to the same hair products is not covered by the SCCS or any other regulatory body. Therefore, our aim was to identify key aspects for improving risk assessments related to reprotoxic chemicals for hairdressers, summarizing literature reviews within the Uni Europa – European Global Services Union project VS/2019/0440. Our main findings were: 1) depending on the task, hairdressers were exposed 4 to 78 times more than consumers to a wide range of hair cosmetics, with the largest difference found for hair colouring with oxidative hair dyes; 2) our systematic review of recent studies did not clearly show that hairdressers are still at reprotoxic risk today, probably reflecting the long-term effects of the ban on mutagenic or carcinogenic aromatic amines for use in hair dyes in the 1980s; 3) the systematic review indicated the *in vitro* and *in vivo* genotoxic potential of oxidative hair dye precursors, which requires further clarification through epidemiological studies. Considering our findings, recommendations to the SCCS were sent from social partners in the hairdressing sector, urging them to include occupational exposure of hairdressers in the process of risk assessment for hair products.

Supported by the project HumEnHealth funded by the European Union – Next Generation EU (Programme Contract of 8 December 2023, Class: 643-02/23-01/00016, Reg.no. 533-03-23-0006).

KEY WORDS: occupational exposure; reprotoxicity; risk assessment; safety at work; Scientific Committee on Consumer Safety; UNI Europa

Closing lecture

IL 14

Unveiling honey origin and authenticity: a comprehensive perspective

Dražen Lušić[1,2,3,4,5]

[1] University of Rijeka, Faculty of Medicine, Rijeka, Croatia
[2] University of Rijeka, Faculty of Health Studies, Rijeka, Croatia
[3] Teaching Institute of Public Health of the Primorje-Gorski Kotar County, Rijeka, Croatia
[4] International Honey Commission (IHC)
[5] Honey platform EU
drazen.lusic@medri.uniri.hr

This work examines the background of a honey's origins, authenticity, and the methods used to ensure its quality. For millennia, honey has been valued not only as a sweetener but also for its cultural, nutritional, and medicinal importance. However, fraud and adulteration increasingly threaten its integrity, requiring expertise from entomology, chemistry, food science, economics, and even ethics. A honey's characteristics depend on its floral and regional origin. Authentic honey mirrors its local ecosystem. Adulterated products often contain cheap sugar syrups, misleading consumers, harming producers, and raising health hazards. Global demand outpaces supply, encouraging fraud. Frail regulatory enforcement and consumer preference for low prices worsen the problem. The entry of such products into the EU (and Croatian) market raises a justified concern: what do they contain? What effects might they have on human health? Questionable origin and composition contribute to potential public health risks. To safeguard authenticity and safety, several techniques are in use: melissopalynology to identify pollen and botanical origin; isotope ratio analysis to detect added sugars; nuclear magnetic resonance spectroscopy (NMR) and chromatography to analyse composition; sensory analysis to assess taste, aroma, and texture. Certification and traceability systems further strengthen trust. Technologies like blockchain, along with emerging standards (e.g. ISO 24607) and government regulations, promote transparency. The EU Honey Platform Expert Group and the Food and Drug Administration (FDA) standards are notable examples. Fraudulent honey undermines true beekeepers, reduces biodiversity, and weakens pollinator protection efforts. Ensuring honey's authenticity benefits consumers, producers, and the environment. Scientific testing, certification, and informed purchasing decisions can protect this ancient, valuable resource and support ethical, sustainable practices for future generations.

KEY WORDS: certification; food fraud; health risk; honey adulteration

Young Scientist Awards

YSL 1

Cellular responses to nuclear receptor-activating chemicals in HepG2 spheroids: effects on viability, proliferation, and genotoxicity

Tim Ravnjak[1,3], Martina Štampar[1], Marc Audebert[2], and Bojana Žegura[1,3]

[1] National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, Ljubljana, Slovenia
[2] National Research Institute for Agriculture, Food and the Environment (INRAE), Toxalim Research Centre in Food Toxicology, Toulouse, France
[3] Jožef Stefan International Postgraduate School, Ljubljana, Slovenia
tim.ravnjak@nib.s

Diuron and 6-formylindolo[3,2-b]carbazole (FICZ) are chemically distinct compounds with different receptor targets. Diuron is a common herbicide and a suspected estrogen receptor (ER) agonist, whereas FICZ is an endogenous photoproduct of tryptophan and a potent aryl hydrocarbon receptor (AhR) agonist. Given the central role of AhR as a ligand-activated transcription factor regulating xenobiotic metabolism through cytochrome P450 enzymes induction, we investigated whether these heterogenous compounds elicit differential cellular responses. To compare cellular responses, 3-day-old *in vitro* HepG2 3D cell models (spheroids) were exposed to Diuron (500–1500 µmol/L for 24 h, and 50–400 µmol/L for 96 h) and FICZ (0.02–9 µmol/L for 24 and 96 h). We assessed cell viability (MTS assay), cell cycle distribution (Hoechst 33258), proliferation (Ki67), DNA double-strand break (γH2AX), and mitotic cell (histone H3 positive events) formation. The results showed that 400 µmol/L Diuron significantly reduced cell viability after 96 h, while FICZ had no effect. Cell cycle distribution was altered by Diuron at the highest concentration at both time points, but not by FICZ. Diuron slightly reduced proliferation after 24 h and significantly suppressed it at 400 µmol/L after 96 h. Conversely, FICZ increased proliferation concentration-dependently after 24 h, but not after 96 h. Neither chemical caused DNA double-strand breaks. However, Diuron significantly increased the percentage of pH3-positive cells after both timepoints, indicating aneugenic potential. These findings show that Diuron exerts broader effects on cellular processes than FICZ, particularly involving cell cycle perturbation and aneugenicity. Ongoing toxicogenomic analysis aims to elucidate the molecular mechanisms underlying these responses.

This study was funded by ARIS (P1-0245), HEU CutCancer (101079113), and HEU PARC (101057014).

KEY WORDS: cell cycle; cytotoxicity; Diuron; FICZ; flow cytometry

YSL 2

Toward the safer use of nanomaterials: (geno)toxicity evaluation of ferrite-based nanoparticles in HepG2 spheroids

Iza Rozman[1,2], Katja Kološa[1], Álvaro Gallo-Cordova[3], María del Puerto Morales[3], Marco A. Morales[4], Gerardo F. Goya[4], Bojana Žegura[1,2], and Alja Štern[1,2]

[1] National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, Ljubljana, Slovenia
[2] University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia
[3] Instituto de Ciencia de Materiales de Madrid, ICMM-CSIC, Madrid, Spain
[4] Institute of Nanoscience and Materials of Aragón (INMA), CSIC-University of Zaragoza, Zaragoza, Spain
iza.rozman@nib.si

Spinel ferrite magnetic nanoparticles (MNPs) doped with transition metals such as zinc and manganese exhibit unique electrical and magnetic properties, which is the reason for their use in various applications, including magnetic field manipulation and microwave absorption, as well as biomedicine. However, data on their safety and potential health risks remain limited. In this study, we investigated the (geno)toxic potential of ferrite NPs with three compositions: γ-Fe_2O_3 (N4), $Zn_{0.7}Fe_{2.3}O_4$ (N8), and $Mn_{0.4}Fe_{2.6}O_4$ (N9). Cytotoxicity and genotoxicity were assessed using a 3D cell model (spheroids) derived from the human hepatocellular carcinoma (HepG2) cell line via ATP quantification and flow cytometric detection of γH2AX and phosphorylated histone H3 (pH3), respectively. ROS production was measured using the DCFH-DA assay, and gene expression related to DNA damage (*CDKN1A, GADD45A, TP53, MYC, OGG1*) and oxidative stress (*SOD1, CAT, GPX1, GCLC, GSR*) was analysed by qPCR. Nanoparticle internalisation was confirmed by transmission electron microscopy (TEM). Spheroids were exposed to concentrations up to 250 μg/mL of MNPs for 2, 4, 24, or 96 hours, depending on the assay. N8 and N9 induced greater cytotoxicity than N4 at 24 and 96 hours, while no significant increase in γH2AX or pH3-positive events was observed. A dose-dependent increase in ROS occurred only with N4 after 4 hours. At the transcript level, *MYC* was the only gene consistently deregulated across all samples after 24 hours. TEM analysis showed MNP penetration into the outer layers of spheroids. Our findings contribute to a better understanding of the biological effects of MNPs and represent another step toward the safer use of MNPs in biomedical applications.

Funding: H2020-MSCA NESTOR (101007629), HE CutCancer (101079113), ARRS P1-0245 and J1-4395, and ARIS MR grant to IR.

KEY WORDS: 3D cell models; genotoxicity; *in vitro*; nanotoxicity; spinel ferrite nanoparticles

YSL 3

Effects of *N*-alkyl quaternary quinuclidines on oxidative stress biomarkers in SH-SY5Y cells

Lucija Marcelić, Antonio Zandona, Suzana Žunec, Josip Madunić, and Maja Katalinić

Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia
lmarcelic@imi.hr

Quinuclidinium compounds, characterised by a rigid bicyclic core, are versatile agents of natural or synthetic origin with unique electrostatic properties that enable selective binding to biological targets. Advances in organic synthesis have led to synthetic analogues with a broad spectrum of biological and pharmacological activities, including anticholinergic, antihistaminic, antiparasitic, antioxidant, and antitumour effects. This study investigated the effects of six *N*-alkyl quaternary quinuclidine derivatives (oximes QNOH-Cn and alcohols QOH-Cn, n=12–16) on oxidative stress biomarkers in SH-SY5Y neuroblastoma cells, with the aim of evaluating their safety profile margins if applied as potential therapeutics. SH-SY5Y cells were treated with the lowest observed adverse effect level (LOAEL) concentrations of quinuclidine derivatives for 4 h to assess their influence on the reactive oxygen/nitrogen species (ROS/RNS), lipid peroxidation, glutathione (GSH) levels, superoxide dismutase (SOD) activity, and DNA damage. The results showed that Q(N)OHs significantly increased ROS and RNS levels, particularly -NOHs, while lipid peroxidation remained unaffected and GSH depletion was minimal. Cytosolic Cu/Zn-SOD activity increased significantly with the increase of the alkyl chain substituent length, while Mn-SOD activity decreased, indicating mitochondrial damage. DNA damage was not elevated. Overall, the observed effects of the tested quinuclidine derivatives on oxidative stress biomarkers appear to be associated with their structural features; compounds containing hydroxyl groups and shorter alkyl chains exhibited lesser impact. Furthermore, this study indicates that even a non-cytotoxic dose of the tested compounds could impact cell homeostasis, pointing to the importance of such testing early on in the evaluation of new potential drugs.

This study was supported by the Croatian Science Foundation (HRZZ-2017-05-7660), by European Union – Next Generation EU (Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006), and performed using the facilities and equipment funded within European Regional Development Fund projects (KK.01.1.1.02.0007, BioMolTox).

KEY WORDS: glutathione; mitochondrial dysfunction; oximes; reactive nitrogen species; reactive oxygen species

YSL 4

Novel cholesterol- and quinoline-based oximes as potential antidotes for organophosphate nerve agent poisoning

Petra Tuksar and Zrinka Kovarik

Institute for Medical Research and Occupational Health, Zagreb, Croatia
ptuksar@imi.hr

The covalent inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), which hydrolyse the neurotransmitter acetylcholine with organophosphate (OP) compounds during poisoning, requires immediate medical treatment based on oxime antidotes that recover the activity of inhibited enzymes. Due to their permanent positive charge, the cationic pyridinium oximes currently in use do not cross the blood-brain barrier (BBB) in sufficient concentrations and prolonged overstimulation of cholinergic receptors can lead to neuroinflammation and permanent brain damage. Thus, new oximes are continuously synthesized, and in this study, we investigated nine uncharged compounds with either a cholesterol or quinoline (QN) base, which have the potential to cross the BBB. Among the tested compounds, human cholinesterases showed binding potential towards 5 oximes, with reversible inhibition constants (K_i) for AChE ranging from 60 to 267 μmol/L, whereas the K_i of a quinoline oxime for BChE was in nanomolar range (5 nmol/L). Furthermore, the reactivating potential of oximes was screened on sarin- and cyclosarin- inhibited AChE and BChE. Our results showed that none of the 0.1 mmol/L oximes induced significant reactivation of AChE within 24 hours. In the case of BChE, the reactivation maxima were slightly higher compared to those observed for AChE. Although the tested oximes did not show ability to reactivate cholinesterases, some exhibited notable reversible inhibition constants, suggesting that their structures could provide a basis for further development of novel cholinesterase ligands.

The authors thank Prof José Luis Marco (Institute of Organic Chemistry, Madrid, Spain) for providing the oximes. This work was funded by the European Union – Next Generation EU (BioMolTox, Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006), and supported by the European Regional Development Fund (project ReC-IMI, KK.01.1.1.02.0007).

KEY WORDS: 2-PAM; cholinesterase; HI-6; inhibition; reactivation

YSL 5

Disruption of ovarian redox balance by bisphenol mixtures: a comparison with individual exposures

Jovana Živanović, Katarina Baralić, Đurđica Marić, Jovana Šljivić, Aleksa Milačić, Mihajlo Bojić, Evica Antonijević Miljaković, Aleksandra Buha Đorđević, Zorica Bulat, Biljana Antonijević, Marijana Ćurčić, and Danijela Đukić-Ćosić

University of Belgrade, Faculty of Pharmacy, Department of Toxicology "Akademik Danilo Soldatović", Belgrade, Serbia
jzivanovic@pharmacy.bg.ac.rs

Bisphenol A (BPA), bisphenol S (BPS), and bisphenol F (BPF) are widespread industrial chemicals associated with reproductive disorders in women, such as polycystic ovary syndrome, endometriosis, ovulatory dysfunction, and reduced fertility, primarily by inducing oxidative stress. This study aimed to examine whether bisphenol mixtures have a more pronounced effect on ovarian oxidative stress than individual exposures using an *in vivo* model. Forty female Wistar rats were divided into eight groups (n=5). One group served as the control, while the remaining groups were treated with individual bisphenols, their binary mixtures, or a combination of all three (MIX), at human-relevant doses. After 28 days of oral exposure, ovarian tissue was analysed for malondialdehyde (MDA), ischemia-modified albumin (IMA), superoxide dismutase (SOD), glutathione (GSH), and total sulfhydryl groups (SH). All bisphenol mixtures elevated MDA levels, with the highest increase observed in the MIX group, indicating enhanced oxidative stress. Similarly, IMA levels were significantly increased only in the MIX and BPA groups, with MIX again showing the strongest effect. SOD activity declined more noticeably in individual bisphenol groups, although significant reductions were also observed in the MIX and BPS+BPF groups. Regarding antioxidant levels, most groups showed reductions in GSH and SH, with the greatest decreases observed in the BPA+BPF and BPS+BPF mixtures. In conclusion, bisphenol MIX exerted the strongest effects on pro-oxidative markers (IMA and MDA). Consequently, compared to individual bisphenols, mixtures, particularly MIX, induced a more pronounced disruption of ovarian redox balance, with the BPA+BPS mixture showing the weakest effect across all of the measured redox parameters.

This study was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (no. 451-03-136/2025-03/200161).

KEY WORDS: endocrine disruptors; oxidative stress; ovarian toxicity; real-life exposure; Wistar rat

YSL 6

Cyto/genoprotective effects of propolis *in vitro*

Mateo Jakac[1], Dražen Lušić[2,3], Andreja Jurič[4], Irena Brčić Karačonji[3,4], and Nevenka Kopjar[4]

[1] Teaching Institute of Public Health of the Istria County, Pula, Croatia
[2] University of Rijeka, Faculty of Medicine, Croatia
[3] University of Rijeka, Faculty of Health Studies, Rijeka, Croatia
[4] Institute for Medical Research and Occupational Health, Zagreb, Croatia
mateo.jakac@gmail.com

Propolis is a well-known bee product used as a natural preventive and therapeutic agent for centuries. The use of this type of nutritional supplements is currently on the rise due to the increasingly frequent turn to complementary and alternative medicine. Due to the exceptional but very complex phenolic composition, depending on the concentration, propolis can exert both pro-oxidant and antioxidant effects. The aim of this study was to explore the cyto-/genoprotective effects of ethanolic extract of a selected Croatian propolis on healthy lymphocyte cells and its ability to protect them from irinotecan-induced cyto/genotoxic effects *in vitro*. Human peripheral blood lymphocytes were isolated and exposed for 3 hours to three concentrations of propolis extract corresponding to an average daily dose of 0.25 mL of extract (propolis: 70 % ethanol=3:7, w/w), as well as a five- and a ten-fold higher concentration. Cyto- and genoprotective effects were assessed using the cytokinesis-block micronucleus cytome (CBMN Cyt) assay. The propolis demonstrated high biocompatibility with lymphocytes and significantly reduced the level of cytogenetic damage induced by irinotecan. The obtained results speak in favour of future investigations of propolis using other exposure scenarios, available cytogenetic methods, and cell models.

This research was funded by the European Union – Next Generation EU (BioMolTox, Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006), the Croatian Academy of Sciences and Arts, the University of Rijeka (grant uniri-iskusni-biomed-23-247 and Prometheus Fund), Hrvatska elektroprivreda d.d., and supported by the European Regional Development Fund (project ReC-IMI, KK.01.1.1.02.0007).

KEY WORDS: bee product; cytokinesis-block micronucleus cytome assay; ethanolic extract; irinotecan; lymphocytes

YSL 7

Toxicological and clinical aspects of paediatric drug poisoning in Vojvodina, Serbia

Jovan Baljak[1,2], Aleksandra Stojadinović[2,3], Maja Đurendić-Brenesel[1], Nikša Ajduković[1], Miljana Poparić[2], David Strilić[2], and Aleksandar Rašković[2]

[1] Clinical Center of Vojvodina, Toxicology and Molecular Genetics, Center for Forensic Medicine, Novi Sad, Serbia
[2] University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia
[3] Institute for Child and Youth Health Care Vojvodina, Novi Sad, Serbia
jovan.baljak@mf.uns.ac.rs

Acute drug poisoning represents a significant public health issue among the paediatric population. The aim of this study was to evaluate the clinical characteristics of drug poisoning in children and adolescents in the Vojvodina region from 2018 to 2023. In a retrospective observational study, 82 patients with confirmed drug poisoning were included, and data were collected regarding demographic characteristics, clinical manifestations, types of drugs involved, and the therapeutic interventions administered. The severity of poisonings was evaluated using the poisoning severity score (PSS), and toxicological analysis was performed using the gas chromatography-mass spectrometry (GC-MS) method. The results showed that poisonings were most prevalent among adolescent girls (72 %), with most cases resulting from intentional ingestion, whereas unintentional poisonings were more common in children. The number of paediatric poisoning cases exhibited an increasing trend from 2019 onward, with a significant rise during the COVID-19 pandemic. Benzodiazepines were the most frequently involved drugs (47 %), followed by antipsychotics (14 %), antidepressants (13 %), anticonvulsants (13 %), and analgesics (13 %). The majority of patients (78 %) experienced mild clinical symptoms, while 9 % of cases were classified as severe, with complications such as aspiration pneumonia and acute renal failure. Toxicological analysis revealed discrepancies between patient-reported drug exposures and confirmed toxicological findings, particularly in children (59 %) and adolescents (28 %). Addressing paediatric drug poisoning in Vojvodina requires a stronger focus on preventive strategies, including parental education and appropriate psychosocial support for adolescents.

KEY WORDS: benzodiazepines; clinical manifestations; gas chromatography-mass spectrometry; paediatric population; poisoning severity score

Arh Hig Rada Toksikol 2025;76(Suppl. 1):55–63

YSL 8

The impact of abandoned coal mines on water and sediment pollution in the Mura River

Zoran Kiralj[1], Željka Fiket[1], Tomislav Kralj[1], Dušica Ivanković[1], Mavro Lučić[1], Ana Puljko[1], Nikolina Udiković-Kolić[1], Damir Valić[1], and Zrinka Dragun[1]

Ruđer Bošković Institute, Zagreb, Croatia
zoran.kiralj@irb.hr

The Mura River basin in Croatia faces complex contamination sources, including the historical coal mines in its upstream section. This study investigated metal contamination in water/sediment at five selected locations, extending from the most upstream to the most downstream part of the river. We additionally analysed local coal samples and assessed the potential effects on the sediment microbial community. Sampling was conducted across two campaigns, capturing high (HWD) and low water discharge (LWD) periods. During HWD, dissolved concentrations of silver (Ag), chromium (Cr), lead (Pb), and tungsten (W) increased in river water near abandoned coal mines, reaching even the downstream location far away from the last coal mine pits. Concurrently, sediment analyses revealed significant enrichment of molybdenum (Mo) and W (EFs=2.5–4). Analysis of the local brown coal confirmed a connection between water/sediment contamination and abandoned coal mines, revealing more than 10-times higher Mo and W concentrations in the coal compared to Clarke values, as well as 2–4 times higher W and 20-times higher Mo concentrations compared to the river sediment at the nearby site. This widespread Mo/W contamination could therefore be attributed to the washout from mine pits during heavy rainfall, together with atmospheric transport of metal-rich dust. Crucially, some of the measured water/sediment concentrations exceeded protective ecological thresholds, suggesting a potential toxicity risk to certain aquatic biota. This was supported by molecular analyses which showed increased Pb-resistance (high levels of *pbr*T genes) in sediment bacteria in the vicinity of the abandoned coal mines, reflecting chronic environmental pressure on microbial communities.

This study was funded by the Croatian Science Foundation under the projects "Metal-binding biomolecules and health disturbances of freshwater organisms exposed to industrial wastes" (METABIOM; IP-2019-04-2636) and "Transport, fate and toxicity of metal(loid)s released from industrial waste deposits" (FORtIS; IP-2019-04-9354).

KEY WORDS: bacterial metal resistance; brown coal; freshwater contamination; molybdenum; tungsten

YSL 9

Effects of pharmaceuticals and personal care products on the fitness of freshwater fauna: a systematic review and meta-analysis

Anita Tarandek[1], Sandra Hudina[2], Marina Veseli[1], Antonia Smolić[1], Jelena Bujan[1], Ana Previšić[2,3], and Antica Čulina[1,4]

[1] Ruđer Bošković Institute, Zagreb, Croatia
[2] University of Zagreb, Faculty of Science, Croatia
[3] University of Bayreuth, Faculty of Biology, Chemistry & Earth Sciences, Collaborative Research Centre 1357 Microplastics, Bayreuth, Germany
[4] Royal Netherlands Academy of Arts and Sciences, Netherlands Institute of Ecology, Wageningen, the Netherlands
anita.tarandek@irb.hr

Pharmaceuticals and personal care products (PPCPs) are being detected at an increasing rate in freshwater ecosystems worldwide due to widespread use and insufficient removal during wastewater treatment. PPCPs include psychoactive drugs, antibiotics, analgesic and anti-inflammatory drugs, antiparasitic drugs, cardiovascular drugs, hormones, chemotherapeutic drugs, disinfectants, fragrances, insect repellents, preservatives, UV filters, plasticizers, and dyes. These contaminants can affect non-target organisms, with reported impacts on survival, reproduction, and development. However, experiments studying the effects of PPCPs on the fitness of freshwater fauna vary widely in exposure levels, studied species, and reported outcomes, making general conclusions difficult. We conducted a systematic review and meta-analysis of such experiments, defining fitness as any direct or indirect measure linked to survival or reproduction. We extracted data from peer-reviewed experimental studies and open datasets, focusing only on environmentally relevant concentrations and stable exposures. We calculated two effect sizes, log response ratio (lnRR) and log variance ratio (lnVR) to evaluate both the magnitude and variability of effects. Moderators such as developmental stage, sex, exposure duration, environmental conditions, and species traits were assessed using meta-regression models. By identifying taxonomic and geographic gaps, we aimed to aid the implementation of better environmental risk assessments and regulatory decisions. Our work also included exploratory analyses of mixture exposures and effect size comparisons across concentration gradients. Furthermore, we aimed to assess whether the included studies influenced policy or regulatory attention by exploring their alternative impact metrics. This study provides a comprehensive quantitative synthesis on PPCP effects on freshwater animals.

This work was supported by the Croatian Science Foundation under project number HRZZ-IP-2022-10-2872.

KEY WORDS: aquatic toxicology; freshwater animals; meta-analyses; PPCP; sublethal effects

Sponsor presentations

INEL – MEDICINSKA TEHNIKA d. o. o.

Simultaneous identification and quantitation of novel psychoactive substances (NPS) in human whole blood using liquid chromatography-tandem mass spectrometry (LC-MS/MS) technology

Jianru Stahl-Zeng[1], Pierre Negri[2], and Scott Krepich[2]

[1] SCIEX, Darmstadt, Germany
[2] SCIEX, Redwood City, CA, United States of America
jianru.stahl-zeng@sciex.com

The proliferation of novel psychoactive substances (NPS) in the recreational drug market presents escalating global health and safety concerns due to their unpredictable potency and purity. This study introduces a robust, targeted drug screening method designed for the accurate quantitation and identification of 130 NPS in human whole blood, spanning a concentration range of 0.1 to 50 ng/mL. The panel included 22 stimulants, 35 benzodiazepines, dissociatives and hallucinogens, 45 synthetic opioids, and 28 synthetic cannabinoids. Sample preparation involved protein precipitation, followed by liquid chromatography using the SCIEX ExionLC AC system. Mass spectrometric analysis was conducted on the SCIEX QTRAP® 5500+ and 7600 platform with positive electrospray ionization. Quantitative performance was assessed using triplicate injections of spiked calibrator samples. The scheduled multiple reaction monitoring (MRM) method achieved precise and accurate quantitation at the lowest calibrator level (0.1 ng/mL), with R^2 values exceeding 0.99 across all of the analytes. For qualitative analysis, multiple reaction monitoring-information-dependent acquisition-enhanced product ion (MRM-IDA-EPI) and sequential window acquisition of all theoretical fragment ion spectra (SWATH) acquisition strategies enabled confident identification of structurally related compounds with overlapping retention times, leveraging automated tandem mass (MS/MS) spectral library matching. This dual approach significantly enhanced selectivity and sensitivity, reducing false positives and negatives commonly encountered in traditional screening workflows. In conclusion, the developed method offers a fast, reliable, and comprehensive solution for NPS detection in forensic and clinical toxicology. Its integration of high-resolution quantitation with spectral library matching sets a new benchmark for analytical accuracy in the evolving landscape of psychoactive substance monitoring.

KEY WORDS: high-resolution MS; NPS detection; Qtrap; qualitative and quantitative analysis

ALTIUM INTERNATIONAL d. o. o.

Altium per- and polyfluoroalkyl substance (PFAS) workflow solutions

Borna Ferčec

Altium International d. o. o., Zagreb, Croatia
borna.fercec@altium.net

Per- and polyfluoroalkyl substances (PFAS) represent one of the most pressing analytical challenges due to their persistence, complexity, and widespread occurrence across environmental and industrial samples. This presentation introduces PFAS solutions by Altium and Agilent, highlighting their comprehensive portfolio of technologies and workflows designed to deliver confident and reproducible results. Central to the workflow is liquid chromatography-tandem mass spectrometry (LC-MS/MS) instrumentation, enabling sensitive and selective quantification of PFAS compounds across diverse sample matrices. Complementary solutions include online solid phase extraction (SPE) systems for efficient sample preparation, direct injection workflows for streamlined analysis, and the PFAS conversion kit for high-performance liquid chromatography (HPLC) pumps and autosamplers to minimize background contamination and ensure data integrity. Additionally, the Agilent PFAS database, covering more than 100 target compounds, provides a robust foundation for method development, screening, and regulatory compliance. Together, these tools form a complete analytical ecosystem for laboratories addressing PFAS detection and monitoring.

KEY WORDS: Agilent; HPLC, LC-MS/MS; PFAS database

Poster presentations

P 1

Assessment of (geno)toxic effects of bisphenol A (BPA) and its analogues bisphenol AP (BPAP) and bisphenol C (BPC) in a 3D *in vitro* HepG2 cell model

Martina Štampar[1], Tim Ravnjak[1,2], Alja Štern[1], Ana-Marija Domijan[3], and Bojana Žegura[1,2]

[1] National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, Ljubljana, Slovenia
[2] Jožef Stefan International Postgraduate School, Ljubljana, Slovenia
[3] University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Pharmaceutical Botany, Zagreb, Croatia
bojana.zegura@nib.si

Bisphenol A (BPA) is a widely recognised environmental contaminant with well-documented endocrine-disrupting, genotoxic, and cytotoxic effects. Its structural analogues, bisphenol AP (BPAP) and bisphenol C (BPC), are considered emerging contaminants, yet their safety profiles, particularly under combined exposure scenarios, remain insufficiently characterised. We applied new approach methodologies (NAMs) using a hepatic 3D *in vitro* model (HepG2 spheroids) to investigate the individual and binary mixture effects of BPA, BPAP, and BPC. Spheroids were exposed to bisphenols for 24 h (10–80 μmol/L) and 96 h (1–8 μmol/L), followed by assessments of cytotoxicity (ATP assay), genotoxicity (Comet assay), oxidative stress (DCFH-DA and MDA assays), and toxicogenomic responses (qPCR analysis). No significant cytotoxicity was observed at the tested concentrations; however, slight reductions in cell viability were detected following exposure to BPAP, BPC, and the BPA+BPAP mixture. DNA damage was induced by all of the compounds, with BPAP showing the highest genotoxic potency (≥0.1 μmol/L). All of the bisphenols and their mixtures triggered oxidative stress responses at both time points. After 24 h, BPA, BPAP, and BPC (up to 40 μmol/L) upregulated *BAX* and *BCL2* gene expression, suggesting activation of early apoptotic signalling pathways. At 96 h, *BAX* expression declined while *BCL2* remained elevated, indicating a potential shift toward cell survival. Notably, *BCL2* was strongly upregulated by BPA and binary mixtures (1–4 μmol/L). Genes involved in xenobiotic metabolism (CYP1A1, CYP3A4, UGT1A1) and oxidative stress responses (HMOX1, SRXN1, OGG1) were also upregulated, whereas no significant changes were observed in DNA repair-related genes. These findings highlight that BPA analogues, especially BPAP, may elicit similar or greater adverse effects than BPA and emphasise the importance of assessing not only the impact of individual bisphenol compounds but also the potential risks associated with combined exposures.

Supported by ARRS (P1-0245, J1-2465, Z1-3191), bilateral projects (BI-HR/18-19-003; BI-HR/20-21-019), project FarmInova (KK.01.1.1.02.0021) University of Zagreb, and HE projects PARC (GA 101057014) and CutCancer (GA 101079113).

KEY WORDS: bisphenol analogues; combined exposure; DNA damage; *in vitro* 3D cell model; oxidative stress

P 2

First insights into the biocompatibility of recycled polyamide 6 (PA 6) polymer at cellular level: *in vitro* study on human keratinocytes and peripheral blood lymphocytes

Anja Ludaš Dujmić[1], Antonio Zandona[2], Vesna Benković[3], Sanja Ercegović Ražić[1], Maja Katalinić[2], and Nevenka Kopjar[2]

[1] University of Zagreb, Faculty of Textile Technology, Zagreb, Croatia
[2] Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia
[3] University of Zagreb, Faculty of Science, Zagreb, Croatia
nkopjar@imi.hr

Modern waste management practices in the textile industry include recycling of polyamides using mechanical and chemical processes. Some involve the use of low-pressure plasma, which changes the surface characteristics of the material. The aim of this study was to establish the biocompatibility of a recycled polyamide 6 polymer (PA 6; ECONYL®, AquafilSLO, Ljubljana, Slovenia), used in granulate (G) and extruded (E) form, both original and processed with low-pressure argon plasma for 5 and 15 minutes. Due to the fact that the toxicological profile is yet unknown, the amount of PA 6 used in the testing (10 mg per mL of cell culture medium) was determined based on the literature. Cytotoxic effects of the 24-h exposure to PA 6 samples were studied using the MTS test on human keratinocyte cell line HaCaT. The level of DNA damage in the same cells was measured using the alkaline comet assay. Using the cytokinesis blocked micronucleus assay, the level of cytogenetic damage was studied on human peripheral blood lymphocytes of a healthy male donor (non-smoker, 22 years). Under the applied experimental conditions, none of the tested samples caused significant cytotoxic, DNA damaging, or cytogenetic damage in HaCaT cells and lymphocytes. Results suggest that the extrusion process reduces the DNA damaging potential of the recycled PA 6 material. The overall findings are of great importance for the production of new textile materials. Nevertheless, in order to obtain as much toxicologically relevant data as possible, further investigations using a broader range of tested mass concentrations and other suitable models are needed.

This study was funded by the Croatian Science Foundation (grant HRZZ-IP-2019-04-6418). It was also supported by the European Union – Next Generation EU (Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006) – grant BioMolTox, and performed using the facilities and equipment funded within the European Regional Development Fund project KK.01.1.1.02.0007.

KEY WORDS: cell culture; cytotoxicity; genotoxicity; new textile materials; waste recycling

P 3

Adverse (geno)toxic effects of benzo[a]pyrene (B[a]P) and dibenz[a,h]anthracene (DB[a,h]A) in a 3D hepatocellular spheroid model

Katja Kološa[1], Tim Ravnjak[1], Eva Kanalec[1], Sonja Žabkar[1], Luka Kazensky[2], Goran Gajski[2], and Bojana Žegura[1]

[1] National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, Ljubljana, Slovenia
[2] Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia
katja.kolosa@nib.si

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants generated by the incomplete combustion of organic material, their main sources being transport, industry, and domestic heating. In addition to outdoor air pollution, PAHs are also present indoors, particularly in areas where cooking, heating, or smoking takes place. Among them, benzo[a]pyrene (B[a]P) is a well-characterised PAH with established carcinogenic properties, where data on dibenzo[a,h]anthracene DB[a,h]A properties remain scarce. To bridge this gap, we analysed and compared the genotoxic potential of DB[a,h]A and B[a]P using a physiologically relevant 3D HepG2 spheroid model. We evaluated the cytotoxic (ATP assay) and genotoxic (comet assay, flow cytometry for γH2AX, pH3 and p21) properties as well as the toxicogenomic effects of both compounds after 24-h (short-term) and 96-h (long-term) exposure. DNA damage was observed for both compounds at non-cytotoxic concentrations of 5 and 0.25 μmol/L after 24- and 96-hour exposure, respectively. Gene expression profiling revealed the upregulation of key genes involved in metabolism (encoding phase I and phase II enzymes), oxidative stress, and DNA damage response, which was further confirmed by flow cytometric analysis of DNA damage markers, such as γH2AX. The consistent results across multiple endpoints suggest that DB[a,h]A has comparable genotoxic potential to B[a]P, emphasising the need for further mechanistic investigation of its effects on human health. A comprehensive understanding of the genotoxic effects of DB[a,h]A is essential for elucidating the underlying mechanisms of action and advancing our understanding of its biological impact.

Supported by HEU EDIAQI (101057497), HEU CutCancer (101079113), Slovenian Research Agency (P1-0245), HRZZ HUMNap (1192) & EU BioMolTox (533-03-23-0006).

KEY WORDS: 3D cell model; DNA damage; genotoxicity; polycyclic aromatic hydrocarbons (PAHs); toxicogenomic effects

P 4

Phytochemical-drug interactions: cellular impact of hydroquinone and nitrofurantoin co-treatment in T24 bladder cells

Ana Huđek Turković[1], Vilena Kašuba[2], Gordana Rusak[3], Marijana Ćurčić[4], and Ksenija Durgo[1]

[1] University of Zagreb, Faculty of Food Technology and Biotechnology, Zagreb, Croatia
[2] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[3] University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia
[4] University of Belgrade, Faculty of Pharmacy, Department of Toxicology "Akademik Danilo Soldatović", Belgrade, Serbia
ana.hudek@pbf.unizg.hr

Natural products are often considered safe, and their potential interactions with synthetic drugs are frequently underestimated. In the treatment of uncomplicated urinary tract infections (UTIs), hydroquinone, the main active compound of the bearberry [*Arctostaphylos uva–ursi* (L.) Spreng.] leaf extract, is commonly used in combination with the antibiotic nitrofurantoin. This study aimed to clarify the interactions between hydroquinone and nitrofurantoin to support safer and more effective UTI therapies. We evaluated the cytotoxic and genotoxic effects of hydroquinone (5–900 μg/mL), nitrofurantoin (167–670 μg/mL), and their combinations *in vitro* using T24 human bladder cells, with exposure times of 2–8 h. Cell viability was measured by *Neutral Red* assay, while genotoxicity was assessed by the Cytokinesis-Blocked Micronucleus (CBMN) *Cytome* Assay. Cytotoxicity analysis demonstrated a statistically significant synergistic reduction in cell viability at all exposure times with 24 h recovery (β_{12}=5.45–9.38×10^{-5}, p<0.01), whereas individual compounds showed no such effect. The CBMN assay showed the highest micronuclei frequencies (31.2/1000 binucleated cells, BNC), nuclear buds (8.5/1000 BNC), and nucleoplasmic bridges (14.2/1000 BNC) following treatment with a mixture of 5 μg/mL hydroquinone and 167 μg/mL nitrofurantoin (compared to negative control: 12.5, 1.3 and 7/1000 BNC, respectively). This combination also produced the lowest nuclear division index (NDI=1.08) and highest cytostasis (64.1 %). The highest apoptotic cell count (25/3000 BNC) was observed after 8 h exposure to 10 μg/mL hydroquinone. These results underscore the importance of assessing the combined effects of phytochemicals and antibiotics to develop safer therapeutic strategies for UTIs.

KEY WORDS: antibiotics; cell proliferation; cytotoxicity; genotoxicity; *in vitro*; synergism

P 5

Assessing genotoxicity and inflammatory response to benzo[g,h,i]perylene and benzo[b]fluoranthene in a lung co-culture model at the pseudo-air-liquid interface

Matjaž Novak[1], Martina Štampar[1], Alja Štern[1], Michael J. Burgum[2], Gillian E. Conway[2], Goran Gajski[3], Shareen H. Doak[2], and Bojana Žegura[1]

[1] National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, Ljubljana, Slovenia
[2] Swansea University Medical School, Faculty of Medicine, Health and Life Sciences, In Vitro Toxicology Group, Swansea, United Kingdom
[3] Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia
matjaz.novak@nib.si

Indoor air pollution poses a growing health risk, causing around 3.2 million premature deaths annually and a range of health problems. Harmful pollutants like polycyclic aromatic hydrocarbons (PAHs), including benzo[g,h,i]perylene (BGP) and benzo[b]fluoranthene (BBF), are especially toxic and widespread. Since inhalation is the main exposure route, understanding their impact on the respiratory system is vital for assessing health risks and developing effective mitigation strategies. We examined the genotoxicity and inflammatory response to BGP and BBF using a co-culture model of human alveolar epithelial (A549) and macrophage-like (d.THP-1) cells at a pseudo-air-liquid interface. After 24 hours of exposure to non-cytotoxic doses (BGP ≤18.1 μmol/L; BBF ≤39.6 μmol/L), neither compound triggered DNA double-strand breaks (*γ*H2AX), mitotic activity (pH3), micronuclei formation (CBMN assay), changes in proliferation (Ki67), nor alterations in cell cycle progression (flow cytometry). BGP exposure led to increased IL-8 secretion and a higher proportion of cells expressing TNF-*a*, IL-6, and IL-1*β*, as shown by ELISA and flow cytometry. BBF similarly elevated the percentage of cells expressing TNF-*a* and IL-6, along with IFN-*γ*. Transcriptomic analysis indicated that neither compound significantly activated DNA damage-responsive genes, except for *CDKN1A*. However, both PAHs upregulated several inflammation-associated genes, including *TNF-a*, *IL-10, IL-17d, IL-8, IL-6*, and *IL-1β*. These results indicate that BGP and BBF can trigger inflammatory responses without inducing DNA damage, likely due to the limited metabolic activity of A549 cells. Their ability to upregulate inflammatory markers suggests a potential role in chronic airway inflammation, highlighting the need for investigating their long-term health impacts.

Supported by HEU CutCancer (101079113), HEU EDIAQI (101057497), ARIS (P1-0245).

KEY WORDS: flow cytometry; gene expression; indoor air pollution; inflammation; micronuclei

P 6

Effect of naturally occurring naphthoquinones on cell viability and oxidative stress in a liver cancer cell line

Ana Marija Marjanović Čermak[1], Krunoslav Ilić[1], Dafine Kastrati Bajçinca[2], and Ana-Marija Domijan[2]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia
adomijan@pharma.hr

Naphthoquinones include naturally occurring and synthetic compounds with different pharmacological properties that enable their anticancer activity and therapeutic application. Depending on structural differences and the type of cell affected, many mechanisms have been proposed regarding their activity at cellular level. The aim of this study was to assess the biological activity of two naturally occurring naphthoquinones, 2-methoxy-1,4-naphthoquinone (MNQ) and 2-hydroxy-1,4-naphthoquinone (HNQ) on liver cancer cells (HepG2) after 24 h of exposure. Cell viability was determined based on their metabolic activity and apoptotic cells were detected by Annexin V-FITC assay kit and quantified by flow cytometry. Fluorescent probe 2'7'-dichlorofluorescin diacetate was used as an indicator of oxidative stress to measure the levels of intracellular reactive oxygen species (ROS). Results showed that increasing concentrations of MNQ inhibit cell viability and increase the percentage of early and late apoptotic cells. MNQ treatment also induced significantly higher levels of intracellular ROS that were concentration-dependent. Compared to MNQ, HNQ produced smaller amount of ROS and appeared to be less toxic to HepG2 cells. In conclusion, MNQ showed greater biological activity toward liver cancer cells by inducing oxidative stress and cell death. Based on the observed results, further studies will be conducted to reveal the exact mechanism behind its toxicity.

This study was funded by the University of Zagreb.

KEY WORDS: apoptosis; flow cytometry; HepG2; *in vitro*; reactive oxygen species

P 7

Nopal-based dietary supplement against rifampicin-induced hepatotoxicity

Zoran Zhivikj[1], Ana Marija Bajatovska[1], Marija Karapandzova[2], Kristina Shutevska[1], Tatjana Kadifkova Panovska[1], Lidija Petrushevska-Tozi[1], and Tanja Petreska Ivanovska[1]

[1] University Ss. Cyril and Methodius in Skopje, Faculty of Pharmacy, Institute of Applied Biochemistry, Skopje, Republic of North Macedonia
[2] University Ss. Cyril and Methodius in Skopje, Faculty of Pharmacy, Institute of Pharmacognosy, Skopje, Republic of North Macedonia
zzivic@ff.ukim.edu.mk

Rifampicin is a widely used antibiotic known to cause hepatotoxicity through oxidative stress and liver cell damage, partly due to its induction of the enzyme CYP3A4, which alters drug metabolism. Nopal (*Opuntia ficus-indica*) is a cactus rich in antioxidants with potential hepatoprotective properties. This study investigates whether a nopal-based supplement exerts a protective effect against rifampicin-induced toxicity in HepG2 cells. To this end, HepG2 cells were exposed to 20 μmol/L rifampicin during 48 hours. Following induction, the cells were treated with the nopal-based supplement at concentrations of 0.05, 0.5, and 1 mg/mL. Cell viability was assessed using the MTT assay after 24, 48, and 72 hours. At the same time intervals, the enzymatic activities of AST, ALT, γ-GT, ALP, and LDH were determined in the cell supernatants. Considering the cell viability results, rifampicin-induced toxicity was suppressed probably due to the nopal antioxidant activity. An increase in cell viability up to 20 % was observed with higher concentrations of the nopal-based supplement and longer exposure durations. The lowest tested concentration (0.05 mg/mL) did not attenuate rifampicin-induced toxicity after 24 hours but slightly promoted cell growth within 72 hours of exposure. Compared to rifampicin untreated cells, enzymatic activities were generally higher. However, a decreasing trend of enzymatic activities with prolonged time of exposure (72 hours) to nopal-based supplement was noticed. These results suggest promising hepatoprotective effects of nopal-based supplements, particularly against rifampicin-induced toxicity. Further studies are needed to elucidate the underlying molecular mechanisms, confirm these effects *in vivo*, and evaluate clinical safety and efficacy.

KEY WORDS: antioxidant; cell viability; enzymatic activity; hepatoprotective effects; HepG2

P 8

In vitro safety evaluation of clinically used HI-6 and two novel oximes, antidotes in the treatment of organophosphate poisoning, in a 3D human liver spheroid model

Ivana Vrhovac Madunić[1], Katarina Fras[2], Josip Madunić[1], Maja Katalinić[1], Bojana Žegura[2], and Martina Štampar[2]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] National Institute of Biology, Ljubljana, Slovenia
ivrhovac@imi.hr

Oximes are key therapeutic agents used in the treatment of organophosphate (OP) poisoning due to their ability to reactivate acetylcholinesterase. While HI-6 is a well-established antidote, the development of novel oximes is essential to improve efficacy, broaden the spectrum of action, and ensure safety. This study aimed to assess the safety profile of HI-6 and two newly synthesized oximes (pyridinium GM508 and quinuclidinium Q5) and HI-6 as a control compound, using a 3D spheroid cell culture model of human hepatocellular carcinoma cells (HepG2). Cytotoxicity was evaluated by cell viability assays, while genotoxic potential was examined via the alkaline comet assay to detect DNA strand breaks. Effects on cell cycle distribution and proliferation were determined by flow cytometry using Hoechst and Ki67 markers, with additional analysis of DNA damage (γH2AX) and mitotic activity (histone H3 phosphorylation). The results demonstrated that both tested novel oximes, as well as HI-6, exhibited low cytotoxicity within therapeutic concentration ranges, with no significant increase in DNA strand breaks compared to controls. Cell cycle analysis showed minimal alterations in phase distribution, and proliferation rates remained comparable to untreated spheroids. Furthermore, no significant induction of γH2AX or pH3 positivity was observed. These findings suggest that, under the tested conditions, the novel oximes GM508 and Q5 displayed a favourable safety profile *in vitro*, supporting their potential for further development as antidotes for OP poisoning.

This research was funded by the Slovenian Research Agency ARIS (P1-0245, J1-2465, Z1-3191), bilateral projects (BI-HR/18-19-003; BI-HR/20-21-019), European Regional Development Fund project KK.01.1.1.02.0007 (ReC-IMI), European Union – Next Generation EU 533-03-23-0006 (BioMolTox) and Croatian Science Foundation [grant UIP-2017-05-7260 (CellToxTargets)].

KEY WORDS: cell cycle; cell proliferation; DNA strand breaks; flow cytometry; pyridinium compounds

P 9

Interpreting the metabolomic signals of polycyclic aromatic hydrocarbons (PAHs) in a 3D liver model

Anja Bošnjaković[1], Thomas O. Eichmann[2], Alja Štern[3,4], Morten Arendt Rasmussen[5,6], Mario Lovrić[1,5,7,8], and Bojana Žegura[3,4]

[1] Institute for Anthropological Research, Centre for Applied Bioanthropology, Zagreb, Croatia
[2] Medical University of Graz, Center for Medical Research, Core Facility Mass Spectrometry, Graz, Austria
[3] National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, Ljubljana, Slovenia
[4] University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia
[5] University of Copenhagen, Department of Food Science, Frederiksberg, Denmark
[6] University of Copenhagen, Herlev and Gentofte Hospital, Copenhagen Prospective Studies on Asthma in Childhood, Gentofte, Denmark
[7] The Lisbon Council, Brussels, Belgium
[8] Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Osijek, Croatia
anja.bosnjakovic@inantro.hr

Does prolonged, low-dose polycyclic aromatic hydrocarbons (PAH) exposure leave a stable and biologically interpretable footprint in a human liver model? To answer that, we used a network-guided interpretation of untargeted liquid chromatography-mass spectrometry (LC-MS) based metabolomics to decode low-dose PAH responses in 21-day HepG2 spheroids. Cells were exposed to non-cytotoxic doses of benzo[a]pyrene (BaP) or benzo[b]fluoranthene (BBF) for 24 or 96 hours. To interpret the data biologically, we combined principal component analysis (PCA) for global structure, the false discovery rate (FDR)-controlled differential testing with a predefined robustness filter, a correlation network to position co-varying metabolites, and pathway enrichment for context. At 24 hours, no shared metabolite passed the robustness threshold. By 96 hours, both compounds converged on a seven-metabolite pattern consisting of 2-aminoadipic acid, *N*-acetylspermidine, glycerophosphorylinositol, orotidine, valerylcarnitine (C5), *S*-adenosylmethionine, and *β*-citrylglutamate. PCA separated high-dose groups from controls, volcano plots highlighted directionally consistent shifts, and the correlation network placed the seven metabolites within coherent communities. Together, the seven metabolites pointed to possible changes in four metabolic areas, including polyamine/one-carbon reactions (*N*-acetylspermidine, S-adenosylmethionine), membrane-lipid turnover (glycerophosphorylinositol), mitochondrial acyl-carnitine handling (valerylcarnitine), and nucleotide pathways (orotidine), with 2-aminoadipic acid and *β*-citrylglutamate suggesting amino-acid/tricarboxylic acid (TCA) connections. Network analysis indicated broader, more interconnected perturbations under BBF than BaP, pointing to compound-specific toxicodynamics. Overall, extended exposure was required to reveal stable low-dose PAH signals in this model, and the data supports a concise panel of candidate biomarkers for follow-up. The workflow is suited for longitudinal, low-concentration studies with potential relevance for health-risk assessments.

Experimental work was funded by Horizon Europe (EDIAQI project #101057497; CutCancer project #10050740). ML received funding from the Croatian Science Foundation (MOBODL-2023-12-5502). AB received funding from the Croatian Science Foundation (MOBDOK-2023-1077. BZ received funding from the Slovenian Research Agency (P1-0245).

KEY WORDS: benzo[a]pyrene; benzo[b]fluoranthene; biomarkers of exposure; enrichment analysis; liquid chromatography-mass spectrometry

P 10

Cyto-/genoprotective effects of homogentisic acid against 24- and 48-hour exposure to benzo[a]pyrene in HepG2 liver cancer cells

Bruno Bekić[1], Vesna Benković[2], Ana Popović[2], Marija Lauc[2], and Mirta Milić[1]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] University of Zagreb, Faculty of Science, Biology Department, Zagreb, Croatia
mmilic@imi.hr

Benzo[a]pyrene (BaP), a polycyclic aromatic hydrocarbon, is one of the primary air pollutants, recognized by the International Agency for Research on Cancer as a Group 1 human carcinogen. Although its mechanisms of action are still not understood, to exert its harmful effects, BaP needs to be metabolised, which occurs mainly in the liver. In our previous studies, we demonstrated that some antioxidants, such as homogentisic acid (HGA), a constituent of a Mediterranean strawberry tree honey, can protect lung cells from toxic effects caused by BaP exposure. Considering that lung cells have limited metabolic competence, we wanted to establish whether a similar effect would be seen in HepG2 liver cancer cells. We used MTT proliferation assay and alkaline comet assay to determine the outcomes of 24- and 48-hour treatments of cells with different BaP concentrations (1, 2, 5, 10, 20, 40, 50, and 60 μmol/L) and three concentrations of HGA that could be possibly used by the general population in daily tea preparations with HGA-rich honey (equal to the use of one, two, or three cups of tea: 1.27, 6.36, and 10 μmol/L). The results demonstrated that HGA can decrease both cell proliferation and DNA damage caused by the lower BaP concentrations. However, its protective potency diminished at BaP concentrations above 20 μmol/L, resulting in increased cell proliferation, especially after 24 h exposure. Still, the addition of HGA slightly diminished DNA damage compared to treatments with single BaP. However, all of the damage descriptors were higher than the control values, both after 24 and 48 hours. The promising results obtained *in vitro* indicate that the use of antioxidants can diminish harmful effects of known air pollutant carcinogens.

Results were obtained using the facilities and equipment funded within the European Regional Development Fund project KK.01.1.1.02.0007. This study was funded by the HUMNap project – Air Pollution and Human Biomarkers of Effect [Croatian Science Foundation (Hrzz-IP-2020-02-1192)], the EDIAQI project – Evidence Driven Indoor Air Quality Improvement (HORIZON-HLTH-2021-ENVHLTH-02), the BioMolTox project – Assessment of efficacy and toxicity of biologically active substances funded by the European Union – Next Generation EU (Program Contract of 8 December 2023, Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006).

KEY WORDS: antioxidant; cell proliferation; DNA damage; DNA protection; polycyclic aromatic hydrocarbons

P 11

Evaluating the genotoxic potential of bisphenol A (BPA) alternatives in an advanced zebrafish liver-based *in vitro* model system

Alja Štern[1], Katja Kološa[1], Špela Rozman[2], and Bojana Žegura[1]

[1] National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, Ljubljana, Slovenia
[2] University of Ljubljana, Biotechnical Faculty, Slovenia
alja.stern@nib.si

Growing concerns over the harmful health effects of bisphenol A (BPA), once widely used in plastics, have led to its gradual replacement in industrial applications. In response, industries have adopted structurally similar alternatives whose toxicological profiles remain largely unknown. This study investigates the (geno)toxic and endocrine-disruptive potential of three emerging BPA substitutes – bisphenol AF (BPAF), bisphenol AP (BPAP), and bisphenol PH (BPPH). Their increased detection and presence in surface waters and sediments, their persistence, bioaccumulation potential, and high biological activity raise serious concerns for aquatic ecosystems. Using 3D liver spheroids derived from zebrafish (*Danio rerio*) liver cells, a highly relevant *in vitro* model that closely mimics *in vivo* liver tissue, ZFL cells, was applied. Cytotoxicity was assessed via the CellTiter-Glo® assay, while genotoxicity was evaluated using the comet assay, flow cytometry-based detection of γH2AX, and toxicogenomic profiling. All three BPA alternatives showed higher cytotoxicity than BPA. At non-cytotoxic concentrations, they induced transient DNA damage without causing double-strand breaks. Gene expression analysis revealed activation of TP53-mediated DNA repair pathways (NER and BER), with no evidence of non-homologous end joining (NHEJ), confirming the absence of significant DSB formation and indicating the formation of bulky DNA lesions and oxidative DNA damage. Additionally, endocrine-related gene expression (*ar*, *esr2a*, *esr2b*, *vtrg4*) was significantly altered. Our findings highlight the elevated genotoxic and endocrine-disruptive risks posed by BPAF, BPAP, and BPPH, underscoring the urgent need for comprehensive risk assessments before these compounds are widely adopted as BPA replacements.

Supported by ARIS (P1-0245) and the HEU projects CutCancer (101079113) and PARC (101057014).

KEY WORDS: comet assay; cytotoxicity; genotoxicity; plastics; ZFL cells

P 12

Effect of weight loss dietary supplements on intracellular glutathione levels in HepG2 cells

Zoran Zhivikj[1], Tanja Petreska Ivanovska[1], Ana Marija Bajatovska[1], Kristina Shutevska[1], Dushan Zhivikj[1], Marija Karapandzova[2], Tatjana Kadifkova Panovska[1], and Lidija Petrushevska-Tozi[1]

[1] University Ss. Cyril and Methodius in Skopje, Faculty of Pharmacy, Institute of Applied Biochemistry, Skopje, Republic of North Macedonia
[2] University Ss. Cyril and Methodius in Skopje, Faculty of Pharmacy, Institute of Pharmacognosy, Skopje, Republic of North Macedonia
tpetreska@ff.ukim.edu.mk

Glutathione is a key intracellular antioxidant that neutralizes reactive oxygen species and detoxifies harmful compounds, serving as an indicator of cellular oxidative stress and detoxification capacity. Weight loss dietary supplements (WLDS), often containing various bioactive herbal compounds, may influence glutathione levels and affect oxidative balance. This study aimed to evaluate the effects of eight WLDS on glutathione levels in HepG2 cells. Total cellular sulfhydryl (SH) content, a proxy for glutathione, was measured spectrophotometrically using dithionitrobenzoic acid in the culture supernatants after treatment with WLDS at three concentrations (0.05, 0.5, and 1 mg/mL) over 24, 48, and 72 hours. Untreated control cultures were analysed under identical conditions. All of the tested supplements significantly affected SH levels. Interestingly, supplements containing well-established antioxidants such as green tea polyphenols led to a depletion of SH levels in a concentration- and time-dependent manner. One supplement containing acai berry, garcinia, aloe, chitosan, and green tea extracts, used at concentrations of 0.5 and 1 mg/mL caused an approximate 80 % reduction in SH levels compared to the control. Three additional supplements with green tea extracts or chitosan also decreased SH levels by more than 15%. These findings indicate that certain herbal WLDS may paradoxically act as pro-oxidants, decreasing intracellular SH levels, thereby increasing oxidative stress, and compromise the antioxidant defence system. While many herbal compounds have recognised antioxidant activity, these results suggest that additional research is required to clarify their cellular effects, understand the mechanisms involved, and assess the safety and efficacy of various WLDS formulations and doses.

KEY WORDS: antioxidant; cell culture; hepatotoxicity; herbal supplements; sulfhydryl content

P 13

Efficacy of apigenin in protection against 24- and 48-hour exposure to benzo[a]pyrene in a HepG2 cell line model: MTT and comet assay study

Mirta Milić[1], Vesna Benković[2], Marija Lauc[2], Ana Popović[2], and Bruno Bekić[1]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] University of Zagreb, Faculty of Science, Biology Department, Zagreb, Croatia
mmilic@imi.hr

As a primary air pollutant and a known human carcinogen, benzo[a]pyrene (BaP) has garnered significant scientific and public interest. In spite of extensive research, its mechanisms of action are still not fully understood. The results of our previous studies in lung cells demonstrated that BaP induced elevated levels of DNA damage and impaired cell proliferation, especially at lower concentrations. Since the lung cell model is limited by its lack of full metabolic activation, this time we aimed to investigate the toxic effects of BaP on the liver cancer cell line HepG2, which expresses the CYP family members and can thus fully metabolise the chemical. As in previous studies we also demonstrated that the flavonoid apigenin can stop cell proliferation, here we wanted to check whether it produces a similar effect in HepG2 cells after 24- and 48-hour treatment with BaP (at concentrations of 1, 2, 5, 10, 20, 40, 50, and 60 μmol/L) and its combinations with apigenin (at 20, 40, and 60 μmol/L). The outcomes of treatments were assessed using MTT and alkaline comet assays. We found that lower BaP concentrations, such as 1, 2, 5, and 10 μmol/L, can increase cell proliferation. Still, apigenin can lower that effect down to 10 μmol/L. At higher concentrations, such as 20, 40, 50, and 60 μmol/L, cell proliferation was higher than that of the control cell line, but still lower than after the single BaP treatments. The highest effect was observed at 60 μmol/L. DNA damage estimation using the comet assay showed that BaP concentrations below 20 μmol/L produced similar levels of damage to those of control cells. At concentrations above 20 μmol/L, an increase in DNA damage levels compared to the control levels, as well as compared to the single BaP treatment at the same concentration, was observed. This slightly decreased the DNA damage caused by single BaP treatment after 24 hours and 48 hours. The obtained results demonstrated the beneficial and protective effects of phytochemicals against known air pollutant carcinogens.

The research results were obtained using the facilities and equipment the European Regional Development Fund project KK.01.1.1.02.0007. The study was funded by the HUMNap project – Air Pollution and Human Biomarkers of Effect [Croatian Science Foundation (Hrzz-IP-2020-02-1192)], the EDIAQI project – Evidence Driven Indoor Air Quality Improvement (HORIZON-HLTH-2021-ENVHLTH-02), the BioMolTox project – Assessment of efficacy and toxicity of biologically active substances funded by the European Union – Next Generation EU (Program Contract of 8 December 2023, Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006).

KEY WORDS: cell proliferation; DNA damage; DNA protection; phytochemicals; polycyclic aromatic hydrocarbons

P 14

Molecular insights into nicotinamide analogue toxicity: redox imbalance, apoptosis, and cell dysfunction

Josip Madunić[1], Antonio Zandona[1], Ana-Marija Lulić[1], Lucija Marcelić[1], Valentina Bušić[2], Dajana Gašo Sokač[2], and Maja Katalinić[1]

[1] Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia
[2] Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Osijek, Croatia
jmadunic@imi.hr

Previous studies have demonstrated the bioactivity of a series of nicotinamide analogues, exhibiting beneficial effects at low concentrations but pronounced cytotoxicity at higher concentrations, as observed in cell models. To better understand these effects, we investigated their mechanisms of action, with a focus on identifying potential molecular targets. Given the relevance of nicotinamide analogues in neurobiology, we explored their impact on neural-like and muscle cells. The tested compounds significantly elevated markers of cell stress, including reactive oxygen (ROS), indicating membrane damage. Simultaneously, they reduced antioxidant defence, exemplified by a decline in glutathione (GSH) levels and mitochondrial membrane potential, suggesting a disruption of redox homeostasis and mitochondrial function. The activation of caspase-8 points to the initiation of extrinsic apoptotic pathways, likely triggered by receptor-mediated stress signalling. Additionally, the inhibition of calcium flux may have reflected impaired cell signalling and ion homeostasis, further leading to cell dysfunction. The suppression of the MAPK signalling pathway, a critical survival signalling node, could contribute to the failure of pro-survival mechanisms, amplifying cytotoxic effects. These findings suggest a cascade of interconnected cytotoxic events, providing novel insights into their mode of action and highlighting key pathways for further exploration in the context of neurotoxicity and therapeutic intervention.

This study was supported by the Croatian Science Foundation (UIP-2017-05-7260) and European Union Next Generation EU (Program Contract of 8 December 2023, Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006). Facilities and equipment used were funded within the ERDF project KK.01.1.1.02.0007.

KEY WORDS: calcium signalling; caspase; MAPK pathway; mitochondrial dysfunction; oxidative stress

P 15

Bacterial aryl sulfotransferases for selective conjugation of polyphenols

Barbora Petránková[1,2], Katerina Brodsky[1], Kristýna Slámová[1], Natalia Kulik[3], Lucie Petrásková[1], and Kateřina Valentová[1]

[1] Institute of Microbiology of the Czech Academy of Sciences, Laboratory of Biotransformation, Prague, Czech Republic
[2] Charles University, Faculty of Science, Department of Genetics and Microbiology, Prague, Czech Republic
[3] Institute of Microbiology of the Czech Academy of Sciences, Centre Algatech, Laboratory of Photosynthesis, Třeboň, Czech Republic
barbora.petrankova@biomed.cas.cz

Polyphenols are abundant dietary compounds with known antioxidant and anti-inflammatory properties. However, their biological effects in humans are largely determined by their metabolism, in particular by phase II biotransformation reactions such as sulphation. These transformations influence both bioavailability and bioactivity, making them essential for toxicological and pharmacokinetic evaluation. The chemical synthesis of sulphated polyphenol metabolites is often inefficient and non-selective. To change this, we investigated bacterial aryl sulfotransferases (ASTs; EC 2.8.2.22) as biocatalysts for the regioselective sulphation of polyphenols. The ASTs were recombinantly expressed in *Escherichia coli*, purified and tested for their activity towards a range of phenolic substrates, including flavonoids, phenolic acids, carbohydrates, and phytocannabinoids. The resulting sulphated metabolites were isolated and structurally characterised by mass spectrometry and nuclear magnetic resonance spectroscopy. These standards can serve as reference compounds in studies on xenobiotic metabolism, toxicokinetic, and biomarker identification. In addition, the structure-guided mutagenesis of selected ASTs led to enzyme variants with improved activity and regioselectivity. Ongoing crystallographic analyses could elucidate substrate binding and catalytic mechanisms. This work emphasizes enzymatic sulphation as a powerful tool for generating defined polyphenol metabolites and supports its application in the study of human exposure, metabolism and safety assessment of dietary xenobiotics.

This study was funded by the MEYS grant (CZ.02.01.01/00/22_008/0004597) and GAČR (23-04654S).

KEY WORDS: bioavailability; biotransformation; flavonoids; metabolite standards; xenobiotic conjugation

P 16

Seed priming: an approach for improving food crop tolerance to soil toxicity

Erna Karalija[1], Alisa Selović[2], Ajna Lošić[1], Arnela Demir[1], Sabina Dahija[1], and Dunja Šamec[3]

[1] University of Sarajevo, Faculty of Science, Department of Biology, Laboratory for Plant Physiology, Sarajevo, Bosnia and Herzegovina
[2] University of Sarajevo, Faculty of Science, Department of Chemistry, Sarajevo, Bosnia and Herzegovina
[3] University North, Department of Food Technology, Koprivnica, Croatia
dsamec@unin.hr

Soil contamination with heavy metals and salinization represents a major global challenge to food security and ecosystem health. Both cadmium (Cd) and excessive salinity impair seed germination, growth, and metabolism, primarily through osmotic stress, ion imbalance, and oxidative damage. Cd accumulation in edible tissues poses a direct risk to human health, while soil salinization threatens long-term agricultural sustainability. Seed priming is a pre-sowing treatment in which seeds are partially hydrated under controlled conditions to activate early metabolic processes without the emergence of radicles. This "prepares" seeds to respond more rapidly and effectively under stress. Benefits include faster germination, improved seedling vigour, enhanced antioxidant defence, better osmotic and ion balance, and reduced uptake of toxic elements. Here, we present the *in vitro* results from studies on seed priming in two food crops: sweet pepper (*Capsicum annuum* L.) and lettuce (*Lactuca sativa* L.). In sweet pepper, priming enhanced tolerance to salinity and activated intrinsic defence mechanisms, including osmolyte accumulation, maintenance of photosynthetic pigments, and antioxidant enzyme activity. In lettuce, 20 mmol/L proline priming maintained photosynthetic capacity and supported zinc (Zn) and iron (Fe) uptake under Cd stress, reducing oxidative damage and limiting heavy metal accumulation. These findings demonstrate that seed priming is a low-cost, sustainable strategy to mitigate the toxic effects of both abiotic (salinity) and chemical (Cd) soil stressors. From a toxicological perspective, priming modulates biomarkers of stress and toxicity, enhancing plant resilience and reducing risks for environmental and human health by limiting contaminant accumulation in the food chain.

KEY WORDS: heavy metals; increased salinity; ion imbalance; lettuce; pepper

P 17

Biosynthesis and biodegradation of antinutritional amino acid nitriles in pulses: distribution, characterisation, and use of key enzymes

Barbora Křístková[1,2], Lenka Rucká[3], Romana Příhodová[1], Natalia Kulik[4], Vladimír Křen[1], Pavla Bojarová[1,5], and Ludmila Martínková[1]

[1] Institute of Microbiology of the Czech Academy of Sciences, Laboratory of Biotransformation, Prague, Czech Republic
[2] University of Chemistry and Technology, Faculty of Food and Biochemical Technology, Prague, Czech Republic
[3] Institute of Microbiology of the Czech Academy of Sciences, Biotechnological Pilot-Plant, Prague, Czech Republic
[4] Institute of Microbiology of the Czech Academy of Sciences, Centre Algatech, Třebon, Czech Republic
[5] Czech Technical University in Prague, Faculty of Biomedical Engineering, Department of Health Care Disciplines and Population Protection, Kladno, Czech Republic
barbora.kristkova@biomed.cas.cz

Pulses are a rich source of valuable proteins, but many contain significant amounts of antinutritional factors such as cyanogenic glycosides, alkaloid glycosides, or non-protein amino acids. Among the latter are *β*-cyano-L-alanine (cyanoalanine) and its dipeptide *γ*-L-glutamyl-*β*-cyano-L-alanine, which are probably the only natural amino acids that contain the nitrile moiety. The use of these pulses as feed or food has its limitations – the content of these compounds should be monitored, safe varieties selected, or the plant material properly treated. In addition, it is necessary to understand the biosynthesis and biodegradation of these compounds in the plant. Cyanoalanine and its dipeptide are present at high concentrations in the common vetch, a popular ingredient in animal feed. However, these compounds have neurotoxic effects in mammals and birds. Here we focus on the key enzymes involved in the biosynthesis and biodegradation of cyanoalanine. Its synthesis and degradation are catalysed by *β*-cyano-L-alanine synthase (EC 4.4.1.9) and nitrilase NIT4 (EC 3.5.5.1), respectively. We studied their distribution in plants and other organisms and characterized selected enzymes. Here we focus on the heterologous production, purification, and characterisation of a CAS from spinach (*Spinacea oleracea*). The enzyme was studied as the full-length protein and the protein truncated at the *N*-terminus, which forms the signal peptide. We propose the use of CAS and NIT4 in the synthesis and determination of cyanoalanine.

The study was funded by the Czech Science Foundation (grant GA22-06785S) and the Ministry of Education, Youth and Sports of the Czech Republic [grant Talking microbes – understanding microbial interactions within One Health framework (CZ.02.01.01/00/22_008 /0004597)].

KEY WORDS: *β*-cyano-L-alanine; *β*-cyano-L-alanine synthase; neurotoxic effects; nitrilase; vetch

P 18

From QSAR to artificial intelligence: the journey of *in silico* approaches in toxicology through case studies on chemical mixtures and phytochemical modulators

Danijela Đukić-Ćosić, Jovana Živanović, Dragica Božić, Katarina Živančević, Jovana Šljivić, Đurđica Marić, Evica Antonijević Miljaković, Aleksandra Buha Đorđević, Marijana Ćurčić, Zorica Bulat, Biljana Antonijević, and Katarina Baralić

University of Belgrade, Faculty of Pharmacy, Department of Toxicology "Akademik Danilo Soldatović", Belgrade, Serbia
danijela.djukic.cosic@pharmacy.bg.ac.rs

In silico toxicology has undergone significant development, from its early foundations in quantitative structure-activity relationship (QSAR) models to modern approaches based on artificial intelligence (AI) and machine learning. Early QSAR models laid the groundwork for understanding the relationship between chemical structure and biological activity, enabling preliminary toxicity assessments without the need for extensive laboratory testing. Over time, the field has expanded to include molecular docking analysis, systems biology, and large omics data sets, allowing for deeper mechanistic insights into toxic effects. Today, AI-based models leverage vast amounts of chemical and biological data to enhance the accuracy and scope of toxicity predictions, including complex endpoints such as endocrine disruption, neurotoxicity, and carcinogenicity. These advancements increasingly support regulatory efforts aimed at reducing animal testing and accelerating chemical safety assessment. Our work presents examples of current *in silico* approaches for evaluating the toxicity of chemical mixtures, with particular focus on combinations of endocrine disruptors, including plasticizers and toxic metals, and the potential protective effects of substances, such as phytochemicals. It also highlights the contribution of these methods to understanding the mechanisms of toxic and protective actions, emphasizing their potential in the development of predictive and personalized toxicology.

This study was funded by the Ministry of Science, Technological Development and Innovation (no. 451-03-136/2025-03/200161 and 451-03-1203/2021-09) of the Republic of Serbia.

KEY WORDS: mixture toxicity; mechanistic modelling; toxicity prediction; omics data; computational methods

P 19

Disruption of redox homeostasis by gestational α-cypermethrin exposure in rats

Suzana Žunec, Dubravka Rašić, and Anja Katić

Institute for Medical Research and Occupational Health, Zagreb, Croatia
suzana@imi.hr

Alpha-cypermethrin (*a*-cyp), a pyrethroid insecticide, primarily targets the nervous system by activating sodium channels, resulting in increased neuronal excitability. Due to its lipophilic nature, it easily penetrates cellular membranes and crosses the blood-brain barrier, leading to potential long-term toxicity. Although some of its harmful effects on endocrine and reproductive function have been recognized, the impact of subtoxic doses on the oxidative-antioxidative balance during gestation remains unclear. This study aimed to evaluate whether gestational exposure to *a*-cyp at 1, 10, and 19 mg/kg b.w./day disrupts redox homeostasis in the blood and brain of pregnant Wistar rats. The results confirmed the hypothesis, showing dose-dependent oxidative stress characterized by increased reactive oxygen species (ROS) and malondialdehyde (MDA) levels. This was accompanied by decreased glutathione (GSH) content and altered antioxidant enzyme activity. The lowest *a*-cyp dose resulted in the highest superoxide dismutase (SOD) activity, suggesting activation of compensatory antioxidant defences, while catalase (CAT) and glutathione peroxidase (GPx) activity decreased with increasing dose, especially in the brain. Given the brain's sensitivity to oxidative damage, even low-dose *a*-cyp exposure may pose physiological risks to the mother as well as to the foetus. Prenatal exposure thus may predispose offspring to oxidative stress and potentially to endocrine dysfunction. Such findings emphasize the need for further research to elucidate the relationship between oxidative stress and potential endocrine disruptive effects of prenatal exposure to *a*-cyp at environmentally relevant levels.

This study was supported by the in-house scientific project "Assessment of the effects of prenatal exposure to α-cypermethrin on epigenetic programming and endocrine disruption of reproduction and development in experimental rats", the European Union – Next Generation EU (Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006; grant BioMolTox), and conducted using the facilities and equipment funded within the European Regional Development Fund project KK.01.1.1.02.0007.

KEY WORDS: endocrine dysfunction; low dose exposure; oxidative stress; pregnant rats; synthetic pyrethroid

P 20

Assessment of primary DNA damage in blood cells of Ehrlich ascites tumour-bearing mice treated with propolis and flavonoids and gamma-irradiated at 4 Gy

Vesna Benković[1], Anica Knežević[1], Fabijan Knežević[2], and Nevenka Kopjar[3]

[1] University of Zagreb, Faculty of Science, Zagreb, Croatia
[2] Catholic University of Croatia, School of Medicine, Zagreb, Croatia
[3] Institute for Medical Research and Occupational Health, Zagreb, Croatia
vesna.benkovic@biol.pmf.hr

Research on natural radioprotectors in experimental animal models with tumours is scarce, but the data obtained from these models are essential for extrapolating expected effects on human populations. In this study, the effects of ionising irradiation combined with ethanolic extract of propolis (EEP), a water-soluble derivative of propolis (WSDP), quercetin, caffeic acid, chrysin, naringin, and established radioprotector *S*-(2-aminoethyl) isothiouronium bromide hydrobromide (AET) on whole-body irradiated Swiss albino mice bearing the Ehrlich ascites tumour (EAT) were studied. Irradiation was performed using a γ-ray source (^{60}Co), and the absorbed dose was 4 Gy. Each experimental group consisted of 5 male mice per tested component. Three days after EAT intraperitoneal (*i.p.*) inoculation (1×10^6 cells), the effectiveness of the test components was evaluated when administered *i.p.* at a dose of 100 mg/kg for three consecutive days, either before or after irradiation. Peripheral blood cells were used to perform the comet assay 1 and 72 hours after the last treatment. The comet descriptors for evaluation of primary DNA damage were tail intensity and tail length. The higher DNA protective efficiency of the tested components was observed when given preventively. One hour after irradiation, EEP, quercetin, and chrysin showed lower DNA damage. However, after 72 hours, all of the tested components diminished primary DNA damage compared to the control. The obtained results provided new insights into the protective potential of the tested compounds and also suggest a possible synergistic effect with gamma irradiation, which warrants further investigation.

The study was funded by institutional funding provided by the Ministry of Science, Education and the Youth of the Republic of Croatia (no. 106-F25-00045) and European Union – Next Generation EU (Program Contract of 8 December 2023, Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006, BioMolTox).

KEY WORDS: chrysin; comet assay; irradiation; primary DNA damage; quercetin

P 21

Comparative study of the effects of individual bisphenols and their mixtures on pancreatic oxidative stress in rats

Anja Radić, Aleksandra Obradović, Jovana Živanović, Katarina Baralić, Đurđica Marić, Jovana Šljivić, Mihajlo Bojić, Aleksa Milačić, Evica Antonijević Miljaković, Aleksandra Buha Đorđević, Zorica Bulat, Biljana Antonijević, Marijana Ćurčić, and Danijela Đukić-Ćosić

University of Belgrade, Faculty of Pharmacy, Department of Toxicology "Akademik Danilo Soldatović", Belgrade, Serbia
anjaradicc@gmail.com

Bisphenols such as bisphenol A (BPA), bisphenol S (BPS), and bisphenol F (BPF) are industrial chemicals that induce cellular stress and alter signalling pathways, leading to insulin resistance, adipose tissue hypertrophy, and steatosis in pancreatic cells. This study aimed to investigate the toxic effects of individual bisphenols and their mixtures on pancreatic tissue damage in rats. Female Wistar rats were divided into eight groups (n=5). One group served as the control, while the others were treated with individual bisphenols, binary mixtures, or the MIX group containing all three investigated bisphenols, administered at human-relevant doses (BPA 0.26 μg/kg b.w./day, BPS 0.60 μg/kg b.w./day, and BPF 0.68 μg/kg b.w./day). After 28 days of oral exposure, malondialdehyde (MDA), ischemia-modified albumin (IMA), superoxide dismutase (SOD), glutathione (GSH), and content of total sulfhydryl groups (SH) were analysed. All individual bisphenols, their binary mixtures, as well as the MIX group, had a statistically significant effect on MDA levels, with the MIX and BPA+BPS groups showing the highest degree of significance. The combination of BPA and BPF caused a statistically significant increase in IMA levels and a decrease in SOD and SH group levels. Additionally, the BPS+BPF treatment caused a significant decrease in SH levels. GSH levels were significantly reduced in the groups treated with individual bisphenols (BPA and BPS), as well as in the MIX group. In conclusion, bisphenol mixtures induced more pronounced pancreatic oxidative stress compared to individual compounds, with the strongest effects observed in the BPA+BPF and MIX groups, as indicated by elevated MDA and IMA levels and significantly reduced antioxidant parameters, including SOD, SH, and GSH.

This study was funded by the Ministry of Science, Technological Development and Innovation (no. 451-03-136/2025-03/200161) of the Republic of Serbia.

KEY WORDS: endocrine disruptors; environmental exposure; *in vivo* study; pancreatic toxicity; redox status

P 22

Functional knockout of the Oatp1d1 membrane transporter differentially alters diclofenac and perfluorooctane sulfonate (PFOS) toxicity in zebrafish embryos

Jelena Dragojević Višević[1], Lana Vujica[2], Ivan Mihaljević[2], Jovica Lončar[2], Dean Karaica[1], Ana Dananić[3], Arvena Bošnjak[4], and Tvrtko Smital[2]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] Ruđer Bošković Institute, Zagreb, Croatia
[3] Blaževdol, Sveti Ivan Zelina, Croatia
[4] University of Zagreb, Zagreb, Croatia
jdvisevic@imi.hr

Organic anion transporting polypeptide 1d1 (Oatp1d1) in zebrafish is a functional ortholog of human hepatic OATPs (OATP1A and OATP1B) and plays an important role in the uptake of xenobiotics and endogenous compounds. This study investigates the role of Oatp1d1 in modulating developmental toxicity of two environmentally relevant toxicants: diclofenac, a widely used non-steroidal anti-inflammatory drug (NSAID), and perfluorooctane sulfonate (PFOS), a persistent perfluoroalkyl substance. Using CRISPR/Cas9, a zebrafish line lacking functional Oatp1d1 was generated and embryos were exposed to increasing concentrations of diclofenac and PFOS. Results revealed opposing toxicity profiles: Oatp1d1 mutants showed increased resilience to diclofenac, with reduced developmental abnormalities and delayed toxic effects, indicating limited hepatic uptake and metabolism. In contrast, PFOS-exposed Oatp1d1 mutants exhibited greater susceptibility, with lower LC_{50} values, more severe malformations (particularly of the swim bladder), increased oxidative stress (reactive oxygen species, ROS), apoptosis, and behavioural alterations. Gene expression analysis showed differential modulation of biotransformation genes (cytochrome P450s, glutathione-S-transferases) and lipid homeostasis. Notably, Oatp1d1 disruption altered the expression of other transporter genes, suggesting compensatory mechanisms. These findings demonstrate that Oatp1d1 can have both protective and sensitizing roles in contaminant toxicity, depending on the chemical nature of the compound. This highlights the complex function of membrane transporters in toxicokinetic and toxicodynamic, with implications for understanding chemical risk in aquatic organisms and for human health extrapolations. Oatp1d1 knockout zebrafish represent a valuable model for dissecting transporter-mediated toxicity and may support development of mechanistically informed environmental risk assessments.

This study was funded by the Croatian Science Foundation (Project no. IP-2019-04-1147 granted to T. Smital), and by the STIMREI Centre for Excellence project, Contract Number: KK.01.1.1.01.0003 funded by the European Union through the European Regional Development Fund – Operational Programme Competitiveness and Cohesion 2014–2020 (KK.01.1.1.01).

KEY WORDS: CRISPR/Cas9; developmental toxicity; gene expression analysis; organic anion transporting polypeptide; transporter-mediated toxicity

P 23

Aquatic toxicity of binary mixtures of perfluorooctanoic acid, diclofenac, and atrazine: experimental, mathematical, and modelling approaches

Josipa Papac Zjačić[1], Hrvoje Kušić[1,2], and Ana Lončarić Božić[1]

[1] University of Zagreb, Faculty of Chemical Engineering and Technology, Zagreb, Croatia
[2] University North, Koprivnica, Croatia
jpapac@fkit.unizg.hr

Atrazine (ATZ), diclofenac (DCF), and perfluorooctanoic acid (PFOA) are among the most detectable persistent contaminants in water, representing key chemical groups with harmful effects. DCF is toxic to aquatic life, ATZ disrupts endocrine systems, and PFOA is bioaccumulative and developmentally toxic. All are regulated under EU legislation including the Water Framework Directive, Priority Substances Directive, and Persistent Organic Pollutants Regulation. When combined in mixtures, these compounds can interact to produce additive, synergistic, or antagonistic toxic effects. This study investigated the aquatic toxicity of ATZ, DCF and PFOA, using purchased standards in order to analyse their individual and combined toxicity in binary mixtures. Overall, 3 single component and 9 binary mixtures toxicities were determined using two aquatic organisms: *Vibrio fischeri* (bioluminescence inhibition) and *Daphnia magna* (immobilization) according to standard procedure (ISO). For the description of mixture toxicities, two common mathematical models were applied: concentration addition (CA) and independent action (IA) model. Binary mixtures of ATZ-PFOA and ATZ-DCF indicated synergism, while mixtures that contained DCF-PFOA exhibited antagonistic behaviour with respect to additive behaviour (CA model). The applicability of the IA model as proof of independent toxic action of the components was not confirmed in any of the cases.

This work was funded by the Croatian Science Foundation (grant HRZZ IPS-2022-02-4780 SoAPperF).

KEY WORDS: *Vibrio fischeri*; *Daphnia magna*; joint toxicity; CA model; IA model

P 24

Spermatogenesis assessment in pubertal male Wistar rat offspring prenatally exposed to a pyrethroid insecticide

Anja Katić[1], Antonia Merčep[2], Vedran Micek[1], Marta Himelreich Perić[3,4], Dajana Krsnik[3,4], Ana Katušić Bojanac[3,4], Marijana Neuberg[5], Goran Kozina[5], and Ana Lucić Vrdoljak[1]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia
[3] University of Zagreb, School of Medicine, Department of Medical Biology, Zagreb, Croatia
[4] Scientific Centre of Excellence for Reproductive and Regenerative Medicine, Zagreb, Croatia
[5] University North, University Centre Varaždin, Varaždin, Croatia
akatic@imi.hr

Endocrine disruptive chemicals (EDCs) may affect the reproductive system, including reproductive organs. Effects on male reproductive organs associated with exposure to synthetic pyrethroids have been reported in laboratory animals as well as in humans. To date, there have been no data about the effects of α-cypermethrin (*a*-cyp), a type II synthetic pyrethroid insecticide, as an EDC on the male reproductive system. Due to the fact that most of the human population is exposed to this insecticide on a daily basis, this presents an important area of research. Thus, the aim of this investigation was to evaluate for the first time whether prenatal exposure to *a*-cyp impairs the development of reproductive organs in pubertal male Wistar rat offspring. Pregnant Wistar rats were exposed *per os* from the 6th day of gestation until delivery to *a*-cyp at 1, 10, and 19 mg/kg b.w./day. Positive control (diethylstilbestrol), solvent control (corn oil) and negative control (water) were kept in parallel. After confirmation of puberty onset, testes and epididymides from male offspring were isolated under general anaesthesia. The sperm was sampled from epididymides for microscopic analyses of sperm count and viability. Testes were fixed in Sainte Marie solution, embedded in paraffin, and thick sections (4 μm) were stained with haematoxylin and eosin for microscopic assessment of spermatogenesis preservation. The number and viability of sperm as well as the preservation of spermatogenesis in pubertal male rat offspring were not impaired by prenatal *a*-cyp exposure. Further research is needed to clarify the effects of prenatal exposure to *a*-cyp as an EDC on the development of male reproductive organs.

This study was funded by the in-house scientific project "Assessment of the effects of prenatal exposure to α-cypermethrin on epigenetic programming and endocrine disruption of reproduction and development in experimental rats", the European Union – Next Generation EU (Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006, BioMolTox) and performed using the facilities and equipment funded within the European Regional Development Fund project KK.01.1.1.02.0007 (ReC-IMI).

KEY WORDS: α-cypermethrin; endocrine disruptive chemicals; reproduction; sperm count; sperm viability

P 25

Protective effects of sulforaphane and curcumin against pancreatic damage induced by bisphenol mixtures: an *in vivo* study

Aleksandra Obradović, Anja Radić, Jovana Živanović, Katarina Baralić, Đurđica Marić, Jovana Šljivić, Mihajlo Bojić, Aleksa Milačić, Evica Antonijević Miljaković, Aleksandra Buha Đorđević, Zorica Bulat, Biljana Antonijević, Marijana Ćurčić, and Danijela Đukić-Ćosić

University of Belgrade, Faculty of Pharmacy, Department of Toxicology "Akademik Danilo Soldatović", Belgrade, Serbia
aleksandra.obradovic.ffub@gmail.com

Bisphenol A (BPA), bisphenol S (BPS), and bisphenol F (BPF), which are known endocrine disruptors, are linked to metabolic disturbances, including insulin resistance and steatosis in pancreatic tissues. In contrast, sulforaphane (SFN) and curcumin (CUR) are phytochemicals with recognized antioxidative and cytoprotective potential, making them promising candidates for mitigating such effects. The aim of this study was to investigate the effects of SFN and CUR in attenuating pancreatic oxidative stress exposed with a mixture of bisphenols using an *in vivo* model. Seven groups of female Wistar rats (n=5) received either no treatment (control), a bisphenol mixture (MIX), SFN, CUR, or combinations of MIX with SFN, CUR, or both compounds (BPA 0.26 μg/kg/day, BPS 0.60 μg/kg/day, BPF 0.68 μg/kg/day, SFN 2 mg/kg/day and CUR 4.13 mg/kg/day). After 28 days of oral exposure, malondialdehyde (MDA), ischemia-modified albumin (IMA), superoxide dismutase (SOD), glutathione (GSH), and total sulfhydryl groups content (SH), were measured. Exposure to the bisphenol mixture significantly increased MDA levels and decreased GSH levels compared to the control group, indicating enhanced oxidative stress. Both SFN and CUR, administered individually or in combination, effectively restored MDA and GSH levels to values comparable to the control, with the SFN+CUR combination showing slightly greater efficacy in reducing MDA. Treatment with SFN+MIX significantly increased SH and SOD levels. These findings suggest that SFN and CUR, both individually and in combination, may mitigate bisphenol mixture-induced pancreatic oxidative stress by restoring redox balance and enhancing antioxidant defenses.

This study was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (no. 451-03-136/2025-03/200161).

KEY WORDS: endocrine disruptors; natural compounds; oxidative stress; pancreas toxicity; Wistar rats

P 26

Effect of low-dose perfluorooctanoic acid (PFOA) exposure on the reproductive hormone balance in male Wistar rats

Đurđica Marić, Jovana Živanović, Katarina Baralić, Evica Antonijević Miljaković, Danijela Đukić-Ćosić, Marijana Ćurčić, Zorica Bulat, Biljana Antonijević, and Aleksandra Buha Đorđevic

University of Belgrade, Faculty of Pharmacy, Department of Toxicology "Akademik Danilo Soldatović", Belgrade, Serbia
dmaric@pharmacy.bg.ac.rs

Per- and polyfluoroalkyl substances (PFAS) are a group of chemicals with carbon chains and functional groups such as sulfonates and carboxylates. Although many PFAS have been restricted, their widespread use and environmental persistence continue to pose risks to human health, including negative effects on the male reproductive system. Some studies link PFAS, particularly perfluorooctanoic acid (PFOA), to disruptions in sex hormone levels. PFOA is essential due to its frequent presence in the human serum. The aim of this study was to investigate the effects of low doses of PFOA on reproductive hormone levels in male Wistar rats. Groups M1 and M2 received doses of 0.015 and 0.15 mg/kg b.w./day, reflecting general population exposure, while group M3 received 0.625 mg/kg b.w./day, a dose considered safe based on the literature. Each group included six animals. PFOA was dissolved in 0.5 % Tween-20 in deionized water. The control group received only the vehicle. After 28 days of exposure, animals were sacrificed, blood collected, and serum isolated. Testosterone, FSH, and LH levels were measured using commercial ELISA kits. Group M1 showed a significant increase in LH and FSH levels, along with a significant decrease in testosterone. No significant hormonal changes were observed in groups M2 and M3 compared to the control. These results suggest that low doses of PFOA reflecting real-life exposure levels can impair testicular function, reduce testosterone production and cause the pituitary gland to increase gonadotropin secretion.

KEY WORDS: endocrine disruption; FSH; LH; PFAS; testosterone

P 27

Dose response modelling between target tissue levels of decabrominated diphenyl ether and oxidative stress markers in rat brains

Marijana Ćurčić[1], Katarina Baralić[1], Jovana Živanović[1], Đurđica Marić[1], Evica Antonijević Miljaković[1], Aleksandra Buha Đorđević[1], Danijela Đukić-Ćosić[1], Ksenija Durgo[2], Saša Janković[3], Petar Lukić[1], Zorica Bulat[1], and Biljana Antonijević[1]

[1] University of Belgrade, Faculty of Pharmacy, Department of Toxicology "Akademik Danilo Soldatović", Belgrade, Serbia
[2] University of Zagreb, Faculty of Food Technology and Biotechnology, Zagreb, Croatia
[3] Institute of Meat Hygiene and Technology, Belgrade, Serbia
marijana.curcic@pharmacy.bg.ac.rs

The aim of this study was to model the dose response between the target tissue concentrations of decabrominated diphenyl ether (BDE-209) and brain levels of glutathione (GSH) and malondialdehyde (MDA). Effects were examined on male Wistar rats, exposed to doses of 1000, 2000, or 4000 BDE-209/kg b.w./day, by gavages, during 28 days. BDE-209 concentration was determined in brain by gas chromatography-electron capture detection (GC/ECD) method after tissue homogenates preparation using the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) approach. For quality control, CIL-EDF-2524 Clean Fish (slurry) – Organic Contaminants (LG standards) was used. Slope and steepness of dose response curve were calculated using PROAST software. The measured concentrations of BDE-209 were 0.3, 0.65, and 0.85 mg/kg of brain, respectively, corresponding to applied doses. Levels of GSH and MDA were measured spectrophotometrically. Tissue concentration of BDE-209 significantly decreased and factorial regression analysis confirmed significant positive influence of BDE-209 on GSH decrease in brain tissue, while MDA levels were not changed for this period of exposure. The derived benchmark dose of 5 % *i.e.* BMDL5 for GSH level was 0.048 mg/kg/day. These findings confirm BDE-209's effect on antioxidative protection systems in the brain (expressed as GSH level). However, potential for lipid oxidative damage (expressed as MDA level) was not confirmed.

This study was funded by the Ministry of Science, Technological Development and Innovation, Republic of Serbia through two Grant Agreements with the University of Belgrade Faculty of Pharmacy no. 451-03-136/2025-03/200161 and no. 451-03-137/2025-03/200161.

KEY WORDS: BDE-209; oxidative stress; PROAST software; Wistar rats

P 28

Toxicity assessment of erectile dysfunction supplements using Brine Shrimp Lethality Assay

Ana Marija Bajatovska, Zoran Zhivikj, Tanja Petreska Ivanovska, and Tatjana Kadifkova Panovska

University Ss. Cyril and Methodius in Skopje, Faculty of Pharmacy, Institute of Applied Biochemistry, Skopje, Republic of North Macedonia
a.m.bajatovska@ff.ukim.edu.mk

Multiherbal supplements marketed for erectile dysfunction (ED) are increasingly popular, yet their safety profiles remain insufficiently studied, raising concerns regarding their complex formulations and unregulated use. The aim of this study was to evaluate the acute toxicity of six commercially available supplements marketed for ED using the Brine Shrimp Lethality Assay (BSLA). The tested supplements primarily contained extracts of *Tribulus terrestris*, *Panax ginseng*, and *Lepidium meyenii*, but also extracts from some other plants such as *Withania somnifera* and *Piper nigrum*. All six samples were prepared in a concentration range of 10–0.01 mg/mL and applied to *Artemia salina* nauplii. Mortality was recorded after 24 hours, and median lethal concentration (LC_{50}) values were determined using probit regression analysis. Five of the six examined supplements exhibited LC_{50} values below 100 μg/mL (94.07, 27, 0.12, 50.54, 0.32 μg/mL), classifying them as toxic and highly toxic according to the Meyer and the Clarkson scale, respectively. The variation in LC_{50} values is likely due to differences in phytochemical composition, concentration of active constituents, and potential interactions between plant extracts. One of the tested supplements showed lower but still notable toxicity (LC_{50} 453 μg/mL). This may have been due to the presence of quercetin stabilising cell membranes or cytoprotective *Cordyceps sinensis* promoting homeostasis, both reducing lethality in *Artemia salina*. The BSLA results, obtained using a reliable *in viv*o model for preliminary toxicity screening, indicate potential cytotoxicity of the examined ED supplements. These findings highlight the necessity of mandatory toxicological evaluations for multiherbal formulations to mitigate health risks.

KEY WORDS: *Artemia salina*; cytotoxicity; herbal supplements LC_{50}; probit analysis

P 29

Accidental cyanide poisoning from apricot seeds: a diagnostic and therapeutic challenge in a resource-limited setting

Zanina Pereska[1,2], Kiril Naumoski[1,2], Aleksandra Babulovska[1,2], Natasha Simonovska[1,2], Kristin Kostadinoski[1,2], and Zoran Zhivikj[3]

[1] University Ss. Cyril and Methodius in Skopje, Faculty of Medicine, Skopje, Republic of North Macedonia
[2] University Toxicology Clinic, Skopje, Republic of North Macedonia
[3] University Ss. Cyril and Methodius in Skopje, Faculty of Pharmacy, Institute for Applied Biochemistry, Skopje, Republic of North Macedonia
zzivic@ff.ukim.edu.mk

Cyanide is a fast-acting poison that impairs cellular respiration, leading to hypoxia and severe metabolic acidosis. This case report presents an incident of accidental cyanide poisoning after apricot seed ingestion, emphasizing the importance of rapid diagnosis and timely management when toxicology analysis and antidotes are unavailable. A male patient was urgently admitted after consuming 30–40 wild apricot seeds. He presented vomiting, dyspnoea, dizziness, watery diarrhoea, hypotension (50/30–60/40 mmHg), tachycardia (130–140 beats/min), hypoxemia (SaO_2 82 %), and tachypnoea. Medical history included hypertension and heavy smoking (40–60 cigarettes/day). Arterial blood gas analysis revealed BE 12.7 mmol/L, pCO_2 2.9 kPa, and serum lactate was elevated at 11.01 mmol/L. Laboratory analysis showed leucocytosis (WBC 21×10^9/L) and neutrophilia (Ne 19×10^9/L). Serum troponin levels ranged from 104 to 115 ng/L, significantly higher than the upper reference limit (<34.2 ng/L). Due to the unavailability of toxicological tests for blood cyanide, a diagnosis was made clinically based on exposure, arterial blood gas analysis with lactates, and biochemical indicators. Symptomatic therapy included oxygen, activated charcoal, and intravenous sodium bicarbonate. Prompt initiation of treatment resulted in complete clinical recovery. In resource-limited settings, clinical signs and surrogate laboratory findings must guide the evaluation and management of suspected cyanide poisoning. Early symptomatic treatment, especially correction of acidosis, can significantly improve the outcome.

KEY WORDS: apricot kernel; cyanide toxicity; lactic acid; metabolic acidosis; supportive care

P 30

False-positive amphetamine/methamphetamine result in urine due to mexiletine: a case report

Andrijana Ščavničar[1], Dubravka Glasovac[1], Sven Komljenović[1], and Mila Lovrić[1,2,3]

[1] University Hospital Centre Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia
[2] University of Zagreb, Faculty of Pharmacy of Biochemistry, Zagreb, Croatia
[3] University of Zagreb, School of Medicine, Zagreb, Croatia
ascavnic@kbc-zagreb.hr

Immunoassays for urine drug screening employ antibodies designed to recognize either the drug of interest or its metabolites. Immunoassays false positive results depend primarily on the cross-reactivity profile of the immunoassays used and the specific substance in question. Confirmatory testing with a secondary specific laboratory technique, such as liquid (LC-MS) or gas chromatography (GC-MS) with mass spectrometry can be of significant help revealing false-positive immunoassay results. A 54-year-old patient with a medical history of heroin and cocaine use with cardiomyopathy was admitted for a cardiological evaluation. Urine drug screening (enzyme multiplied immunoassay technique, EMIT) revealed a positive test for amphetamines/methamphetamines. A confirmatory test performed by GC-MS revealed mexiletine and caffeine in urine. Amphetamines/methamphetamines were not present in the patient's urine. The hospital doctor confirmed that mexiletine was part of the patient's therapy. Mexiletine is a antiarrhythmic drug and is structurally similar to amphetamine and methamphetamine, containing a benzene ring with a hydrocarbon tail that terminates with a functional amine ring. It can cause false-positive urine drug screenings for amphetamines/methamphetamines but the manufacturer does not list it among the substances that cause cross-reactivity. Additionally, we confirmed it by spiking the urine with a certain amount of mexiletine and screening this urine. Urine was positive for amphetamines/methamphetamines with immunoassay and GC-MS analysis was negative for amphetamines/methamphetamines and positive for mexiletine. This case clearly demonstrated the importance of information on a patient's therapy because of possible false-positive urine drug screenings and confirmatory investigations to rule out false-positive tests.

KEY WORDS: antiarrhythmic drug; urine drug screening; gas chromatography-mass spectrometry; immunoassay; liquid chromatography-mass spectrometry

P 31

The importance of comprehensive toxicological analyses in addressing false positive amphetamine screening results: a case study approach

Marija Bujaroska Perkovikj, Stefanija Ubavkova, Zorica Bozhinoska, Ana Ivcheva, Viktorija Belakaposka Srpanova, Zlatko Jakjovski, and Aleksandar Stankov

University Ss. Cyril and Methodius in Skopje, Medical Faculty, Institute of Forensic Medicine, Criminology and Medical Deontology, Skopje, Republic of North Macedonia
marija.bujaroska@medf.ukim.edu.mk

Immunoassays are commonly employed as the initial urine screening for drugs of abuse. These assays, particularly those targeting amphetamines are susceptible to false positive results. The simple molecule of these drugs challenges the development of specific antibodies leading to cross-reactivity, which requires the employment of additional techniques. A urine sample was submitted for amphetamine analysis, without details regarding medical history. The sample was analysed using test strips (Multi-Drug Rapid Test Panel, MedNet GmbH), enzymatic immunoassay (EIA) (Architect c4000, Abbott), and high-performance liquid chromatography-photodiode array detection with on-line solid phase extraction (TOX.I.S, Shimadzu). Analysis with test strips showed a positive result (cut-off: 300 ng/mL). The amphetamine assay on EIA measured 800 ng/mL, nonetheless it was interpreted as negative (cut-off: 1000 ng/mL). No analytical response was observed at the retention times for amphetamine, methamphetamine, N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB), 3,4-methylenedioxymethamphetamine (MDMA) or 3,4-methylenedioxyamphetamine (MDA) on the TOX.I.S system. However, the analysis revealed the presence of tramadol and its metabolites, confirmed using gas chromatography mass spectrometry (GC/MSQP2010, Shimadzu) as tramadol, *N*-desmethyltramadol and *O*-desmethyltramadol. While the literature does indicate a 4–9 % false positive rate in amphetamine screenings, tramadol was identified in only a few cases. A previous study indicates that a tramadol concentration of 2,500 g/L does not result positive for amphetamine assay (cut-off: 300 ng/mL), suggesting that tramadol metabolites are likely responsible for the cross-reactivities. In the case presented herein, both tramadol and its metabolites were detected. Therefore, comprehensive toxicological analysis is essential not only for clarifying potential cross-reactivity, but also for advancing scientific understanding and ensuring more accurate and reliable results.

KEY WORDS: cross-reactivity; drugs of abuse; immunoassay; tramadol; urine

P 32

Life-threatening unintentional methadone poisoning in a 13-year-old girl: a case report

Arnes Rešić[1,2], Sandra Alavuk Kundović[1], Nikolina Benco Kordić[1], Julijana Rula[1], and Mila Lovrić[3,4]

[1] Children's Hospital Zagreb, Zagreb, Croatia
[2] University of Split, Faculty of Health Sciences, Split, Croatia
[3] University Hospital Centre Zagreb, Zagreb, Croatia
[4] University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia
aresic@pharma.hr

Over the past decade, the United States has seen a marked increase in hospitalizations due to heroin and methadone poisoning among adolescents aged 15–19 years. We present a case of severe methadone poisoning in a 13-year-old girl who allegedly mistook methadone for melatonin. A 13-year-old girl was admitted to the resuscitation unit of the emergency department in a comatose state, exhibiting agonal respiration, no response to painful stimuli, and pinpoint pupils unreactive to light. She was immediately intubated and mechanically ventilated. It was later revealed that she had ingested approximately 50 mg of oral methadone, a medication prescribed to her stepfather, who is a known opioid user. According to the psychiatric evaluation, there were no suicidal intentions; the girl reportedly took the substance mistakenly, intending to fall asleep. Naloxone was administered as a bolus, resulting in prompt pupil dilation and subsequent extubation, although oxygen therapy was continued. Due to methadone's long half-life, continuous naloxone infusion was maintained for 30 hours until respiratory stability was achieved. Urine toxicology confirmed the presence of methadone and its metabolites, with a measured concentration of >3.2 μmol/L [enzyme-multiplied immunoassay technique (EMIT), Siemens], considered elevated. Following clinical recovery, psychological support was provided, and child protective services were engaged to ensure family oversight. Although mortality is highest among adults aged 45–54, adolescents and young adults more frequently present to emergency services due to opioid misuse and experimentation with medications. In this case, the poisoning was assessed to be accidental, likely due to confusion between similarly named substances.

KEY WORDS: accidental poisoning; children; medications; melatonin; opioids

P 33

Polysubstances in drug-induced deaths: a case report

Marija Definis[1,2], Antonio Alujević[2], and Tanja Visković[2]

[1] University of Split, School of Medicine, Split, Croatia
[2] Clinical Hospital Centre Split, Split, Croatia
marija.dg@gmail.com

Estimating drug-induced mortality is essential for understanding the impact of drug use on public health, how it may change over time, and what types of effective responses and prevention activities need to be developed. According to published data, opioids, usually in combination with other substances, are still most frequently identified in drug-induced deaths. The aim of this study was to present a case of polysubstance intoxication that had almost been unreported due to a lack of interest in further investigation by the competent authorities. A man, 40 years old, undergoing interrupted drug addiction treatment in a psychiatric service, was found dead in his apartment in Split. Several half-filled small vials of various medications were found next to the body. The coroner requested an autopsy, which showed severe pulmonary oedema as the immediate cause of death. Toxicological analysis of blood and urine, using gas chromatography-mass spectrometry, revealed the presence of opioids (buprenorphine, methadone, tramadol), antipsychotics (clozapine), benzodiazepines (alprazolam, diazepam), and antidepressants (Mirtazapine), as well as large quantities of pregabaline (antiepileptic, analgesic, anxiolytic). This recent case represents a continuation of the trend observed in the last few years in the Split-Dalmatia County and once again calls for improvements to the current surveillance and communication system in Croatia, which currently provides inadequate monitoring of drug trends and lacks a timely response to drug-related dangers. The general situation with drug addiction in our country needs to be closely monitored and an interdisciplinary study of the morbidity and mortality of drug addicts is urgently needed.

KEY WORDS: epidemiology; forensic autopsy; polysubstance use; toxicology

P 34

Liver disease caused by inhalation of aromatic hydrocarbons in a worker involved in the maintenance of fuel tanks: a case report

Jagoda Mandić[1], Željka Babić[1], Almenka Balenović[2], and Jelena Macan[1,3]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] Occupational Medicine Practice, Ivanić Grad, Croatia
[3] Polyclinic Medicina rada Instituta za medicinska istraživanja i medicinu rada d. o. o.
jmacan@imi.hr

Organic solvents (OS) have dose-dependent adverse effects in humans in the form of skin and mucous membrane irritation and central nervous system toxicity. Some OS also have specific toxic effects on other target organs. Here we present a case of work-related illness caused by excessive exposure to aromatic hydrocarbons with hepatotoxic effects. A male worker, 37 years old, is an industrial mechanic. Since 2017, he has been employed in the anti-corrosion protection of underground tanks for petroleum products, where he is exposed to OS vapour above the threshold limit value, including toluene and xylene as known hepatotoxic chemicals. Respiratory protection equipment (facial mask with filter for OS) was used but not adequately maintained. The worker was healthy until he started employment here. From the first periodical examination by an occupational health physician (OHP) in 2018, an increase in the pathological values of liver enzymes was recorded until 2025, with an ALT/AST ratio > 1, typical for the hepatotoxic effect of toluene/xylene. The worker reported general weakness, diarrhoea, hair loss and seborrheic dermatitis, and irritation of the nose and throat. Diagnostic work-up showed signs of early liver fibrosis with negative tumour and viral hepatitis markers and negative history of alcohol abuse. The worker's health condition completely normalised after 3 months of sick leave without additional therapy. The OHP banned him from working in OS exposure and initiated the procedure for recognising an occupational liver disease. Exposure to OS was stopped in the early, reversible phase of the liver disorder, thanks to the monitoring of the worker's health at mandatory periodic examinations by an OHP.

KEY WORDS: hepatotoxicity; occupational disease; preventive medical examination; toluene; xylene

P 35

Does resveratrol provide protective benefits to patients undergoing heart valve replacement surgery?

Dubravka Rašić[1], Mislav Planinc[2], Kristina Mutabdžić[3], and Maja Peraica[1]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] University Hospital Centre Zagreb, Zagreb, Croatia
[3] Medis Adria, Zagreb, Croatia
rasic@imi.hr

Cardiovascular diseases are the leading cause of mortality and hospitalization in Croatia. Resveratrol, a natural polyphenol, has been studied thoroughly and is recognized for its cardiovascular protective properties. This study investigated the potential benefits of resveratrol use in patients (n=34) hospitalized for heart valve replacement surgery. Upon admission, patients were randomized into two groups: one group received resveratrol (Solgar Resveratrol capsules, 2×400 mg/day), while the control group received a placebo (lactulose). Treatment began 48 hours before surgery and continued until 78 hours postoperatively. Blood samples were collected at five time points: T1 – upon hospital admission, T2 – after induction of anaesthesia, T3 – upon arrival in the intensive care unit (ICU), T4 – 24 hours post-surgery, and T5 – 78 hours post-surgery. Oxidative stress was assessed using two biomarkers: glutathione (GSH) and malondialdehyde (MDA). The highest MDA concentrations were observed in plasma samples collected at T1, with levels decreasing over time; however, these changes were not statistically significant. GSH levels significantly increased in plasma samples taken after ICU admission (T3). No significant differences in MDA or GSH concentrations were found between the resveratrol and placebo groups. These findings suggest that oxidative stress levels undergo only minor fluctuations during aortic valve surgery and that both resveratrol and lactulose may exert antioxidant effects. Further research involving a broader spectrum of oxidative stress markers is needed to fully elucidate the impact of resveratrol in surgical settings.

This study was supported by the European Union – Next Generation EU (Program Contract of 8 December 2023, Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006, BioMolTox) and performed using the facilities and equipment funded within the European Regional Development Fund project KK.01.1.1.02.0007.

KEY WORDS: antioxidants; biomarkers; cardiovascular diseases; oxidative stress; polyphenol

P 36

Verification of a fully automated analyser using the biochip array technology immunoassay

Dubravka Glasovac[1], Andrijana Ščavničar[1], Sven Komljenović[1], Mila Lovrić[1,2,3], and Dunja Rogić[1,2]

[1] University Hospital Centre Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia
[2] University of Zagreb, Faculty of Pharmacy of Biochemistry, Zagreb, Croatia
[3] University of Zagreb, School of Medicine, Zagreb, Croatia
dglasova@kbc-zagreb.hr

The Randox Evidence MULTISTAT is a fully automated analyser that enables the simultaneous preliminary detection of up to 29 drugs of abuse from a single sample using biochip array technology. The measurement is based on a competitive immunoassay coupled with a chemiluminescent reaction. We analysed 59 samples, 28 of which were urine samples and 31 were blood samples. The samples were analysed using four different panels: DOA urine and blood-qualitative analysis, and ToxPlex urine and blood-semi-quantitative analysis. The data were compared with results obtained by the enzyme-multiplied immunoassay (Siemens Dimension EXL, Siemens Viva ProE) and by gas chromatography-mass spectrometry (GC-MS, Shimadzu). The compared immunochemistry results showed that 24 samples were mutually comparable (40.68 %), 12 samples were not comparable mutually due to different sensitivities of the tests (20.34 %), 8 samples were not comparable mutually but their results obtained by the GC-MS method were positive (possibly due to the different specificity of antibodies to drugs and their numerous metabolites such as THC, benzodiazepine, amphetamine/methamphetamine, and cocaine). The remaining 15 samples could not be compared mutually due to a lack of results from Siemens analysers. The analyser can generate a large amount of toxicological data in a short time, but cross-reactivity poses a significant challenge (amphetamine/methamphetamine and opiate tests). In conclusion, for a correct result, it is necessary to use more than one test panel, and for a confirmatory test, it is necessary to use a specific alternative method, such as GC-MS. DOA test panels can also detect analytes from the group of new psychoactive substances (NPS).

KEY WORDS: biochip array technology; drugs of abuse; multiplex testing; screening drugs

P 37

Unconventional abuse of carbamazepine: investigating the intranasal administration of crushed tablets

Stefanija Ubavkova, Marija Bujaroska Perkovik, Zorica Bozhinoska, Natasha Bitoljanu, Ljupcho Chakar, Goran Pavlovski, and Aleksandar Stankov

University Ss. Cyril and Methodius in Skopje, Medical Faculty, Institute of Forensic Medicine, Criminology and Medical Deontology, Skopje, Republic of North Macedonia
stefanija.ubavkova@medf.ukim.edu.mk

The anticonvulsant carbamazepine may evoke feelings of euphoria, a side effect that appears to elevate its risk of misuse and overdose particularly among adolescents. In this study, we present a case involving the unusual abuse of carbamazepine. Two boys and two girls, aged between 15 and 17, were found in a drowsy state after a night out. Four blood and three urine samples were submitted for toxicological analysis following their hospitalization at the University Clinic of Toxicology. The exact time interval between intoxication and sample collection was unknown. Additionally, a powdered substance recovered from the scene was submitted for analysis. All of the samples were tested for alcohol [gas chromatograph coupled with flame ionization detector (GC-FID) 2010 Plus, Shimadzu], screened for drugs of abuse (Architect c4000 Analyzer, Abbott) and analysed on a gas chromatograph coupled with a mass spectrometer (GC-MS-QP2010 Ultra, Shimadzu). The results showed no presence of alcohol in any of the samples. All of the urine samples tested negative for drugs of abuse. Using GC-MS, carbamazepine and its metabolites, as well as diazepam and its metabolites were detected in all samples. The antidepressant citalopram was found only in the urine samples. The powdered substance was confirmed to be carbamazepine. The findings, combined with anamnestic data, suggest that carbamazepine tablets had been crushed and taken intranasally to achieve a euphoric effect. This work represents the first reported case of carbamazepine abuse through an unconventional route of administration in our country. Although rare, similar cases have been described in the literature. Cases alike should draw attention as another threat to substance abuse in addition to traditional recreational drugs.

KEY WORDS: adolescents; carbamazepine abuse; euphoria; gas chromatography-mass spectrometry; snorting

P 38

PlastRepRisk–Effects of exposure to plasticizers and microplastics on men's reproductive health: a project overview

Blanka Tariba Lovaković[1], Andreja Jurič[1], Marija Dvoršćak[1], Darija Klinčić[1], Karla Jagić[1], Zorana Kljaković-Gašpić[1], Dinko Hauptman[2], Anja Katić[1], and Irena Brčić Karačonji[1,3]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] University Hospital Centre, Zagreb, Croatia
[3] University of Rijeka, Faculty of Health Studies, Rijeka, Croatia
btariba@imi.hr

Phthalates (PAEs) and organophosphate esters (OPEs) are classes of compounds widely used as chemical additives in plastics. Due to their physicochemical properties, they are commonly incorporated into consumer products, which contributes to their broad environmental dispersion. The detection of these substances in human biological samples has raised concern among scientists and regulatory agencies responsible for public health protection. Although the health effects of these compounds are not yet fully understood, certain PAEs, OPEs, and microplastics are suspected to act as endocrine-disrupting chemicals, potentially impacting reproductive health negatively. One of the primary objectives of the newly initiated scientific research project PlastRepRisk is to establish an analytical platform for detecting plasticizers (OPEs and PAEs) and microplastic particles in complex biological matrices, such as human blood and urine, and to generate preliminary data on exposure levels in the general Croatian population. The PlastRepRisk project will also assess potential reproductive health risks by comparing men diagnosed with infertility to fertile controls. Associations between the concentrations of specific OPEs, PAEs, and microplastics in blood and urine and indicators of male infertility, such as reduced sperm count or quality and altered sex hormone levels, will be assessed in order to investigate the hypothesis that exposure to plasticizers and microplastics may adversely affect male reproductive function. Ultimately, the research within this project aims to provide critical insights into the combined mechanisms of action of plasticizers and microplastics and contribute to the development of strategies for protecting both human health and the environment.

This study was funded by the Croatian Science Foundation (grant HRZZ-IP-2024-05-8815, PlastRepRisk), European Union – Next Generation EU (Program Contract of 8 December 2023, Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006, BioMolTox) and performed using the facilities and equipment funded within the European Regional Development Fund project KK.01.1.1.02.0007.

KEY WORDS: blood serum; mass spectrometry; organophosphate esters; phthalates; urine

P 39

Preliminary assessment of organophosphate ester levels in blood serum from the Croatian population

Marija Dvoršćak, Darija Klinčić, Karla Jagić, Andreja Jurič, and Blanka Tariba Lovaković

Institute for Medical Research and Occupational Health, Zagreb, Croatia
mdvorscak@imi.hr

Organophosphate esters (OPEs), extensively employed as plasticizers and flame retardants in diverse consumer products such as plastics, foams, electronic equipment, textiles, and building materials, are pervasive environmental contaminants. Their additive nature facilitates continuous release from product matrices. Growing evidence links OPE exposure to various adverse health outcomes. While certain OPEs are relatively rapidly metabolised and excreted through the urine, there are studies reporting OPE concentrations in serum samples, possibly due to their binding to serum proteins. This study aimed to develop and preliminarily apply a sensitive and efficient analytical method for quantifying the five selected OPEs in human serum. Our method utilised a streamlined liquid-liquid extraction (LLE) with a methanol/acetonitrile mixture for efficient analyte isolation from the complex serum matrix. Ultrahigh-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) ensured robust specificity and sensitivity for quantification and identification. This pilot application provides the first preliminary data on OPEs in human serum from Europe. Considering that OPEs are metabolised relatively quickly, their presence in serum indicates constants long-term human exposure. The absence of measurable OPE concentrations in our research suggests significantly lower population exposure in Croatia compared to China, a primary global OPE producer and consumer. This study's value lies in providing novel regional biomonitoring data, highlighting the need for larger cohort analyses to comprehensively assess OPE exposure patterns in European populations.

The study was funded by the Croatian Science Foundation (grant HRZZ-IP-2024-05-8815).

KEY WORDS: contaminants; mass spectrometry; OPEs; plasticizers; ultrahigh-performance liquid chromatography

P 40

Dietary exposure to pyrethroid insecticides in Croatian adolescents

Jelena Kovačić[1], Marija Macan[1], Andreja Jurič[1], Adriana Gustovarac[1], Antonija Sulimanec[1], Martina Pavlić[2], Breige McNulty[3], Anne Nugent[4], Jim Garvey[5], and Veda Marija Varnai[1]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] Croatian Agency for Agriculture and Food, Osijek, Croatia
[3] University College Dublin, UCD Institute of Food & Health, Dublin, Ireland
[4] Queens University Belfast, Institute for Global Food Security, Belfast, United Kingdom
[5] Department of Agriculture, Food and the Marine, Food Chemistry Division, Celbridge, Ireland
jkovacic@imi.hr

This study aimed to provide preliminary estimates of dietary exposure to pyrethroid insecticides in the Croatian adolescent population. A multiresidue analysis of food samples collected in the Zagreb region Total Diet Study (2021–2023) was performed using the standard miniLuke extraction method and quantification by gas chromatography-high resolution accurate mass spectrometry and liquid chromatography-mass spectrometry. A protocol for estimating dietary intake using 24-hour dietary recall data, regional summary consumption data for male adolescents, and national dietary guidelines was developed and applied to estimate the intake of pyrethroids for 456 participants (age 11.6±0.4 years) of the first wave (2022–2023) of the PyrOPECh study (http://pyropech.imi.hr). The estimated median dietary intake of pyrethroids was 52.02 ng/kg b.w./day (interquartile range: 30.04–85.04 ng/kg b.w./day). The highest contributor with a median intake of 44.10 ng/kg b.w./day (on average 81 % of the total pyrethroid dietary intake) was lambda-cyhalothrin, whose residues were found in chicken meat, bacon, and several types of salami/sausages. For all participants, the estimated daily intakes were below the acceptable daily intake (ADI) values for cypermethrin, deltamethrin, bifenthrin, resmethrin, and lambda-cyhalothrin (95th percentile of intake in the range of 0.004–7 % of ADI), while a risk assessment for permethrin and tetramethrin was not performed due to the lack of ADI values.

The study was funded by the Croatian Science Foundation (grant codes: HrZZ IP-2019-04-7193, DOK-2020-01-1312), aided by collaborative efforts with the Department of Agriculture, Food and the Marine, Ireland, and supported by the European Union – Next Generation EU funding Program Contract 533-03-23-0006 (HumEnHealth).

KEY WORDS: 24-hour dietary recall; pesticide residues; pesticide risk assessment; protocol for dietary intake; total diet study

P 41

Monitoring pyrethroid metabolites in school children – preliminary data from the PyrOPECh project

Adriana Gustovarac, Andreja Jurič, Marija Macan, Jelena Kovačić, Željka Babić, and Veda Marija Varnai

Institute for Medical Research and Occupational Health, Zagreb, Croatia
agustovarac@imi.hr

This study presents preliminary results from biomonitoring conducted during the follow-up of the PyrOPECh project among seventh-grade boys in primary schools in the Zagreb region (n=203). This is the first biomonitoring study in Croatia that measured urinary pyrethroid metabolite 3-phenoxybenzoic acid (3-PBA). The urine samples were collected during the second wave (2024–2025) of the project, and the obtained data were compared with European data. A gas chromatograph coupled with a triple mass spectrometer was used for the analysis of 3-PBA. The limit of quantification (LOQ) was 0.62 ng/mL. For values below the LOQ, a corrected value of 0.31 ng/mL (half of the LOQ) was used in the statistical analysis. In total, 80 % (162 out of 203) of the values were below the LOQ, with a median <LOQ and a 95th percentile of 1.85 ng/mL. For 41 samples with concentrations above LOQ, the median was 1.13 ng/mL with an interquartile range of 0.93–1.86 ng/mL. Compared to medians and 95th percentiles of 3-PBA distributions in populations of similar age in other European countries, the values recorded in the PyrOPECh cohort were on average 3 to 4 times lower, which may have been a consequence of different time period of sampling in our study, different patterns of pesticide use, dietary habits, and socioeconomic and environmental factors.

The study was funded by the Croatian Science Foundation (HrZZ IP-2019-04-7193; HrZZ DOK-2020-01-1312), with the support of the Department of Agriculture, Food and the Marine, Ireland, and EU – Next Generation EU funding (Contract 533-03-23-0006; HumEnHealth).

KEY WORDS: 3-PBA; gas chromatography-mass spectrometry; pesticides; urinary metabolites; toxicological assessment

P 42

PROMETHEUS – Promoting Effective Transport through Healthy Bus Drivers

Viviane Remy[1], Marko Gerić[2], Jelena Macan[2], Pascal Wild[3], Marina Otelea[4], Irina Guseva Canu[1], Goran Gajski[2], and the PROMETHEUS Consortium

[1] University of Lausanne, Center for Primary Care and Public Health (Unisanté), Lausanne, Switzerland
[2] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[3] PW Statistical Consulting, Laxou, France
[4] University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania
ggajski@imi.hr

The public transport sector is vital for sustainable living, but is currently facing a severe bus driver (BD) shortage. Despite medical check-ups, BDs experience declining health, high stress, and absenteeism, which demands urgent action. The goal of the PROMETHEUS project is to improve the preventive value of the statutory occupational health surveillance by integrating biomonitoring coupled with the health education of BDs. We aim to investigate its feasibility and effect on BDs' health and wellbeing in a 2-year follow-up. Our innovative approach is the combination of strong epidemiological, biological, and medical expertise with occupational health promotion frameworks. This integrates work-related safety and health hazards prevention with health promotion and disease prevention for the advancement of the wellbeing of BDs. PROMETHEUS is structured into five work packages (WPs). WP1 adapts Swiss expertise to assess Romanian and Croatian BDs' exposure to physical, chemical, and ergonomic hazards, producing inventories and Bus-Exposure Matrices. WP2 establishes harmonized BD cohorts across three countries for pooled analysis of health outcomes, ensuring contextual relevance with local stakeholders. WP3 pilots expanded medical exams with biomarkers of exposure and effect to evaluate biomonitoring's role in occupational health. WP4 develops guidance, training, and educational materials to improve communication between occupational health providers and BDs. WP5 coordinates all of the activities and disseminates results. PROMETHEUS will serve as a tool for providing scientifically based data on occupational exposures. It will generate new knowledge on the BDs' occupational risks, allowing EU and Swiss policymakers to make better-informed decisions about future regulations in this particular area.

This study was funded within the frame of the Multilateral Academic Projects (MAPS) by the Swiss National Science Foundation, Croatian Science Foundation, and Executive Agency for Higher Education, Research, Development and Innovation Funding (Grant no. IZ11Z0_230941).

KEY WORDS: air pollution; biomonitoring; exposure assessment; occupational health; public transport

P 43

Aflatoxin M1 and the dairy trap: what's lurking in your yoghurt?

Marija Kovač Tomas[1], Tomislav Rot[2], Lara Arnautović[1], Mirjana Lenardić Bedenik[1], and Iva Jurčević Šangut[1]

[1] University North, Department of Food Technology, Koprivnica, Croatia
[2] Inspecto d. o. o., Osijek, Croatia
markovac@unin.hr

Aflatoxin M1 (AFM1), a toxic metabolite resulting from the biotransformation of aflatoxin B1 in dairy animals, poses a persistent food safety concern due to its thermal stability and ability to remain in milk and dairy products even after pasteurization or ultra-high temperature processing. This study aimed to assess the presence of AFM1 in fermented milk products available on the Croatian market, an area of research that has thus far primarily focused on liquid milk. Given the established carry-over effect of aflatoxins from contaminated feed to milk, combined with the influence of changing weather patterns on feed contamination, fermented dairy products may represent an underrecognized risk pathway. A total of 81 samples of yoghurt and other fermented milk products were collected and analysed using a validated ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method, with immunoaffinity column clean-up. The results revealed AFM1 presence in 42.0 % of the samples, with the highest found concentration of 0.044 μg/kg approaching the maximum limit for liquid milk in the European Union legislation, raising concerns regarding potential health risks, particularly among vulnerable groups. These findings underscore the limitations of current legal regulations, which apply only to milk, and highlight the need for expanded monitoring and stricter control measures across a wider range of dairy products.

KEY WORDS: climate impact; feed quality; fermented milk products; food safety; mycotoxin; UHPLC-MS/MS

P 44

Assessment of heavy metal contaminations in different oral dosage forms of aloe vera dietary supplements

Petra Bajt[1], Ilija Klarić[2], Jelena Kovačić[1], Ana Mornar[1], and Daniela Amidžić Klarić[1]

[1] University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia
[2] Public Health Brčko DC, Brčko, Bosnia and Herzegovina
pbajt@pharma.hr

Due to their cumulative toxicity and potential adverse effects on human health, the presence of heavy metals in dietary supplements represents a significant public health concern. The aim of this study was to evaluate the content of metal impurities, lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg), in commercially available dietary supplements containing aloe vera, considering their increasing market presence and frequent consumption. A total of 19 samples, classified as either botanical mono-preparations (n=4) or multi-preparations (n=15), were analysed in various oral dosage forms (tablets, soft, and hard capsules), collected from health food stores, pharmacies, and online retailers. Determination of Pb, Cd, and As was conducted using graphite furnace atomic absorption spectrometry (GF-FAAS) after microwave digestion with hydrogen peroxide and nitric acid, while Hg content was measured using a mercury analyser (AMA-254) without prior sample preparation. The contents of As, Cd, and Hg in the soft capsules and tablets forms were below the limit of quantification (LOQ). Mercury was quantified only in one multi-preparation hard capsule (49 μg/g; RSD 3.71 %). Arsenic was detected in 31.6 % and Cd in 84.2 % of the samples, while Pb was found in all of the analysed products. The highest content of Pb (8.6 μg/g) and As (0.9 μg/g) was recorded in the same sample (hard capsule multi-preparation). The detected Cd content was within the range of 0.02–0.14 μg/g, and the highest value was also found in a multi-preparation product. These results underscore the importance of regularly monitoring heavy metals in dietary supplements to ensure consumer safety.

KEY WORDS: As; atomic absorption spectrometry; Cd; Hg; Pb

P 45

Characterisation of nitazenes as novel synthetic opioids in the European context

Andreja Jurič[1], Blanka Tariba Lovaković[1], Dubravka Rašić[1], Ivana Novak Jovanović[1], and Irena Brčić Karačonji[1,2]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] University of Rijeka, Faculty of Health Studies, Rijeka, Croatia
ajuric@imi.hr

Nitazenes are extremely potent synthetic opioids that belong to the one of the fastest-growing groups of new psychoactive substances (NPS) around the world. Some of them are hundreds of times more potent than heroin. Similarly to other opioids, nitazenes can cause fatal respiratory depression. Since nitazenes have been detected in substances sold as benzodiazepines or cannabis products, many consumers are unaware of the risks they are facing. Since 2019, 22 nitazenes have been identified in Europe and new forms of these drugs are continually being synthetized and reported on the drug markets of at least 21 EU Member States. Protonitazene and metonitazene are the most commonly seized nitazenes in Europe. More than 350 deaths were associated with nitazenes in Europe during 2023 and 2024. The majority of deaths involved the use of nitazenes combined with stimulants such as amphetamine, methamphetamine, and cocaine. The main concerns regarding the use of nitazenes are extreme potency, supply-level adulteration, complicating overdose management, high variability in drug concentration, and unintentional use of potent substances. Taking into consideration all of the aforementioned, strengthening national early warning systems, improving monitoring and detection, and law enforcement coordination are key in preventing and mitigating further harm. In this context, a pilot project has been launched in Zagreb to monitor psychoactive substance intoxications. The main objectives of the project are to systematically analyse available data on current intoxication cases, identify monitoring gaps, and develop a structured proposal for implementing a local monitoring system.

This study was supported by the European Union – Next Generation EU (Program Contract of 8 December 2023, Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006, BioMolTox) and performed using the facilities and equipment funded within the European Regional Development Fund project KK.01.1.1.02.0007.

KEY WORDS: early warning system; metonitazene; overdose; protonitazene synthetic opioids

P 46

From treat to threat: are semi-synthetic cannabinoids in edibles a new health concern?

Irena Brčić Karačonji[1,2], Andreja Jurič[1], Blanka Tariba Lovaković[1], Ivana Novak Jovanović[1], and Dubravka Rašić[1]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] University of Rijeka, Faculty of Health Studies, Rijeka, Croatia
ibrcic@imi.hr

Semi-synthetic cannabinoids (SSCs) are a class of new psychoactive substances, primarily produced from cannabidiol extracted from low-Δ^9-tetrahydrocanabinol (THC) cannabis (hemp). They emerged on the European market in 2022 as legal alternatives to cannabis. Hexahydrocannabinol (HHC) was the first identified SSC, but the European Union Drug Agency (EUDA) is currently monitoring 24 SSCs on drug markets in Europe. Despite their growing prevalence, toxicological data on humans remain scarce. Consumption of SSC-containing edibles, such as gummies, gums, and brownies, has been associated with neurological and gastrointestinal symptoms. This study examined the Rapid Alert System for Food and Feed (RASFF) database notifications concerning the detection of HHC and its derivatives in edibles across EU member states. Since 2023, 27 notifications have been recorded. The Czech Republic was the origin country in the highest number of notifications (56 % of the total reported cases). Most alerts originated from official control on the market (21 cases), while the rest (monitoring of media, food poisoning, consumer complaint, and whistle-blower information) accounted for a total of six notifications. HHC and derivatives were most frequently detected in fruit gummies (67 %), followed by fruit gums (30 %), and chocolate or cookies (15 %). The predominance of flavoured confectionery products raises particular concern for accidental consumption by children. These findings highlight an urgent need to raise awareness among healthcare professionals and the public about the potential health risks associated with SSC-containing edibles. Strengthened monitoring, timely communication, and regulatory measures are essential to mitigate risks and protect public health.

This study was supported by the European Union – Next Generation EU (Program Contract of 8 December 2023, Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006, BioMolTox) and performed using the facilities and equipment funded within the European Regional Development Fund project KK.01.1.1.02.0007.

KEY WORDS: fruit gummies; hexahydrocannabinol; new psychoactive substances; public health; RASFF database

P 47

Clinical presentation of acute gamma-hydroxybutyrate (GHB) poisoning – experience of the Poison Control Center Serbia

Snežana Đorđević[1,2], Gordana Brajković[1], Jelena Džudović[1], Marko Antunović[1,2], Vladan Lukić[1,2], Marijana Ćurčić[3], and Nataša Perković Vukčević[1,2]

[1] Military Medical Academy, Poison Control Center, Belgrade, Serbia
[2] University of Defense, Military Medical Academy, Medical Faculty, Belgrade, Serbia
[3] University of Belgrade, Faculty of Pharmacy, Belgrade, Serbia
ivezicnela@gmail.com

Gamma-hydroxybutyrate (GHB) is a central nervous system depressant synthetized normally in the human organism. Because of its effects, it is abused as a recreational drug, as well as a rape drug. Recently, an increase of abused in recreational settings has been reported. Its toxicity is significant even at relatively low doses, with a thin line separating euphoria and coma. Due to its rapid metabolism and short half-life, the diagnosis of acute GHB poisoning is often challenging, especially in the absence of a reliable heteroanamnestic data. The aim of this work was to present cases of acute GHB poisoning recorded at the Poison Control Center, Serbia within the last seven years. Data on age, sex, poison severity score, presence of co-intoxicants, and outcome were analysed. Laboratory confirmation was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) on plasma and/or urine samples. A total of 64 cases were analysed, with patients aged between 17 and 44 years. There were 54 male and 10 female patients. The most common symptoms included sedation, bradycardia, and coma. An abrupt awakening during hospitalization occurred in most cases. Mixed intoxication (GHB+alcohol, benzodiazepines or other drugs of abuse) was identified in 21 cases, resulting in severe clinical presentations. One patient required hospitalization twice. No fatal outcomes were recorded. GHB poisonings are still primarily diagnosed based on one's clinical picture. Due to the short half-life and short detection window in biological samples, early sampling and applying specific analytical methods are essential. Healthcare professional education and improved toxicological diagnostics are key to adequate patient management.

KEY WORDS: LC-MS/MS; plasma; rape drug; recreational drug use; urine

P 48

Rodenticide poisonings and approach to treatment: experiences from the Croatian Poison Control Centre

Franka Šakić[1,2,3], Željka Babić[1,2], and Jelena Macan[1,2,3]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] Institute for Medical Research and Occupational Health, Croatian Poison Control Centre, Zagreb, Croatia
[3] Polyclinic Medicina rada Instituta za medicinska istraživanja i medicinu rada d. o. o.
jmacan@imi.hr

Rodenticides are chemical agents used to control rodents that may cause serious poisoning in humans and animals if ingested. In Croatia, the use of anticoagulant rodenticides is allowed, which in cases of poisoning can cause blood coagulation disorders, spontaneous bleeding, and, in severe cases, hypovolemic shock, coma, and death. A data analysis from the Croatian Poison Control Centre (1 Jan 2019–30 June 2025) recorded 151 poisoning cases with rodenticide: 59 % involved children/adolescents, 32 % adults, and 13 % animals. In 79 % of the cases, the poisoning was accidental, and in 21 % a suicide attempt. In two-thirds of cases, the rodenticide type was unknown. The remaining third involved baits with small amounts of active substances (brodifacoum: 0.029 g/kg, bromadiolone: 0.05 g/kg) or concentrated bromadiolone liquid (0.25 %). Patients with accidental ingestion were mostly asymptomatic, and those with intentional ingestion showed mild symptoms such as bruising or vomiting. Two suicide attempts involving concentrated bromadiolone required antidote treatment with vitamin K1. In cases of unintentional ingestion of small amounts of bait, lavage, and/or antidote are often routinely used in practice, which is not in line with current treatment guidelines. Current recommendations state that asymptomatic individuals who ingest <1 mg of active substance do not require evaluation or treatment, and for ingestion ≥1 mg of active substance coagulation should be assessed 48–72 hours post-exposure. If clinically relevant ingestion occurs, treatment includes application of activated charcoal within one hour after ingestion, intravenous vitamin K1 if international normalized ratio (INR) >1.4, and prothrombin complex or fresh frozen plasma if indicated.

KEY WORDS: anticoagulants; antidote; brodifacoum; bromadiolone; vitamin K1

Abstracts of the 7th International Congress of the Croatian Society of Toxicology – Poster presentations
Arh Hig Rada Toksikol 2025;76(Suppl. 1):66–124

P 49

The toxicological risks of nicotine: a review of calls received by the Croatian Poison Control Centre

Silvija Bošković, Adriana Gustovarac, and Željka Babić

Institute for Medical Research and Occupational Health, Croatian Poison Control Centre, Zagreb, Croatia
sboskovic@imi.hr

Between 1 January 2023 and 30 June 2025, the Croatian Poison Control Centre received 85 calls related to nicotine poisoning, highlighting the ongoing presence of this public health issue. Nicotine is an alkaloid with a strong addictive potential found in nearly all tobacco products, responsible for toxic symptoms following exposure. The reported cases of nicotine poisoning involved infants (n=53), adolescents (n=16), preschool children (n=8), adults (n=7), and school-aged children (n=1), indicating the extreme vulnerability of the youngest population. Of all of the cases, 53 were asymptomatic, 18 presented mild symptoms (nausea, vomiting, tachycardia), eight moderate symptoms (dizziness, headache, tremor, sweating, pallor, hypertension), and four severe symptoms (seizures, respiratory depression), while in two cases the symptoms were not specified. The most common sources of nicotine exposure were cigarettes and cigarette butts, e-cigarette refill liquids, and nicotine pouches (snus). In children, poisonings were most often the result of accidental ingestion of concentrated nicotine products (electric cigarette liquids), which are frequently available in households without child-resistant packaging. Particularly concerning is the high incidence of nicotine poisoning among infants and the substantial proportion of symptomatic cases (n=30). Key recommendations for reducing poisoning incidents include safe storage of nicotine-containing products out of children's reach, proper parental education, and the continued role of the Poison Control Centre as a frontline provider of expert assessment and guidance.

KEY WORDS: child poisoning; e-cigarettes; nicotine pouches; prevention; public health

P 50

Biocidal products as the cause of poisoning: five-year report from the Croatian Poison Control Centre

Emin Veledar, Željka Babić, and Jelena Macan

Institute for Medical Research and Occupational Health, Zagreb, Croatia
eveledar@imi.hr

Data collected by the Croatian Poison Control Centre (CPCC) in 2020 showed that surface disinfectants and hand sanitizers were two types of biocidal products (BP) that led to a significant increase in poisoning cases compared to the same period in 2019. The aim of this study was to determine the prevalence of poisoning cases with the same BPs in the CPCC after the COVID-19 pandemic. Analysis of all poisoning cases involving BPs recorded in CPCC over the last five years (2020–2024) was conducted. During this period, a total of 777 cases involving BPs was recorded (5.5 % of a total of 14,069 calls), among which 465 cases involved surface disinfectants and 312 hand sanitizers. For surface disinfectants, a yearly increase in number of cases was observed in 2020–2024 (n=71, 61, 93, 118, and 122, respectively), while an opposite trend was observed for hand sanitizers (n=78, 71, 47, 64, and 52, respectively). Most of the incidents were accidental (83.0 %), asymptomatic or with mild to moderate symptoms. Severe symptoms were mostly observed in suicide attempts accounting for 5.1 % of cases. Adults were involved in 60 % of exposures to surface disinfectants, and infants/preschool children in 61 % of poisonings with hand sanitizers. In conclusion, BPs do not contribute notably to the total number of cases in CPCC after the COVID-19 pandemic, but the number of poisonings involving surface disinfectants slightly continue to rise. Preventive efforts including adequate labelling and packaging of BP, and promotion of safety behaviour in households and workplaces should continue.

KEY WORDS: biocides; COVID-19 pandemic; disinfectant; hand sanitizer; prevention

P 51

Risk assessment of chemical hazards: principles, methods, and application

Marija Kujundžić

Institute for Medical Research and Occupational Health, Zagreb, Croatia
kujundic@imi.hr

The risk assessment of a chemical substance is a key component in monitoring the health of workers exposed to hazardous chemicals during work processes. This presentation shows the essential steps involved in conducting a chemical risk assessment, with particular focus on the sustainable application of prevention principles to ensure a high-quality and effective outcome. An effective risk assessment begins with collecting core documentation: evaluation of the existing workplace risk assessment, a detailed job process description and a list of chemicals to which workers may be exposed. Hazardous substances, particularly those with toxic, carcinogenic, mutagenic, or reprotoxic properties are identified according to hazard categories and warning labels as defined by Regulation (EC) No 1272/2008 and/or safety data sheets. Exposure levels are determined based on the measured concentrations of chemicals in workplace air and the frequency and duration of exposure in specific time frames (daily, weekly, or monthly). All collected data are systematically analysed to evaluate potential hazards and exposure risks. This approach enables the identification of high-risk substances and the continuous development of targeted recommendations to reduce exposure. These include the implementation of personal protective equipment, technical and organisational measures and the substitution of hazardous substances with safer alternatives. Additionally, guidelines for future monitoring and continuous safety improvement are established. Such a proactive prevention strategy not only meets regulatory requirements but also emphasizes professional responsibility in promoting safety and sustainability in increasingly chemical-intensive industries.

KEY WORDS: chemical risks; prevention; risk assessment; toxicology; occupational safety

P 52

Gaussian Mixture Modelling reveals metal-specific biomarker profiles in great cormorant nestlings from the Kopački rit Nature Park

Dora Bjedov[1], Jorge Bernal-Alviz[2], Lucija Sara Kovačić[1], Nataša Turić[1,3], Alma Mikuška[1], and Mirna Velki[1]

[1] Josip Juraj Strossmayer University of Osijek, Department of Biology, Osijek, Croatia
[2] Independent Researcher, Jamundí, Colombia
[3] Teaching Institute of Public Health Osijek-Baranja County, Osijek, Croatia
mvelki@biologija.unios.hr

Avian apex predators have proven their significance as bioindicators for different environmental pollutants. In this study, cadmium (Cd), mercury (Hg), lead (Pb), arsenic (As), and selenium (Se) concentrations were analysed in whole blood from 10 great cormorants (*Phalacrocorax carbo*) nestlings at the Kopački Rit Nature Park, Croatia, during the spring of 2023. Additionally, reactive oxygen species (ROS), proteins, reduced glutathione (GSH), and the activities of acetylcholinesterase (AChE), carboxylesterase (CES), glutathione S-transferase (GST), and glutathione reductase (GR) were measured. To assess the relationship between biomarker responses and metal(loid) and Se accumulation patterns, a non-supervised probabilistic clustering using a Gaussian Mixture Model (GMM), preceded by dimensionality reduction via Randomized Singular Value Decomposition Principal Component Analysis (RSVD-PCA), was performed. The GMM revealed three distinct mixture components (MC) or clusters. The first cluster (MC 1) (27.30 % from total) grouped two bioactive pollutants and the essential element concentrations in whole blood (As, Hg, and Se), suggesting a general antagonistic interaction that might be closely related to Hg and As hematotoxic and cytotoxic effects. The second cluster (MC 2) (45.60 % from total) featured both pollutant accumulation in whole blood (Cd and Pb) along with CES and GST activities linked to ROS presence, indicating a natural antioxidative detoxification process. The third cluster (MC 3) (27.30 %) comprised AChE activity, GSH presence, and proteins, all probably associated with GSH-repairing effects. Our results highlight the utility of these novel statistical approaches in characterizing the complex pollution/response interactions between metal(loid)s, essential elements, and biomarkers in high-relevance bioindicators like *P. carbo* nestlings.

This study was funded by the Department of Biology, Josip Juraj Strossmayer University of Osijek (project "Research of native and non-native fauna of birds, fish and insects, and bioaccumulation of heavy metals in their food webs").

KEY WORDS: biomarkers; GMM; heavy metals; ROS; water birds

P 53

Characterization of microplastics in seawater, fish, and mussels from the Adriatic Sea

Sara Šariri[1], Marija Kuštro[1], Martina Ugrinović[1], Fabio Faraguna[2], Dajana Kučić Grgić[2], Kristina Pikelj[3], and Vlatka Filipović Marijić[1]

[1] Ruđer Bošković Institute, Zagreb, Croatia
[2] University of Zagreb, Faculty of Chemical Engineering and Technology, Zagreb, Croatia
[3] University of Zagreb, Faculty of Science, Department of Geology, Zagreb, Croatia
ssariri@irb.hr

Microplastic (MP) accumulation in marine animal tissues may cause harmful biological effects, but research is still limited. While it is known that fish and bivalves can take up MPs through ingestion and water filtration, data on tissue-specific distribution are still limited. This study aims to isolate and characterize MPs, by shape, size, colour, and polymer type, in the seawater and marine organisms sea bream (*Sparus aurata*) and mussels (*Mytilus galloprovincialis*) from the Šibenik Bay (Adriatic Sea). Gills and gonads of sea bream, and gills and mantle (overgrown with gonads) of mussels were analysed. A total of 1000 L of seawater was filtered through sieves to collect five size fractions (>1 mm, 1 mm–500 μm, 500–125 μm, 125–63 μm, <63 μm). MP particles were rinsed from each sieve with Milli-Q water and isolated by vacuum filtration. Tissues were digested using HNO_3 at 85 °C for 3.5 h followed by vacuum filtration over gold-coated polyester filters (5 μm pore size). After drying, samples were analysed using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). Contamination was controlled by using blank samples. MPs were detected in all seawater samples, with higher abundance in smaller size fractions. Fibres were the most common shape. In fish and mussel tissues, MP particles were rare and the detected polymers included polyamide and poly(ethylene-co-acrylic acid), thereby demonstrating exposure to plastics. These findings confirm the presence of MP in both seawater and marine organisms in the Adriatic, underlining the need for continued monitoring of their exposure, accumulation, and potential biological impacts.

This study was funded by the Croatian Science Foundation project PlastOrgAnoTox (HRZZ-IP-2024-05-8918).

KEY WORDS: attenuated total reflectance Fourier transform infrared spectroscopy; *Mytilus galloprovincialis*; *Sparus aurata*; tissue accumulation

P 54

Safety assessment of the agricultural use of sewage sludge from a large Croatian wastewater treatment plant

Sanja Stipičević[1], Marina Bubalo Kovačić[2], Jelena Horvatinec[2], René Matthies[3], Predrag Samardžija[3], Radovan Savić[4], Iva Smoljo[1], and Gabrijel Ondrašek[2]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] University of Zagreb, Faculty of Agriculture, Zagreb, Croatia
[3] Zagrebačke otpadne vode, Zagreb, Croatia
[4] University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia
stipicevic@imi.hr

Sewage sludge (SS) from urban wastewater treatment is classified as non-hazardous under Regulation (EC) no. 2001/118, and it is encouraged for agricultural reuse under Council Directive 86/278/EEC, in line with sustainability, zero pollution, and climate goals. However, the SS intended for use as farmland fertilizer must undergo rigorous evaluation to ensure it meets treatment standards and safety requirements. The large-scale wastewater treatment plant (WWTP) in Zagreb, Croatia, designed to serve a population of 1.2–1.5 million people, generates approximately 50,000 tonnes of stabilised SS annually, representing nearly 20 % of the total national SS production. The current agricultural reuse is minimal (around 6 % in 2017), with most SS diverted to landfilling. This long-term monitoring study evaluated the anaerobically digested SS from Zagreb's WWTP to ensure compliance with national and international safety standards for agricultural application and to assess potential environmental and health risks. SS composite samples were collected monthly and analysed in a licensed laboratory. Regulated contaminants were monitored over the periods: heavy metal(oid)s (2009–2024), indicator polychlorinated biphenyls and organochlorine pesticides (2008–2024), and polycyclic aromatic hydrocarbons (2012–2024). Environmental risk was assessed using the EU Technical Guidance Document on Risk Assessment, while the health risk of dermal exposure and inhalation was evaluated according to the US EPA methodology. The agricultural use of treated sludge from the Zagreb WWTP can be considered safe within the existing Croatian and EU regulations. However, further studies should address the monitoring of emerging contaminants with unquantified risks to evaluate the cumulative effects of sludge applications.

The study was funded by the European Union – NextGenerationEU (grant NPOO.C3.2.R3-I1.04.0143).

KEY WORDS: heavy metal(loid)s; organic micropollutants; risk assessment; soil amendment; waste management

P 55

Oxidation state of anti-biofouling copper nanoparticles is a key predictor of toxicity in marine macroalga *Ericaria crinita*

Lara Krasulja[1], Edi Gljušćić[2], Andrea Bilajac[2], Ljiljana Iveša[2], Petra Peharec Štefanić[1], and Daniel Mark Lyons[2]

[1] University of Zagreb, Faculty of Natural Sciences, Zagreb, Croatia
[2] Ruđer Bošković Institute, Center for Marine Research, Rovinj, Croatia
lyons@irb.hr

Copper has found widespread use as an additive to ship hull antifouling paints, particularly after the ban on use of organometallic compounds such as tributyl tin. Copper can be present in ultrafine particulate form as copper or copper oxide. However, the chemical identity of these nanoparticles can have significant implications for their stability which may ultimately drive toxicity towards non-target organisms. While the impact of copper nanoparticles on a number of later-diverging marine organisms has been noted, there is little corresponding research on macroalgae, a key ecosystem component providing habitat, shelter and food for numerous marine organisms. Therefore, this study investigated the effects of copper nanoparticles on macroalga *Ericaria crinita* to determine if copper speciation (oxidation states 0, I, and II) can modulate toxicity, and if the macroalga shows seasonal susceptibility to toxicants. The glutathione detoxification pathway showed strong activation in growing macroalga (spring), with greater reduction of glutathione and increased glutathione-*S*-transferase activity, particularly for Cu_2O nanoparticles. Antioxidant content significantly increased with greater proline concentrations noted after Cu_2O treatment in spring and autumn (low-growth phase) compared to controls. In addition, polyphenol content significantly increased with Cu_2O treatment in spring. Copper-based nanoparticles did not have a significant effect on lipid peroxidation. Overall, the greatest impact on *E. crinita* was caused by nanoparticles containing copper in the +I oxidation state (Cu_2O), followed by those in the +II state (CuO), while zero-valent copper nanoparticles (Cu) had the least effect. Actively growing macroalga were more susceptible to the negative effects of the nanoparticles.

This study was funded by the Croatian Science Foundation (grant HRZZ-IP-2018-01-5351).

KEY WORDS: biomarkers of oxidative stress; glutathione; lipid peroxidation; polyphenol; seasonal susceptibility

P 56

Long-term seasonal monitoring of radionuclides in mussel *Mytilus galloprovincialis* along the Croatian northern Adriatic coast

Dijana Pavičić-Hamer, Ivana Čarapar, Lara Jurković, and Daniel Mark Lyons

Ruđer Bošković Institute, Center for Marine Research, Rovinj, Croatia
dpavicic@irb.hr

The marine environment represents the final sink for both naturally occurring radionuclides (^{7}Be, ^{40}K, ^{232}Th, ^{226}Ra, and ^{238}U) and anthropogenic ^{137}Cs, with atmospheric deposition, underground freshwater discharges and river flows as primary inputs. Due to the potential harm to biota, the radioecological condition of the northern Adriatic Sea has been assessed by long-term measurement (2008–2024) of radioactivity in the mussel *Mytilus galloprovincialis*. Mussels are widely used as an indicator organism in pollution monitoring programmes as their high filtering capacity (≈3 L/h) enables the accumulation of dissolved/particulate matter including radionuclides. Activity concentrations of ^{137}Cs were mostly below the detection limit (<0.3 Bq/kg). Low activity concentrations (maximum value of 0.86 Bq/kg) were detected in only a few samples, independently of sampling season and location. The estimated committed annual effective dose for humans via mussel consumption was 0.3 μSv/yr, which is significantly below the maximum permitted dose (MPD) of 1 mSv/yr recommended by the International Commission on Radiological Protection. In contrast, the activity concentrations of ^{7}Be in mussels were regularly higher in spring (26.01 Bq/kg) than in autumn (19.90 Bq/kg) at all of the locations, which may have been related to the impact of rain and freshwater inflow. ^{40}K activity concentrations did not vary significantly with season or location (average activity 321 Bq/kg) and can be attributed to its high and uniform concentration in the sea. Activity concentrations of other naturally occurring radionuclides were generally below the detection limits. This indicated that the radioecological condition in northern Adriatic waters is satisfactory and without significant radionuclide input from fallout and rivers.

KEY WORDS: air pollution; biomonitoring; exposure assessment; occupational health; public transport

P 57

Passive monitoring of lead exposure in griffon vultures from the Kvarner archipelago, Croatia: bone and liver as indicator tissues

Maja Lazarus[1], Tatjana Orct[1], Dubravko Dender[2], Bolesław Jan Słociński[2], Ivana Ribić[2], Tomislav Anić[3], Krešimir Severin[4], and Magdalena Palić[4]

[1] Institute for Medical Research and Occupational Health, Zagreb, Croatia
[2] BIOM Association, Zagreb, Croatia
[3] "Priroda" Public Institution for Protected Area Management of the Primorje-Gorski Kotar County, Rijeka, Croatia
[4] University of Zagreb, Faculty of Veterinary Medicine, Zagreb, Croatia
mlazarus@imi.hr

The conservation of critically endangered Eurasian griffon vultures (*Gyps fulvus*) in Croatia is threatened by anthropogenic poisoning and electrocution. While scavenging carcasses, vultures may ingest fragments of spent lead (Pb)-based ammunition, leading to the gradual release and absorption of Pb into the bloodstream. Lead is subsequently deposited in bones and, to a lesser extent, in liver, the primary organ responsible for chemical metabolism. Growth, development, and reproductive periods are particularly vulnerable to Pb toxicity, posing a risk to both individuals and population viability. Numerous raptor species globally exhibit adverse effects from Pb absorption. We conducted Pb monitoring using inductively coupled plasma mass spectrometry on femur, sternum, and liver tissues from 17 griffon vultures found dead in the Kvarner archipelago in 2024. The average Pb levels were 10.6±6.4 (n=16, range 1.21–28.4 mg/kg dry mass) in femur, 20.3±21.2 (n=4, range 6.89–51.8 mg/kg dry mass) in sternum, and 1.73±1.70 (n=13, range 0.173–6.21 mg/kg dry mass) in liver. One individual exhibited Pb bone levels indicative of clinical poisoning, while eight others showed levels suggestive of subclinical toxicity. Femoral Pb levels were consistent with sternum levels. Compared to other Croatian raptors and mammalian scavengers, vultures exhibited substantially higher tissue levels compared to the Eurasian eagle-owl (*Bubo bubo*), approximately double the femoral levels observed in brown bears (*Ursus arctos*), and similar hepatic levels to those of golden jackals (*Canis aureus*) and brown bears. Although elevated Pb levels were found in 50 % of the sampled griffon vultures, a broader study is necessary to assess the status across a representative portion of the national population.

This study was funded by the European Union (101074212-LIFE21-NAT-HR-LIFE SUPport), European Union – Next Generation EU (HumEnHealth, Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006), and performed using the facilities and equipment funded within the European Regional Development Fund project KK.01.1.1.02.0007.

KEY WORDS: bird; bone; *Gyps fulvus*; liver; scavenger; toxic elements

P 58

Combined information on the dissolved and nanoparticulate metals in surface river-water leads to a more complete recognition of water pollution

Zrinka Dragun, Mavro Lučić, Zoran Kiralj, Karla Bogdan, Tomislav Kralj, and Damir Valić

Ruđer Bošković Institute, Zagreb, Croatia
zdragun@irb.hr

The section of the Mura River that flows through Croatia, forming the state border with Slovenia and Hungary, is impacted by several diffuse pollution sources, including highly developed agricultural acitivities and abandoned coal mine pits, which could lead to water/sediment contamination, potentially endangering its diverse biota. The aim of this study was to investigate river-water contamination with selected dissolved and nanoparticulate metals during prolonged period of stable water discharge at five sites, covering the entire river-section within Croatia. The methods applied were inductively coupled plasma mass spectrometry (ICP-MS) and single particle inductively coupled plasma mass spectrometry [(SP)-ICP-MS]. The dissolved concentrations were rather uniform, with somewhat increased Mn and Pb concentrations at Goričan, and Pb at Mursko Središće. Moreover, a slight decrease was observed for Fe, Cs, La, and Ce towards downstream sites, as opposed to the slight increase of Mo, W, and U. Contrary, nanoparticulate metals revealed a clear concentration increase of several elements (La, Ce, Pb, Cs, W, Fe, U) at Mursko Središće (in front of former coal mine pits), Cu and Zn at Podturen, and Fe and Cs additionally at Kotoriba. The nanoparticle sizes, which could have potentially influenced their reactivity, were also determined, in the following decreasing order: Fe, Mn (~150 nm)>Zn (~130 nm)>Cr (~110 nm)>Cs, Mo (~100 nm)>Cu (~70 nm)>W (~50 nm) >Ce (~40 nm)>Pb (~35 nm)>La, U (~30 nm). As metal nanoparticles can cause toxicity to biota, the obtained results indicated the importance of their inclusion in water pollution assessment in addition to dissolved metal concentration measurement.

Funded by the European Union – NextGenerationEU.

KEY WORDS: agriculture; coal mines; diffuse pollution; lead; tungsten

P 59

Innovative Problem-Based Learning in toxicology through internationalization at home: insights from the PRODIC project

Javier Esteban[1], Serçin Karataş[2], Asuman Aşık[3], Marijana Ćurčić[4], Ksenija Durgo[5], and Gonca Çakmak[6]

[1] Universidad Miguel Hernández de Elche, Instituto de Bioingeniería, Elche, Spain
[2] Gazi University, Gazi Faculty of Education, Department of Computer Education and Instructional Technology, Ankara, Türkiye
[3] Gazi University, Gazi Faculty of Education, Department of English Language Teaching, Ankara, Türkiye
[4] University of Belgrade, Faculty of Pharmacy, Department of Toxicology "Akademik Danilo Soldatović", Belgrade, Serbia
[5] University of Zagreb, Faculty of Food Technology and Biotechnology, Zagreb, Croatia
[6] Gazi University, Faculty of Pharmacy, Department of Toxicology, Ankara, Türkiye
jesteban@umh.es

The evolving landscape of higher education requires innovative teaching methods to encourage student engagement, intercultural competence, and global awareness. In toxicology education, Problem-Based Learning (PBL) has emerged as a powerful student-centred methodology, promoting critical thinking, teamwork, and practical application of knowledge. This work combines two case studies integrating internationalization strategies into toxicology teaching: ERASMUS staff mobility and the PRODIC initiative under the Internationalization at Home (IaH) framework. The PRODIC project, implemented across four European universities [University of Zagreb (Croatia), University of Belgrade (Serbia), Miguel Hernández University of Elche (Spain), and Gazi University (Türkiye)] enabled collaborative PBL activities in a virtual international environment. Using collaborative tools, students engaged in lectures, tasks, and peer interactions, across three academic years. Despite challenges in coordination, language proficiency, and technology access, adaptive measures such as recorded lectures and flexible deadlines enhanced accessibility and inclusivity. Students reported increased motivation, broader perspectives, and appreciation for the international learning experience. These findings highlight the potential of IaH to complement traditional mobility programs, democratize access to global education, and enrich toxicology curricula. The PRODIC model offers a scalable and sustainable framework for integrating international dimensions into health sciences education.

This study was funded by the Miguel Hernández University (grants UMH_RR_01643_2024, UMH_ RR_02619_2023, UMH_ RR_00511_2022).

KEY WORDS: ERASMUS; Internationalization at Home (IaH); PRODIC project; toxicology education; virtual international environment

AUTHOR INDEX

L, LJ

M

N

O

P

R

S, Š

T

U

V, W

Z, Ž

NOTE TO SUBSCRIBERS

Arhiv za higijenu rada i toksikologiju – Archives of Industrial Hygiene and Toxicology is published four times a year. Annual subscription is EUR 17 for individual subscribers and EUR 56 for institutions in Croatia, EUR 30 for the members of the Slovenian Society of Toxicology, EUR 70 for other subscribers in Europe, and EUR 85 for overseas countries, postage included. Students and the retired are entitled to a 50% discount on respective rates. For payment details, other inquiries, purchase order, and address changes please contact the Editorial Office *Archives of Industrial Hygiene and Toxicology*, Institute for Medical Research and Occupational Health, Ksaverska cesta 2, HR-10000 Zagreb, Croatia. Phone: (+385 1) 4682 500, Fax: (+385 1) 4673 303, E-mail: *arhiv@imi.hr*, web: *http://arhiv.imi.hr*

INSTRUCTIONS TO AUTHORS

are provided in the first issue of each volume and are available at
https://sciendo.com/journal/aiht
http://hrcak.srce.hr/aiht

Arhiv za higijenu rada i toksikologiju

Archives of Industrial Hygiene and Toxicology

Arh Hig Rada Toksikol • Vol. 76 • Suppl.1 • ZAGREB, CROATIA 2025

ISSN 0004-1254

IZDAVAČ / PUBLISHER:

Institut za medicinska istraživanja i medicinu rada Zagreb, Hrvatska

Institute for Medical Research and Occupational Health Zagreb, Croatia