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toksikologiju**

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**Abstracts of the 6<sup>th</sup> Croatian Congress of Toxicology with  
International Participation**

**CROTOX 2021**

Rabac, Croatia, 3 to 6 October 2021

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Abstracts of the 6<sup>th</sup> Croatian Congress of Toxicology with International Participation

CROTOX 2021

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## EDITORIAL

Dear Reader,

It is with great pleasure that I present to you the Supplement to the *Archives of Industrial Hygiene and Toxicology* which comprises abstracts of all the presentations at the 6<sup>th</sup> Croatian Congress of Toxicology with International Participation, which is taking place in Rabac, Croatia in the period 3-6 October, 2021. The rich conference programme is showcased herein by all the abstracts which encompass a wide range of plenary and invited lectures and oral and poster presentations.

It must be admitted that the circumstances of holding this edition of the conference are significantly different from years past. The adage, and not meant in a positive sense, "May you live in interesting times" seems particularly apt to describe the period which we are currently passing through. Indeed, the rapid spread of a coronavirus and the accompanying declaration of a pandemic have dramatically altered the way in which we have lived our lives for the last year and a half, with concomitant effects on many planned public events which have either been drastically curtailed, postponed or cancelled. It is against this backdrop that the Croatian Congress of Toxicology with International Participation has endeavoured to realise its quadrennial conference and overcome all the challenges associated with holding such an event in a period of lockdowns and disrupted national and international travel.

It is with some resonance that these global events highlight the vital role that toxicology and toxicologists play in the modern world. In particular, the full spectrum push to rapidly roll out population-wide experimental treatments against coronavirus symptoms, and hence any potentially deleterious effects that may arise over short to long time scales, emphasises the need to support increased monitoring and research efforts in the field of human and clinical toxicology. The conference addresses not only coronavirus related issues such as the impacts of increased disinfectant and alcohol use but broader exposure scenarios ranging from exposure of foetuses to tobacco smoke, children taking analgesics and anti-inflammatory pharmaceuticals to adults consuming psychoactive substances. An aspect oftentimes overlooked is exposure through poisoning from accidental consumption of potentially harmful materials such as plants, which this conference addresses in addition to investigating potential benefits such as the anti-cancer, anti-oxidative, and anti-bacterial potential of plant-derived bioactive substances. Hand in hand with clinical toxicology goes exposure risk assessment and a broad variety of studies focussing on issues such as neonicotinoid and legacy pesticides use and hidden exposure pathways such as that through house dust provide an ideal base to support discourse among all the experts and participants at the conference. Advances in these areas will be underpinned by new developments in

laboratory test systems, and a range of data is presented on emerging approaches incorporating 3D cell models and *in silico* computation methods.

Of course, there is an array of other modern challenges present in the world around us underscoring the continuing need for new approaches and efforts, for example addressing issues ranging from the application of genetically modified organisms to release of micro- and nanoscale materials in the environment, in the fields of food and environmental toxicology, respectively. It is entirely appropriate that attention is given to the area of ecotoxicology as our integration in the biome inevitably puts us at risk due to consumption of foods that may have bioaccumulated, trophically transferred and biomagnified toxic substances of anthropogenic origin such as herbicides and heavy metals. Indeed, this overlaps to a significant degree with food toxicology, where the safety of foodstuffs, ranging from fresh fruit and vegetables to honey and fermentation products, is of primary concern. In addition to the presentation of these studies, the recent rapid increase of interest in anthropogenic materials with micro- and nano-dimensions reaching the environment is also addressed, with data on microplastics, nanoparticles, and nanotubes impact on plant and animal models presented. Unfortunately, these few brief comments can only give a mere glimpse of the rich programme on offer at the 6<sup>th</sup> Croatian Congress of Toxicology with International Participation, and the important fundamental and applied data that is being presented.

While the presenters are rightly the stars of the show, it should be mentioned that a large number of people have been working quietly in the background to organise and bring to life this conference, and I feel it is appropriate to mention at this point, with thanks, the contributions of the Editor-in-Chief, Assistant Editors and Editorial Board of the *Archives of Industrial Hygiene and Toxicology* who have helped us realise this Proceedings book as a supplement to the journal. The enormous contribution of the members of the Organising and Scientific boards is also gratefully acknowledged.

In conclusion, the new data and approaches presented at the 6<sup>th</sup> Croatian Congress of Toxicology with International Participation, and outlined in this issue, are timely as they address an enormous wealth of both legacy and contemporary issues in all the fields of toxicological research. Looking around us, it seems we are indeed living in interesting times, and it is clear that the role toxicologists play will remain as important as ever as we continue into the future.

Guest Editor  
Prof Daniel Mark Lyons, PhD



## WELCOME ADDRESS

Dear Friends and Colleagues,

It is my great pleasure and honour to be able to welcome you on behalf of CROTOX 2021 and the Croatian Society of Toxicology (CST) at this 6<sup>th</sup> Croatian Congress of Toxicology with international participation in the charming town of Rabac.

Our plan was to organize CROTOX in October last year, but because of the COVID-19 pandemic we had to postpone it on three separate occasions. In the meantime, while we were waiting for the permission of health authorities for holding the congress, our Scientific and Organising Committees prepared plans for the Congress, invited the lecturers, edited our book of abstracts and organised each detail of the Congress. I am always delighted by the enthusiasm of our committees because the results of their efforts are amazing: we all have the opportunity to hear eminent toxicologists presenting the results of their most recent research in a joyful, smooth, and relaxed atmosphere.

In spite of all the problems, we can proudly say that the CST is very active and that our activities provide an adequate response to the modern challenges in toxicology. Due to the pandemic, we could not invite all of the members of our and other similar societies to give lectures in various fields of toxicology. However, we organised online lectures and gave fellowships to our members for participation at online scientific meetings.

The Croatian Society of Toxicology made use of the lockdown(s) to publish the monograph "25 Years of the Croatian Society of Toxicology". In this monograph, nine toxicologists from various institutions (medical schools, institutes, toxicology agencies, and public health agencies) gave an overview of the scientific, educational, and practical position of toxicology in Croatia. This monograph, written

in Croatian and English, covers the 25-year history of the CST.

The systematic education of toxicologists in our country is still lacking and therefore we decided to organise a Continuing Education Course (CEC) before the beginning of the Congress. This CEC, financially supported by EUROTOX is entitled "Old methods, new perspective: new regulation and approach to toxicity testing of food chemicals". Eminent Croatian and international scientists agreed to share their knowledge with young scientists. As usual, in order to help young scientists to participate at CROTOX, the Organising Committee gave eight fellowships to young scientists. They will hold oral presentations of their works together with eminent scientists from various fields of toxicology. The abstracts of the oral and poster presentations will be published in the Abstract Book as a Supplement to the journal *Archives of Industrial Hygiene and Toxicology*, the official journal of the Croatian Society of Toxicology. I would like to thank the Editor-in-Chief and the Editorial Board of the *Archives of Industrial Hygiene and Toxicology* for accepting the abstracts of CROTOX 2021 to be published.


The Congress is held under the auspices of the Institute for Medical Research and Occupational Health, which is greatly acknowledged. We would like to thank our sponsors for supporting the organisation of the Congress.

On behalf of the Organising and Scientific Committees, I wish to thank all the participants of the Congress and all sponsoring organisations that made this Congress possible.


*President of the Congress  
Maja Peraica, MD, PhD, ERT*

CROTOX 2021, Rabac, Croatia, October 3-6


**Programme**

<b>Sunday, 3 October 2021 (Day 1)</b>		
12:00 – 18:00	Registration of participants	
12:00 – 18:00	<b>Continuing Education Courses (CEC), including coffee &amp; lunch breaks</b> <b>Moderator: Emanuela Corsini (Milan, Italy)</b>	
19:30 – 20:00	<b>Opening ceremony</b>	
<b>PLENARY LECTURE</b> <b>Chairs: Maja Peraica, Daniel Mark Lyons</b>		
20:00 – 20:45	<b>IL – 1</b>	<b>Andrew Collins</b> (Oslo, Norway) The comet assay; from nanoparticles to human populations
<b>21:00</b>	<b>Welcome reception</b>	
<b>Monday, 4 October 2021 (Day 2)</b>		
8:00 – 9:00	<b>Posters will be put up by presenters</b>	
<b>SESSION 1 CLINICAL TOXICOLOGY</b> <b>Chairs: Arnes Rešić, Željka Babić</b>		
9:00 – 9:35	<b>IL – 2</b>	<b>Ines Potočnjak</b> (Zagreb, Croatia) Intoxications in clinical settings
9:35 – 10:10	<b>IL – 3</b>	<b>Mila Lovrić</b> (Zagreb, Croatia) The diagnostic needs and possibilities of detecting new psychoactive substances in clinical practice
10:10 – 10:25	<b>OP – 1</b>	<b>Jelena Macan</b> (Zagreb, Croatia) Toxicological aspects of the increased use of disinfectants during the COVID-19 pandemic in Croatia
10:25 – 10:40	<b>OP – 2</b>	<b>Zrinka Franić</b> (Zagreb, Croatia) Plant poisoning reported to the Croatian Poison Control Centre during a ten-year period (2010-2019)
10:40 – 11:00	Silver sponsor presentation	<b>Labtīm d.o.o.</b> <b>Ivica Blažević</b>
<b>11:00 – 11:30</b>	 <b>Poster viewing and coffee break sponsored by Medic d.o.o</b>	
<b>SESSION 2 ANALYTICAL TOXICOLOGY</b> <b>Chairs: Davorka Sutlović, Alica Pizent</b>		
11:30 – 12:05	<b>IL – 4</b>	<b>Snežana Đorđević</b> (Belgrade, Serbia) Unconventional psychoactive substances – big analytical challenges
12:05 – 12:20	<b>OP – 3</b>	<b>Tanja Živković Semren</b> (Neuchâtel, Switzerland) Real-time (on-line) chemical characterization of thermal aerosols by super secondary electrospray ionization coupled with high-resolution mass spectrometry (Super SESI–HRMS)

12:20 – 12:50	Golden sponsor's presentation	<b>Alphachrom d.o.o.</b> <b>Matea Kovač &amp; Ines Topalović Piteša</b> Microplastic – reality or science fiction?
<b>12:50 – 14:00</b>	<b>Lunch break</b>	
<b>SESSION 3 EMERGING APPROACHES IN TOXICOLOGY</b>		
<b>Chairs:</b> Davor Želježić, Biljana Antonijević		
14:00– 14:35	<b>IL – 5</b>	<b>Bojana Žegura</b> (Ljubljana, Slovenia) <i>In vitro</i> 3D cell cultures in genetic toxicology
14:35 – 15:10	<b>IL – 6</b>	<b>Ivica Dimkić</b> (Belgrade, Serbia) A new insight into <i>Bacillus</i> lipopeptides in terms of cytotoxic, genotoxic, and embryotoxic potential in correlation with synthetic pollutants
15:10 – 15:25	<b>OP – 4</b>	<b>Marijana Čurčić</b> (Belgrade, Serbia) The effects of decabrominated diphenylether (BDE-209) and cadmium (Cd) mixture on thiol groups (SH) and copper (Cu) balance in Wistar rat's brain
15:25 – 15:40	<b>OP – 5</b>	<b>Ivan Ožvald</b> (Zagreb, Croatia) Micronucleus cytome assay results in obese patients with body mass index (BMI)≥35 on a 500-kcal-3-week diet controlled in hospital
15:40 – 16:10	<b>Poster viewing and coffee break</b> sponsored by <b>Medic d.o.o</b>	
<b>SESSION 4 YOUNG SCIENTISTS AWARDS</b>		
<b>Chairs:</b> Nevenka Kopjar, Andreja Prevendar Crnić		
16:10 – 16:25	<b>YSL – 1</b>	<b>Antonio Zandona</b> (Zagreb, Croatia) Cytotoxic effects of vitamin B3 derivatives in cultured cells
16:25 – 16:40	<b>YSL – 2</b>	<b>Milan Gavrilović</b> (presenter: <b>Pedja Janačković</b> ) (Belgrade, Serbia) <i>In vitro</i> toxicology screening of <i>Centaurea calcitrapa</i> (Asteraceae) extracts, their phenolic profiles, and bioactivity
16:40 – 16:55	<b>YSL – 3</b>	<b>Martina Štampar</b> (Ljubljana, Slovenia) <i>In vitro</i> 3D cell model for detection of genotoxic effects
16:55 – 17:10	<b>YSL – 4</b>	<b>Renata Biba</b> (Zagreb, Croatia) Differently coated silver nanoparticles cause oxidative stress and induce cellular damage in tobacco ( <i>Nicotiana tabacum</i> ) seedlings
17:10 – 17:25	<b>YSL – 5</b>	<b>Carina Lackmann</b> (Frankfurt am Main, Germany/ Osijek, Croatia) The effects of chronic exposures of four commercial pesticide preparations on multiple levels of biological organisation in earthworm ( <i>Eisenia andrei</i> )
17:25 – 17:40	<b>YSL – 6</b>	<b>Vedran Micek</b> (Zagreb, Croatia) Individual and combined subchronic oral exposure to ochratoxin A and citrinin affect the expression of organic cation transporters in rat kidneys
17:40 – 17:55	<b>YSL – 7</b>	<b>Zuzana Redžović</b> (Zagreb, Croatia) Adenylate energy charge (AEC) as a useful indicator of environmental stress in <i>Synurella ambulans</i> (Müller, 1846) from the hyporheic zone of the Sava River
17:55 – 18:10	<b>YSL – 8</b>	<b>Karla Jagić</b> (Zagreb, Croatia) Polybrominated diphenyl ethers in Croatian house dust and assessment of human exposure

19:30 – 21:30	<b>Poster &amp; beer party sponsored by Carlsberg Croatia</b>	
21:30 – 22:00	<b>Posters will be taken down by presenters</b>	
<b>Tuesday, 5 October 2021 (Day 3)</b>		
<b>SESSION 5 ECOTOXICOLOGY &amp; EXPOSURE ASSESSMENT</b>		
<b>Chairs:</b> Maja Peraica, Bojan Šarkanj		
9:00 – 9:35	<b>IL – 7</b>	<b>Doris Marko</b> (Vienna, Austria) <i>Alternaria</i> toxins in food – an underestimated hazard?
9:35 – 10:10	<b>IL – 8</b>	<b>Maja Šegvić Klarić</b> (Zagreb, Croatia) Aspergilli in damp dwellings – how diverse and dangerous are they?
10:10 – 10:25	<b>OP – 6</b>	<b>Zdenko Franić</b> (Zagreb, Croatia) Toxicity and radiotoxicity of honey and other beehive products
10:15 – 10:30	<b>OP – 7</b>	<b>Marija Kovačević</b> (Osijek, Croatia) Effects of strobilurins (azoxystrobin, pyraclostrobin, and trifloxystrobin) on reproduction and hatching delay in <i>Enchytraeus crypticus</i>
<b>10:45 – 11:15</b>	 <b>Coffee break</b>	
<b>SESSION 6 GENOTOXICOLOGY AND AGING</b>		
<b>Chairs:</b> Andrew Collins, Goran Gajski		
11:15 – 11:50	<b>IL – 9</b>	<b>Vanessa Moraes de Andrade</b> (Santa Catarina, Brazil) Melatonin supplementation over different time periods until aging modulates genotoxic parameters in mice
11:50 – 12:05	<b>OP – 8</b>	<b>Marko Gerić</b> (Zagreb, Croatia) Toxicological assessment of wastewater treatment processes: impact of pressure boat washing
12:05 – 12:20	<b>OP – 9</b>	<b>Gonca Çakmak</b> (Ankara, Turkey) Investigation of genotoxicity in buccal epithelial cells and determination of urinary metal levels of children with exposure to urban and industrial air pollution
12:20 – 12:40	Bronze sponsor presentation	<b>VWR International, LLC</b> <b>Cassandra Rusher</b> HTP-MS solutions from Avantor
<b>12:40 – 14:00</b>	<b>Lunch break</b>	
<b>15:00 – 23:00</b>	<b>Excursion and congress dinner</b>	
<b>Wednesday, 6 October 2021 (Day 4)</b>		
<b>SESSION 7 COMPUTATIONAL TOXICOLOGY</b>		
<b>Chairs:</b> Daniel Mark Lyons, Predrag Putnik		
9:00 – 9:35	<b>IL – 10</b>	<b>Goran Klobučar</b> (Zagreb, Croatia) Toxicity prediction and prioritization of pharmaceuticals in the aquatic ecosystems
9:35 – 10:10	<b>IL – 11</b>	<b>Tin Klanjšček</b> (Zagreb, Croatia) Beyond descriptive modelling – predictive ecotoxicology using dynamic energy budgets

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10:10 – 10:25	<b>OP – 10</b>	<b>Ines Haberle</b> (Zagreb, Croatia) Dynamic Energy Budget theory in (eco)toxicological research
<b>10:25 – 10:55</b>	 <b>Coffee break</b>	
<b>SESSION 8 NANOTOXICOLOGY &amp; MICROPLASTICS</b>		
<b>Chairs:</b> Bojana Žegura, Marko Gerić		
10:55 – 11:30	<b>IL – 12</b>	<b>Daniel Mark Lyons</b> (Rovinj, Croatia) The humble sea urchin in the Nano-cene: the gift that keeps on giving
11:30 – 12:05	<b>IL – 13</b>	<b>Mirta Smodlaka Tanković</b> (Rovinj, Croatia) Microplastics in the marine environment – distribution, availability, and risk assessment
12:05 – 12:20	<b>OP – 11</b>	<b>Ivana Hazdovac</b> (Rovinj, Croatia) The adverse impact of copper nanoparticles and role of copper speciation in the embryogenesis of sea urchin <i>Sphaerechinus granularis</i>
12:20 – 12:35	<b>OP -12</b>	<b>Petra Burić</b> (Pula, Croatia) Interaction of microplastics with silver nanoparticles and cypermethrin and their effect on early embryonal development of the sea urchin <i>Arbacia lixula</i>
12:35 – 13:00	<b>Closing lecture</b>	<b>Ivica Prlić</b> (Zagreb, Croatia) Toxicity of “5G”!?
<b>13:00 – 13:20</b>	<b>Closing ceremony</b>	

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## **ABSTRACTS**

IL-1

## **The comet assay; from nanoparticles to human populations**

Andrew Collins

*Department of Nutrition, University of Oslo, Norway*

The comet assay is now well established as a convenient and reliable method for measuring DNA damage. In its original form, strand breaks were detected, but the inclusion of digestion with a lesion-specific endonuclease has allowed the additional analysis of various base modifications. The measurement of oxidised purines and pyrimidines has been particularly useful in human biomonitoring, reflecting the involvement of oxidative stress in many human disorders, and also in genotoxicology, since many mutagens and carcinogens act via an oxidative process. Recently, light has been shed on the mechanism of action of numerous nanomaterials, using this assay. The COST Action hCOMET was set up to collect comet assay data from as many human trials as possible and to carry out a pooled analysis to define the methodological, demographic, genetic, and exposure variables that determine levels of DNA damage as measured in white blood cells. A secondary aim was to identify and control the experimental factors that affect the performance of the assay and to produce standard operating procedures that we hope will be widely adopted. This should reduce the variability that is a problem when results from different laboratories are compared. While there is an OECD test guideline for the *in vivo* comet assay, a modification to include the enzyme digestion step would be desirable. No such guideline exists for the *in vitro* comet assay, but a proposal for a guideline, including use of enzymes, is in preparation.

KEY WORDS: DNA damage; hCOMET; human biomonitoring; oxidative stress; pooled analysis

IL-2

## Intoxications in clinical settings

Ines Potočnjak<sup>1</sup>, Iva Klobučar<sup>2</sup>, and Vesna Degoricija<sup>3,4</sup>

<sup>1</sup> Institute for Clinical Medical Research and Education, University Hospital Centre Sestre Milosrdnice, Zagreb, Croatia

<sup>2</sup> Department of Cardiology, University Hospital Centre Sestre Milosrdnice, Zagreb, Croatia

<sup>3</sup> University of Zagreb School of Medicine, Zagreb, Croatia

<sup>4</sup> Department of Medicine, University Hospital Centre Sestre Milosrdnice, Zagreb, Croatia

Treating intoxicated patients is a daily challenge in hospital emergency departments (ED). Intoxicated patients often require toxin-specific diagnostic and medical treatment protocol. Our aim was to investigate the demographic characteristics, intentions, toxic agents, and outcomes in acutely intoxicated patients. We performed a prospective, observational study that included acutely intoxicated patients treated in the ED at the University Hospital Centre Sestre Milosrdnice, Zagreb, Croatia, during the years 2001, 2010, and 2015. A total of 1593 patients were included in the study (331 in 2001, 618 in 2010, 644 in 2015). According to gender, men were predominant (55.9, 65.2, and 70.7 %, respectively). Ethanol was the main intoxication substance (40.2, 69.4, and 75.8 % respectively). Anxiolytics were the main agent used in a suicide attempt, and the second most commonly used agent (46.5, 32, and 18.5 % respectively). During the years, there was a decline in suicide attempts (46.2, 22.2, and 17.1 % respectively). A total of 160 acutely intoxicated patients were treated in the Intensive Care Unit, i.e. 67 (20.2 %), 49 (7.9 %), and 44 (6.8 %), respectively. In-hospital mortality was low (0.9 % of all patients in 2001, 0.8 % in 2010, and 0.8 % in 2015). Interestingly, this study had a low mortality rate due to the good safety profile of substances. It is important to know which substances are commonly used in a population in order to apply diagnostic and treatment protocols. However, the challenge are new substances for which there are still no quick diagnostic tests or specific treatments available.

KEY WORDS: acute intoxication; overdose; poisoning; substance abuse; toxicology

IL-3

## The diagnostic needs and possibilities of detecting new psychoactive substances in clinical practice

Mila Lovrić

*Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia*

The term “new psychoactive substance” (NPS) is used for many substances that have rapidly emerged during the past twenty years and are designed to mimic the effects of classic drugs. For most NPS there are limited or no pharmacologic and toxicologic data in humans. Because their structure is frequently different enough from common illicit drugs, they are often not detected on commonly used drug screenings. This presents a challenge in recognizing and treating emergency cases with NPS as a cause. Clinical testing of common illicit drugs is routinely done in two stages: urine drug screening that typically uses immunoassays, followed by a confirmatory assay (gas chromatography-mass spectrometry or liquid chromatography-mass spectrometry). The rapid turnover of NPS drug composition is not suitable for this type of drug testing. Although NPS are derivatives or analogues of common illicit drugs, their structures may differ enough to evade cross-reaction with the immunoassays used to screen for common drugs of abuse. Several studies have evaluated the cross-reactivity of NPS to commonly used immunoassays in clinical and forensic laboratories and have demonstrated that NPS do not cross-react with immunoassays for common drugs of abuse. The prompt analytical identification of a newly introduced NPS is affected by the absence of analytical procedures or certified reference materials to perform the analysis. Clinicians should consider an NPS as a cause when a patient presents physical signs and symptoms that may be similar to drug intoxication. Identifying and confirming NPS in emergency cases poses a unique and almost insurmountable challenge to clinical laboratories.

KEY WORDS: confirmatory assay; cross-reactivity; drugs of abuse; intoxication; screening assay



IL-4

## **Unconventional psychoactive substances – big analytical challenges**

Snežana Đorđević

*National Poisoning Control Centre, Belgrade, Serbia*

*Medical Faculty Military Medical Academy, University of Defence, Belgrade, Serbia*

The number of abuses of controlled and newly synthesized psychoactive substances (NPS) is increasing every year. Analytical confirmation in biological samples can facilitate diagnosis and treatment of patients. It requires modern analytical equipment with mass spectrometric spectrums library, as well as analytical standards of NPS and their metabolites. The aim of this work was to present the clinical picture of patients and provide an analysis of biological samples after ingestion of unconventional psychoactive substances. From 2013, when we first noticed an abuse of newly synthesized cannabinoids (SC) we have treated many patients at the National Poisoning Control Centre (NPCC) for the effects of different types of SCs, tryptamines, DMT from Ayahuasca, GHB, ibogaine, myristicin from nutmeg or atropine, etc. In most cases, acute poisonings were mild or without symptoms. Management was mainly supportive and involved nonspecific detoxification therapy. Differential diagnosis of intoxication in these cases is difficult, especially because the standard urine test for psychoactive substances is usually negative. Toxicological screening of unconventional psychoactive substances requires appropriate sample preparation and using sensitive analytical techniques. We used liquid chromatography with photodiode array (HPLC/PDA) and mass spectrometric (MS) detection as a screening and confirmation of the identity of the toxic substance. There are numerous problems in the detection and identification of unconventional psychoactive substances, but their identification can facilitate the treatment of patients. Modern analytical equipment and well trained analysts are key to solve these problems.

KEY WORDS: acute poisoning; biological samples; liquid chromatography; National Poisoning Control Centre

IL-5

## ***In vitro* 3D cell cultures in genetic toxicology**

Martina Štampar, Metka Filipič, and Bojana Žegura*Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Ljubljana, Slovenia*

Genetic toxicology plays an essential role within hazard identification and risk assessment during the development of drugs as well as chemicals, cosmetic products, food and feed additives, pesticides, and others. Throughout the early stage of drug development, a substance's ability to damage DNA through genotoxic mechanisms must be fully investigated. For the purpose of safety evaluations, hepatic two-dimensional (2D) cell cultures are used, but they have several constraints, including low expression of metabolic enzymes. Therefore, there is high demand to develop physiologically more relevant *in vitro* cell-based systems that can restore this highly complex microenvironment and provide more predictive results for *in vivo* conditions. In this respect, three-dimensional (3D) cell cultures have gained increased interest in drug discovery and tissue engineering as they have improved cell-cell and cell-matrix interactions, preserved complex *in vivo* cell phenotypes and exhibited higher levels of liver-specific functions including metabolic enzymes compared to 2D cell models. Thus, recently tremendous effort has been put into the development of a variety of 3D models, which hold promise for application in drug discovery, cancer cell biology, stem cell research, safety studies and many other cell-based analyses by bridging traditional 2D monolayer cell cultures and whole-animal systems. In the present study, an *in vitro* 3D cell model (cell viability and proliferation) developed from human hepatocellular carcinoma (HepG2) cells was characterised by applying classical genotoxicity endpoints (DNA single and double strand breaks) combined with toxicogenomic analyses (hepatic markers). Acknowledgement to project ARRS (P1-0245, grant MR-MŠtampar) and CA16119 COST Action.

KEY WORDS: alternatives to animal testing; genotoxicity; risk assessment; spheroid; toxicogenomics

IL-6

## **A new insight into *Bacillus* lipopeptides in terms of cytotoxic, genotoxic, and embryotoxic potential in correlation with synthetic pollutants**

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Excessive and uncontrolled use of chemical pesticides has led to an increase in crop yield losses due to the development of pathogen resistance, atypical pest infestations, and pathogen outbreaks. Contrary to this, many *Bacillus* strains exhibit almost all biocontrol and biostimulation mechanisms by producing antimicrobial substances, including non-ribosomally synthesized cyclic lipopeptides. The biocontrol potential of ethyl-acetate extracts (EAE<sub>bsp</sub>) from the *Bacillus* spp. strains, previously described as the most significant antimicrobial, antioxidant, and anti-enzymatic agents, was tested *in vitro* and *in vivo* as an assessment of their cytotoxicity, genotoxicity, and myelotoxicity along with commercially available biopesticides, EAE<sub>cb</sub> (Bacillomix and Ekstrasol) and chemical pesticides, and CPs (Mankogal 80 and Antracol WP-70). Our results showed that the most substantial cytotoxic effect *in vitro* were caused by CPs (starting from 2 µg/mL), while EAE<sub>bsp</sub> resulted in 17.3 times lower toxicity and without DNA damage of treated MRC-5 cells, even at a dose of 150 µg/mL. The EAE<sub>bsp</sub> (20 % higher) and CPs showed a statistically significant higher genotoxicity at 10 times lower concentration. Also, the CPs significantly suppressed neutrophil development (at 25 µg/mL), in contrast to EAE<sub>bsp</sub> without causing neutropenia, inflammation, cardiotoxicity, nor hepatotoxicity in treated zebra embryos even at a dose 40 times higher compared to CPs (inhibited the hatching of all embryos at all of the applied doses) and EAE<sub>cb</sub> which showed *in vivo* toxicity at a very low concentration. These data indicate a significantly better toxicologically-safe profile of EAE<sub>bsp</sub> compared to commercially available and widely used (bio)pesticides in Serbia's agriculture.

KEY WORDS: antimicrobial substances; biopesticides; chemical pesticides; secondary metabolites; zebrafish model

IL-7

## ***Alternaria* toxins in food – an underestimated hazard?**

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*Alternaria* toxins are formed by black moulds of the genus *Alternaria*, which occur ubiquitously and grow under varying temperature and moisture conditions on a large range of substrates including grain and grain-based products, apples, oilseeds, sunflower oil, and tomato products. Although *Alternaria* species are known to generate a spectrum of secondary metabolites, toxicological studies have focused on the commercially available toxins, e.g. alternariol (AOH), its monomethyl ether (AME), tenuazonic acid, and tentoxin. In 2011, the European Food Safety Agency (EFSA) performed the first risk assessment on only these four *Alternaria* toxins due to the limited amount of data. However, no regulation has yet been implemented, thus these potential food contaminants remain so-called “emerging mycotoxins”. The EFSA repeatedly calls for more data on the occurrence and toxicity of metabolites such as AOH and AME as they have *in vitro* genotoxic, mutagenic immunomodulatory, and endocrine disruptive potential. *Alternaria* spp. may also produce perylene quinone derivatives such as alterperyleneol (ALP), altertoxins (ATX) I-III, and stemphyliotoxin III (STTX-III). ATX-II was found to greatly exceed the genotoxicity of AOH. STTX-III was also found to be a potent inducer of DNA damage, indicating a mechanism involving a common functional epoxy moiety of STTX-III and ATX-II that may react directly with nucleic acids, thus forming DNA adducts. This hypothesis was recently confirmed by finding two guanine-ATX-II adducts and one cytosine-ATX-II adduct in cell-free conditions. Although the exact chemical structure of the adducts has not been elucidated, the epoxy-bearing perylene quinones were shown to directly attack DNA. Taken together, *Alternaria* toxins comprise a spectrum of mycotoxins with different molecular targets and therefore with important implications for the food chain.

KEY WORDS: alternariol; altertoxin II; DNA damage; endocrine disruption; perylene quinone

IL-8

## **Aspergilli in damp dwellings – how diverse and dangerous are they?**

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Aspergilli section *Nidulantes* series *Versicolores*, producers of the mycotoxin sterigmatocystin (STC), dominated the fungi isolated from air and dust in flood-affected homes (Gunja) and control houses (Gornji Stupnik) in Croatia. The majority of isolates were able to produce STC and some isolates also produced its derivate 5-methoxysterigmatocystin (5-MET-STC). How can this affect human health? Here we present: a) Aspergilli series *Versicolores* species diversity in air and dust (calmodulin sequence-based methods); b) occurrence of STC and metabolites from the STC biosynthesis pathway in dust (LC-MS/MS); and c) immunomodulatory and genotoxic effects of STC and 5-MET-STC in lungs of male Wistar rats upon intratracheal instillation in concentrations found in dust. Most of the *Versicolores* isolates were from Gunja (91/125). Most isolates belonging to *A. jensenii*, *A. creber*, *A. puulaauensis*, *A. tennesseensis*, *A. venenatus* produced both STC and 5-MET-STC. *A. amoenus*, *A. fructus*, *A. griseoaurantiacus*, *A. pepii* and *A. protuberus* produced STC but not 5-MET-STC, while *A. sydowii* did not produce any of these two toxins. Among the 75 fungal compounds detected in dust, metabolites from the STC pathway including STC, 5-MET-STC, veriscolorin C, averufin and averantin were among the most abundant metabolites, which prevailed in repaired houses in Gunja. The highest concentrations of STC (0.59 µg/g) and 5-MET-STC (7.70 µg/g) in dust were detected in Gunja. In rat lungs, the immunomodulatory effects of STC (0.4 µg/mL) and 5-MET-STC (5.0 µg/mL) were insignificant. The levels of DNA damage were similar although STC was applied at a 10-fold lower dose. The results of combined treatment suggested possible antagonistic interactions. This work was fully supported by the Croatian Science Foundation under the project MycotoxA (IP-09-2014-5982).

KEY WORDS: 5-methoxysterigmatocystin; *Aspergillus* series *Versicolores*; fungal metabolites; genotoxicity; sterigmatocystin

IL-9

## **Melatonin supplementation over different time periods until aging modulates genotoxic parameters in mice**

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The aging process is a multifactorial phenomenon associated with decreased physiological and cellular functions and an increased propensity for various degenerative diseases. Studies on melatonin (N-acetyl-5-methoxytryptamine), a potent antioxidant, are gaining attention, as melatonin production declines with advancing age. Hence, the aim of this study was to evaluate the effects of chronic melatonin consumption on genotoxic and mutagenic parameters of old Swiss mice. Herein, 3-month-old Swiss albino male mice (N=240) were divided into 8 groups and subdivided into 2 experiments: 1<sup>st</sup> (3 groups): natural ageing experiment; 2<sup>nd</sup> (5 groups): animals that started water or melatonin supplementation at different ages (3, 6, 12, and 18 months) until 21 months. After 21 months, the animals from the 2<sup>nd</sup> experiment were euthanized to perform a comet assay, micronucleus test, and western blot analysis. The results demonstrated that melatonin prolonged the life span of the animals. Relative to genomic instability, melatonin was effective in reducing DNA damage caused by aging, presenting antigenotoxic and antimutagenic activities, independently of the initiation age. The group receiving melatonin for 18 months had high levels of APE1 and OGG1 repair enzymes. Conclusively, melatonin presents an efficient antioxidant mechanism aiding modulating genetic and physiological alterations due to aging.

KEY WORDS: antioxidants; comet assay; genome instability; micronuclei; oxidative stress

IL-10

## Toxicity prediction and prioritization of pharmaceuticals in aquatic ecosystems

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Thousands of chemicals enter the aquatic environment contributing to a complex chemical eco-exposome. In the last few decades, particular attention has been given to contaminants of emerging environmental concern (CEEC): pharmaceuticals, personal care products, illicit drugs, pesticides, and industrial chemicals with poorly characterized hazards and increasing input. Many CEECs have a specific mode of action and are environmentally persistent. However, current risk assessments of freshwater pollution using traditional ecological and chemical analyses remain incomplete, fragmented, and often contradictory while exhibiting low potential for prioritizing chemicals of concern. Recent progress from target-based analytical approaches to the broad spectrum chemical screening of hundreds of contaminants with highly sensitive analytical techniques and development of high throughput *in vitro* toxicity testing as well as *in silico* toxicology methods has enabled integration of chemical and biological data for comprehensive environmental assessments of complex mixtures in the aquatic environments. We implemented such an approach in the analysis of water, sediment, and blood plasma of fish from the Sava River, Croatia, and the results pointed to the rising threat of pharmaceuticals to freshwater ecosystems. Antibiotics, allergy/cold medications, and analgesics had the highest plasma (and sediment) concentrations. The applied prioritization methods indicated stimulants (nicotine, cotinine) and allergy/cold medications (prednisolone, dexamethasone) as having the highest potential biological impact on fish. The Fish plasma model also prioritized psychoactive substances (hallucinogens/stimulants and opioids) and psychotropic substances in the cannabinoids category. Therefore, although present at low concentrations, their adverse effect on aquatic communities, due to chronic exposure and additive effects, should be taken with great caution.

KEY WORDS: allergy/cold medications; Fish plasma model; freshwater pollution; illicit drugs

IL-11

## Beyond descriptive modelling – predictive ecotoxicology using dynamic energy budgets

Tin Klanjšček

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Relating exposure to processes of individual survival, growth, and reproduction is key to understanding the ecotoxicological effects of toxicant exposure. Developments in the Dynamic Energy Budget (DEB) theory over the past few decades have shown that the processes can be captured by a relatively small set of equations, state variables, and parameters applicable to all known organisms. These developments were largely driven by problems in ecotoxicology, and resulted in a comprehensive, general theory that enables the inclusion of multiple stressors, streamlines assimilation of disparate data, fosters cross-species comparisons, provides plausible explanations for confusing observations, and has considerable predictive powers. After a short introduction to the DEB theory, the lecture will demonstrate explanatory and predictive powers of the DEB approach using problems I have been working on. We will briefly discuss how (i) DEB can be used to differentiate between nano- and ionic- toxicity in CdSe quantum dots and predict the toxicity of exposures order of magnitude greater than those used to fit the model, (ii) DEB-based *in-silico* testing can identify ecological mechanisms leading to hormesis during exposure of soybeans to nano-cerium despite clear negative physiological effects on the plant, and (iii) demonstrate the ability of DEB models to set hypotheses on the effects of cross-generational transfer of polychlorinated biphenyls in whales. Finally, I will suggest how the DEB theory could be used to assimilate data from omics to foster further development of the theory, as well as our understanding of relationships between pollution and ecosystem-wide effects.

KEY WORDS: bioenergetics; data mining; mechanistic modelling; no-effect concentration; reactive oxygen species

IL-12

## The humble sea urchin in the Nano-cene: the gift that keeps on giving

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The sea urchin has found widespread use as a model in fields ranging from biology to immunology. For example, not only has it provided fundamental insight into developmental biology since the 19<sup>th</sup> Century but aspects of the functioning of its immune system have been noted to be analogous to that of humans. Urchins had also found an important niche in toxicology research for indicating the broad-spectrum presence of pollutants in aquatic environments. Over recent decades research on the impact of a broad range of chemicals and materials of anthropogenic origin has increasingly turned to cell lines or cultures, including their new incarnation as 3D cultures, which have gradually come to the fore. As such approaches have begun to supersede testing at the whole organism level it has become tempting to overlook reasons for investigating the toxicological impacts of chemicals at the organism level. While testing chemicals on cell cultures has its advantages, not least of which the ability to achieve relatively good reproducibility and high-throughput, a vast wealth of information can be lost should testing at higher levels of biological organisation be ignored. Indeed, adverse outcomes at the cellular level do not always reflect in similar outcomes at the whole organism level, particularly as data from cells do not take into consideration all of the diverse metabolic pathways and defence mechanisms in the organism *in toto*. Addressing this, results deriving from a wide range of endpoints in urchin bioassays, including embryotoxic, spermiotoxic and cytotoxic outcomes that provide important, unique and complementary data to those that may be derived from cell-based assays are highlighted.

KEY WORDS: adverse outcome pathways; particles; pesticides; whole organism testing

IL-13

## Microplastics in the marine environment – distribution, availability, and risk assessment

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Over the past few decades, microscopic plastic litter termed ‘microplastics’ (plastic particles or fibres < 5 mm in size) have been accumulating in seas and oceans becoming a growing global concern. Their increased widespread occurrence corresponds to the growth in manufacturing plastic materials and includes sources such as cosmetic exfoliates, polyester fibres from fabrics, polyethylene fragments from plastic bags, and other larger plastic items. The ubiquity microplastics in marine environments is shown by its presence throughout the world’s oceans and seas from the Arctic to the Antarctic and in samples taken from the sea surface and shoreline to the seafloor. The amount and distribution of microplastics in the sea depends on several factors, from the proximity of urban centres and the coast to the wind and sea currents. The Adriatic is a dynamic ecosystem with numerous and steep ecological gradients, but also a relatively long seawater retention time, which makes it a particularly vulnerable area for negative impact, with concentrations of microplastics up to 600,000 particles/km<sup>2</sup> noted. The omnipresence and size of microplastics renders particle ingestion by marine organisms unavoidable and can cause sub-lethal physical injury, accumulation of chemical contaminants (including both additives and reversibly sorbed waterborne pollutants) within the body, and the toxicity to ultimately be transferred to the upper trophic level (including humans). Microplastics in natural systems are evident worldwide and therefore call for an in-depth understanding of the implications of these pollutants studying sources, releases, uptake, and toxicity of microplastics to perform environmental risk, as well as human health risk, assessments.

KEY WORDS: Adriatic Sea; ingestion; pollutant; toxicity; trophic transfer

Closing lecture

## Toxicity of “5G”!?

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Modern physics and biogenetics research into radiation covering the electromagnetic (EM) spectrum frequencies used in information and communications technology (ICT) are mainly focused on delivered energy interactions with living matter using animal models and, as of recently, artificial intelligence computational modelling and simulations. Results are not easily transposed to a real absorption human model. One of the physical reasons for this is the fact that ICT frequencies lie well below visible light frequencies which are natural to humans and to which they are constantly exposed, whilst ICT frequencies are not. This, additional, non-natural global non-stop energy exposure to non-ionising radiation has been the source of much public controversy. The terminology used while explaining the possible harmful effects of exposures is mainly bombastic, scientifically inadequate, and provocative; for example, “Is EM radiation ‘toxic’?” Some countries are performing large scale EM research under the “toxicity” paradigm in order to harmonise independent research in the ICT field. Societal factors are often not included. Among others, all these were reasons why our research group implemented an interdisciplinary research project covering physical measuring (dose delivered from EM source) and biological interaction protocols. These were incorporated into a computational modelling of real human exposure situations and simulating expected real exposure situations and social behaviour according to the use of ICT by school children in Croatia. Preliminary results are presented with a focus on encouraging a broad academic and public discussion. A necessary short overview of the “5G” industrial revolution and its toxicity is also presented.

KEY WORDS: electromagnetic radiation; EM toxicity; exposure; ICT; social behaviour

OP-1

## Toxicological aspects of the increased use of disinfectants during the COVID-19 pandemic in Croatia

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Prevention strategies for COVID-19 include regular use of surface disinfectants and hand sanitisers. We analysed records of suspected/symptomatic poisonings with disinfectants and sanitisers from the Croatian Poison Control Centre during the first 6 months of 2019, 2020, and 2021 to determine the effect of the COVID-19 pandemic in this regard. Cases of exposure to disinfectants doubled in the first 6 months of 2020 in comparison with 2019 (41 vs 21 cases, respectively), but decreased in the first 6 months of 2021 (14 cases). Compared to 2019, exposure to sanitisers increased about 9 times in 2020 (5 vs 46 cases, respectively) and 2021 (5 vs 44 cases, respectively). The most common ingredients of the disinfectants and sanitisers involved in poisoning incidents were hypochlorite/glutaraldehyde and ethanol/isopropanol, respectively. In 2020 and 2021, exposure to disinfectants was recorded mostly in adults (55 %), as unintentional exposure (80 %) via ingestion or inhalation (87 %), with mild symptoms in 45 % (gastrointestinal or respiratory irritation) and severe symptoms in 2% (gastrointestinal corrosion) of cases. Exposure to sanitisers was recorded mostly in toddlers and preschool children (69 %), as unintentional exposure (88 %) via ingestion or eye contact (97 %), and mild symptoms developed in 24 % of cases (gastrointestinal or eye irritation, somnolence, and excitation). In conclusion, the increased availability and use of disinfectants and sanitisers in Croatia during the course of the COVID-19 pandemic resulted in a significant increase of potentially toxic exposures. Measures to avoid the toxic effects of biocides should be included in the epidemiological recommendations for the prevention of COVID-19.

KEY WORDS: corrosive injury; ethanol; hand sanitisers; hypochlorite; preschool children

OP-2

## Plant poisoning reported to the Croatian Poison Control Centre during a ten-year period (2010-2019)

Zrinka Franić<sup>1,2</sup>, Željka Babić<sup>1,2</sup>, Rajka Turk<sup>1,2</sup>, and Jelena Macan<sup>1,2</sup><sup>1</sup> *Occupational Health and Environmental Medicine Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia*<sup>2</sup> *Croatian Poison Control Centre, Institute for Medical Research and Occupational Health, Zagreb, Croatia*

Published data on human plant poisonings in Croatia are scarce. Therefore, a retrospective review of plant exposures during a ten-year period (from January 1, 2010 to December 31, 2019) from telephone consultations with the Croatian Poison Control Centre (CPCC) was performed with the aim of gaining a better understanding of the general characteristics of the poisoned patients. We identified a total of 803 cases; the median age was 2 years (2 months-83 years), 70.8 % being preschool children, 52.7 % male. Ingestion (90.9 %) and dermal exposure (3.86 %) accounted for most of the cases. As for plant origin, 35.2 % were house plants (mostly *Alocasia odora*, *Dieffenbachia* and *Ficus* sp.), 33.9 % garden plants (mostly *Nerium oleander* and *Prunus laurocerasus*) and 15.4 % wild plants (mostly *Arum maculatum* and *Colchicum autumnale*). In general, *Alocasia odora* (15.8 %) and *Nerium oleander* (10.1 %) were most commonly involved. In 19.5 % of cases there was exposure to berries, seeds or fruits. The majority of exposures were unintentional (95.7 %), with an additional 13 suicide attempts (mostly *Nerium oleander*) and 15 abuse cases (mostly *Hyoscyamus* sp.). Mild symptoms developed in 39.3 % and severe symptoms developed in 3.2 % of patients. There were three fatal outcomes following ingestion of *Colchicum autumnale*. Cases were reported mostly during summer (39.2 %). In conclusion, plant exposures were typically unintentional ingestions of house or garden plants in preschool children, although serious morbidity occurred with exposure to wild plants. The need for education on how to prevent such unnecessary incidents is indicated.

KEY WORDS: *Alocasia odora*; *Colchicum autumnale*; *Nerium oleander*; poison control; poisonous plants



OP-3

## **Real-time (on-line) chemical characterization of thermal aerosols by super secondary electrospray ionization coupled with high-resolution mass spectrometry (Super SESI-HRMS)**

Tanja Živković Semren, Shoib Majeed, Maria Fatarova, Csaba Laszlo, Claudius Pak, Sandro Steiner, Arkadiusz Kuczaj, Anatoly Mazurov, Manuel Peitsch, Nikolai Ivanov, Julia Hoeng, and Philippe Alexandre Guy

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Thermally produced aerosols have been chemically characterized by both off-line and on-line approaches. We used a programmable dual syringe pump (PDSP) connected to a super secondary electrospray ionization (SESI) system interfaced with a Q Exactive HF mass spectrometer (MS) to detect the main constituents of thermally generated aerosols in real time. The benefits of Super SESI–HRMS for detecting drug aerosolisation from variably prepared in-house formulations with anatabine, azithromycin, chloroquine, favipiravir, and hydroxychloroquine were evaluated. Additionally, various commercially available vaping products with main constituents such as caffeine, cyanocobalamin (B12), and melatonin were evaluated similarly. The presence of these compounds in aerosol was confirmed by exact mass measurements from protonated and/or deprotonated species as well as their respective tandem mass spectra (MS<sup>2</sup>). To confirm the Super SESI-HRMS data and obtain information on the transfer rate, the generated aerosol samples were trapped on a Cambridge filter pad and extracted with water or ethanol. These extracts were analysed by liquid chromatography (LC) coupled to HRMS, and the target compounds were quantified using calibration curves. The LC-HRMS data confirmed the Super SESI online data. Most of the compounds could be aerosolized, except azithromycin and vitamin B12. The vitamin B12 data showed that the conditions employed for thermal aerosol generation strongly impacted aerosolization efficiency, and the breakdown products suggested that vitamin B12 underwent thermal degradation. This fast coupling technique shows a strong potential for helping us rapidly investigate the possibility of aerosolising chemicals and derive information on their liquid-to-aerosol transfer rate.

**KEY WORDS:** accurate mass; aerosolisation; aerosol trapping; analytical chemistry; compound identification

OP-4

## The effects of decabrominated diphenylether (BDE-209) and cadmium (Cd) mixture on thiol groups (SH) and copper (Cu) balance in Wistar rat's brain

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The growth and development of the nervous system are targets for chemicals BDE-209 and Cd. However, the toxicity mechanisms of these compounds have not been fully examined, nor is there data on the mechanisms of their *in vivo* interactions. This study examined the influence of a BDE-209 and Cd mixture on –SH groups and Cu balance in the brain of male Wistar rats. Animals weighing 200-240 g were exposed by gavage to doses of BDE-209 (1000, 2000, or 4000 BDE-209/kg bw/day) and Cd (2.5, 7.5, and 15 mg/kg bw/day) (as a mixture or individually) during 28 days. After preparation, the samples of brain tissue homogenates were spectrophotometrically evaluated for the content of thiol groups (SH). Cu level was measured by atomic absorption after brain tissue mineralisation with nitric and perchloric acid (8:1). BDE-209 alone did not influence SH groups significantly. The same doses of Cd given alongside BDE-209 induced an increase in the concentration of SH groups in the brain in relation to BDE-209 alone. The highest level of Cu in brain was induced by a medium dose of Cd. A decrease in SH groups could indicate that BDE-209 decreases the production of the metallothionein initially induced by Cd, with a simultaneous decrease in Cu levels in brain caused by the BDE-209/Cd mixture. It could be assumed that the balance between SH groups and Cu, i.e. the simultaneous decrease of both parameters, mediates the toxicity of the BDE-209/Cd mixture, however further experiments would be necessary to reveal the exact mechanisms of their toxicity.

KEY WORDS: copper and thiol group balance; nervous system; rats

OP-5

## **Micronucleus cytome assay results in obese patients with body mass index (BMI) $\geq 35$ on a 500-kcal-3-week diet controlled in hospital**

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As the country with the highest obesity prevalence in the EU and 5<sup>th</sup> in the EU according to adult obesity percentage, Croatia requires a systematic and serious approach, especially among the (severely) obese. Previously, and for the first time, our use of a short-term restricted diet in controlled conditions gave good results and kept the interest of the severely obese for life style and dietary habits change with significant improvements in primary DNA damage and oxidative stress measured by the comet assay, and anthropometric and biochemical parameters related to DNA damage and obesity (BMI reduction, 10-35 % excessive weight loss, decrease in TSH, fT3, fT4, basal metabolism rate, waist-hip ratio, visceral fat level, body fat mass, percent body fat, skeletal muscle mass, energy intake, significant decrease in insulin, glucose, homeostatic model assessment for insulin resistance, urea, cholesterol, HDL and LDL levels). We wanted to ascertain whether a similar effect on genomic stability can be seen in permanent DNA damage measured with cytochalasin B-blocked micronucleus cytome assay. Whole blood of 22 volunteers with BMI  $\geq 35$  was analysed before and at the end of the 3-week medically-controlled 500 kcal diet (50-60 % complex carbohydrates, 20-25 % protein and 25-30 % fat, carbo-glycaemic index attention). Results demonstrated correlation with oxidatively damaged DNA, significant decrease in micronucleus and nucleoplasmic bridge frequency, nuclear division index and apoptosis. There were correlations with vitamins B<sub>12</sub> and D. This approach can lead to significant health improvements and permanent genomic stability in the severely obese.

KEY WORDS: apoptosis; calorie restricted diet; comet assay; DNA damage; obesity

OP-6

## Toxicity and radiotoxicity of honey and other beehive products

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There are numerous toxic and radiotoxic substances found in honey and other beehive products that may pose health risks for beekeepers as well as for humans who consume them. The aim of this work was to present an overview of the main toxic contaminants found in beehive products and the pathways of their introduction. Honeybees forage food within their flight range, which extends for about 3.2 km around the beehive. By foraging nectar and pollen from blooming plants, bees also unintentionally collect various toxic environmental contaminants. However, many substances toxic to humans have no effect on bees. Environmental beehive contaminants are heavy metals, organic pollutants, pesticides, pathogenic bacteria, radioactive isotopes etc. Honey can be contaminated by natural (e.g. radium, potassium-40) and anthropogenic radioactive isotopes (e.g. radiocaesium) which can pose radiological health risks. Likewise, if bees obtain nectar from flowers of certain toxic plants (e.g., *Rhododendron*, *Nerium oleander*), the resulting honey, called “mad honey”, can be toxic or psychoactive. Furthermore, beekeepers who do not follow good apiculture practice can cause chemical contamination of beehives and beehive products with acaricides, chemical repellents, organic acids and other chemicals. Additionally, antibiotics, where used for prevention of bee diseases, may be toxic to humans as well as contribute to antimicrobial resistance. In conclusion, to minimise potential health risks, for beekeepers it is recommended to follow good apiculture practice and for consumers to buy attested beehive products, advisably from beehives situated far from highways and industry zones, and most advisably, to buy certified ecological honey.

KEY WORDS: beehive contamination; beekeeping; health risk; radiotoxic isotopes; toxic substances

OP-7

## Effects of strobilurins (azoxystrobin, pyraclostrobin, and trifloxystrobin) on reproduction and hatching delay in *Enchytraeus crypticus*

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Strobilurins, a commonly used class of fungicides, are known to impact the embryonic development of aquatic organisms and show toxicity to different soil organisms. Standard tests for soil invertebrates are usually limited to the assessment of endpoints like survival and number of juveniles, while hatching and embryotoxicity tests have emerged recently. Besides earthworms, enchytraeids are the most commonly used bioindicators of soil contamination. Their size and short life cycle makes them suitable for this type of research. The main aim of the study was to assess the impact of strobilurins on the reproduction and hatching delay of enchytraeid *Enchytraeus crypticus*. Standardised OECD reproduction tests with azoxystrobin (AS), pyraclostrobin (PS) and trifloxystrobin (TS) were conducted. A decreased number of juveniles and an increased number of unhatched cocoons in strobilurin treatments were recorded. TS was the most toxic in terms of reproduction ( $EC_{50}$  0.04 mg a.i./kg soil), followed by PS ( $EC_{50}$  2.22 mg a.i./kg soil) and AZ ( $EC_{50}$  57.45 mg a.i./kg soil). The use of synchronised cocoons enabled monitoring of hatching dynamics. After a standard 11-day exposure, more than 90 % of cocoons were hatched in the control, while those values were lower in treated soil. However, as hatching was monitored after that period (up to the 19<sup>th</sup> day) the percentage of hatched cocoons significantly increased with the time of exposure. Consequently,  $EC_{50}$  values also changed. These results show that supplementary endpoints such as hatching success included in standard tests could increase test sensitivity and accuracy.

KEY WORDS: bioindicators of soil contamination; ecotoxicology; enchytraeids; OECD reproduction test; pesticides

OP-8

## Toxicological assessment of wastewater treatment processes: impact of pressure boat washing

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Submerged boat surfaces are susceptible to fouling by biological material which reduces performance and increases shipping costs. One of the most common approaches to prevent fouling is the application of metal-based antifouling paints. Periodically, these paints are washed off, generating wastewater that might impact the marine environment and human health. The aim of this study was to perform a chemical analysis of such wastewater, determine its toxicological profile, and provide an appropriate water purification method. The wastewater contained a mixture of heavy metals: Cu (48 mg/L), Zn (31.2 mg/L), Fe (10.3 mg/L), Cr (1.43 mg/L), V (0.92 mg/L) and Pb (0.87 mg/L), with most of them at concentrations exceeding permitted levels. Using *Salmonella typhimurium* strains TA98 and 100, no mutagenic potential of the wastewater was found. However, studies on human blood cells indicated cytotoxicity (~35 % decrease in cell viability) and genotoxicity based on chromosome aberrations (higher frequency of total aberrations), micronucleus test (higher frequency of micronuclei), and the comet assay (higher tail intensity). A full-scale treatment plant was installed in the vicinity of a dry dock collecting released wastewater, enabling electrochemical and ozonation purification processes that reduced concentrations of water contaminants by up to 99.9%. After performing toxicological assessments on purified samples, their profiles did not differ from negative control samples. To conclude, we present an efficient way to improve waste management at marinas and minimise the potential negative impact on marine ecosystems.

KEY WORDS: Ames test; comet assay; heavy metals; micronucleus test; wastewater purification

OP-9

## Investigation of genotoxicity in buccal epithelial cells and determination of urinary metal levels of children with exposure to urban and industrial air pollution

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Environmental exposure to air pollution has been shown to be related with adverse health effects such as respiratory and cardiovascular system diseases and cancer. The chemical composition, temporal and spatial variations, and pollution sources are among the main determinants of the toxicity. Since children are assumed to be the most susceptible group of a population, increasing efforts are being made to design children-based environmental studies. Around 150 children, living in the traffic dense city center of Kütahya (KC) as an urban site, and the Tunçbilek Region (TC), near the Tunçbilek and Seyitömer Thermal Power Plants, as a rural/industrial site were selected for a biomonitoring study to evaluate genotoxicity with buccal epithelial cell (BEC) micronucleus (MN) assay and analyse urinary metal levels (U-ML). The frequencies of cytome parameters were evaluated in 2000 cells for each child, while 17 U-ML was determined in urine samples of children by an inductively coupled plasma mass spectrometer. The MN frequencies were similar between the two regions ( $p > 0.05$ ). Children from one of the schools located in TC had the highest frequencies of cytome parameters and as well as increased levels of individual ozone and sulphur dioxide ( $p < 0.05$ ). According to the U-ML, arsenic and nickel levels in children indicated residence in the TC rural/industrial region, whereas vanadium, manganese, iron, and lead were noted in those from the traffic intense city centre. The information gained from these studies could be crucial in the hazard identification step of the risk assessment approach for regulatory actions.

KEY WORDS: air pollutant; biomonitoring; genotoxicity; toxic elements

OP-10

## **Dynamic Energy Budget theory in (eco)toxicological research**

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The ability to understand and generalise effects of exposure from experiments is essential for predicting toxic effects in untested conditions and/or natural environments. Chemical effects initiate molecular-level responses that ultimately – through effects on individual growth, reproduction and survival – have ecosystem-level effects. Transcending these scales of organisation requires modelling, in particular of individual-level effects because individuals are units of natural selection and fundamental elements of populations and ecosystems, allowing translation of effects to higher organisational levels. The Dynamic Energy Budget (DEB) theory offers a general, comprehensive, tested framework for capturing toxic effects of exposure on individual physiological processes. A complete DEB ecotoxicological (DEBtox) model consists of three main modules: (i) the DEB model describing the acquisition and utilisation of energy as a function of environmental conditions, (ii) a toxicokinetic module describing the assimilation and distribution of the toxicant, and (iii) a toxicodynamic module describing the effects of the toxicant based on toxicokinetics. The simplest toxicokinetic model considers the organism as a single compartment, but much more elaborate schemes are possible. The toxicodynamic module applies a dose-dependent stress function to a metabolic flux by affecting one or more DEB parameters. As metabolic fluxes fully describe the oncology (and, therefore, the ecological role) of an individual, the resulting DEBtox fully captures the ecotoxic effects of exposure. Such a description is also general because DEB models have been created for more than 2200 species. We present the general concepts of the DEBtox and of the underlying DEB theory, and give an overview of existing modelling toolboxes with examples.

KEY WORDS: DEBtox model; individual; metabolic parameters; modelling toolboxes; stress function

OP-11

## The adverse impact of copper nanoparticles and role of copper speciation in the embryogenesis of sea urchin *Sphaerechinus granularis*

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Harbours have long been known as pollution hotspots due to a range of anthropogenic inputs such as, for example, tributyltin which was historically used in ship paints to prevent biofouling but also resulted in toxicity to non-target organisms. Recently the use of copper, as copper nanoparticles (CuNP), in anti-fouling formulations has been gaining popularity. However, there is relatively little data available on the behaviour of such particles in marine waters and how they impact on biota. We report herein on the behaviour and fate of CuNP in marine waters and their impact on embryogenesis of sea urchin *Sphaerechinus granularis*. The colloidal stability of CuNP in seawater (S-38), and hence potential residence time in the water column, was investigated as a function of various types of natural organic matter (NOM). NOM discouraged CuNP aggregation over a wide concentration range, e.g. humic acid (0.1-10 mg/L) and alginate (1-10 mg/L). Further, NOM decreased Cu<sup>+</sup> ion release from nanoparticles, with Cu<sup>+</sup> concentrations increasing 36 % for uncoated nanoparticles compared to 18 % in the presence of NOM. Exposure of *S. granularis* zygotes to CuNP nanoparticles showed distinctly different outcomes after 72 h. Larval malformations and delayed development increased with increasing concentrations, with CuO showing the lowest EC<sub>50</sub> (concentration inducing 50 % abnormal development) at 51.47±14.74 µg/L, while Cu<sub>2</sub>O and Cu<sup>0</sup> were less toxic with EC<sub>50</sub> values of 106.73±26.28 and 193.86±45.39 µg/L, respectively. These results contribute to a better understanding of the CuNP persistence in harbour waters and how subsequent copper speciation may modulate toxicity to biota.

KEY WORDS: biofouling; Cu<sup>+</sup> ion; dissolution kinetics; developmental defect; marine waters

OP-12

## Interaction of microplastics with silver nanoparticles and cypermethrin and their effect on early embryonal development of the sea urchin *Arbacia lixula*

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Microplastics represent one of the most significant environmental issues in today's world. In addition to mechanical damage, microplastics can have deleterious effects on organisms due to the leaching of residual monomers, oligomers, and additives but also as vectors for other pollutants that may be simultaneously present in urban wastewater streams. Thus, the effects of two microplastics, polystyrene (PS; 10 µm) and polymethylmethacrylate (PMMA; 10 µm) with either adsorbed bactericidal silver nanoparticles (AgNPs; 40 nm) or the pyrethroid insecticide cypermethrin (Cyp), on fertilisation and embryogenesis in the sea urchin *Arbacia lixula* were investigated. Physicochemical analysis indicated high concentrations of AgNPs and Cyp adsorbed on the microplastics. Adsorbed AgNPs (10 and 50 µg/L) or Cyp (10 and 1000 µg/L) on either of the two microplastics (50 mg/L) did not significantly affect either the embryonal development of the treated zygotes or the fertilisation ability of pre-treated sperm. However, a modest reduction in the quality of the offspring of pre-treated sperm was noted, suggesting transmissible damage to the embryos. The unexpectedly low toxicity of AgNPs and Cyp may derive from the sequestration of these toxicants, including silver ions released from the AgNPs, by the polymer microparticles. Interestingly, while ingestion of virgin nanoparticles was noted, for embryos co-exposed to AgNPs and microparticles no such ingestion was observed. While antagonistic effects may be ascribed to reduced toxicity of surface adsorbed AgNPs, the cause of reduced ingestion is less clear and may be related to mechanisms that suppress feeding rather than the ability to differentiate between microplastics and natural food.

KEY WORDS: adsorption; insecticide; polymethyl methacrylate; polystyrene; urban wastewater

YSL-1

## Cytotoxic effects of vitamin B3 derivatives in cultured cells

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Vitamin B3 derivatives are today investigated for their antioxidative, anticancer, anti-angiogenic activities as well as cholinesterase inhibitors for potential use in treatment of neurodegenerative disorders such as Alzheimer's disease. They share a structural similarity with nicotinamide, which participates in vital and important physiological processes in the organism as the nicotinamide adenine dinucleotide (NAD). With that fact in mind, we tested a series of nine vitamin B3 derivatives, previously described as inhibitors of cholinesterases, with the aim to determine their influence on cell viability and homeostasis, membrane integrity and oxidative status. All research was conducted by standard methods: activity of mitochondrial succinate dehydrogenase by the tetrazolium salt MTS, lactate dehydrogenase leakage by fluorescent resazurin and induction of reactive oxygen species and level of glutathione by fluorescent dyes DCFDA and mCB, respectively. As our results showed, four out of nine analogues were cytotoxic and displayed a time-dependent toxicity mechanism on neural-like and kidney cells, indicating cell death by apoptosis. Only one compound triggered significant LDH release at higher concentrations, indicating a necrotic-like impact. More detailed analysis also revealed a significant disturbance in cell homeostasis. Derivatives influenced cell GSH level and the expression of the oxygen sensitive hypoxia-inducible transcription factor-1 $\alpha$ . Furthermore, the tested analogues modulated nicotinamide-linked pathways such as AMPK-, Akt- and mTOR signalling with an important role in multiple cellular functions by standard western blot technique. Although the concentrations inducing the observed toxic effects were far greater than predicted in medical practice, they should not be neglected in the future design of new nicotinamide structures as potential drugs. This research was supported by the Croatian Science Foundation UIP-2017-05-7260, Croatian-Slovenian Bilateral project BI-HR/20-21 and Slovenian Research Agency P3-0043 in J7-8276.

KEY WORDS: AD-drugs; cells; ChE-inhibitors; cytotoxicity; nicotinamide

YSL-2

## *In vitro* toxicology screening of *Centaurea calcitrapa* (Asteraceae) extracts, their phenolic profiles, and bioactivity

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The genus *Centaurea* is one of the largest genera in the Asteraceae family comprising approximately 400-700 species. Ethnobotanical and ethnopharmacological studies have shown that many *Centaurea* species have been used in folk medicine for treatment of various diseases (such as diabetes, to treat fever, high blood pressure diseases, etc.). In the search for potentially bioactive natural plant compounds, special attention needs to be undertaken to identify their toxic or potentially toxic effects. In this regard, a study on the cytotoxicity of *C. calcitrapa* extracts on MRC-5 cells was performed. The main objective of the present study was to determine and evaluate the bioactivity, phenolic profiles and cytotoxicity of methanol (MeOH), 70 % ethanol (EtOH), ethyl-acetate (EtOAc), 50 % acetone (Me<sub>2</sub>CO), and dichloromethane:methanol (DCM:MeOH, 1:1) *C. calcitrapa* leaf extracts. The bioactivity against different human pathogenic and phytopathogenic strains was tested using well diffusion method and minimum inhibitory concentration (MIC) assay. A total of 55 phenolic compounds were identified: 30 phenolic acids and their derivatives and 25 flavonoid glycosides and aglycones. The highest cytotoxicity was recorded for EtOAc and Me<sub>2</sub>CO extracts with the lowest relative and absolute IC<sub>50</sub> values between 88 and 102  $\mu$ g/mL, while the EtOH extract was the least toxic with a predicted relative IC<sub>50</sub> value of 1578  $\mu$ g/mL. Our results indicate that all of the tested extracts, at a non-toxic concentration and simultaneously harmful for human pathogenic and phytopathogenic strains (methicillin-resistant *Staphylococcus aureus*; *Xanthomonas campestris* pv. *campestris*, *Erwinia amylovora* and *Pseudomonas syringae* pv. *syringae*), can be considered natural sources of novel bioactive phytochemicals.

KEY WORDS: antimicrobial and biocontrol agents; leaf extracts; liquid chromatography–mass spectrometry; MRC-5 cell line; phytochemicals



YSL-3

## ***In vitro* 3D cell model for detection of genotoxic effects**

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For the purpose of safety evaluations, there is high demand for the development of physiologically more relevant *in vitro* cell-based systems that can provide more predictive results for human exposure. In this study, we developed an *in vitro* HepG2 3D cell model using the forced floating method. The spheroids were cultured under static conditions for 3 days and validated for genotoxicity assessment by testing the genotoxic activity of the indirect acting compound, benzo(a) pyrene (BaP). Spheroids were exposed to BaP (0.1, 1, 10 and 20  $\mu\text{mol/L}$ ) for 24 h. The influence of BaP on spheroid growth was monitored by planimetry, while live/dead cells were determined by FDA/PI staining and evaluated by confocal microscopy. The results revealed that BaP decreased the spheroid surface area to  $\geq 10 \mu\text{mol/L}$  and affected cell viability at 20  $\mu\text{mol/L}$ . The effect of BaP on cell proliferation and cell cycle alterations was assessed by flow cytometry, and its genotoxic activity was determined with the comet and  $\gamma\text{-H2AX}$  assays. At the applied conditions, BaP (10  $\mu\text{mol/L}$ ) reduced the number of Ki67 positive cells and arrested HepG2 cells in the S phase of the cell cycle. BaP induced the formation of DNA single (comet) and double ( $\gamma\text{-H2AX}$ ) strand breaks. On the mRNA level, BaP deregulated the expression of phase I and II enzymes and DNA damage responsive genes. In summary, the newly developed HepG2 3D cell model provides a suitable model for genotoxicity assessment due to its improved metabolic capacity. Acknowledgement to the Slovenian Research Agency, Program P1-0245 and Grant to MŠ and COST Action 16119.

KEY WORDS: cell cycle; cell proliferation; comet assay; gene expression; HepG2 3D cell model

YSL-4

## **Differently coated silver nanoparticles cause oxidative stress and induce cellular damage in tobacco (*Nicotiana tabacum*) seedlings**

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The novel characteristics of silver nanoparticles (AgNPs) have broadened their application in various industrial sectors, increasing the chance of their release into the environment and posing a risk for human health. Although AgNPs tend to oxidize and aggregate in aqueous media, they may be stabilized through surface coatings that can change their physicochemical properties and affect their toxicity. This study compares effects of two differently coated AgNPs [polyvinylpyrrolidone (AgNP-PVP) and cetyltrimethylammonium bromide (AgNP-CTAB)] and silver nitrate ( $\text{AgNO}_3$ ) on tobacco (*Nicotiana tabacum*) seedling oxidative stress parameters. Proline, glutathione (GSH) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) levels were spectrophotometrically measured in seedlings treated with AgNPs or  $\text{AgNO}_3$  (25, 50 and 100  $\mu\text{mol/L}$ ). Cellular effects were analysed in roots and tobacco seedlings leaves treated with AgNPs or  $\text{AgNO}_3$  (100  $\mu\text{mol/L}$ ) by *in situ* detection of reactive oxygen species (ROS) using dihydroethidium for superoxide radical ( $\text{O}_2^{\cdot -}$ ) and 2',7'-dichlorodihydrofluorescein diacetate for  $\text{H}_2\text{O}_2$ . Cell viability was determined using propidium iodide. Only AgNP-CTAB treatments caused a significant increase in proline and  $\text{H}_2\text{O}_2$  content in extracts, while AgNP-PVP and  $\text{AgNO}_3$  induced higher GSH production. Increased cellular  $\text{O}_2^{\cdot -}$  accumulation was observed in roots of all of the treated seedlings and AgNP-CTAB treated leaves. On the contrary,  $\text{H}_2\text{O}_2$  production increased in AgNP-PVP treated roots and leaves exposed to all silver forms (PVP- and CTAB-coated AgNPs, and ionic silver). Cell death was severe in both roots and leaves in all treatments. The obtained results show that both AgNP-PVP and AgNP-CTAB induce oxidative stress causing cellular damage through mechanisms that cannot be completely assigned to  $\text{Ag}^+$  release.

KEY WORDS: AgNP-CTAB; AgNP-PVP; cell viability; glutathione; reactive oxygen species

YSL-5

## The effects of chronic exposures of four commercial pesticide preparations on multiple levels of biological organisation in the earthworm (*Eisenia andrei*)

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In the last decades, the negative effects of pesticides on the environment have been increasingly observed worldwide. A widely known example is the overall biodiversity decline often due to effects on non-target organisms. A vast biodiversity decline not only affects ecosystems and food chains but consequently also ecosystem services. In this study, we assessed the effects of four pesticides on the earthworm (*Eisenia andrei*) in standardised soil: namely, the insecticides thiacloprid (Calypso) and esfenvalerate (Sumialfa) and herbicides prosulfocarb (Filon) and dimethenamid-p (Frontier). As current regulations only include standardised toxicity testing of active substances, important data is missing on the different modes of action and possible varying toxicity of commercial formulations. Hence, we investigated the effects of the commercially available pesticide formulations after 7, 14, and 28-day exposures. After determining the sublethal concentrations in preliminary tests, a comprehensive test-battery, including biomarkers, fluorescence-based oxidative stress-related markers, multixenobiotic resistance and reproduction, was used to determine the sublethal toxicity of the investigated pesticides. All of the pesticides showed effects on oxidative stress-related markers and multixenobiotic resistance. Prosulfocarb (Filon) and esfenvalerate (Sumialfa) both showed significant effects on the multixenobiotic metabolism and cocoon production.

KEY WORDS: biomarker; multixenobiotic metabolism reproduction; soil; toxicity

YSL-6

## Individual and combined subchronic oral exposure to ochratoxin A and citrinin affect the expression of organic cation transporters in rat kidneys

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Previous studies have shown that transporters for both organic anions (Oat) and organic cations (Oct) provide a possible entrance pathway for mycotoxins including ochratoxin A (OTA) and citrinin (CIT) with nephrotoxic effects in mammals. However, compared to Oat, the individual and combined exposure effects of these mycotoxins on Oct expression in mammalian kidneys is still unknown. We used immunofluorescence microscopy and Western blot analysis to investigate individual and combined effects of OTA (0.125 and 0.250 mg/kg bw) and CIT (20 mg/kg bw) exposure on renal localisation and protein expression of rat Oct (rOct1 and rOct2) following 21-day subchronic oral exposure of Wistar rats. In addition, we studied the potential protective effects of resveratrol (RSV) (20 mg/kg bw) on renal localisation and protein expression of the studied rOct. Results showed that protein expression of rOct1 (but not rOct2) was significantly downregulated by both of the individual OTA doses used in this study. The combination of both mycotoxins (OTA+CIT) had a downregulating effect exclusively on the rOct1 protein expression when the higher experimental OTA dose (0.250 mg/kg bw) was used. In an individual manner, citrinin did not affect the expression of both rOct1 and rOct2 proteins. Interestingly, the combination of tested mycotoxins (OTA+CIT) and RSV caused a significant decrease of renal rOct1 and rOct2 protein expression. In conclusion, OTA has an Oct- and dose-dependent effect on the protein expression of studied rOcts, whereas RSV does not protect but does potentially enhance the nephrotoxic effects of studied mycotoxins in rat kidneys. Acknowledgement to Croatian Science Foundation project IP-09-2014-5982.

KEY WORDS: mycotoxins; proximal tubules; resveratrol; Western blot analysis; Wistar rat

YSL-7

## Adenylate energy charge (AEC) as a useful indicator of environmental stress in *Synurella ambulans* (Müller, 1846) from the hyporheic zone of the Sava River

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Groundwater organisms are exposed to progressive pollution and multi-xenobiotic stressors that influence the organism's physiology, metabolism and finally ecosystem functioning and biodiversity. A useful index of stress response at the cellular level is the adenylate energy charge (AEC). AEC is determined as the ratio of adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) through the formula  $AEC = ([ATP] + 1/2[ADP]) / ([ATP] + [ADP] + [AMP])$ . The aim of the study was to for the first time quantify adenine nucleotides in stygophilous crustacean *Synurella ambulans* and compare the AEC at two sampling sites with various levels of pollution in four seasons. Separation and quantification of nucleotides was performed using ion-pair reversed phase HPLC, with UV detection at 260 nm. Amphipods were collected from the hyporheic zone (HZ) of the Sava River at Jarun-Zagreb and Medsave-Zaprešić (upstream of a pharmaceutical wastewater outlet) sampling sites in December 2018 and April, July, and October 2019. The Medsave population had significantly higher AEC values (0.41; 0.45) than the Jarun population (0.35; 0.31) in the winter and spring sampling campaigns, indicating higher energy supply and thus lower metabolic stress. The possible impact of concentrations of metals/metalloids in interstitial water and in amphipods on seasonal and spatial variation of the AEC are discussed. AEC was shown as a useful index of environmental stress in *S. ambulans*, as it can directly measure the change in available energy and thus the metabolic stress experienced by an organism. In conclusion, it appears that the seasonal and spatial variations of the AEC values reflect the ecological state in the HZ. The financial support of the European Regional Development Fund for the Qua/Qua Protein project (KK.01.1.1.07.0023) and of Croatian Science Foundation for the AQUAMAPMET (IP-2014-09-4255) are highly acknowledged.

KEY WORDS: adenine nucleotides; amphipod; groundwater ecosystems; multi-xenobiotic stressors; pollution

YSL-8

## Polybrominated diphenyl ethers in Croatian house dust and assessment of human exposure

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Polybrominated diphenyl ethers (PBDEs) are organic compounds used as flame retardants in a wide variety of consumer and industrial products from which they are released into surrounding environments through abrasion, volatilisation, and leaching. Growing evidence of their adverse effects in ecosystems and human health have led to a global ban on their production and use, as these chemicals can cause thyroid disorders, neurobehavioral and developmental disorders. PBDEs accumulate in indoor dust, and dust ingestion is one of the main human exposure pathways to these chemicals. Studies have shown that toddlers are the most exposed population group due to their frequent hand-to-mouth activity and extensive contact with dusty surfaces. At this important developmental stage they also have reduced metabolic capacity for contaminant elimination and are therefore especially vulnerable. Seven PBDE congeners (BDE-28, -47, -99, -100, -153, -154, and -183) were analysed in five house dusts collected in Zagreb. PBDEs were extracted using microwave-assisted extraction, and purified extracts were analysed by gas chromatography with electron capture detectors. The mass fractions obtained for positive samples were used to calculate estimated daily intakes (EDI) for toddlers in central and worst case scenarios. EDI for ΣPBDEs ranged from 0.003 to 55.04 and from 0.01 to 110.07 ng/kg bw/day at the central and worst case scenarios. For the most contaminated dust sample, the EDI for BDE-99 (accounting for 63 % of ΣPBDEs) at the worst case scenario was 68.99 ng/kg bw/day, which is very close to the prescribed reference dose (RfD) value of 100 ng/kg bw/day. Supported by Croatian Science Foundation project UIP-2017-05-6713 (*DeValApp*).

KEY WORDS: brominated flame retardants; children exposure; congeners; daily intake; dust ingestion

P-1

## Unintentional exposures of preschool children to nonsteroidal anti-inflammatory drugs and analgesics

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The aim of the study was to characterize unintentional exposures of preschool children to nonsteroidal anti-inflammatory drugs (NSAIDs) and non-opioid analgesics, with the purpose of guiding future preventive actions. We analysed data records collected by the Croatian Poison Control Centre (CPCC) and Children's Hospital Zagreb (CHZ) for 2009-2019. In total, there were 466 cases (411 CPCC and 55 CHZ) of mono-ingestions by children aged 0-5 years. Median age was 2.5 years, of which 53 % were boys. The most frequent drug ingested was ibuprofen (47 %), followed by paracetamol (19 %), ketoprofen (15 %), and diclofenac (11 %). The study outcomes indicating the greatest preventive potential were as follows: 1) In 94 % of all cases, an unsupervised child had access to a drug, indicating that more effort is required in educating parents on safe home storage of medications; 2) according to CPCC data, the share of calls from the general population related to unintentional exposures of preschool children to NSAIDs and non-opioid analgesics in total calls grew from 2 % to 27 % between 2009 and 2019, indicating the increasing importance of the CPCC's role in pre-hospital patient disposition; 3) gastric lavage was performed in 11 % of cases treated in healthcare facilities (the subsample was obtained by pooling 344 CPCC cases reported by healthcare professionals and 55 CHZ cases). Healthcare professionals should be encouraged to use the PCC service for risk assessment and toxicological consultation to prevent unnecessary medical procedures.

KEY WORDS: drug ingestion; poison control centre; preschool; prevention; toxicological consultation

P-2

## Late-presenting acetaminophen self-poisoning: a case report

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Acetaminophen is one of the most commonly used analgesics and antipyretics due to its low price, over the counter availability, and big packaging. Therefore, acetaminophen overdoses occurs often and are the most common drug-related toxicity reported to poison centres. Ingestion of over 200 mg/kg or 10 g in a single dose is considered toxic and overwhelms the conjugation pathways of the acetaminophen metabolism in the liver, resulting in an increased formation of N-acetyl-*p*-benzoquinone imine which causes hepatic injury. We report the case of a 15-year-old adolescent girl admitted to the Intensive Care Unit of the Children's Hospital Zagreb 38 hours after having ingested 13.5 g acetaminophen as a suicide attempt. Due to the ingestion of a toxic acetaminophen dose, N-acetylcysteine (NAC) was initiated following the 21-hour NAC protocol. Blood tests revealed elevated levels of transaminase, high ammonia values, and coagulopathy. Abdominal ultrasound reported a diffusely enlarged hypoechoic liver with an hypoechoic area, which may indicate a necrosis zone. Serum acetaminophen levels were measured daily with immunoassay (EMIT<sup>®</sup>tox<sup>™</sup> Siemens). In the initial sample, 36 hours after ingestion, the level was 557 µmol/L. Acetaminophen was detectable until the seventh day after ingestion, while NAC was discontinued after 6 days of treatment, when concentrations were undetectable and liver enzymes decreased. She did not develop any criteria for liver transplantation and was discharged on day 15 after ingestion. At her check-up one month later, transaminase levels were in normal range. In this case, late administration of NAC proved to be beneficial in the treatment of acetaminophen-induced liver injury.

KEY WORDS: acute hepatic injury; immunoassay; N-acetylcysteine; suicide; toxicity

P-3

## Intravenous acetaminophen overdose: a case report of a therapeutic error

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Intravenous acetaminophen, as a 10 mg/mL solution, is commonly used in hospital settings as an analgesic and antipyretic when oral administration is not possible. Unintentional overdoses, errors in dose calculation, and therapeutic errors often occur and can cause acute hepatic injury. We present the case of an 8-month-old female infant, weighing 7 kg, with numerous comorbidities, admitted to the Department of Nephrology of the Children's Hospital Zagreb for a urinary tract infection. Due to clinical deterioration, she underwent a central venous catheter placement. After the procedure, due to high fever a 100 mL intravenous acetaminophen solution was administered. Shortly thereafter she became hypothermic and the nurse admitted making a therapeutic error – instead of 100 mg of acetaminophen, she administered 100 mL (i.e. 1000 mg) of intravenous acetaminophen solution (142 mg/kg). Four hours after the administration, serum acetaminophen concentration was 465 µg/mL. She was transferred to the intensive care unit and intravenous N-acetylcysteine (NAC) therapy was started immediately, following the 21-hour NAC protocol. Blood tests (liver and kidney functions, ammonia, prothrombin time, blood gas analysis) were performed daily and all values were in normal range. The infant remained well and without hepatic impairment. Caution should be exercised when prescribing and administering the drug to avoid dosing errors caused by confusion between milligrams (mg) and millilitres (mL), which may lead to accidental overdoses and death. When prescribing the drug, it is necessary to indicate the total dose in mg and the total dose in volume in mL.

KEY WORDS: accidental overdose; hepatic injury; infant; intravenous administration; N-acetylcysteine

P-4

## Alcohol consumption during the COVID-19 lockdown

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The aim of this study was to present the results of an anonymous survey on alcohol consumption during the so-called COVID-19 lockdown. The lives of most people around the world have changed since the start of the SARS-CoV-2 pandemic (SARS-CoV – severe acute respiratory syndrome corona virus). According to WHO data, the first registered cases in Croatia were on 26<sup>th</sup> February, in Bosnia and Herzegovina on 5<sup>th</sup> March, and in Serbia on 10<sup>th</sup> March 2020. The presumption was that, due to the stress caused by the imposed isolation, the consumption of alcohol would start to increase. To gather information on alcohol consumption in the period before and during the COVID-19 lockdown, an on-line anonymous survey was conducted in which a total of 930 persons participated: 542 from Croatia, 219 from Bosnia and Herzegovina, and 169 from Serbia. Among those who filled out the survey, there were 659 females and 271 males, mostly between 20-25 years of age, with high or middle education. The majority were employed and living in urban centres with a higher number of inhabitants. The results showed no influence of isolation on consumption of alcohol. Quite the opposite, 20.2 % of examinees who previously consumed alcohol responded that during the lockdown they stopped using it. Only 0.4 % of examinees started to consume alcohol exactly because of isolation. Thus, this research shines a new light on the everyday practice of people during the COVID-19 pandemic in western Balkan countries, a region known for its considerably widespread alcohol consumption.

KEY WORDS: alcohol beverage; anonymous survey; isolation; SARS-CoV; western Balkan countries

P-5

## Mixed intoxication during the Ultra Europe music festival in Split, Croatia

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This study was aimed at presenting the clinical picture of a young person admitted to the Psychiatry Emergency Department of the Clinical Hospital Centre Split during the Ultra Europe music festival. Upon admission to hospital, anamnesis data were taken and the patient was clinically examined. A form, specifically created for persons suspected of being under new psychoactive substance (NPS) intoxication, was filled out and biological samples (blood and urine) were taken for biochemical and toxicological analysis. The anamnesis data were not informative. According to the clinical evaluation, the patient was self-conscious, disorientated, complained of blurred vision, had skin redness, and muscular tension, while psychological symptoms were the most prominent ones. The results of biochemical analyses did not deviate from reference values. Four substances were detected by toxicological analysis: benzoyl-ecgonine, cocaine, coca ethylene and piperazine (NPS). In addition, he was alcohol intoxicated with a blood alcohol level (BAC) of 0.18. The patient was treated with supportive symptomatic therapy only. In conclusion, recreational usage of products with psychoactive effects has become popular among young people, especially during music festivals. Taking mixtures of psychoactive compounds can result in acute health problems and additionally with unpredictable remote effects. An integrated approach to diagnosis, correct identification and quantification are necessary for an effective diagnosis and therapy of such patients. Where indicated by drug history or suspicious clinical or analytical findings, specialised methodologies for detecting NPS not originally covered in routine toxicology screening are recommended.

KEY WORDS: clinical evaluation; forensic toxicology; new psychoactive substance; piperazine; supportive symptomatic therapy

P-6

## New psychoactive substances in pooled urine samples

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An analysis of pooled urine samples, collected in the period from 2016 to 2018 during the Ultra Europe music festival in Split, Croatia, was carried out for detecting the presence of new psychoactive substances (NPS). Thirty urine samples, collected from 14 portable chemical toilets located at or close to the venue of the festival (9 different locations in total), were analysed. Samples were qualitatively analysed by gas chromatography-mass spectrometry (GC/MS) using full-scan mode. Data were compared with the Wiley Mass spectra of the designer drugs library (DD2015) as well as with an in-house library containing about 1000 compounds and metabolites. Forty-seven different substances were found in the analysed samples. Among them, 26 were classical substances, mostly from the stimulants group, while 21 substances were from the NPS group. In the latter group, most of the substances were from the phenethylamines and cathinones groups. In each observed year, on the first day of the festival, compared to the other days, more substances were detected. Contrary to 2016 and 2017, in 2018 the number of detected substances was significantly smaller. The highest single concentration was of MDMA and tramadol. An analysis of pooled urine samples collected from chemical portable toilets was useful in detecting recreational drugs used during these music festivals where more than 150,000 people from over 150 countries were present. With this efficiency in mind, it is possible to uncover more information about worldwide NPS trends.

KEY WORDS: designer drugs; forensic toxicology; gas chromatography-mass spectrometry; music festivals

P-7

## Association between exposure to toxic metals and the disruption of thyroid function in cancer patients: preliminary results

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Exposure to toxic metals, as well as mixtures of metals, represents a ubiquitous environmental exposure capable of causing adverse health effects in humans. Various thyroid disruptors and their mechanisms have been studied *in vitro*, but a knowledge gap exists regarding the endocrine disruption effects of metals and their mixtures on thyroid function. In the context of the DecodExpo study, financed by the Science Fund of the Republic of Serbia, cancer patients (prostate, testes, breast, pancreatic) and healthy controls were recruited and blood samples were taken to measure hormone and metal levels. T3 levels were 1.8 nmol/L and 2.0 nmol/L in cancer patients and healthy controls, respectively, and this difference was statistically significant ( $p < 0.001$ ). FT3 levels were 4.7 and 5.1 pmol/L in cancer patients and healthy controls, respectively, and this difference was also statistically significant ( $p < 0.001$ ). There were no statistically significant differences in T4, FT4, and TSH between patients and controls. Statistically significant differences between patients and controls were found in Cd, Cr, Pb, Ni, and Hg levels. Statistically significant correlations were found between T3 and As ( $r = -0.379$ ), T3 and Ni ( $r = -0.200$ ), T3 and Pb ( $r = -0.204$ ), T4 and Cd ( $r = -0.185$ ), T4 and Ni ( $r = -0.164$ ), and T4 and Pb ( $r = -0.159$ ). Interestingly, once stratified by disease status, stronger correlations were found in patients than in controls. Exposome wide association studies can help better understand the role of exposure to a mixture on endocrine-mediated health effects.

KEY WORDS: adverse health effects; endocrine disruption; exposome; mixtures; thyroid hormones

P-8

## Valproic acid increases retinol-binding protein 4 levels in the serum of psychiatric inpatients

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Treatment with psychiatric drugs is associated with the onset of metabolic syndrome components such as dyslipidaemia, diabetes, and hypertension. These medical conditions reduce the survival of patients with severe mental illness. The retinoid system has a key role in the homeostasis of tissues and the metabolism, so that its disruption is involved in the pathogenesis of several diseases. In fact, the elevation of the retinol-binding protein 4 (RBP4), whose function is the distribution of retinol into extrahepatic tissues, has been considered an early biomarker of the components of metabolic syndrome. Therefore, the aims of this work were to (1) assess RBP4 levels in serum of 74 psychiatric inpatients enrolled in an observational cross-sectional study, (2) evaluate the relationship between RBP4 levels and psychiatric treatments, and (3) correlate RBP4 levels with biochemical parameters. Participants treated with valproic acid showed higher RBP4 levels ( $p = 0.009$ ). RBP4 was positively correlated with gamma glutamyl transferase (GGT) and cardiac frequency, whereas negatively correlated with aspartate aminotransferase (AST) and folate levels. This is the first study showing a significant elevation of RBP4 levels in valproic-acid-treated psychiatric inpatients. Nevertheless, due to the small sample size and cross-sectional design, further studies are required to corroborate our results.

KEY WORDS: anticonvulsants; biomarker; mental disorders; metabolic diseases; plasma

P-9

## Effects of two types of anaesthesia on oxidative stress in plasma in patients with hydronephrosis

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Human studies on oxidative stress induced by general anaesthesia are rather scarce. It is important to study the effects of anaesthetics on oxidative stress, particularly in surgical procedures with high reactive oxygen species (ROS) production, because their effect may be pivotal for the outcome of surgery. In this study, patients with hydronephrosis underwent balanced anaesthesia or Total Intravenous Anaesthesia (TIVA) with target-controlled infusion (TCI) during surgery. Blood samples from patients (N=32) were taken before induction of anaesthesia, 30 minutes and 24 hours after beginning of surgery. Measured parameters of oxidative stress were compared between two types of anaesthesia. Malondialdehyde (MDA) was measured using a HPLC with a fluorescence detector. Glutathione (GSH), ROS, total antioxidant capacity (TOC), and protein carbonyl concentration were measured spectrophotometrically. Activity of superoxide dismutase (SOD) was measured spectrophotometrically using commercial kits. The observed data were analysed by mixed modelling with the oxidative stress parameter as a dependent variable and sampling time nested within an anaesthetic group as fixed effects and subject id as a random effect. *Post-hoc* analyses were performed and the false discovery rate was controlled by the Benjamini-Hochberg method. SOD activity was significantly higher in patients that underwent balanced anaesthesia than anaesthesia with TIVA/TCI 30 minutes after beginning of surgery. There was no statistically significant difference in the other measured parameters between the balanced and TIVA/TCI type of anaesthesia. Results of the majority of the measured parameters of oxidative stress were significantly different 30 minutes or 24 hours after surgery from those measured before anaesthesia (balanced and TIVA/TCI). Results from this study contribute to general knowledge about oxidative stress caused by anaesthesia as an important factor in causing higher rates of postoperative complications and delayed recovery after surgery.

KEY WORDS: balanced anaesthesia; glutathione; malondialdehyde; surgery; target-controlled infusion

P-10

## The impact of the therapeutic use of nonsteroidal analgesics on preliminary psychoactive substances analysis

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Commercial immunochromatographic test strips are known as a fast method for routine control of people suspected of abusing psychoactive substances (PAS). Due to the non-specificity of these test strips, interference with compounds structurally similar to psychoactive substances could be expected. The aim of this study was to investigate the influence of the therapeutic use of selected analgesics on the occurrence of false positive results in PAS urine analyses. The first morning urine of four healthy volunteers who had consumed naproxen (550 mg twice daily), ibuprofen (400 mg three times per day), ketoprofen (100 mg twice daily) or dexketoprofen (25 mg twice daily) the day before, were used as samples. During the experiment, the participants did not use any other medicines or supplements. The specimens' integrity was checked using pH strips and biochemical strips (URIT 11G Urine Reagent Strips®). After the integrity check, drug detection was performed with one-component immunochromatographic test strips (AmeriTek, Inc.®) for the detection of methamphetamine, phencyclidine, methylenedioxymethamphetamine, cocaine, benzodiazepine, tetrahydrocannabinol, opioids, and barbiturates. All of the samples passed the integrity check. An analysis of the urine samples using phenylpropionic acid derivatives (ibuprofen, ketoprofen, and dexketoprofen) showed a false positive for benzodiazepines, while a positive test result for tetrahydrocannabinol was determined in all four specimens. Additionally, a false positive result for opioids was observed in a patient taking dexketoprofen. Based on the data obtained, it can be concluded that there is a possibility of obtaining false positive PAS test results in case of a therapeutic use of the tested analgesics. However, such a result needs to be confirmed by another method.

KEY WORDS: anti-inflammatory drugs; false positive; test strips; urine analyses



P-11

## Effects of irinotecan and $\Delta^9$ -tetrahydrocannabinol on tumour growth and weights in a mouse syngeneic model of colon cancer

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Colon cancer is one of the most common cancers and causes of death worldwide. The aim of this study was to investigate the effects of the concomitant intake of  $\Delta^9$ -tetrahydrocannabinol (THC) and cytostatic irinotecan (IRI) on tumour growth and body and organ weights in mice with experimental colon cancer induced by an injection of the CT26 cell line. Male BALB/c mice (N=5 per group) were randomly assigned to the following groups: IRI (60 mg/kg bw, *i.p.*, 1<sup>st</sup> and 5<sup>th</sup> day of the experiment), THC (7 mg/kg bw, *p.o.*, consecutively during the 7-day experimental period), combination of IRI and THC, solvent control (sesame oil, *p.o.*, consecutively during the 7-day experimental period), and cancer control (without treatment). Weight and tumour measurements were performed every other day throughout the experiment. Mice were sacrificed on the seventh day and tissue samples were dissected and weighed. Body weights decreased significantly in IRI and IRI-THC treated mice on the third day as well as in IRI-THC treated mice on the seventh day compared to THC-treated and cancer control mice. There were no significant changes in brain and liver relative weights between the experimental groups. The tumours grew constantly over time with significant differences in growth between the third and seventh day in all groups. Differences in tumour size between experimental groups were not observed. The present study showed that IRI exposure affected body weights when applied alone or in combination with THC and that neither single IRI, single THC or combined exposure affected tumour growth under these experimental conditions.

KEY WORDS: body weights; cancer therapy; cannabinoid-based preparations; organ weights; tumour size

P-12

## Prenatal exposure to $\alpha$ -cypermethrin and endocrine disruption in mother rats and foetuses at term

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$\alpha$ -cypermethrin ( $\alpha$ -cyp) is a synthetic pyrethroid insecticide widely used in and around households, as well as in agriculture and veterinary applications due to its rapid degradation rate and low mammalian toxicity. Due to the fact that pyrethroids are one of the most used groups of insecticides worldwide and can act as endocrine disrupting chemicals, we investigated the effects of prenatal exposure to  $\alpha$ -cyp on endocrine disruption parameters in rat mothers and foetuses at term. Pregnant Wistar rats were orally exposed from the 6<sup>th</sup> day of gestation (DG) to the 21<sup>st</sup> to  $\alpha$ -cyp at 1, 10, and 19 mg/kg bw/day, diethylsilbestrol as positive control, corn oil as solvent control, and water as negative control. At the 21<sup>st</sup> DG, gravid uterus and ovaries were dissected under general anaesthesia and parameters of endocrine disruption were monitored according to adequate OECD protocols. No differences were found in the number of corpora lutea in the ovaries. The number of implants, live or dead foetuses, as well as of early and late resorptions did not change between groups. No changes in the number and weight of male and female foetuses were noticed. Male to female foetal sex ratio (% per litter) was approximately 70 to 30 % in the rats treated with  $\alpha$ -cyp at 1 and 10 mg/kg bw/day and diethylsilbestrol, while about 40 to 60 % in negative and solvent control. Our findings suggest that  $\alpha$ -cyp at the applied exposure scenarios and doses did not act as an endocrine disruptor in mother rats and foetuses.

KEY WORDS: endocrine disrupting chemical; gravid uterus; *in utero* exposure; ovaries; pyrethroid insecticide

P-13

## Histological assessment of foetal rat testes prenatally treated with $\alpha$ -cypermethrin

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$\alpha$ -cypermethrin ( $\alpha$ -cyp) is a widely used insecticide with proven reproductive toxicity in male rat offspring, thus acting as an endocrine disrupting chemical. Limited animal data indicate transplacental transfer of  $\alpha$ -cyp. With the aim of investigating the effects of prenatal exposure to  $\alpha$ -cyp on the histology of foetal rat testes, we treated pregnant Wistar rats subsequently p.o. from the 6<sup>th</sup> day of pregnancy with 1, 10, and 19 mg/kg bw/day of  $\alpha$ -cyp. The doses were calculated according to NOAEL and LOAEL values for  $\alpha$ -cyp reproductive toxicity in rats. On the 21<sup>st</sup> day of pregnancy, foetal testes were microsurgically removed, fixed in Sainte Marie solution, and embedded in paraffin. Then, 4- $\mu$ m thick sections were stained with haematoxylin and eosin. Proliferation of Sertoli cells, present at that specific foetal period, was assessed with staining for the rabbit antibody against mitotic marker phospho-histone H3 (Ser10+Thr11) (1:500, ab32107, Abcam). The histological assessment revealed no changes in the foetal testes histology, considering the basal membrane integrity or disruption of seminiferous tubules or interstitial spaces. Sertoli cells and gonocytes were present inside tubules, with no vacuolization or multinucleation observed. Leydig cells appeared histologically normal. Furthermore, a medium to strong intensity of staining for mitotic marker phosphorylated histone H3 antibody was observed predominantly in Sertoli cells, located in the periphery of seminiferous tubules. The signal was present in all of the treated groups, as well as in controls showing no negative effect of  $\alpha$ -cyp on Sertoli cell proliferation. We concluded that  $\alpha$ -cyp had no effect on the foetal testes histology after *in utero* exposure. However, further molecular analyses are needed to fully assess the effect of  $\alpha$ -cyp on reproductive endocrine disruption.

KEY WORDS: *in utero* exposure; insecticide; male foetuses; reproductive effects; testicular histology

P-14

## Effects of the concomitant use of $\Delta^9$ -tetrahydrocannabinol and the cytostatic irinotecan on cholinesterase activities in a mouse syngeneic model of colon cancer

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Colorectal cancer patients treated with irinotecan (IRI) often manifest acute cholinergic syndrome, which is the reason behind the growing use of legally prescribed cannabis preparations as well as illicit ones that can contain very high  $\Delta^9$ -tetrahydrocannabinol (THC) levels. IRI-induced cholinergic syndrome originates from the inhibition of acetylcholinesterase (AChE), while butyrylcholinesterase (BChE) plays an important role in the hydrolytic metabolism of this prodrug. On the other hand, existing reports of THC effects on cholinesterases are inconclusive. In this study, we aimed to investigate how the concomitant use of a high THC dose with IRI affects cholinesterase activities in blood of mice with experimental colon cancer induced by injection of CT26 cell line. Male BALB/c mice were assigned randomly to five study groups: (i) negative control, (ii) cancer control, (iii) irinotecan (*i.p.* 60 mg/kg; 1<sup>st</sup> and 5<sup>th</sup> day of the experiment), (iv) THC (*p.o.* 7 mg/kg; repeatedly for 7 days), (v) IRI + THC. ChE activity in blood was assayed by the spectrophotometric Ellman method, using acetylthiocholine as a substrate and inhibitors to distinguish between AChE and BChE activities. The results showed a time-dependent increase in total ChE and BChE activities in all experimental groups, including cancer control. In contrast, AChE activities decreased, with some minor fluctuations in the THC and IRI+THC group. Although there was a lack of statistically significant differences, the time course of changes for AChE and BChE activities suggested that these esterases may not only be indicators of metabolic status but also functionally important in neoplastic cell transformation.

KEY WORDS: acetylcholinesterase; blood; butyrylcholinesterase; cannabis; tumorigenesis

P-15

## Application of the alkaline comet assay to evaluate DNA instability in different cell types of Wistar rats exposed to $\Delta^9$ -tetrahydrocannabinol

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The ever-growing use of illegal highly concentrated  $\Delta^9$ -tetrahydrocannabinol (THC) preparations, frequently used as supportive therapies for various malignancies and neurological disorders, motivated us to perform an assessment of THC toxicity on a Wistar rat model. Considering the well-established sensitivity of the alkaline comet assay for detection of DNA damage in single cells, this study aimed to clarify (i) the dynamics of DNA instability in liver cells, leukocytes, and brain cells of rats that were repeatedly administered THC *per os* over seven days at 7 mg/kg doses, and (ii) to establish the differences between levels of DNA damage produced in the studied cell types. THC treatment slightly affected total body weight, as well as liver and brain weights compared to controls of rats after 1, 3, and 7 days of treatment. Repeated THC exposure resulted in DNA instability in all of the studied cell types. While liver cells showed similar levels of primary DNA damage, leukocytes and brain cells tended to accumulate DNA lesions in a time-dependent manner. The observed pattern of DNA damage could be related both to the induction of lesions and their repair, which created additional DNA damage detectable by the alkaline comet assay. The obtained results suggest induction of apoptosis in brain cells. Differences in cell susceptibility may be associated with inherent defence mechanisms in the tested tissues. Although the majority of primary DNA lesions detected by the alkaline comet assay are subject to repair, increased levels of DNA damage in brain cells after repeated THC exposure call for concern.

KEY WORDS: brain cells; DNA damage; illegal THC preparations; leukocytes; liver cells

P-16

## Aucubin administered by either oral or parenteral route protects against cisplatin-induced acute kidney injury in mice

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Aucubin, an iridoid glucoside isolated from the leaves of *Aucuba japonica* and other herbs is a pharmacologically active natural compound that possesses anti-inflammatory, antioxidative, and other beneficial properties. This study aimed to evaluate the protective effect of aucubin against cisplatin (CP)-induced acute kidney injury in mice and the mechanism of its action. Aucubin was administered to mice orally or intraperitoneally (i.p.) (1.5 and 5 mg/kg) for two consecutive days, two days after i.p. injection of cisplatin (11 mg/kg). Treatment with aucubin by both routes of administration ameliorated histopathological changes and reduced elevated serum markers of kidney injury (blood urea nitrogen and creatinine). CP administration increased renal expression of heme oxygenase-1 (HO-1) and 4-hydroxynonenal (4-HNE), as well as tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), determined by Western blot analysis, which was dose-dependently ameliorated by aucubin showing attenuation of CP-induced oxidative stress in kidneys. Moreover, aucubin reduced increased renal expression of cleaved caspase-3 and -9 and decreased poly (ADP-ribose) polymerase (PARP) cleavage, indicating the attenuation of CP-induced apoptosis in kidneys. Mechanistically, aucubin suppressed the activation of several signalling pathways involved in inflammation and apoptosis, including the immunohistochemically determined nuclear localisation of nuclear factor-kappa B (NF- $\kappa$ B) and by western blot analysis of signal transducer and activator of transcription 3 (STAT3), Akt, extracellular signal-regulated kinase 1/2 (ERK1/2) and forkhead box O3a (FOXO3a) showing that aucubin suppresses CP-induced renal inflammation and suppresses CP-induced activation of ERK1/2, Akt/mTOR, and FOXO3a signalling. The parenteral application was marginally but statistically more effective in reducing CP-induced kidney injury than oral administration. These findings suggest that aucubin acts as a protective agent against CP-induced nephrotoxicity, which should be further investigated.

KEY WORDS: apoptosis; inflammation; oxidative stress; protective effect; Western blot analysis

P-17

## Antitumour activity of luteolin in human colon cancer SW620 cells is mediated by the ERK/FOXO3a signalling pathway

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The aim of this study was to investigate the mechanism of the anticancer activity of luteolin, a member of the flavonoid family, in metastatic human colon cancer SW620 cells. Studies have shown that luteolin possesses many beneficial effects *in vitro* and *in vivo*, including antioxidant, anti-inflammatory, antibacterial, antidiabetic and antiproliferative. Luteolin dose-dependently (1, 2, 5, 10  $\mu$ M) reduced the viability (XTT Cell Viability Kit) and proliferation of SW620 cells and increased the expression of antioxidant enzymes. Expression of proteins was determined by Western Blot. The expression of antiapoptotic protein Bcl-2 decreased, whereas the expression of proapoptotic proteins Bax and caspase-3 increased by luteolin treatment, resulting in increased poly (ADP-ribose) polymerase (PARP) cleavage and terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) positivity. Luteolin also increased the expression of autophagic proteins Beclin-1, autophagy-related protein 5 (Atg5) and microtubule-associated protein 1A/1B-light chain 3 beta-I/II (LC3B-I/II), while the usage of 3-methyladenine suggested a prosurvival role of autophagy. Moreover, treatment with luteolin induced reversal of the epithelial-mesenchymal transition process through the suppression of the wingless related integration site protein (Wnt)/ $\beta$ -catenin pathway. The cytotoxic activity of luteolin coincided with the activation of extracellular signal-regulated kinase 1/2 (ERK1/2) and forkhead box O3a (FOXO3a). Treatment with the mitogen-activated protein kinase (MEK) inhibitor PD0325901 inhibited ERK-dependent FOXO3a phosphorylation, resulting in increased FOXO3a expression and apoptosis, with the suppression of autophagy. The results of the current study suggest the antitumour activity of luteolin in SW620 cells through the ERK/FOXO3a-dependent mechanism, as well as its antimetastatic potential.

KEY WORDS: anticancer activity; apoptosis; autophagy; flavonoid; forkhead box O3a

P-18

## DNA-binding, DNA-damaging, and DNA-protecting activities of 1,4-dihydropyridine derivatives

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Numerous compounds widely used as drugs directly interact with DNA, for example the Ca<sup>2+</sup> antagonists from the 1,4-dihydropyridine (1,4-DHP) group: nifedipine, lercanidipine, and amlodipine interact with the minor groove of DNA. We performed a series of studies on the DNA binding, DNA damaging, and DNA protecting capabilities of 1,4-DHP synthesised at the Latvian Institute of Organic Synthesis in Riga. The docking analysis predicted intercalation of the antimutagenic AV-153 sodium salt in the DNA molecule in a place of a single-strand break, and the model was proven experimentally: the compound triggered extrusion of the ethidium bromide out of a complex with DNA, while the induction of DNA breaks increased affinity to DNA. Using the comet assay and ExSy-SPOT assays, it was shown that the compound protects DNA from damage by peroxynitrite and increases activity of the DNA excision repair enzymes. Both the DNA-binding capacity and ability to protect DNA was dependent on metal ions forming salts with the AV-153 residue. When administered to rats, the water-soluble 1,4-DHP increased expression of the genes encoding DNA repair enzymes and proteasome subunits. 1,4-DHP with promising antidiabetic and neuroprotective activities – cerebrocrast and its analogue etafitorone acting mainly on mitochondria, turned out to be DNA binders and DNA protectors. It should be noted that the Ca<sup>2+</sup>-channel blocker nitrendipine also could reduce the level of DNA damage induced by peroxynitrite. Representatives of this group can also bind unusual DNA structures – the G-quadruplexes. It seems that the DNA binding capacity is an important feature of 1,4-DHP derivatives, depending on their structure.

KEY WORDS: Ca<sup>2+</sup> antagonist; comet assay; DNA repair; docking analysis; ExSy-SPOT assay

P-19

## Antibacterial, cytotoxic, and antioxidative effects of benzoxanthene lignans

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Benzoxanthene lignans are natural compounds that have been isolated from various plants, but not much is known about their biological activity. This study investigated the antibacterial, cytotoxic and antioxidant effects of eight different benzoxanthene lignans synthesised by the oxidative coupling of caffeic acid and methyl caffeate. Results showed that all benzoxanthene lignans exhibited significant antibacterial effects against Gram positive bacteria at concentrations of 0.5 and 0.05 mmol/L. Seven of them showed significant cytotoxic effects on human liver cancer cells at a concentration of 0.05 mmol/L, with phenyl and hydroxyl groups in the structure being responsible for their effective tumour cell growth inhibition. All benzoxanthene lignans showed significant antioxidative effects at 0.05 mmol/L. The C-6, C-9, and C-10 hydroxyl groups were proven to be important for their antioxidative activity.

KEY WORDS: caffeic acid; Gram positive bacteria; human cell line; methyl caffeate; tumour cell growth inhibition

P-20

## Cytotoxic and antiproliferative effect of transgenic *Senna obtusifolia* hairy root extract against the human glioma cell line

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*Senna obtusifolia* is an annual or perennial plant, belonging to the Fabaceae family. It is found in South America, Africa, Asia and Oceania. Among the many valuable compounds, pentacyclic triterpenes and many anthraquinone derivatives can be found in this plant. Most of them show a wide biological effect, very important in many areas of human life. In this work, we present cytotoxic and antiproliferative effect of *Senna obtusifolia* transgenic hairy roots extracts derived from roots cultivated in bioreactor and overexpressed squalene synthase in comparison to the roots obtained after transformation with the wild-type *Agrobacterium rhizogenes*. In this *in vitro* study, two different extracts were used; the transgenic root with overexpression of squalene synthase and roots obtained by *A. rhizogenes* transformation. Chromatographic analysis was carried out using an HPLC LC-MS/MS. MTT assay was employed to measure the viability of glioma cell line. Antiproliferative effect has been tested by clonogenic assay. Our results showed a higher content of selected secondary metabolites and a stronger cytotoxicity and antiproliferative effect of the extract derived from transgenic hairy roots grown in a bioreactor against human glioblastoma cell lines compared to the extract obtained from hairy roots without overexpression of squalene synthase. These results indicate that transgenesis and the use of bioreactor can be an effective tool for obtaining alternative *in vitro* plant systems for production of valuable secondary metabolites that may contribute to a more effective fight against many serious diseases in the future.

KEY WORDS: bioreactor; clonogenic assay; HPLC LC-MS/MS; *in vitro*; MTT assay

P-21

## Toxic effects of polybrominated diphenyl ethers on cultured human lung and neuronal cells

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Polybrominated diphenyl ethers (PBDEs) are flame retardants that used to be widely used in many consumer products, including plastics, textiles, furniture and electronic devices. They are globally ubiquitous in all abiotic compartments (air, soil, water) as well as in animals and humans due to their persistency and ability to bioaccumulate in lipid rich matrices. Despite measures taken to reduce further PBDE contamination, humans are exposed daily via food consumption, ingestion of indoor dust and inhalation of contaminated air and particle-bound PBDEs. Children are the most exposed population group due to extensive contact with dusty surfaces as well as hand-to-mouth behaviour. Studies have revealed that exposure to these chemicals is associated with disruption of thyroid hormone and oestrogen homeostasis, developmental and reproductive toxicity, neurotoxicity and carcinogenicity. As the primary concern is health risk due to long-term and multi-pathway exposure to PBDEs, particularly for children, we monitored the viability of human adherent alveolar basal epithelial and neuronal cells exposed to selected PBDEs (concentration range 375 ng/mL – 12 µg/mL) for 24 hours. Several of the tested PBDEs significantly induced cytotoxicity at higher concentrations. Interestingly, lung cells were more sensitive to the tested chemicals than neuronal cells. Furthermore, the BDE-99 congener was the most toxic with an  $IC_{50}$  value of  $8.96 \pm 1.06$  µg/mL. Although this concentration lies at the higher end of the range expected in exposure scenarios for the general population, the exact mechanism of the observed effects should be investigated in future research. Supported by Croatian Science Foundation projects UIP-2017-05-6713 (*DeValApp*) and UIP-2017-05-7260 (*CellToxTargets*).

KEY WORDS: A549; cytotoxicity; flame retardants; human exposure; SH-SY5Y

P-22

## Insights on the toxicity and detoxication of organophosphorus pesticides *in vitro*

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Toxic organophosphorus (OP) pesticides are still widely used in agriculture and are the cause of great risk of human exposure. Such exposure can lead to distinct neurotoxic effects depending on dose and frequency. The main mechanism of action of OP pesticides is inhibition of cholinergic enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), causing an accumulation of the neurotransmitter acetylcholine in the synaptic cleft inducing cholinergic crisis. This progressive inhibition occurs by a phosphorylation of their active centre leading to an inactive enzyme species. After exposure, to prevent a cholinergic crisis, quick dephosphorylation of AChE's active centre needs to be affected by a strong nucleophile with an oxime group to avoid severe health effects and illnesses, ranging from respiratory problems, muscle twitches, convulsions, etc. We evaluated the *in vitro* inhibition rate of AChE by methamidophos and the potency of three pyridinium oximes, 2-PAM analogues, to reactivate OP-inhibited AChE and BChE. The overall inhibition rate of AChE for methamidophos was  $2760 \pm 119$  min<sup>-1</sup> L/mol, which was almost 10-fold higher than for BChE ( $386 \pm 16$  min<sup>-1</sup> L/mol). The reactivation experiments showed that the selected oximes at 0.1 mmol/L possessed an almost 2-fold higher observed reactivation rate than the standard oxime 2-PAM in case of AChE. Furthermore, an absence of cytotoxicity in human astrocytoma (1321N1) and human liver (HepG2) cells makes these oximes good candidates for future research as reactivators of OP-pesticide inhibited cholinesterases. This study was supported by Croatian Science Foundation projects IP-2018-01-7683 and UIP-2017-05-7260.

KEY WORDS: 2-PAM; acetylcholinesterase; butyrylcholinesterase; cholinergic crisis; fenamidophos; methamidophos

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## **Pralidoxime analogues and acetylcholinesterase mutants in counteracting tabun poisoning**

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Acetylcholinesterase (AChE) is a pivotal enzyme with a role in the degradation of nerve impulses. Its activity can be irreversibly inhibited by organophosphates (OPs) acting as nerve agents and pesticides. Compounds with an oxime group (2-PAM, HI-6, obidoxime) can restore phosphorylated AChE activity and the success of reactivation depends on the OP conjugated to AChE. Tabun is one of the most dangerous nerve agents and is known to cause severe symptoms of poisoning often with deadly outcomes. The electron pair located on tabun's phosphoramidate group and the steric hindrance arising within the AChE active centre gorge upon formation of the AChE-tabun conjugate unfavourably affects the oxime orientation and its embedding in the vicinity of the phosphorylated catalytic serin, disabling AChE reactivation. Pralidoxime (2-PAM) is the oxime of choice of many armies worldwide, but with limited potency. The efficacy of nerve agent exposure therapy is supplemented by AChE mutants, which could in combination with the oxime degrade the OP in cycles before it inhibits the native AChE. In this study, a series of 2-PAM analogues were tested as a reactivator of tabun-inhibited AChE and its mutants. The most promising was the analogue with a hexyl chain which *in vitro* and *ex vivo* restored a high percent of phosphorylated AChE activity within a short time. Nevertheless, experiments on mice failed to prove the efficacy of this treatment *in vivo* and investigations of further adjustment of the dose or route of administration to enhance it are warranted. The authors thank Professors P. Taylor and K. B. Sharpless for the generous gift of cholinesterases and oximes. The study was supported by Croatian Science Foundation projects IP-01-2018-7683 and IP-11-2013-4307.

KEY WORDS: cholinesterase; detoxification; nerve agent; oxime; reactivator

P-24

## Toxicological Risk Assessment Centre – mission and vision

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The Toxicological Risk Assessment Centre (TRAC) at the Faculty of Pharmacy, University of Belgrade was established in 2013 and is a unique institution of its kind in the Republic of Serbia. In 2017, the TRAC became a member of the WHO Chemical Risk Assessment Network. The overall objective of the TRAC is to improve chemical risk assessments on the national level. The specific objectives of the TRAC are to: identify critical science issues, develop and apply state of the art scientific research to characterise impacts on human health and ecological systems, and provide training courses related to human health chemical risk assessment. The TRAC focuses on two broad research topics: (1) science assessments and translation and (2) advancing the science and practice of risk assessment. The Centre is a partner in the project “Development of the National Road Map for Enhancing Health Sector Engagement/Contribution to Sound Management of Chemicals in Serbia to 2030”. The Road Map has been developed within the framework of the WHO HQ project and under the leadership of the Institute of Public Health of Serbia. The mission of the TRAC is to increase public awareness regarding toxic chemicals and promote the implementation of the best methodologies for chemical risk assessments.

KEY WORDS: chemical safety; health promotion; networking; science and practice; risk training

P-25

## HUMNap – Air pollution and human biomarkers of effect: an overview

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Air pollution is becoming recognized as one of the most serious public health issues worldwide. Rising industrial and energy productions, the burning of fossil fuels and biomass, as well as the rise in road traffic frequency contribute to air pollution in our cities. Urban air is a complex and variable mixture of many different chemicals whose exact mechanisms of action is not known, although oxidative stress and inflammation are suspected. Human biomonitoring is an essential tool for assessing whether and to what extent environmental substances affect the human population and as such can provide valuable data on environmental exposure and help identify potential health risks. Therefore, HUMNap will determine possible associations between the air pollutants and biomarkers of exposure and early biological effects. The project will start with investigations at multiple geographical locations with different air pollution levels and origin followed by measurements of various environmental airborne pollutants. The next step will be a detailed assessment of different biomarkers of exposure and early effects (genomic instability and oxidative stress) in human populations living in those geographical locations. HUMNap will promote state-of-art techniques and research approaches to develop risk assessments of human exposure to airborne pollutants. The results from HUMNap will demonstrate how airborne pollutants affect early molecular events important for disease development in different human blood and epithelial cells. It will also provide an assessment of possible cancer risk among human populations affected by polluted urban areas. Furthermore, HUMNap aims to draw the attention of many stakeholders such as leading scientists, policy makers, industry and the public in order to raise awareness regarding air pollution and develop monitoring regimes. Finally, HUMNap will provide new datasets necessary for scientifically based risk assessments of human populations exposed to urban air pollution. Supported by the Croatian Science Foundation project HUMNap.

KEY WORDS: comet assay; micronucleus assay; oxidative stress; particulate matter; polycyclic aromatic hydrocarbons; risk assessment



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## Effects of sub-chronic exposure to $\alpha$ -cypermethrine or imidacloprid on oxidative stress and concentration of essential elements in testes and epididymis of adult rats

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The use of pyrethroid and neonicotinoid insecticides has been increasing during the past decade. Although considered a good alternative to organophosphate pesticides, there are reports indicating their adverse health effects. Our aim was to assess the effects of exposure to low doses of  $\alpha$ -cypermethrin or imidacloprid on the oxidative stress parameters and concentration of essential elements in testes and epididymis of adult Wistar rats. Rats were orally treated with doses comparable to currently proposed health-based reference values ( $\alpha$ -cypermethrin: 0.02 (ADI), 0.15 (OEL), 0.80 (5 $\times$ OEL) or 2.20 (REL) mg/kg bw/day; imidacloprid: 0.06 (ADI), 0.80 (10 $\times$ AOEL) or 2.25 (1/200 LD<sub>50</sub>) mg/kg bw/day) for 28 consecutive days. Exposure to  $\alpha$ -cypermethrin or imidacloprid significantly decreased body weight gain in the treated groups compared to the controls. The weight of testes was significantly lower in animals treated with the highest dose of imidacloprid, while treatment with  $\alpha$ -cypermethrin had no effect on testicular/epididymal weight. Both insecticides significantly increased the level of reduced glutathione in epididymis and the activities of epididymal glutathione peroxidase and superoxide dismutase. Additionally, treatment with  $\alpha$ -cypermethrin increased the activity of glutathione peroxidase in testes at the highest applied dose. A significantly lower concentration of Fe, Mo and Se, and higher concentration of Ca was measured in testicular tissue of animals treated with  $\alpha$ -cypermethrin, while treatment with imidacloprid resulted in a higher concentration of Mo and Na in testes compared to control animals. The fact that such low doses had the potential to produce measurable biological effects calls for further evaluations of these widely used insecticides.

KEY WORDS: antioxidative enzymes; glutathione; neonicotinoids; pyrethroids; testicular/epididymal weight

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## Oxime impact on oxidant/antioxidant status in diaphragms of rats exposed to dichlorvos

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Oxime compounds are used as causal antidotes in intoxication by organophosphate (OP) insecticides and nerve agents. They are able to directly reactivate acetylcholinesterase (AChE), specifically inhibited by organophosphates, therefore preventing the toxic effects of acetylcholine (ACh) accumulated in synapses. The latest research on the mechanisms of action of oxime also takes into account the finding that OPs, in addition to AChE inhibition, interfere with redox processes in cells, and it has been hypothesised that the antioxidant properties of oxime reactivators may contribute to OP-intoxication therapy. Therefore, we investigated *in vivo* the effect of two experimental (K027, K203) and four conventional oximes (obidoxime, trimedoxime, pralidoxime and HI-6) on dichlorvos (DDVP)-induced oxidative changes. Oxime (5 % LD<sub>50</sub> *i.m.*) was injected to Wistar rats (5/group) immediately after DDVP injection (75 % LD<sub>50</sub> *s.c.*). Total oxidant status (TOS) and total antioxidant status (TAS) were measured 60 min after the treatment in homogenised diaphragm samples by spectrophotometric methods. When compared to controls, DDVP induced a significant increase in TOS (p<0.01) and decrease in TAS (p<0.01). All of the evaluated oximes significantly decreased TOS elevated by DDVP (p<0.01), whereby obidoxime, trimedoxime and oxime K203 showed levels of TOS in diaphragm samples similar to control samples. A significant increase in TAS was obtained by oxime K027, K203, pralidoxime, and HI-6 (p<0.05). These promising results support the hypothesis on antioxidant properties of oxime reactivators along with the necessity of further research. Supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (451-03-9/2021-14/200161).

KEY WORDS: antidote; *in vivo*; oxidative stress; oxime K027; oxime K203

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## Effects of subchronic exposure of rat to individual and combined ochratoxin A and citrinin on the expression of renal water channels and sodium-glucose cotransporters

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Ochratoxin A (OTA) and citrinin (CIT) are nephrotoxic mycotoxins that frequently co-contaminate food and feed, thus representing a serious health threat to humans and animals. Studies have shown that these toxins deregulate an array of genes/proteins leading to their expressional changes. However, the individual and combined effect of OTA and CIT on the expression of renal proteins facilitating the transport of biologically important molecules such as water and glucose, have been poorly studied. Herein, we used immunochemical methods (immunofluorescence microscopy and Western blotting) to reveal the individual and combined effects of OTA (0.125 and 0.250 mg/kg bw) and CIT (20 mg/kg bw) on the localisation/expression of renal water channels (rAqp1 & rAqp2) and sodium-glucose co-transporters (rSglt1 & rSglt2) after a 21-day subchronic oral exposure of Wistar male rats. Additionally, we investigated if the antioxidant resveratrol (RSV; 20 mg/kg bw) can counteract the OTA- and CIT-related nephrotoxic effects on the localisation/expression of the studied proteins. In the rat kidney, the higher individual doses of OTA significantly downregulated the protein expression of rAqp1 glycosylated isoforms and upregulated the protein expression of rSglt1, whereas a combination of higher OTA dose and CIT downregulated the expression of rAqp1 non-glycosylated isoforms. Interestingly, the protein expression of rAqp1 glycosylated isoforms and rSglt1 was significantly downregulated in the kidneys of rats exposed to the combination of both mycotoxins (OTA+CIT) and RSV. Thus, OTA and CIT seem to have had a selective effect on the protein expression of the studied transporters in rat kidneys, whereas RSV does not counteract their nephrotoxic effects. Funded by Croatian Science Foundation project IP-09-2014-5982.

KEY WORDS: kidney; mycotoxins; oral exposure; proximal tubules; resveratrol

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## Estimation of urinary cotinine cut-off value to discern pregnant at term cigarette smokers from non-smokers

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Prenatal exposure to cigarette smoking has been extensively studied as one of the factors that may have adverse health outcomes later in life. Reliance on self-reported smoking behaviour may lead to understatements of nicotine exposure due to negative social connotations related to maternal smoking. To overcome the uncertainty of self-reporting, cotinine, the major metabolite of nicotine in urine, is widely used as a reliable biomarker of tobacco smoke exposure. Since half-life of cotinine is much shorter in pregnant (8.8 h) vs. non-pregnant women (16.6 h), cut-off level to classify non-smokers and active smokers must be established for pregnancy. The aim of this study was to determine a cut-off value to discern pregnant non-smokers from pregnant smokers. Spot urine samples from pregnant women self-reported as non-smokers ( $n=117$ ; never smoked or smoked >12 months before the last pregnancy) or smokers ( $n=39$ , smoking any time during pregnancy or within 12 months before last pregnancy) were collected in the maternity ward before delivery. Cotinine was extracted from urine by headspace solid phase microextraction and quantified using gas chromatography-mass spectrometry. A receiver operating characteristic (ROC) curve analysis showed that the optimal cut-off value of urinary cotinine to discern pregnant non-smokers from smokers was 24.1 µg/L (42.6 µg/g creatinine). Misclassification rate for current smokers who reported no smoking was 5.1 %. The presented cut-off value for urinary cotinine ensures a more accurate categorisation of the smoking habit among pregnant women than by self-reporting. The study was funded by the Croatian Science Foundation grant HRZZ-IP-2016-06-1998.

KEY WORDS: biomarker of cigarette smoking; gas chromatography-mass spectrometry; ROC curve; solid phase microextraction; spot urine

P-30

## Thermal comfort analysis in nursing homes with an Atlantic climate

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Increased longevity and ageing populations are one of the greatest achievements and social challenges of this century, especially in developed countries. Being classified as the most important parameter in comparison with visual and acoustic comfort and indoor air quality, thermal comfort is highly significant for the elderly and, consequently, for all decisions regarding the well-being of the elderly in such spaces. This project aims to develop several analytical models for thermal comfort analysis in elderly care institutions in Porto's metropolitan area. The proposed methodology belongs to a study being carried out in Portugal and Spain, thus encompassing the Atlantic and Mediterranean climates. Monitoring in different spaces in 8 residential structures for the elderly, three times per season, from February 2019 to March 2020, the field work was developed by data collection of physical parameters (air temperature, radiant temperature, air velocity, and relative humidity), personal parameters (clothing insulation and metabolic heat), and responses to 925 questionnaires regarding 61 people (residents and non-residents) of thermal sensation, using the ASHRAE 7-point scale. Through a statistical analysis of all information and ascertaining the main relationships between variables, it will be possible to develop new mathematical models based on PMV/PPD and adaptive models, for the evaluation and analysis of thermal comfort specific for this type of population. The methodologies presented, both for data collection during fieldwork and for the treatment, analysis, and development of mathematical models, will contribute to the development of knowledge on the topic of thermal comfort in the elderly.

KEY WORDS: analytical model; ASHRAE 7-point scale; data collection; elderly; questionnaire

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## Nutrition and teeth mineral content through the centuries

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Nutrition, among other factors, significantly affects the quality and mineral composition of teeth. Dental health depends on systematic factors, among which digestion, absorption and metabolism of trace elements as well as local factors in the oral cavity. Diet and lifestyle are linked with teeth quality, the focus of dentistry. Having the possibility to compare teeth originating from different periods of history and different lifestyles, we were curious to determine whether there is a difference in their mineral composition. Results showed that the mineral content in teeth has changed over the centuries. Among the samples analysed, encompassing the Medieval period, World War II and recent times, there were significant differences in elemental content as well as in the interrelationship of the elements. The largest differences were observed in the mean values of magnesium and zinc concentrations, while the mean values of calcium and phosphorus varied less between the teeth groups. Since one's diet is the most important route of intake of minerals and trace elements, the obtained results can be viewed in light of changes in eating habits over the centuries.

KEY WORDS: diet; eating habits; elemental content; food; medieval period

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## Prenatal exposure to tobacco smoke: H2AX phosphorylation levels in umbilical cord blood

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Tobacco smoking is still the leading preventable cause of human morbidity and mortality. Exposure to tobacco smoke and other environmental pollutants in the prenatal period has been shown to affect the aetiology of and susceptibility to diseases later in life, suggesting that further attention is needed on early-life environmental exposures. The current study, carried out within the frame of the NeoGene project, examined the effects of prenatal tobacco smoke on phosphorylation levels of the histone variant H2AX, a reliable biomarker of the formation of DNA double strand breaks in umbilical cord blood. This cross-sectional study enrolled pregnant women receiving prenatal care in a public hospital. At birth, along with other samples, umbilical cord blood samples ( $n = 573$ ) were collected and peripheral blood mononuclear cells were isolated and cryopreserved. Participants' smoking status and exposure to environmental tobacco smoke were defined on the basis of cotinine concentrations in urine (ELISA). H2AX phosphorylation was determined in all cord blood samples by flow cytometry. Results presented herein are expected to widen knowledge about the risks of prenatal exposure to tobacco smoke, and provide scientific support for the employment of health promotion campaigns in order to increase pregnant women's awareness on the risks of tobacco smoke to their health status as well to the health of their children, which may constitute an important stimulus for smoking cessation. This work was supported by FCT and FAPESP (FAPESP/19914/2014). JM, CC and AIS supported by FCT SFRH/BPD/115112/2016, SFRH/BPD/96196/2013 and SFRH/BD/145101/2019 grants, respectively.

KEY WORDS: cotinine; cross-sectional; flow cytometry; genotoxicity; newborns

P-33

## Exposure assessment to dioxins originating from domestic fireboxes and burning waste dumps in Serbia

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The objective of this study was to assess the inhalation intake of dioxins through developing exposure scenarios which cover a lifetime, i.e. the lifetime average daily dose – LADD (mg/kg/day). Dioxins predominantly originated from domestic fireboxes and burning waste dumps. Data on the annual dioxin emission for the period 2006-2012 were taken from the Updated National Implementation Plan for the Stockholm Convention. For different scenarios, quantities of 100, 1, and 0.001 % of the total annual production were taken. The assumed volume of inhaled air during exposure period was 60 m<sup>3</sup>, while for exposure duration and frequency we used data on 8 hours daily during the heating season of 180 days. The calculated LADDs in scenarios dioxins/burning waste dumps were higher than in the scenario dioxins/domestic fireboxes. The highest calculated LADD was 0.0619 mg TEQ/kg/day for females exposed to dioxins from burning waste dumps in 2007, while the lowest was 1.043 pg TEQ/kg/day for males exposed from domestic fireboxes. For risk characterization, we used the value proposed by the European Food Safety Authority of 2 pg TEQ/kg/week, which can be converted into 0.3 pg TEQ/kg/day. For most of the scenarios, inhalation exposure was higher than the safe one and risk could be considered unacceptable. Having in mind the exposure by food consumption calculated for Serbia in 2008 of 3.14 pg TEQ/kg/day, risk must be managed by a set of activities. Human biomonitoring would be of high importance, however discovering and putting wild waste dumps under supervision would be essential.

KEY WORDS: human biomonitoring; inhalation exposure; lifetime average daily dose; lifetime exposure; wild waste dumps

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## **BMDL5 for the effect of the cadmium and decabrominated diphenyl ether mixture on reactive oxygen species production: indication for co-exposure interactions?**

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The objective of this study was to assess the effect of cadmium (Cd) and decabrominated diphenyl ether (BDE-209) co-exposure on the formation of reactive oxygen species (ROS) by calculating the lower confidence dose limit that can cause a significant increase in effect of 5 %, i.e. the benchmark dose (BMDL5). The calculated BMDL5s and dose response curves steepness and slope were used as indicators of possible interactions. The human colon carcinoma cell line (SW 480) was used as a model for the intestinal system. The concentration range used in the experiment included 2.5, 7.5, or 15 µg Cd/mL (corresponding to 22, 67 or 134 µM, respectively), 2.5, 5, and 10 µg BDE-209/mL (corresponding to 2.61, 5.21, or 10.42 µM, respectively), all nine co-exposure combinations, and control. The formation of ROS in cells treated with single chemicals or co-exposed to Cd and BDE-209 was determined by DCF-DA assay. The dose-response relationship was assessed using the PROAST software (RIVM, The Netherlands). The derived BMDL5 for ROS production was 2.57 for BDE-209, while for single Cd, the effect on ROS could not be determined. However, with the increase of the Cd concentration in mixture with BDE-209, BMDL5 also increased. A possible explanation for the more potent BDE-209-than Cd-induced ROS production could be explained with the fact that Cd not only inhibits antioxidative enzymes, but also influences the non-enzyme compounds of this protective system. Moreover, chronic exposure to high doses of Cd could cause a decrease in ROS production through damage to and dysfunction of mitochondria, the most important source of intracellular ROS. For the first time, we derived a lower confidence limit of BMD5 for the mixture of Cd and BDE-209. Interestingly, the slope and steepness of dose response curves implied a reduction in ROS production with an increase of Cd as a covariance to the BDE-209, i.e. an antagonistic type of interaction.

KEY WORDS: BDE-209; benchmark dose for co-exposure; mitochondria dysfunction; PROAST; SW480 cells

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## Use of veterinary drugs in the region of Sjenica as a potential source of environmental pollution

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The objective of this study was to evaluate the use and potential environmental contamination with veterinary drugs and their metabolites in the region of Sjenica (Serbia) by examining the views of livestock breeders and veterinary medicine doctors. Contamination is expected from the abuse of veterinary drugs, lack of consultations with veterinarians, and improper handling of waste from animals. The survey questionnaire was used to obtain information on the most frequently used veterinary drugs, use of manure, reasons for calling doctors of veterinary medicine by the breeder, as well as use of veterinary medicines without the supervision of a veterinary MD. Veterinary drugs enter the environment mostly due to inadequate management of pharmaceutical waste and their widespread use, but also through metabolite elimination from the organisms. The obtained data show that the attitudes of the veterinary medicine doctors and domestic animal breeders on the use of veterinary drugs are very similar and in accordance with the data obtained by the Sjenica Veterinary Station. The most commonly used veterinary drug was a combination of antibiotics, streptomycin, and benzylpenicillin-procaine, antihelmintic, albendazole, which has long been used in veterinary practice, while unexpectedly, the third was formaldehyde, a chemical widely used as a disinfectant in poultry. The results have shown that breeders frequently treat animals with drugs on their own, without control by a veterinarian. Veterinary drugs are applied in the Sjenica region for the adequate health care of a large number of cattle, but it is necessary to monitor and measure their concentrations in water and soil for the purpose of establishing levels of environmental and human exposure.

KEY WORDS: antibiotics; antihelmintics; disinfectant; formaldehyde; veterinary medicine misuse

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## Occupational poisonings recorded at the Croatian Poison Control Centre during a five-year period (2015-2019)

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The aim of this study was to investigate the characteristics of occupational poisonings referred to the Croatian Poison Control Centre (CPCC), in relation to current safety at work measures and prevention of adverse health effects. Data from the CPCC records for the period 2015-2019 were analysed. There were 233 workplace incidents resulting in symptomatic or suspected poisonings during the studied period. The most common causes were toxic gases/fumes (64 cases, yearly average 27 %), followed by organic solvents (53 cases, 23 %) and corrosives (45 cases, 19 %), with a notable increase in the proportion of cases caused by gases/fumes from 14 % in 2017 to 32 % in 2019. The main route of exposure was inhalation (149 cases, yearly average 64 %). Overall, 183 workers had mild symptoms (gastrointestinal, skin or respiratory irritation, or headache; yearly average 79 %), 23 were asymptomatic (10 %), while 27 had severe symptoms (12 %), mostly disturbances of the central nervous system and respiratory tract due to organic solvents or welding fumes exposure. The majority of cases involved men (67 % of total cases), with an additional declining trend of the percentage of females in the total number of occupational poisonings from 38 % in 2017 to 28 % in 2019. Although men prevailed in cases of occupational poisonings caused by all 3 groups of hazards, women were involved in a higher proportion of cases caused by corrosives than gases/fumes, and solvents (47 %, 17 %, and 21 % of total cases, respectively). Results indicate that safety policies at the workplace should focus on lowering inhalation exposure, improving respiratory protection measures and worker education.

KEY WORDS: corrosives; organic solvents; respiratory protection measures; safety at work; toxic gases and fumes

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## Contact allergy to bisphenol F epoxy resins in an occupational setting

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Diglycidyl ethers of bisphenol A and F epoxy resin (DGEBA-ER and DFEBF-ER, respectively) products are widely used in various industries. DGEBA-ER is among the most prevalent industrial contact allergens, but independent contact allergy to DGEBA-ER is considered rare due to the significant cross-reactivity between DGEBA-ER and DGEBA-ER. we present here a diagnostic approach to a group of workers occupationally exposed to epoxy paint. A group of 9 workers had been working on anticorrosive protection of oil tanks, including sand blasting, washing with acetone, and spraying with two-component epoxy-based paint preheated to 30 °C. After 5 months of work, one worker developed eczematous changes (erythema, itchy vesicles, induration) on the face, neck, hands, and forearms. The company's occupational physician initiated diagnostic procedures for a suspected occupational disease. All 9 workers were patch tested (PT) with the commercial DGEBA-ER allergen (1 % in petrolatum) with negative results. Analysis of safety data sheets determined that the relevant paint contains >70 % DGEBA-ER. Additional PT was performed in workers and two control subjects with in-house 0.5 % and 1 % paint preparations provided from the workplace. The worker with skin symptoms showed a positive skin reaction, with an intensity of reaction following increase in concentration, while PT was negative in others. The worker was diagnosed with occupational allergic contact dermatitis caused by DGEBA-ER. As cross-reactivity between DGEBA-ER and DGEBA-ER is not equally presented for all DGEBA-ER isomers, independent sensitisation to DGEBA-ER should be evaluated in cases with typical exposure and clinical appearance, and negative PT with DGEBA-ER allergen.

KEY WORDS: anticorrosive protection; cross-reactivity; diglycidyl ether of bisphenol A epoxy resin; diglycidyl ether of bisphenol F epoxy resin; patch test

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## Toxic substances in cosmetic preparations

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Cosmetic preparations contain a large number of toxic ingredients. These compounds can have a number of negative consequences, most often in the form of allergic reactions, skin irritations, hormonal disorders, and increased risk of cancer. Chemicals are most often added to protect, soften or otherwise make the product more usable. The aim of this study was to determine the presence of certain toxic substances by qualitative analysis of cosmetic products using gas chromatography-mass spectrometry (GC-MS) and to compare them with the data stated on the product declaration. A total of 66 samples were analysed: 10 baby shampoos and bath products, 26 cosmetic products for the axillary area and 30 samples of creams and serums. In 4 samples of baby shampoos and bath products (40 %), the presence of harmful ingredients was proven (parabens and phenoxyethanol), while diethyl phthalate was detected in all samples but was not stated in any of the declarations of these samples. Parabens were detected in 7.7 % of the products for application in the axillary area and diethyl phthalate was detected in 19 % of these samples. In 24 samples of creams and serums (80 %), the presence of toxic substances butylhydroxytoluene, cyclopentasiloxane, cyclotetrasiloxane, benzophenone, triethanolamine, phenoxyethanol, diethylphthalate was noted. These studies have proven the deviation of the actual undesirable substances in the samples from those declared. Phthalates were most often not declared on products. Better regulation of product declarations is needed, as well as stricter controls regarding the impact of primary packaging on product composition after packaging.

KEY WORDS: cosmetics; GC-MS method; parabens; phthalates; toxic ingredients

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## Effects of low-level exposure to a mixture of organic solvents on oxidative stress in shoe industry workers

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Occupational exposure to a mixture of organic solvents is more common than exposure to a single solvent. This study aimed to investigate the relationship between the inhalation exposure to a mixture of organic solvents and its capacity to induce oxidative stress and liver damage in men employed in the shoe industry. The study included 16 male workers and 30 healthy unexposed male controls. The superoxide anion ( $O_2^-$ ), advanced oxidation protein products (AOPP), total oxidative status (TOS), prooxidative-antioxidative balance (PAB), oxidative stress index (OSI), and antioxidative defence parameters [superoxide dismutase (SOD) enzyme activities, values of SH groups, and total antioxidant status (TAS)] were determined in all of the subjects. Additionally, liver enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transpeptidase (GGT)] and bilirubin direct (BD) were also analysed in the workers. The obtained results showed that the investigated oxidative stress parameters ( $O_2^-$ , PAB, TOS, and OSI), as well as the antioxidative defence parameters, were significantly higher ( $p < 0.001$ ) in exposed workers compared to controls. However, AOPP was not different in workers compared to controls. Bilirubin direct and AST values were elevated in 68.75 % of workers and AST and GGT in 37.50 % of workers. Furthermore, AST, ALT, and GGT were significantly positively correlated with TOS, while ALT was correlated with OSI ( $p < 0.05$ ). These results suggest that occupational exposure to even the permissible levels of individual solvents present in the mixture may induce oxidative stress in the plasma of workers, proving that oxidative stress is among the mechanisms that positively contribute to hepatotoxicity development.

KEY WORDS: antioxidative defense; hepatotoxicity; liver enzymes; mixture toxicity; occupational toxicology



P-40

## Strawberry tree (*Arbutus unedo* L.) honey extract ameliorates the genotoxic effects of irinotecan but leads to cell cycle delays in human peripheral blood lymphocytes *in vitro*

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This study assessed the genotoxic profile of strawberry tree honey extract (STHE) (*i.e.* extracted phenolic fraction of STH) and its potential protective effects against cytogenetic damage induced by the anticancer drug irinotecan (IRI) in human peripheral blood lymphocytes *in vitro*. Isolated lymphocytes were exposed for 2 h to three different concentrations of STHE (corresponding to an average daily portion of 50 g of honey, as well as five- and ten- fold higher concentrations) alone and in combination with IRI. The concentration of IRI corresponded to its therapeutic dose of 350 mg/m<sup>2</sup>. The outcomes of the treatments were evaluated using an analysis of chromosomal aberrations and the cytokinesis-block micronucleus (CBMN) cytome assay. Although STHE increased the incidence of chromosomal aberrations compared to negative controls at all of the tested concentrations, when administered with IRI, it showed significant protective effects. Single STHE did not produce significant changes in the number of MN and nuclear buds (NB) compared to negative control. When given in combination with the tested anticancer drug, STHE effectively diminished the incidence of both MN and NB compared to single IRI. The most prominent effect of STHE observed both after single and combined treatments was its cell-cycle delaying activity, which resulted in significantly lowered values of the replication index. The observed cytostatic effect could be associated with the chemical composition of the tested extract, whose constituents exhibited both prooxidative and antioxidative properties. The convincing results obtained *in vitro* speak in favour of future investigations of STH, as well as further development of STH-based nutraceuticals, potentially useful even in cancer patients. This study was financially supported by the Croatian Academy of Sciences and Arts.

KEY WORDS: anticancer drug; chromosomal aberrations; cyto/genoprotective effects; micronucleus assay; white blood cells

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## Influence of bearberry extract (*Arctostaphylos uva ursi* L.) on gastrointestinal bacteria adhesion

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Bearberry extract has for centuries been used in traditional and modern medicine to treat and alleviate problems caused by urinary infections. Nevertheless, the exact mechanism of action of biologically active compounds from bearberry extract to date has not been fully defined. In this study the cytotoxic effect of bearberry water extract, including a comparison between fresh and freeze-dried extract effects on bacteria *Escherichia coli*, *Staphylococcus aureus*, *Lactobacillus plantarum* and *Lactobacillus fermentum* was studied. Furthermore, the influence of the extract on the adhesion of the aforementioned bacteria to human epithelial gastrointestinal tract cells in an *in vitro* system was studied. Before the adhesion tests, cytotoxicity tests using the Neutral red method were carried out together with tests of potential antioxidant activity of the extract using the DCHF-DA method on human tongue squamous cell carcinoma cell line (CAL 27) and human epithelial colorectal adenocarcinoma cells (Caco-2). The results obtained showed a strong antimicrobial effect of this extract towards Gram-positive bacteria as well as its ability to cause changes in the adhesion of bacterial cells to the epithelial cells of the digestive system. Antioxidant activity of the extract was established and stimulation of human cell proliferation was determined, all of which depended on the concentration of the extract and the type of treated cell. No significant difference was observed by comparing the biological activity of freshly prepared and freeze-dried bearberry water extract.

KEY WORDS: antioxidant activity; Caco-2; CAL 27; toxicity; urinary infection

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## Trace metal(loid)s and synthetic acaricides in organic vs. conventional chestnut honey

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Honey is a nutritionally valuable natural product of honey bees which can be contaminated by anthropogenic chemicals from the surrounding environment and beekeeper practices. Good beekeeping in conventional practice and regulations on organic beekeeping aim to reduce harmful residues in honey for human consumption. We compared prominent metal(loid) contaminant (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) and acaricide (pesticides used for treatment of beehives for Varroa mite control) levels in 40 samples of Croatian chestnut honey (*Castanea sativa*) from the Banovina region (Banski med, Croatia; 4 organic, 26 conventional) and Ozalj (2 organic, 8 conventional). Metal(loid)s were quantified using an Agilent 7500cx inductively coupled plasma mass spectrometer and acaricides using an Agilent Technology 1260 HPLC system coupled with a Triple Quad LC/MS 6410 mass spectrometer. All honey samples were safe for consumers regarding contaminant residues. Anti-varroa drug residues were not quantified in certified organic honeys (ORG), while amitraz (sum of parent compound and metabolites; range: 0.007-0.035 mg/kg), DMF (N-(2,4-dimethylphenyl) formamide; 0.005-0.018 mg/kg) and coumaphos (0.005-0.031 mg/kg) were detected in 9, 8, and 25 samples of 34 conventional honeys (CONV), respectively. The potentially toxic Cr was the only metal(loid) that showed a statistically significant difference between ORG and CONV (median 5.74 vs. 1.98 µg/kg, respectively; U=31, p=0.009, Mann-Whitney U test) honey groups. On average, this small scale study found organic chestnut honey less burdened with acaricides and more contaminated with Cr residues than conventional honey.

KEY WORDS: amitraz; anthropogenic contaminants; coumaphos; pesticide; toxic metal

P-43

## Food-drug interactions: focus on fruit juices

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Fruit juices contain numerous phytochemicals and bioactive compounds, which, in combination with certain drugs (food-drug interactions), can pose a risk for human health and have unwanted effects on the desired pharmacological outcome. Depending on the nature, such interactions are categorized as pharmacokinetic or pharmacodynamic. In other words, drug activity is changed due to a change in absorption, distribution, metabolism, and elimination of a certain drug, or due to a change at its site of action. In most cases, the mechanisms behind food-drug interactions are related to fruit juice interference with the activity of cytochrome P450 metabolizing enzymes (CYPs) or drug transporters, whereas phytochemicals can act as inhibitors (or inducers). The clinical relevance of food-drug interactions precipitated by fruit juices is especially important with drugs that have a narrow therapeutic index, as relatively small changes in the drug plasma concentrations can lead to adverse effects, i.e. drug toxicity. Unfortunately, the potential for food-drug interactions caused by fruit juices, in addition to clinical relevance, cannot be easily predicted. However, examples of potentially relevant drug-fruit juice interactions with suggested/known mechanism in humans noted so far would be presented. By summarizing, the best advice is to take the prescribed medication with water, and/or consult a specialized healthcare professional about the correct administration of drugs.

KEY WORDS: adverse events; CYP enzymes and drug transporters; inhibition and induction

P-44

## Mercury and selenium concentrations and their molar ratios in commonly consumed wild, farmed, frozen, and canned marine fish in Croatia

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Fish is a highly valuable staple food and a main source of mercury (Hg) exposure in the general population. Among the trace elements contained in fish, selenium (Se) has the potential to sequester Hg and reduce its toxicity. Our study aimed to determine concentrations of total Hg and Se, as well as their molar ratios in muscle tissue of commonly consumed marine fish species in Croatia to evaluate consumer health risk. Based on the EUROFISH survey (2017), six species of fresh and frozen (pilchard, hake), wild and farmed (gilthead seabream, seabass) and canned fish (pilchard, mackerel, tuna) were selected for analysis. Fresh wild specimens from the Adriatic Sea ( $n=97$ ) were purchased in fish markets, and farmed fish ( $n=24$ ), frozen ( $n=8$ ) and canned ( $n=37$ ) products in supermarkets. Elements were analysed by ICP-MS. Concentration (mg/kg wet wt) ranges were 0.009-1.98 for Hg and 0.050-1.09 for Se, with the highest Hg obtained in wild gilthead seabream and the highest Se in canned tuna. Only in wild gilthead seabream, mean Hg exceeded the European regulatory limit (0.50 mg/kg). Element levels significantly differed between species. In all fish species, a mean Se:Hg molar ratio  $>1$  was obtained, which is considered safe. Given the common intake of two fish servings per week and mean Hg of 0.192 mg/kg, there is no risk of increased dietary Hg exposure except from wild gilthead seabream, but this nevertheless requires systematic Hg monitoring. This study was partially funded by Croatian Science Foundation grant HRZZ-IP-2016-06-1998.

KEY WORDS: Adriatic Sea; consumer health risk; food safety; toxic metal; trace element

P-45

## Mycotoxin levels in Croatian brewer's and feed barley

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Barley is a widely spread cereal sown for the malting and brewing industry (*Hordeum vulgare* convar. *distichum*), and as feed (*Hordeum vulgare* convar. *hexastihum*). The main difference in the chemical composition between these two is protein content, which is higher in the latter. In the feed industry, maximum concentrations of mycotoxins are regulated by the EU (Commission Regulation (EC) No 1881/2006), while maximum concentrations of mycotoxins in the malting and brewing industry are not. In this study, we compared the concentrations of the following mycotoxins in 10 samples of brewer's and 10 samples of feed barley by ELISA tests: aflatoxins (AFT), deoxynivalenol (DON), and zearalenone (ZEN). All samples were collected in the harvest season 2019, which had a lot of rain in the barley flowering period, when cereals are susceptible to *Fusarium* attacks. Analysis showed that DON was a prominent contaminant in both barleys with an average contamination level of 1011 µg/kg; followed by ZEN with an average contamination level of 127 µg/kg, while aflatoxins were not detected. When comparing the two types of barley by Mann-Whitney *U* test, there were no statistically significant differences, although feed barley had higher mean concentrations of both of the detected mycotoxins. Six samples in total exceeded the maximum allowed concentrations for DON in raw cereals, and three samples exceeded the maximum allowed ZEN concentrations. Due to the specific processing of brewer's barley which includes malting, drying and mashing that increases *Fusarium* contamination, modification of DON (masking), and increases DON extraction rate, it is recommended to include beer in DON legislation.

KEY WORDS: aflatoxins; beer brewing; deoxynivalenol; ELISA; zearalenone

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## Cytotoxic and genotoxic activity of *Fusarium* mycotoxins, deoxynivalenol and zearalenone in HepG2 cells

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The aim of the study was to evaluate the cytotoxic and genotoxic activity of mycotoxins deoxynivalenol (DON) and zearalenone (ZEA), two common contaminants of food and feed found worldwide. Human hepatocellular carcinoma (HepG2) cells were treated with DON (0.25-16 µmol/L), ZEA (1.25-80 µmol/L) or their combination (1 µmol/L+5 µmol/L; 1 µmol/L+10 µmol/L or 1 µmol/L+20 µmol/L, respectively) for 24 h, and cell viability was assessed with the MTS assay. For genotoxicity experiments, cells were treated with DON (0.5, 1, and 2 µmol/L), ZEA (5, 10, and 20 µmol/L) or their combination (1 µmol/L+5 µmol/L; 1 µmol/L+10 µmol/L or 1 µmol/L+20 µmol/L, respectively) for 24 h, and a comet assay was performed. DON (2 µmol/L) and ZEA (80 µmol/L) reduced cell viability to 70.5 and 55.2 %, respectively, while combined exposure increased cell survival from 16.2 to 24.5 % (P<0.05). Results imply that both mycotoxins are cytotoxic to HepG2 cells. DON and ZEA in single and combined exposures did not increase the percentage of tail DNA (P>0.05), suggesting that these two mycotoxins do not induce DNA single strand breaks. Due to climatic changes and the global food trade, humans can be repeatedly exposed to DON and ZEA as well as their combinations; therefore, the underlying mechanisms of their single and combined toxicity have to be further explored. Acknowledgement to bilateral collaborations between the Republic of Slovenia and Republic of Croatia BI-HR/18-19-003; BI-HR/20-21-019.

KEY WORDS: cell survival; co-exposure; comet assay; *in vitro*; single and combined exposures

P-47

## Evaluating the toxicity of selected tyrosine kinase inhibitors in HepG2 cells

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Tyrosine kinase inhibitors (TKIs) are successfully used chemotherapeutics with more than 30 drugs applied clinically. They are small molecules directed against specific mutated tyrosine kinases to interfere with the signalling pathways deregulated in certain cancers. Most of these drugs are rationally designed to directly inhibit the catalytic activity of the mutated kinase by blocking the ATP binding site and consequently inhibiting proliferation, migration, differentiation and growth of cancer cells. Due to an increasing number of successful therapies, with less frequent and mostly of mild or moderate severity and usually reversible side effects, they are becoming one of the most consumed anticancer drugs. Therefore, the unintentional exposure of healthy healthcare workers and patient's family members to the residues of these drugs is expected to increase. However, the information that allows any hazard characterisation and risk assessment of indirectly exposed healthy population to TKI residues is limited. Therefore, we explored the cytotoxicity and genotoxicity of three TKIs; dasatinib, sorafenib and regorafenib, in HepG2 cells. Genotoxicity was determined using the comet assay and γH2AX assay (marker for DNA double strand breaks). Additionally, the effects on cell proliferation (Ki67), cell cycle (Hoechst staining) and phosphorylation of histone H3 (ser28, marker of mitotic cells) were studied. The results showed different cytotoxic potential, with regorafenib being the most toxic followed by sorafenib and dasatinib. The study indicates that certain TKIs may interfere with DNA, presenting a higher risk for unintentionally exposed healthy populations. Therefore, there is an urgent need to assess their risk for healthy humans. *Supported by Slovenian Research Agency (P1-0245 and Z1-1854).*

KEY WORDS: cell cycle; cell proliferation; cytotoxicity; genotoxicity; histone H3

P-48

## Genotoxic effects of the cyanobacterial hepatotoxin cylindrospermopsin in an advanced *in vitro* hepatic model system

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Toxic cyanobacterial blooms are increasing globally due to climate change and water eutrophication. The cyanotoxin cylindrospermopsin (CYN) is considered an emerging health threat worldwide. It was shown to exert numerous harmful effects *in vitro* and *in vivo*. *In vitro* studies describing the genotoxic activity of CYN in metabolic competent test systems are accumulating. However, traditional *in vitro* test systems lack several phenotype specifics of the corresponding *in vivo* cell types and hence have low predictive value for human health hazard assessments. Therefore, the cyto/genotoxic activity of CYN was evaluated in an advanced 3D cell model developed from the human hepatoma HepG2 cell line. HepG2 spheroids were formed by a forced floating method and were cultured under static conditions for three days prior to exposure to CYN (0.125, 0.25 and 0.5 µg/mL) for 72 h. Cell viability was determined daily by the MTS assay and confocal microscopy using live/dead staining. The influence on cell proliferation, cell cycle alterations and induction of DNA double strand breaks (DSB) was determined using flow cytometry (γH2AX). The results showed that CYN dose-dependently reduced the size of spheroids and induced G1 phase cell cycle arrest. No increase in the induction of DSBs could be determined at the applied conditions. The advanced 3D HepG2 cell model provides more physiologically relevant information and increased data predictivity for adverse effects in humans due to its higher structure complexity and improved cellular interactions and can thus contribute to a more reliable genotoxicity assessment of chemicals, including cyanobacterial toxins.

KEY WORDS: 3D hepatic model; cell cycle; cyanotoxin; cytotoxicity; DNA double strand breaks

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## Utilisation of red LED light significantly reduces inadvertent DNA damage in plant comet assay

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The last decade has witnessed the revival of the comet assay as a method for evaluation of DNA damage and DNA repair in single-cells. When performed in plant cells, it may serve as an invaluable tool in environmental monitoring. However, due to issues related to nuclei extraction, inadvertent DNA damage due to photochemical reaction, lack of standardisation, and results interpretation, many laboratories have terminated their research. We were not able to find any data on the effect of red LED light on DNA damage in plant nuclei. Therefore we evaluated DNA damage in two parallel lines of the plant comet assay, where the entire procedure was performed either under red LED light or office light. The omnipresent and widely available ryegrass, *Lolium perenne* L. was chosen as a model. The leaves were collected in the urban area of Sarajevo (Bosnia and Herzegovina). Additional modifications to the original protocol were: use of freshly prepared PBS, increase in nucleus extraction buffer volume to reduce plant debris, increase in the electric field strength by 33.34 % in electrophoresis, use of thermocycler, and thin-wall PCR tubes to maintain semiliquid consistency of low-melting agarose until the embedment of isolated plant nuclei. The slides were stained with fluorescent dye DAPI (4',6-diamidino-2-phenylindole; 1 µg/mL) and analysed using Comet Assay IV software (Instem, UK). The results showed significant difference ( $p < 0.001$ ) in the cell tail intensity between the two experimental lines. We concluded that combining red LED light with the quick performance of the routine can significantly reduce inadvertent DNA damage in plant comet assays. This work was supported by the Environmental Protection Fund of the Federation of Bosnia and Herzegovina grant No: 01-09-2-1469/2021.

KEY WORDS: *Lolium perenne*; plant nuclei; protocol optimisation; SCGE assay; tail intensity

P-50

## Radioprotective effect assessment of arbutin in human leukocytes

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Ionising irradiation (IR) causes genotoxic effects in human cells by different mechanisms of action. It is documented that certain plant derivatives can offer protection for cells exposed to IR. Arbutin is a simple phenolic glucoside biosynthesised in various plant species and shows a range of beneficial properties in animal cells and tissues. The aim of this study was to assess the possible radioprotective effect of arbutin in human leukocytes exposed to a therapeutic dose of IR. Four different concentrations of arbutin (11.4 µg/mL, 57 µg/mL, 200 µg/mL, and 400 µg/mL) were applied to whole blood samples obtained from three consenting donors as a one-hour pretreatment before exposure to 2 Gy γ-irradiation and as treatment of blood samples without exposure to IR. Amifostine (250 µg/mL), a commonly applied radioprotector, was used in the same manner to compare the efficacy of radioprotection. Genotoxic and radioprotective effects were assessed by alkaline comet assay. Arbutin did not exhibit significant genotoxic effects toward human leukocytes. However, it caused more DNA damage than amifostine. When used as pretreatment to IR exposure, most of the arbutin concentrations lowered the values of all comet descriptors. Considering that the lowest tested concentration (11.4 mg/mL) efficiently lowered DNA damage in these experimental settings, we believe that further research should be conducted using even lower doses in order to clarify the mechanisms of arbutin and IR interaction.

KEY WORDS: amifostine; comet assay; genotoxicity; ionising irradiation; radioprotector

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## Assessment of water-pipe induced DNA damage in oral leukocytes among young adults from Sarajevo

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Hookah, also known as a water pipe or narghile, is a type of tobacco product that has seen increasing popularity in recent years in Bosnia and Herzegovina and countries in the region. Hookah users are commonly adolescents and young adults, who often believe that smoking a water pipe is a less harmful alternative to cigarettes. This study aimed to determine the genotoxic effects of hookah smoking using the comet assay in oral leukocytes of individuals who had used a hookah for more than one year. The study group consisted of 22 individuals who did not smoke cigarettes but who regularly smoked a hookah. The majority of participants (73 %) reported smoking at least one water pipe per week. As a control, 22 non-smoking subjects were selected to match smokers for age and geographical area. All participants were healthy adults aged between 18 and 29 years. DNA damage was analysed in 200 comets per each participant using Comet Assay IV software (Instem, UK). Log-transformed values of tail intensity (%) were compared among groups. Results of independent t-tests showed significantly increased DNA damage among hookah consumers compared to non-smokers ( $t=19.903$ ;  $p<0.0001$ ). According to the results, additional assessments of hookah smoking genotoxicity conducted in a larger group of participants using other biomarkers and also considering general air pollution content is recommended.

KEY WORDS: biomarkers; comet assay; genotoxicity; hookah smoking; tail intensity

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## Genotoxicity of $\alpha$ -cypermethrin in Wistar rats and their offspring during gestation

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Low-dose, long-term exposure of higher organisms to man-made chemicals including insecticides can contribute to cancer development by induction of primary DNA damage and its fixation in the form of mutations. *In utero* development is a particularly sensitive period for these adverse effects. In accordance with guidelines for sustainable agriculture, many synthetic insecticides have been replaced with substances based on naturally occurring insecticidal agents. Despite the wide use of these new substances, there is little knowledge regarding possible toxic effects at low doses presenting real-life exposure.  $\alpha$ -cypermethrin, a synthetic pyrethroid insecticide, is one such example. In this study, adult female Wistar rats were treated with  $\alpha$ -cypermethrin during the whole gestation period by gavage at doses representing the acceptable daily intake (ADI) (0.010 mg/kg bw/day), acceptable operator exposure level (AOEL) (0.015 mg/kg bw/day), and 1/100 LD<sub>50</sub> (0.57 mg/kg bw/day). The dams and their offspring were sacrificed within 24 h after birth and blood and liver samples were processed by alkaline comet assay. The results showed significantly elevated tail length at 1/100 LD<sub>50</sub> and tail intensity at AOEL in dam leukocytes. No significant changes were observed in dam liver cells. In male F1 offspring, tail intensity was significantly increased at ADI in leukocytes and at all doses in liver cells. Female F1 offspring demonstrated no changes of comet assay parameters in leukocytes but rather significant changes at all doses in liver cells.  $\alpha$ -cypermethrin might compromise genetic integrity via induction of primary DNA damage both in adult animals and their offspring, hinting at transplacental transfer.

KEY WORDS: alkaline comet assay; DNA damage; *in utero* development; pyrethroid insecticide; real life exposure

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## Background DNA damage in oral leukocytes in the group of healthy individuals from Sarajevo – preliminary data

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Human biomonitoring studies are used in assessing exposure to environmental genotoxins. The comet assay, used for measuring level of DNA damage in single cells, has applications in human biomonitoring and genetic ecotoxicology. The assay enables analysis of DNA strand breaks in different types of cells including oral leukocytes, which are suitable samples, as their collection is non-invasive and convenient especially for vulnerable populations. The main objective of this study was to commence comet assay-based evaluation of the background level of DNA damage in healthy individuals from Sarajevo (Bosnia and Herzegovina), analyse inter-individual variation, and correlate it with lifestyle habits or environmental pollution effects. Oral leukocytes were collected and isolated from 30 healthy individuals during the summer of 2019. Alkaline comet assay was performed and at least 200 comets were visually categorised. The total score of DNA damage was calculated and arbitrary units (0-800) were assigned to each total score. Results revealed high background DNA damage, even within the subgroup of individuals from 20-30 years old, with only 5 individuals in total having DNA damage lower than 10 %. This may be influenced by the constant air pollution and lifestyle factors that should be further investigated. However, as expected, age differences were significantly associated with the total score of DNA damage. Independent t-test revealed that smokers *versus* non-smokers did not significantly differ, as well as females *versus* males, although females had lower DNA damage. This work was supported by the Federal Ministry of Science and Education grant No: 05-39-2553-1/19.

KEY WORDS: biomonitoring; lifestyle; environmental genotoxins; visual comet scoring

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## **Biomonitoring occupational exposure to a mixture of organic solvents: genotoxic effects according to the workplace**

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Styrene and xylene are used in large quantities as organic solvents in a variety of industries. The aim of this study was to evaluate occupational exposure to a styrene and xylene mixture through environmental monitoring and identify the potential genotoxic effects using cytokinesis blocked micronucleus assay and comet assay in workers. The workers may be allocated to the following workplaces: (1) Shift Chief; (2) Production and Analysis Technician; (3) Reactor Operator; (4) Cargo Preparer; (5) Filling Operator. The results obtained by air monitoring were below the occupational exposure limits for both substances and the urinary metabolites for all workers exhibited values below the detection limit of the method (mandelic and phenylglyoxylic acids=0.16 g/L; methylhippuric acid=0.55 g/L). In the general analysis, all biomarkers of effect, except nucleoplasmic bridges, had higher mean values in workers (n=17) compared to the corresponding control group (n=12). Specifically regarding the workplaces occupied by the exposed individuals, the Shift Chief has a higher average of micronuclei (11.67±3.93). On the other hand, the Cargo Preparer has the higher average for nuclear protusions (3.00±2.04) and DNA damage (32.32±10.08). A higher mean of nucleoplasmic bridges was found in the Fill Operator (1.00±1.00) and the highest mean of oxidative damage was observed in the Reactor Operator (39.14±17.27). The obtained results demonstrate that effect biomarkers can support the identification of higher risk groups of workers and shape/guide the definition of health surveillance programs.

KEY WORDS: biomarkers; genotoxicity; monitoring; occupational health; workstation



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## Polyoxopalladates as potential antitumor drugs: *in vitro* toxicity assessment

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Polyoxopalladates (POPs) are a subclass of polyoxometalates (POMs) comprising discrete, anionic palladium(II)-oxo complexes. Although numerous studies have been conducted on the biological activity of POMs, their toxicity (e.g. non-selectivity) is frequently a limitation for real-world biomedical applications. Thus, the aim of this study was to evaluate the *in vitro* safety of the three POPs Pd<sub>3</sub>As<sub>8</sub>, SrPd<sub>12</sub>As<sub>6</sub>, and Pd<sub>13</sub>(PhAs)<sub>8</sub>, which exhibited strong antitumor activity against human neuroblastoma cell line SH-SY5Y, by performing a cyto/genotoxicity study on human healthy blood. Blood samples obtained from a healthy female donor were treated with three different concentrations (12.5, 25, and 50 μmol/L) of the POPs, and incubated at 37 °C for 4 and 24 h. A cytotoxicity (cell viability) assay was performed on isolated human peripheral blood lymphocytes stained with acridine orange and ethidium bromide. A genotoxicity test was carried out on whole blood by alkaline comet assay (microgel electrophoresis), and the percentage of tail DNA was used to assess the level of DNA damage. Pd<sub>13</sub>As<sub>8</sub> did not affect neither cell viability nor DNA damage, related to the control, at either of the investigated concentrations (after both 4 and 24 h). On the contrary, higher concentrations (25 and 50 μmol/L) of both SrPd<sub>12</sub>As<sub>6</sub> and Pd<sub>13</sub>(PhAs)<sub>8</sub> induced a statistically significant decrease in cell viability after 24 h (up to 42 %), and a relative increase of tail DNA (up to 3×) was observed at 50 μmol/L, after 24 h. Therefore, Pd<sub>13</sub>As<sub>8</sub> could be regarded as non-toxic to human healthy cells, whereas SrPd<sub>12</sub>As<sub>6</sub> and Pd<sub>13</sub>(PhAs)<sub>8</sub> require additional toxicity analysis.

KEY WORDS: antitumor activity; cell viability; comet assay; cyto/genotoxicity; polyoxometalates

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## Cyto/genotoxicity evaluation of promising antileukemic palladium-based drugs

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Polyoxometalates (POMs) are discrete, negatively charged metal-oxo clusters of early transition metal ions in high oxidation states. Their biological significance has greatly increased in recent years because of their approved anticancer, antibiotic, and antidiabetic properties. However, toxicity studies have reported adverse effects after *in vivo* POM studies, which limits their potential application in biomedicine. The aim of this study is to evaluate the *in vitro* cyto- and genotoxic properties of two polyoxopalladates(II) containing tetravalent metal ion guests, SnPd<sub>12</sub> and PbPd<sub>12</sub>, that were found to possess potent antitumor activities against the human acute promyelocytic cell line HL-60. For this purpose, blood samples obtained from a healthy female donor were treated with three different concentrations (12.5, 25, and 50 μmol/L) of the tested POPs, and incubated at 37 °C for 4 and 24 h, respectively. Cytotoxicity studies were performed on isolated human peripheral blood lymphocytes which were stained with acridine orange and ethidium bromide, and then viewed under a fluorescence microscope. The genotoxicity was tested in whole blood by alkaline comet assay (microgel electrophoresis). The percentage of tail DNA was used to determine the level of DNA damage. The obtained cytotoxicity results indicated that neither SnPd<sub>12</sub> nor PbPd<sub>12</sub> induced statistically significant alterations of cell viability related to the control, at all of the investigated concentrations. Moreover, the results of the comet assay showed that none of the tested POPs resulted in a statistically significant relative increase of tail DNA. Accordingly, both SnPd<sub>12</sub> and PbPd<sub>12</sub> could be considered as safe promising antileukemic drugs from a cyto/genotoxicity point of view.

KEY WORDS: antitumor drugs; cell viability; comet assay; *in vitro* toxicity; polyoxopalladates

P-57

## Selenium and trace metal levels in tissues of wild birds from an area contaminated with superhigh-organic-sulphur Raša coal and ash

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The Labin (Croatia) city area is a locality where local Raša coal had been mined at six localities from the 17<sup>th</sup> century until the late 1990s, while significant quantities of the coal were used in a local coal-fired power plant during the period 1970-2000. Raša coal belongs to a class of superhigh-organic-sulphur coals, as it contains high levels of sulphur and selenium, which was the reason for conducting ecotoxicological studies previously. Selenium is of great concern as it is characterised by a narrow range between dietary essentiality and toxicity. The aim of this study was to determine the concentrations of Se and some trace metals (iron, copper, arsenic, cadmium, and lead) in different tissues of wild pigeons (*Columba livia*) collected within the vicinity of the mentioned industrial areas and to compare them with those originating from an uncontaminated area. Element analysis was carried out by inductively coupled plasma mass spectrometry. Statistically significant differences in the concentrations of the investigated element in edible tissue (i.e. muscles) were found only for selenium. The average values of Se in muscle, liver, kidney, and brain of birds were 0.344, 0.575, 1.011, and 0.233 μg/kg fresh weight (fw), respectively. Only values measured in muscle tissue were significantly higher in pigeons from the area in comparison to those from the uncontaminated area. Still, these values were below the quantity intended for the consumption as a single meal and maximum levels permitted in food, respectively (50-100 μg/kg). These results are consistent with the increased concentration of selenium in soil, water, and vegetables from the study area, which means that wild pigeons are good biomarkers and can provide additional insight into the environment in which they live.

KEY WORDS: coal mining; ecotoxicology; element levels in food; wild pigeons

P-58

## **Environmental lead (Pb) exposure in captive and free-ranging European brown bears: whole blood monitoring 2011-2019**

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Lead (Pb), as a ubiquitous environmental pollutant of natural and anthropogenic origin, enters a bear's organism mostly via food and water. It is initially found in the bloodstream bound to red blood cells and reflects recent exposure. As a non-essential metal, lead causes adverse health effects on developing nervous systems (maternal transfer), renal, cardiovascular, haematological, immunological and reproductive systems. Unlike in humans or domestic animals, there is no threshold for ursid blood Pb levels. Information about lead exposure in this predator species is of high interest to conservationists. Our aim was to compare Pb levels across captive and free-ranging brown bears from two European populations (Dinara-Pindos and Carpathian) sampled between 2011 and 2019 using inductively coupled plasma mass spectrometry (ICP-MS). The median (range) level of lead in 32 bears from Croatia and 15 from Poland was 64.0 µg/L (5.08-187 µg/L) and did not differ between countries. Levels were similar to Scandinavian and Canadian brown bears, three-times higher than in Yellowstone black bears and three times lower than reported for giant pandas. Free-ranging bears had higher Pb (median 67.8 µg/L) compared to captive individuals (median 32.8 µg/L), although not significantly ( $t(47)=-1.94$ ,  $p=0.058$ ). Blood lead also displayed gender differences ( $t(47)=-2.26$ ,  $p=0.028$ ) with higher levels in female (median 80.6 µg/L) than in male (median 45.1 µg/L) bears. Eleven of the 47 individuals had blood levels over the threshold for an adult human (100 µg/L), but none surpassed the levels considered toxic for large domestic animals.

**KEY WORDS:** adverse health effects; ICP-MS; mammal; non-essential metal; pollutant

P-59

## Antioxidant enzymes and metallothionein in mother-newborn pairs exposed to cigarette smoke during pregnancy

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Maternal cigarette smoking may impair the pro/antioxidant balance, affect bodily element disposition and disturb nutrient and oxygen transport to the developing foetus. The aim of this study was to assess the effect of active maternal smoking during pregnancy on superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities and metallothionein (MT), cadmium (Cd), and zinc (Zn) concentrations in samples of maternal and umbilical cord blood and placenta of healthy mother-newborn pairs collected after term vaginal delivery. We compared results for non-smokers and smokers ( $n = 37$  each), grouped based on cotinine urine levels as a biomarker of tobacco smoke exposure ( $<0.25$  ng/mL in non-smokers and  $>100$  ng/mL in active smokers). Activity of SOD was 14 % lower in maternal plasma, 91 % higher in placenta and 12 % lower in cord plasma of smokers compared to non-smokers, whereas GPx and MT did not differ between the groups. These results accompanied decreased maternal blood Zn and increased placental Zn in smokers who also had significantly higher Cd in maternal blood and placenta than non-smokers. Cord blood Cd was low and did not differ between the groups. Although active maternal smoking contributed to Cd levels in maternal blood and placenta and Zn in placenta, there was no induction of increased placental MT synthesis. Overexpression of placental SOD can increase the susceptibility of offspring to oxidative stress and risk of disease development, even later on in life. The study was funded by the Croatian Science Foundation grant HRZZ-IP-2016-06-1998.

KEY WORDS: cadmium exposure; maternal blood; placenta; umbilical cord blood; zinc

P-60

## The effects of the *MT2A* –209A/G gene polymorphism and maternal smoking habits on trace elements in mother-infant pairs

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Metallothioneins (MTs) are a family of cysteine-rich metal-binding proteins with primary functions in the homeostasis of essential elements (especially Zn and Cu) and protection against metal toxicity. They also have a role in free radical scavenging and oxidative stress protection. In humans, of the four known MT isoforms, MT1 and MT2 are the most prevalent. However, MT2 synthesis seems predominantly induced by toxic metal ions. In the *MT2A* gene, 24 single nucleotide polymorphisms were identified, but only several were shown to affect physiological and pathophysiological processes as well as element levels in the body. The aim of this study was to assess the genotype frequencies of *MT2A* –209A/G for the first time in Croatia (postpartum women,  $n = 401$ ) and its effects on element levels in maternal blood, placenta, and cord blood. PCR-RFLP was used for *MT2A* –209A/G genotyping and ICP-MS for trace element analysis. Results showed that 60.6 % of subjects were homozygous dominant (AA), 36.2 % heterozygous (AG), and 3.2 % homozygous recessive (GG). Maternal smoking increased Cd and Pb in all of the analysed samples, Fe and Cu in cord blood, and Zn in the placenta. In smokers, G allele carriers (AG/GG) vs. AA genotype had lower Cu in maternal blood and placenta and no differences were found for other elements. Maternal smoking habit affected element levels in all compartments of the maternal-placental-foetal unit; however, the effect of the *MT2A* –209A/G polymorphism on trace element levels should be further explored in a larger population. This study was funded by Croatian Science Foundation grant HRZZ-IP-2016-06-1998.

KEY WORDS: maternal blood; metallothioneins; placenta; single nucleotide polymorphism; umbilical cord blood

P-61

## Bioaccumulation and intracellular distribution of trace elements in the liver of northern pike (*Esox lucius* L., 1758) from the Mrežnica River: the METABIOM project

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The METABIOM project was designed to detect the consequences of increased metal exposure in aquatic biota in the Mrežnica River at the site of historical contamination originating from the cotton industry in Duga Resa in comparison to upstream and downstream locations. In the northern pike (*Esox lucius*), increased hepatic bioaccumulation was observed at the former factory site for Ag, Bi, Cd, Cu, Rb, V, and Zn, which could be associated to higher concentrations of several elements in the river sediment and with a higher portion of the finer sediment fraction (<2 mm) at that site. Increased levels of Mo, Tl, Mn, Co, and As at the upstream site indicated a possible agricultural origin. Cs and Fe were highest at the downstream location. High metal presence in hepatic cytosols indicated higher potential for toxic effects of Cd, Cs, Cu, Rb (>90 %), Ag, Bi, Co, and Zn (71-90 %). For ten elements, analysis by size exclusion HPLC revealed the molecular masses of cytosolic biomolecules which bind specific metals in the fish liver. The results indicated the predominant binding of Ag, Cd, and Cu to metallothioneins (MTs) and, thus, their presumable complete detoxification under the studied conditions. Probable binding to MTs was also observed for Bi and Zn, which were, however, additionally present in the high (>100 kDa) and medium (30-100 kDa) molecular mass protein fractions. These results will contribute to a better understanding of the impact of legacy contamination sources on aquatic biota, as well as metal bioaccumulation patterns in *E. lucius*.

KEY WORDS: fish; metallothionein; metals; proteins; size exclusion HPLC

P-62

## Characterization of the water and sediment quality of the lower course of the Mrežnica River: the effect of long-term historical contamination

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The lower course of the Mrežnica River has long been exposed to different types of industrial loads. In Duga Resa, the cotton industry has been active for more than a century, and a few kilometres downstream, industrial wastewaters of Karlovac entered the river until approximately a decade ago. Our aim was to determine the main inorganic and organic contaminants of river-water and sediments at two differently polluted sites compared to an upstream reference site. Turbidity, chemical oxygen demand, and total nitrogen were somewhat increased in Karlovac, whereas total phosphorus was increased at the reference site. Although dissolved metal concentrations in the water were rather low, indicating a pristine river, an increasing trend towards Karlovac existed for several elements (e.g., Fe, Mn), where the highest metal concentrations in the finer sediment fraction (<2 mm) were also observed. The highest portion of finer fraction in total sediment observed in Duga Resa (100 %) compared to the other sites (reference 13 %, Karlovac 54 %) probably lead to a higher metal bioavailability at both industry-impacted sites compared to the reference site. The screening of 626 organic compounds in the river-water revealed ten-fold higher total concentrations in Karlovac compared to the other sites, and their types varied by sampling site. Herbicides used in agriculture (atrazine) were detected upstream, whereas herbicides applied in water tanks (neburon) were found in Karlovac. The results indicated mild agricultural impact at the reference site and still visible industrial contamination at downstream sites, especially Karlovac, despite the cessation of industrial activities and wastewater effusion into the river.

KEY WORDS: herbicides; metals; nutrients; organic contaminants; river-water

P-63

## Ecotoxicity assessment of samples during advanced oxidation processes

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The presence of pharmaceuticals in water is an emerging environmental problem due to their potential impact on human health and the environment, even at low concentrations. Effluents from municipal wastewater treatment plants have been identified as major sources of pharmaceuticals in aquatic environments and can be characterised as highly toxic, thus requiring efficient treatment. The technologies based on advanced oxidation processes (AOPs) may offer a viable solution in terms of effluent detoxification. However, potential adverse effects during treatment can possibly be associated with the formation of degradation by-products and should be taken into consideration. In this study, photo-oxidative and photo-catalytic AOPs were applied for the degradation of polar and non-polar pharmaceuticals in water, oxytetracycline (OTC) and memantine (MEM), respectively. The obtained results were correlated with biodegradability and toxicity in assessing the environmental aspects of the applied treatment processes. Acute toxicity bioassays were performed according to standard procedures using the freshwater planktonic crustacean *Daphnia magna* (ISO 6341:2012) and freshwater microalgae *Selenastrum capricornutum* (ISO 8692). For the bioassay on *D. magna*, a light table and was used, while the bioassay on *S. capricornutum* used a spectrophotometer with a long-cell holder for optical density measurements. All of the experiments were carried out in a laboratory incubator. Among both pharmaceuticals, OTC has shown higher toxicity for *Selenastrum capricornutum* than MEM, while both compounds showed a similar level of toxicity in *Daphnia magna*. The toxicity levels determined for samples collected through the studied AOP-processes therefore depended on the test organism chosen for the bioassay. This study was supported by the European Structural and Investment Fund project Water Purification and Energy Conversion using Novel Composite Materials and Solar Irradiation (KK.01.1.1.04.0001).

KEY WORDS: *Daphnia magna*; ecotoxicity; pharmaceuticals; *Selenastrum capricornutum*; wastewater treatment

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## Assessment of cytotoxic and genotoxic effects of tyrosine kinase inhibitors on cultured zebrafish liver cells

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Tyrosine kinase inhibitors (TKIs) are small molecules used as a first- or second-line therapy in different types of cancer. Due to the widespread use of TKIs, they represent a potential risk for aquatic non-target organisms, as they are excreted through a patient's body fluids. Therefore, we aimed to investigate the cytotoxic and genotoxic activity of three frequently used TKIs, namely erlotinib (10, 20 and 40  $\mu\text{M}$ ), nilotinib (5, 10 and 20  $\mu\text{M}$ ) and sorafenib (1, 2 and 4  $\mu\text{M}$ ) on the zebrafish (*Danio rerio*) liver (ZFL) cell line. Cytotoxicity was determined with flow cytometry by live/dead cell discrimination and cell cycle analysis, while genotoxicity was evaluated by two modifications of the micronucleus test; (i) high content screening micronucleus determined by flow cytometry and (ii) cytokinesis-block micronucleus (CBMN) assay. After 72 h of exposure, both nilotinib (20  $\mu\text{M}$ ) and sorafenib (4  $\mu\text{M}$ ) reduced the viability of ZFL cells, while none of the TKIs significantly affected the cell cycle of ZFL cells. Moreover, TKIs did not affect genomic stability of the cells as no micronuclei formation was determined by any of the method variants. In addition, none of the TKIs induced the formation of nucleoplasmic bridges and nuclear buds, yet nilotinib and sorafenib significantly influenced the nuclear division index. In conclusion, the results indicate that although TKIs at non-cytotoxic concentrations had no genotoxic effect on ZFL cells, they did affect ZFL cells viability, thus representing a potential risk for aquatic organisms. This study was supported by the Slovenian Research Agency projects P1-0245 and J1-8140.

KEY WORDS: cytokinesis-block micronucleus assay; cytotoxicity; *Danio rerio*; flow cytometry; genotoxicity

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## Effects of herbicides and fungicides on behaviour and biomarker responses in the earthworm

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Intensive use of pesticides in agriculture can have a detrimental effect on soil ecosystems and soil organisms. Despite the frequent use of pesticides and high number of studies conducted on the adverse effects of pesticides, there is still a lack of information on the effects of some pesticides in non-target organisms. Therefore, the aim of this study was to assess the impact of a herbicide and a fungicide on the earthworm *Eisenia andrei*. The commercial herbicide formulation Koban T contains the active substances pethoxamide (300 g/L) and terbuthylazine (250 g/L), and the commercial fungicide formulation Pyrus 400 SC contains pyrimethanil as its active ingredient (400 g/L). The effects of these pesticides on earthworms were assessed using an artificial soil test and assessing the changes in behaviour of earthworms, activities of several enzymes [acetylcholinesterase (AChE), carboxylesterase (CES), catalase (CAT), glutathione-reductase (GR), glutathione S-transferase (GST)], and the multixenobiotic resistance activity (MXR). Both of the investigated pesticides caused the avoidance of treated soil. Changes in the measured enzyme activities were more prominent after exposure to herbicide. Both pesticides caused changes in MXR activity – the herbicide caused an inhibition of MXR activity, whereas exposure to the fungicide resulted in an inhibition of MXR activity at lower concentrations and induction of MXR activity at higher concentrations. The results showed that both of the investigated pesticides affected the measured parameters in earthworms and exposure to these pesticides could have harmful effects on earthworms.

KEY WORDS: avoidance response; *Eisenia andrei*; enzyme activity; multixenobiotic resistance activity; pesticides

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## Harnessing the filtration proficiency of Mediterranean mussel *Mytilus galloprovincialis* for water column microplastics biomonitoring

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Microplastics have become ubiquitous in the aquatic environment and represent a potentially significant threat to marine organisms not only by physically blocking their gut upon ingestion but also impacting at the cellular level by acting as vectors for other pollutants. In recognition of the growing threat from microplastics, the multilateral project "Marine litter cross-border awareness and innovation actions" has kicked-off. Within this framework, a Mussel Microplastic biomonitoring and Purification System (MMPS) has been manufactured and deployed in coastal waters with the aim of using the Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819 as a bioaccumulator of microplastics both for biomonitoring and purification purposes. In laboratory studies, MMPS reliably enabled the uptake of cigarette butt microfibres (CBMs), one of the most common plastic fibers found in the marine coastal ecosystem. While not showing adverse effects, the mussels within MMPS efficiently removed CBMs from the water column with a high turnover rate (uptake/release). Specifically, high levels of CBMs were found in the faeces and pseudofaeces of mussels within 4 h from the start of exposure to CBMs while nearly complete egestion of microfibers was noted during mussel recovery in the first 48 h after removal of the source of pollutant. The installation of MMPS at five Rovinj and two Dubrovnik coastal locations in Croatia, and bimonthly microplastic collection and analysis over a period of 1 year (July 2021-August 2022), are providing some of the highest density temporal data in the Adriatic for the type of microplastics present, with subsequent chemical analysis aimed towards developing a toxicity profile for those locations based on microplastics as a vector for other pollutants.

KEY WORDS: biomonitoring; cigarette butt; microfibers; microplastics; purification



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## Comparison of metal accumulation, intracellular metal distribution, and metal-binding biomolecules in the digestive gland of two freshwater bivalve species from the Mrežnica River

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Metals are common pollutants in aquatic systems, but they are also crucial for the normal functioning of organisms. Freshwater bivalves, as filter-feeders, are suitable bioindicators of metal contamination but their accumulation capacity and intracellular distribution may vary by species. Therefore, the aim of this study was to compare the bioaccumulation and intracellular distribution of nine selected metals/metalloids (essential: Cu, Zn, Fe, Co, Mo and Se; non-essential: Cd, Ag and As) in the digestive gland of two freshwater bivalve species, *Anodonta anatina* and *Unio pictorum*, collected from a reference site on the Mrežnica River (Croatia). Higher total metal concentrations were found in *U. pictorum* for all of the elements studied, except for Ag where concentrations were comparable in both species. The largest differences were found for Cd being 13 times higher, and for Co and Fe with about 3.5 fold increased values in *U. pictorum*. In both species, Cd and Se were primarily accumulated in the cytosol, while Fe was found mainly in the insoluble tissue fraction. The distribution of Cd and Cu did not differ significantly between species, while for all other elements except As, a significantly higher presence in the cytosolic fraction was found in *A. anatina*. Analysis of metal distribution among cytosolic biomolecules by size exclusion HPLC allowed the definition of molecular masses of biomolecules involved in binding of particular metals. The obtained metal profiles were comparable in both of the studied species for all of the studied elements. Our results will contribute to a better understanding of accumulation and metal metabolism in the studied freshwater bivalve species.

KEY WORDS: bioaccumulation; bivalves; metabolism; metal profiles; proteins

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## A first investigation of mercury in body feathers of nestling white-tailed eagle *Haliaeetus albicilla* from Croatia

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The Kopački rit Nature Park is the most important breeding site for white-tailed eagles (*Haliaeetus albicilla*) in Croatia. The present study therefore investigated the exposure of local eagle nestlings to mercury (Hg), an ubiquitous contaminant well-known to cause toxic effects in apex bird species. Nestling body feathers ( $n = 72$ ) showed significant variation in Hg concentrations over the studied years (2014-2019), with the highest median concentration recorded in 2015 (8.55  $\mu\text{g/g dw}$ ). Hg concentrations were not significantly different between sexes, nor between siblings. However, Hg concentrations were found to vary spatially: higher median Hg concentrations were found for nestlings within the active Danube river floodplain (7.46  $\mu\text{g/g dw}$ ) compared to those from the former floodplain (3.38  $\mu\text{g/g dw}$ ). We expect this to be due to different prey availability: fish, known to be high in Hg content, are less available than terrestrial prey, low in Hg content, in the former floodplain due to extensive drainage works carried out in the past. The stable isotope data will allow us to further shed light on this hypothesis. Finally, the observed Hg concentrations were considered to exceed natural background levels (generally considered to be below 5.00  $\mu\text{g/g dw}$ ), warranting continued monitoring of the Hg exposure and its pathways in this population in Kopački rit.

KEY WORDS: biomonitoring; dietary pathway; Hg; Kopački rit; non-destructive sampling

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## Acute exposure to silver nanoparticles and ionic silver triggers oxidative stress in tobacco plants

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The antimicrobial properties of silver and its enhanced reactivity when applied in nanoparticle form (AgNPs) have led to the increased utilisation of AgNPs in consumer products. However, their increasing release into water or soil represents a potential environmental hazard. AgNPs can give rise to detrimental effects in plants, mainly through an excess generation of reactive oxygen species (ROS), leading to the induction of oxidative stress. In this work, detached roots of *in vitro* grown tobacco (*Nicotiana tabacum*) plants were exposed to AgNPs stabilised with cetyltrimethylammonium bromide (CTAB) or polyvinylpyrrolidone (PVP) coating, or to ionic silver (AgNO<sub>3</sub>) applied at the same concentration (100 µmol/L) in ultrapure water for 24 h. The aim was to investigate *in situ* the early physiological responses as the first signs of stressful conditions. Generation of ROS in root cells was monitored by confocal laser scanning microscopy using highly sensitive and specific fluorescent probes: dihydroethidium (DHE) to detect O<sub>2</sub><sup>-</sup> and 2',7'-dichlorofluorescein-diacetate (H<sub>2</sub>DCF-DA) to detect H<sub>2</sub>O<sub>2</sub>. Propidium iodide was used to verify cell viability. Increased amounts of H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub><sup>-</sup> were observed in roots exposed to either AgNPs or AgNO<sub>3</sub>, compared to non-exposed roots, which was correlated to an increased percentage of dead cells, while the changes were most prominent after AgNP-CTAB treatment. Additionally, AgNPs were visualised in root cells and their uptake and accumulation could be linked to excess generation of ROS. Results show that AgNPs not only compromised cellular redox-homeostasis, but also possibly caused cell necrosis, while a different level of impact was observed depending on the form of applied silver and possibly on the intrinsic properties of the AgNP coatings.

KEY WORDS: confocal microscopy; fluorescent probes; nanosilver coatings; phytotoxicity; reactive oxygen species

P-70

## Effects of PMMA microplastic on the Mediterranean mussel *Mytilus galloprovincialis* after an experimental exposure

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The widespread occurrence of plastic is becoming an increasing problem in the natural environment. Microplastic particles (MPs) in the marine environment have subtler effects than larger fragments, as their size range overlaps with the size of particles preferentially ingested by filter-feeders. In this context, the effect of two-particle sizes (10 µm and 50 µm) of PMMA (polymethyl methacrylate, known as plexiglass) on the ecologically significant mussel *Mytilus galloprovincialis* was investigated. This experimental study focused on the determination of PMMA particles uptake by mussel gills and the subsequent transport of these microparticles to the haemolymph and digestive system causing adverse effects on mussel health. Mussel health status was evaluated by measuring the condition index (CI) and survival in air (SOS, stress on stress test) after 72 h exposure to low (0.1 mg/L), medium (1 mg/L) and high (10 mg/L) concentrations of PMMA microparticles. The effects of exposure to 10 µm and 50 µm PMMA particles were noted as increased total haemocytes counts and reduced cell viability at the 10 mg/L concentration of both particle sizes. Results also indicated a significant increase in levels of vacuolised haemocytes as a result of PMMA exposure. Only 10 µm PMMA was detected in the gills and digestive gland of exposed mussels by histological analysis and correlated with physiological damage as decreased condition and fitness indices in the exposed mussels. This study provides proof of the principle that MPs are taken up into tissue causing significant effects on mussel health.

KEY WORDS: condition index (CI); gills; haemocytes; microplastics particles (MPs); SOS test

P-71

## Impact of polystyrene and polymethylmethacrylate microplastics on sea urchin *Paracentrotus lividus* embryogenesis

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Microplastics in the marine environment has become an important issue in contemporary ecotoxicology due to its potential to cause harm to biota. There is still relatively little data available on the roles polymer microparticle size and chemical identity play in causing deleterious effects in a range of aquatic organisms. Thus, the present study was focused towards determining if microplastics could negatively impact early embryo development in the sea urchin species *Paracentrotus lividus*. Urchin sperm or zygotes were exposed to virgin polymethylmethacrylate and polystyrene microparticles in the size range 1-230 µm of diameter and at concentrations of 0.1 to 10 mg/L. The zygotes brought in contact with the microplastics only after fertilisation did not display a significant increase of developmental defects after 72 h. In contrast to embryo exposures, exposure of *P. lividus* sperm to the microplastics for 1 h resulted in modest, yet significant, effects. The spermotoxicity test indicated a decreased fertilisation rate, and increase of transmissible damage to offspring after 72 h. Plutei larvae were noted to have ingested 10 µm polymethylmethacrylate microparticles although ingestion of polystyrene microparticles was not observed. Such ingestion may play a role in polymethylmethacrylate microparticles causing greater toxicity than the corresponding polystyrene microparticles as noted in the present work. Ultimately, the relatively mild effects of these polymer microparticles on embryogenesis in *P. lividus* indicated that species sensitivity is an important factor to consider in view of the greater toxicity reported for similar microplastics on embryonal development in the sea urchin *Sphaerechinus granularis*.

KEY WORDS: developmental anomaly; embryo; offspring quality; spermotoxicity; transmissible damage

P-72

## Effects of two types of microplastics (car tire abrasion and polystyrene) on earthworms *Eisenia andrei* at multiple levels of biological organisation

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Microplastics are small plastic fragments of less than 5 mm in size, which are widely distributed in marine and terrestrial environments. Due to their key role in the soil ecosystem, earthworms are often used as model organisms in soil ecotoxicology. Therefore, the aim of this study was to evaluate the effects of the two most common types of microplastics on the earthworm *Eisenia andrei*. The tested microplastics included car tire abrasion products and polystyrene at 1 mg/kg and 1000 mg/kg, and 0.1 mg/kg and 100 mg/kg, respectively. The study was conducted by exposing earthworms to microplastics in natural soil for 2, 7, 14, and 28 days. Acute toxicity for these exposure times as well as avoidance behaviour after 48 h were evaluated. Further investigation included various biomarkers and subcellular endpoints; namely, carboxylesterase (CES), acetylcholinesterase (AChE), glutathione S-transferase (GST), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione (GSH) and reactive oxygen species (ROS) fluorescence, and multixenobiotic resistance activity (MXR). Since no mortality was observed, the same concentrations were chosen for all of the tested endpoints. Effects on subcellular level for both microplastics could be observed for all oxidative stress indicators (e.g., ROS and GSH fluorescence, CAT, GPx, and GR activity). Reproduction tests showed lower production of cocoons and juveniles compared to controls. The observed changes indicate the potential negative effect of microplastics on soil organisms. Our findings provide new insights regarding the toxicological effects of microplastics on the earthworm as a key organism of terrestrial ecosystems.

KEY WORDS: biomarkers; multixenobiotic resistance activity; oxidative stress; plastic fragments; reproduction

P-73

## ***In silico* prediction of the fate and toxic effects of IARC Group I anticancer drugs in the environment**

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Anticancer drugs and their metabolites discarded in wastewater upon excretion may negatively impact the reproduction and survival of many freshwater organisms. The aim of this *in silico* study was to predict the ecotoxic profile of the IARC Group I anticancer drugs (n=12) and elucidate their impact on the environment and health using four ADMET Predictor™ models: the fathead minnow acute toxicity model based on lethal effects on *Pimephales promelas* (Minnow LC<sub>50</sub>, TOX\_FHM), the concentration needed to inhibit 50 % of growth in the protozoa species *Tetrahymena pyriformis* (Th\_pyr\_pIGC<sub>50</sub>), the lethal concentration that results in the death of 50 % of *Daphnia magna* (water fleas) (Daphnia LC<sub>50</sub>, TOX\_DM) and the bioconcentration factor (BCF). The obtained results revealed the non-biodegradability of all molecules with the bioconcentration factor (BCF) computed in the range from 1.781 (thiotepa) to 175.844 (tamoxifen). Toxicities to the fathead minnow were computed in the range of Minnow LC<sub>50</sub> 0.002 (4-OH-tamoxifen and 4-OH-desmethyltamoxifen) to 3611.004 mg/mL (busulfan), toxicities to *Tetrahymena pyriformis* were computed in the range of Th\_Pyr\_pIGC<sub>50</sub> from 0.503 (thiotepa) to 2.366 mmol/L (tamoxifen), whereas toxicities to *Daphnia magna* were in the range of Daphnia LC<sub>50</sub> 0.069 (4-OH-desmethyltamoxifen) to 2047.628 mg/L (busulfan). QSAR analysis revealed a linear relationship between lipophilicity (MlogP) and Th\_pyr\_pIC50 ( $y=2.039x-0.204$ ,  $R^2=0.704$ ), and hyperbolic relationship with BCF. Furthermore, mutagenicity was predicted for alkylating agents (melphalan, cyclophosphamide, busulfan, thiotepa, and semustine), carcinogenicity for cyclophosphamide, etoposide, thiotepa and semustine, cardiotoxicity for tamoxifen and its OH- and N-dealkylated metabolites, with hepatotoxicity calculated only for semustine.

KEY WORDS: ADMET; bioconcentration factor; *Daphnia magna*; *Pimephales promelas*; *Tetrahymena pyriformis*; toxicity prediction tests

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## **The extra-trees regressor model in predicting autonomic neurotoxicity**

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Existing *in silico* models for predicting neurotoxicity of chemicals still have numerous limitations. Among the recently published models, the extra-trees regressor (ETR) model was recommended by Jiang et al. (2020) for quantitative prediction of autonomic nervous system toxicity. Our study aimed to assess the applicability of this model, developed on mouse data after intraperitoneal application of various chemicals, on the same species exposed via intravenous (*i.v.*), subcutaneous (*s.c.*) and peroral route (*p.o.*), for the same neurotoxic endpoint. Mouse LD<sub>50</sub> data related to autonomic neurotoxicity were collected from the ChemIDplus database by webchem R package. Molecular descriptors were calculated by PyBioMed package for 86 organic chemicals for *i.v.*, 65 for *s.c.* and 100 for *p.o.* routes. A separate ETR model for each route was trained to predict pLD<sub>50</sub> [ $-\log_{10}(\text{LD}_{50} \text{ mol/kg})$ ] related to autonomic neurotoxicity, using 19 molecular descriptors selected as most suitable by Jiang et al., and evaluated by leave-one-out cross-validation. Good agreement between the predicted pLD<sub>50</sub> values and those stated in the ChemIDplus database was found for the *s.c.* route (intra-class correlation 0.82, 95 % confidence interval 0.72-0.89,  $P<0.001$ ), although root mean square error was three times higher than in the model by Jiang et al. Moderate agreement was observed for the *i.v.* route (ICC 0.64, 95 % CI 0.50-0.75,  $P<0.001$ ), and poor for the *p.o.* route (ICC 0.41, 95 % CI 0.23-0.56,  $P<0.001$ ). The results indicate that the model developed by Jiang et al. has limited applicability for other exposure routes, especially non-parenteral, even when model parameters are learned specifically for a respective exposure route in the same species.

KEY WORDS: autonomic nervous system; *in silico*; intra-class correlation coefficient; LD<sub>50</sub>; mice

P-75

## Cadmium and autophagy: a toxicogenomics data mining approach

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Autophagy, a self-degradative process with an important role in removing dysfunctional cell components, is increasingly recognised in cadmium (Cd) toxicity. It is considered crucial in Cd-caused tissue injury, acting both as a protector and executioner of cell death. The aim of this study was to explore the relationship between Cd and autophagy using an *in silico* toxicogenomics data mining approach. The Comparative Toxicogenomics Database (CTD; <http://ctd.mdibl.org>) served as the main data source in this research, along with its Set Analyzer tool (<http://ctdbase.org/tools/analyzer.go>). Default settings were used for the analysis, meaning that 0.01 was set as the p-value threshold. It was shown that Cd interacted with 33, 34, and 55 genes linked with an autophagy increase in the cerebral cortex, proximal tubular, and bronchial epithelial cells, respectively. Some of these genes were *BECN1*, which encodes Beclin-1 protein and regulates autophagy, and *MAP1LC3B*, which encodes light chain 3 (LC3), one of the central proteins in autophagy. Cadmium was found to increase autophagosome assembly in adrenal medulla and mesenchymal stem cells, interacting with 11 genes. The top 5 of these genes included *PIK3C3*, associated with autophagosome preassembly machinery and formation of phosphatidylinositol 3-phosphate, as well as autophagy related genes, *ATG101*, *ATG12*, *ATG13*, and *ATG5*. These results provide a basis for further *in vitro* and *in vivo* investigation in order to better understand autophagy as one of the molecular mechanisms of Cd toxicity. This work was partially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia project 451-03-9/2021-14/200161.

KEY WORDS: CTD; genes; *in silico*; molecular mechanisms; toxic metal

P-76

## Investigating sulforaphane safety: *in silico* toxicogenomic data mining approach

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Sulforaphane is an organosulfur compound found in cruciferous vegetables including broccoli, Brussel sprouts, and cabbage. Depending on the amount and intended application, it is used as a dietary supplement, while increasing data even suggest its use as a medication for various diseases, including neurodegenerative, cardiovascular, and even cancer. This compound is regarded as safe and well-tolerated. However, studies on animals have shown that high doses of sulforaphane may induce sedation, hypothermia, motor coordination impairment, decrease in skeletal muscle strength, leucopenia, and even death. Thus, the aim of the present study was to further explore the potentially harmful effects of sulforaphane by applying toxicogenomic data mining. *Comparative Toxicogenomics Database* (CTD; <http://CTD.mdibl.org>) was used to identify the genes linked to sulforaphane toxicity. The *GeneMania* tool (<https://genemania.org>) was used to determine the interactions between the identified set of genes, while the *ToppGene* portal (<https://toppgene.cchmc.org/>) (p-value: 0.05) was used to determine the related molecular functions, biological processes, and molecular pathways. In the CTD, 6 genes were responsible for the sulforaphane toxicity (*ABCB1*, *BCHE*, *CDKN2A*, *CST3*, *CYP4F3*, *GSK3B*). The majority of the interactions between the genes indicated possible co-expression (95.77 %), while the rest were genetic interactions (4.23 %). Extracted binary interactions showed that sulforaphane increased the mRNA expression of *BCHE* and *CYP4F3* and decreased mRNA expression of *ABCB1* and *CDKN2A*. The top ranked molecular functions, biological processes, and molecular pathways were NF-kappaB binding, tissue remodelling, and C-MYC pathway, respectively. In conclusion, these preliminary results indicated that the sulforaphane safety profile and risk-to-benefit ratio require further evaluation. This project was funded by the bilateral Serbia-China project 451-03-1203/2021-09.

KEY WORDS: gene ontology; mechanisms; sulforaphane; toxicity

P-77

## The impact of air pollution on COVID-19 severity: *in silico* toxicogenomic data mining

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The new coronavirus (SARS-CoV-2) pandemic was followed by numerous complications of the COVID-19 disease, with indications that severe cases might correlate with air pollution exposure. The aim of this research was to determine the link between air pollutants [sulphur dioxide (SO<sub>2</sub>), carbon monoxide (CO), PM<sub>x</sub> – particulate matter, nitrogen dioxide (NO<sub>2</sub>), and ozone (O<sub>3</sub>)] and COVID-19 complications by toxicogenomic data mining. The *Comparative Toxicogenomics Database* (CTD; <http://CTD.mdibl.org>) was used to identify the set of common genes impacted by the examined pollutants and linked to COVID-19, while the *ToppGene* portal (<https://toppgene.cchmc.org/>) (p-value: 0.05) was used to determine biological processes, molecular pathways and diseases linked to the identified set of genes. SO<sub>2</sub>, CO, PM<sub>x</sub>, NO<sub>2</sub>, and O<sub>3</sub> were found to interact with 148, 104, 11573, 138, and 3578 genes, respectively. Out of these, 6, 6, 18, 9, and 12 were related to COVID-19, respectively, while *IL10*, *IL6*, *IL1B*, and *TNF* were in common for all of the investigated substances. The most significant molecular function extracted for these genes was cytokine activation, while the crucial biological processes were biosynthesis of NO and cytokine production, which might have contributed to the „cytokine storm” – an excessive immune inflammatory response found in severe cases of COVID-19. The identified gene set correlated with diseases of the respiratory, immune, cardiovascular, and endocrine system and lung cancer, indicating that COVID-19 patients afflicted by these diseases are more susceptible to additional complications. Further research on COVID-19 patients is needed to elucidate this relation. The study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia project 451-03-9/2021-14/200161.

KEY WORDS: air pollutants; bioinformatics; COVID-19 complications; *in silico*; respiratory diseases

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## Using dynamic energy budgets to account for eco- and nano-toxicity

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With the steep rise in both the variety and volume of nano-materials in use, ascertaining their toxicity has become a regulatory priority – especially since the effects of nano-materials can be orders of magnitude different from those of their ‘classic’, more researched counterparts. However, distinguishing ‘nano’ from ‘classic’ toxicity may be difficult; for example, bacteria exposed to nano-materials such as cadmium-selenium quantum dots (QDs) may be reacting just to the ionic cadmium released by dissolution of QDs, rather than the nano-material itself. In the example of *Pseudomonas aeruginosa* exposed to QDs, we show how Dynamic Energy Budget (DEB) models can be used to quantify effects attributable to the nano-scale of the toxicant. Even when differentiating between ‘nano’ and ‘classic’ toxicity may not be an issue, real-world experiments sometimes yield contradictory data; for example, indicators of toxic stress in soybeans exposed to ceria nanoparticles (nano-CeO<sub>2</sub>) increase with exposure, but yield – rather than decreasing due to stress – actually also increases for low exposures. We show how a DEB model can accommodate these data by helping to explore and select mechanisms leading to the measured outcomes. Exploring contradictory results using such modelling not only provides additional insights and value to data, but can also reduce the temptation of declaring an experiment a failure.

KEY WORDS: mechanistic modelling; *P. aeruginosa*; soybeans; toxicodynamics; toxicokinetics

P-79

## Physiologically-based model of organismal response to oxidative stress, applicable to ecotoxicology, and nanotoxicology

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Toxicants, radiation, and other stressors profoundly affect cell function and, consequently, the energy fluxes of an organism. Currently, the most advanced and complete metabolic theory – the Dynamic Energy Budget (DEB) theory – captures some of these effects by subjecting one or more DEB parameters to a stressor density as experienced by an organism, e.g. the body burden of a toxicant. In most applications, one or more DEB parameter values increase linearly with a stressor density below the no-effect concentration, which represents the capacity of an organism to mitigate adverse stressor effects. This approach neither explains the roots of no-effect concentration, nor has a mechanistic way of estimating energy costs of countering stress. The approach is also inconsistent with data where, although effective levels of exposure are reduced or even stopped, effects continue to escalate. Starting by considering an example of biochemical and regulatory network of a cell, we suggest a theoretical framework that abstracts the basic cellular processes to model dynamics of damage-inducing compounds and cellular damage, thus addressing many of the current shortcomings of the theory by: (i) relating state variables to data gathered through -omics approaches, (ii) capturing dynamics of damage and repair based on metabolism, (iii) offering a mechanistic description of the no-effect concentration, and (iv) describing tipping points leading to a runaway increase in damage as a function of exposure level and duration. Our models have a stronger mechanistic foundation than current formulations and may provide a process-based platform for modelling sublethal toxic effects and model-mediated data mining.

KEY WORDS: bioenergetics; data mining; mechanistic modelling; no-effect concentration; reactive oxygen species

P-80

## Elucidating the effects of plastics ingestion on individuals and populations – mechanistic modelling in risk assessments

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Plastic debris is widespread in marine environments, and interactions with plastics have been recorded for over 700 marine species. The two most frequent modes of interaction are ingestion and entanglement, both of which may result in the death of the organism. Non-lethal interactions, however, can be equally harmful, while taking longer to detect. This is especially true for animals with a naturally long life-cycle, where changes in life-cycle patterns can take decades to become apparent. Such species are sea turtles, which are also one of the most affected (by plastic debris) groups of organisms. We used the loggerhead turtle as a model organism to study the sublethal effects of plastic ingestion. The effects were studied at individual and population level. To achieve this, we linked, using physiological energetics, a certain quantity of plastics in the turtle's digestive contents to the effects that the ingested quantity would exert on the ontogeny of a turtle, i.e. its growth, maturation, and reproduction. These effects on the individual level should, in turn, affect the whole population of turtles because the population will be stable only if mortality losses within a population are offset by reproduction. The method is mechanistic and modular, so that it can be modified and applied in a straightforward and consistent way to any species of interest, for which sufficient (especially physiological) data is available. Such a broad and consistent approach can be a powerful tool for risk assessment, either while focusing on a specific species or an ecosystem/niche.

KEY WORDS: bioenergetics; dynamic energy budgets; green marine turtle; ontogeny; population modelling

CEC-1

## Exposure to endocrine disruptors is associated with alterations of the retinoid system in both experimental animal and human studies

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Retinoids are involved in essential functions such as vision, reproduction, development, and metabolism among others, insomuch as retinoid receptors regulate gene expression for the homeostasis of tissues. In fact, retinoid supplementation programmes are implemented globally against vitamin A deficiency. Currently, the retinoic acid signalling pathway is being evaluated for enhancing test methods aimed at identifying endocrine disruptors. Additionally, key elements of the retinoid system have already been proposed in adverse outcome pathways for developmental disorders. Herein, the effects of endocrine disruptors and persistent organic pollutants on key elements of the retinoid system in experimental animal studies and human observational studies are described as, for example, altered concentrations of all-*trans*-retinoic acid in the liver and retinol in adipose tissue as well as increased ratios of the retinol binding protein 4/retinol in serum. Although the mechanisms of toxicity have not been fully elucidated, these retinoid endpoints have been associated with the onset of chronic conditions of high public concern such as obesity, diabetes, and cardiovascular diseases, etc. Taking into consideration the importance of the retinoid system, increasing the amount of information on key elements of the retinoid system of human populations would allow a better understanding of the effects derived from human exposure to endocrine disruptors and hence protect human health.

KEY WORDS: endocrine disruptors; persistent organic pollutants; retinoic acid; testing methods



CEC-2

## Fate and effects of cytostatic pharmaceuticals in environmental and occupational settings

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Cytostatics have been used in cancer treatment since the 1940s. However, many of them are also carcinogenic, mutagenic and teratogenic, triggering widespread concerns about the risks they pose to the environment and to healthcare workers in occupational settings. Here, we focus on the toxicity of the most commonly used cytostatics (5-fluorouracil, cisplatin, etoposide, imatinib mesylate, cyclophosphamide and paclitaxel) with different modes of action (MoA) using different experimental models and biomarkers both *in vitro* and *in vivo*. Depending on the cytostatic used, these pharmaceuticals, as single substances, proved to be cyto/genotoxic at environmentally relevant concentrations. Moreover, in both environmental and occupational settings, simultaneous exposure to cytostatics may occur creating a higher risk than that of a single substance. By testing different cytostatic mixtures, they also showed their potential to induce cell and genome damage even at very low concentrations. The obtained results indicated not only that such mixtures may pose a risk, but also that single compound toxicity data are not always sufficient for predicting toxicity in a complex environment. The presence of pharmaceuticals in different amounts and with different MoA suggests the need to study the relationship between the presence of genotoxic components in the mixture and the resulting effects, taking into account the MoA of each component by itself. Altogether, the cumulative data emphasise the need for further toxicological screening of cytostatics and other pharmaceuticals to avoid any possible adverse effects on the environment and human health and provide new data sets necessary for scientifically-based risk assessments.

KEY WORDS: cyto/genotoxicity; cytostatic drugs; environmental and occupational exposure; risk assessment; toxicology of mixtures

CEC-3

## Genotoxic properties of low doses of cadmium and phthalates

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Cadmium and phthalates are often found as environmental contaminants. Cadmium enters the food chain both naturally and from industrial and agricultural sources. Foodstuffs are the main source of cadmium exposure for the non-smoking general population (cereal products, vegetables, nuts and pulses, starchy roots or potatoes, and meat and meat products). Phthalates are not directly acting genotoxic compounds but are associated with a variety of health outcomes. Diet is considered a significant exposure pathway for these compounds. Poultry, cooking oils, and cream-based dairy products are the main food groups in which these compounds can be found. In this presentation, the genotoxic potential of both compounds will be presented. Different human cell lines will be used as biological test systems; human larynx carcinoma (HEp2) as a representative of the epithelial cells which are first in contact with these contaminants after ingestion; human colon carcinoma (Caco-2) cells, and human hepatocellular carcinoma (HepG2) cells in which metabolic reactions and presence of certain endogenous compounds can influence the overall (geno)toxicity of investigated compounds. Ames strains *Salmonella typhimurium* TA98 and TA100 are useful in the determination of the potential of these two chemicals to cause point mutations.

KEY WORDS: environmental contaminants; genotoxicity; human cell lines; point mutations; test systems

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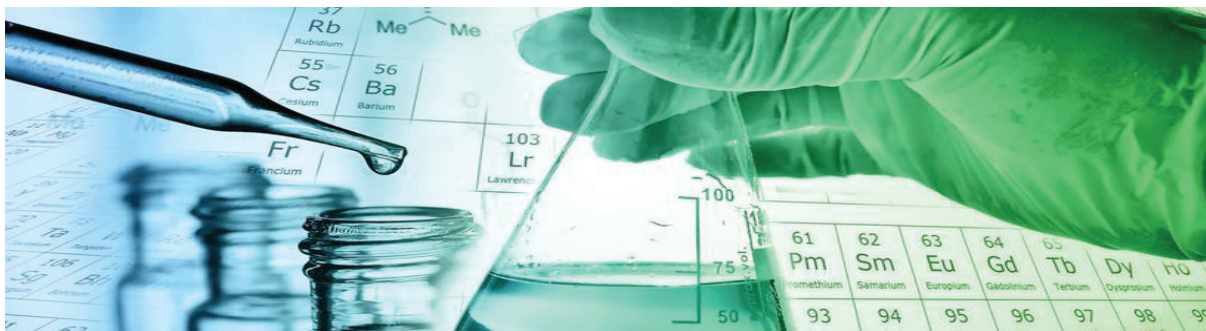
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